OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

47

Microfiche No. (7) •	Ø421	1	No. of Pages 2
Doc I.D.	FYI-OTS-0785-0421 FW	3	Old Doc I.D. 4
Case No.(s)	—		5
Date Produced (6)	— 6	Date Rec'd (6) 071885 7	Conf. Code • N 8

Check One: ☐ Publication ☐ Internally Generated ☒ Externally Generated

Pub/Journal Name — 9

9

Author — 10

Organ. Name DEPT OF HEALTH & WELFARE 11

Dept/Div HEALTH PROTECTION BRANCH 12

P.O. Box — 13 Street No./Name TUNNEYS PASTURE 14

City OTTAWA 15 State ONTARIO 16 Zip — 17 Country CA 18

MID No. (7) — 19 D & B NO. (11) — 20

Contractor — 21

Doc Type • X.G • U.P • F.Y.I.V.S F.W D. 22

Doc Title 23

LONG-TERM TOXICITY OF OCTACHLOROSTYRENE IN THE RAT WITH COVER LETTER 070285

Chemical Name (300 per name) 25	CAS No. (10) 24
OCTACHLOROSTYRENE	29082-74-4

1A

RM 8/19/85

Health and Welfare Canada
Santé et Bien-être social Canada

Health Protection Branch
Direction générale de la protection de la santé

Environmental Health Centre
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

7-16-85
47

INFO. CONTROL EPA
1985 JUL 16 AM 10: 14

July 2, 1985

FYI-OTS-0785-0421 FLWP
SEQUENCE D

Mr. T. O'Bryan
Document Control Officer
Information Management Division
(TS-793)
Office of Toxic Substances
401 M. Street, SW
Washington, D.C. 20460
U.S.A.

RECEIVED 7/18/85 TOB/CSB

Dear Mr. O'Bryan:

In reply to the June 6, 1985, letter from Frank D. Kover, Chief, Chemical Screening Branch/ECAD, soliciting unpublished information concerning polychlorinated styrenes and triphenyl phosphite, I am pleased to be able to provide you with copies of a recent unpublished study and three recently published articles dealing with toxic effects of octachlorostyrene.

Please find enclosed copies of the following publications:

Chu, I., Villeneuve, D.C., Secours, V.E., Valli, V.E., Leeson, S., and Shen, S.Y. Long-Term Toxicity of Octachlorostyrene in the Rat. Fundamental and Applied Toxicology (in press).

Chu, I., Villeneuve, D.C., Secours, V.E., Yagminas, A., Reed, B., and Valli, V.E. Octachlorostyrene: A 90-Day Toxicity Study in the Rat. Fundamental and Applied Toxicology, 4, 547-557.

Chu, I., Villeneuve, D.C., Secours, V., Benoit, F.M., and Viau, A. (1982) The Tissue Distribution, Metabolism, and Excretion of Octachlorostyrene in the Rat. Drug Metabolism and Disposition, 10(6), 632-635.

Chu, I., Secours, V.E., Villeneuve, D.C., and Valli, V.E. (1982). Acute and Subacute Toxicity of Octachlorostyrene in the Rat. J. Toxicology and Environmental Health, 10, 285-296.

Also enclosed is a draft report, prepared by staff of this Directorate, entitled "Polychlorinated Styrene: An Environmental Health Perspective" which deals with the environmental health implications of Polychlorinated styrenes. This report has as yet not been approved for publication and I would therefore be grateful if this draft was not quoted, cited, or circulated.

Canada

FYI-0421 D

I hope that the information provided will be useful for your review. I regret, however, that I am not able to provide any relevant information regarding triphenyl phosphate.

Yours sincerely,

P. Toft

P. Toft
Chief
Monitoring and Criteria Division

613-990-9071

Enclosures

** Spoke with Dr. Toft concerning this matter. We can cite the information using their address. He didn't want the document cited as a "personal" [illegible]. Therefore, we can cite it as a draft document until further notice.

Also phone conversation P. Toft on 29 July 85

I hope that the information provided will be useful for your review. I regret, however, that I am not able to provide any relevant information regarding triphenyl phosphate.

Yours sincerely,

P. Toft

P. Toft
Chief
Monitoring and Criteria Division

Enclosures

Accepted for publication by the Fundamental & Applied Toxicology

LONG-TERM TOXICITY OF OCTACHLOROSTYRENE IN THE RAT

I. Chu[1], D.C. Villeneuve[1], V.E. Secours[1], V.E. Valli[2]
S. Leeson[3] and S.Y. Shen[4]

[1]Environmental and Occupational Toxicology Division,
Bureau of Chemical Hazards,
Environmental Health Directorate,
Ottawa, Ontario. Canada
K1A 0L2

[2]Biopath Analysts Limited,
Guelph, Ontario.
N1E 2X7

[3]Department of Animal Poultry,
University of Guelph,
Guelph, Ontario.
N1G 2W1

[4]Department of Public Health,
Shanxi Medical College,
Taiyuan, The Peoples Republic of China.

ABSTRACT

Long-term toxicity of octachlorostyrene in the rat, Chu, I., Villeneuve, D.C., Secours, V.E., Valli, V.E. and Leeson, S., Shen, S.Y. (0000) Fundam. Appl. Toxicol. (000000000).

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.

INTRODUCTION

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 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 byproduct 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). 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 hea[illegible] [illegible]ty change in liver was determined in frozen sections as previously [illegible] 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 b 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 6-12 months of experiment. Based on the body weight and dietary consumption data, the OCS ingested ranged from 0.0003-3.1 mg/kg b.w./day and 0.0004-4.4 mg/kg b.w./day for the males and females respectively (Table 1).

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; $p < 0.05$). 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 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 towards 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 a moderate to marked accentuated 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 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 density 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 and frequently occurred in aging rats. 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.

(Kuntzman, 1969). A comparison was made on 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 of the similar nature in the 12 month exposure. The dose levels at which these changes occurred were around 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 one 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, 1984b). 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, it ws 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

GC analysis showed a dose-dependent accumulation of OCS residue in fat, liver and 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 a previous 28-day (Chu et al., 1982a) and 90-day studies (Chu et al., 1984a) in which rats were treated with OCS in the diet 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 myeloid hypertrophy reported in the 90-day study were not seen in the long-term experiment (Chu et al., 1984a). 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

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 the histological evaluation is the most sensitive index of OCS exposure since changes were observed in the target organs at levels between 0.005 - 0.5 ppm levels. However, these changes are considered to be mild and adaptative in nature. Significant histological changes only 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; Chu et al., 1984a) the no adverse effect level was judged to be 0.5 ppm in the diet (31 μg/kg b.w./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 b.w./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.

ACKNOWLEDGEMENT

The authors are indebted to Drs. T. Kuiper-Goodman and L. Whitehouse for useful discussion, N. Beament, A. Viau, J. Kelly, A. Yagminas and B. Reed for technical assistance, M. Beaudette for data handling and J. Ireland for typing the manuscript.

REFERENCES

Berg, B. (1967) "Pathology of Aging Rats" in Pathology of Laboratory Rats and Mice, ed. Cotchin, E. and Roe, F.J. Blackwell Scientific Publications, Oxford and Edinburgh.

Bieniek, D., and Korte, F. (1978) Einfache synthese des octachlorstyrols. Chemosphere, 7, 729-730.

Chu, I., Secours, V.E., Villeneuve, D.C., and Valli, V.E. (1982a) Acute and subacute toxicity of octachlorostyrene in the rat. J. Toxicol. Environ. Health, 10, 285-296.

Chu, I., Villeneuve, D.C., Secours, V.E., Benoit, F.M., and Viau. A. (1982b). The tissue distribution, metabolism and excretion of octachlorostyrene in the rat. Drug Metab. Dispos. 6, 632-635.

Chu, I., Villeneuve, D.C., Secours, V.E., Yagminas, A., Reed, B., and Valli, V.E. (1984a) Octachlorostyrene: A 90-day toxicity study in the rat. Fundam. Appl. Toxicol. 4, 547-557.

Chu, I. (1984b) Unpublished data.

Cochin, J., and Axelrod, J. (1959) Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. J. Pharmacol. Exp. Ther. 125, 105-110.

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

Furth, S., Nakane, P., and Pasteels, J.C. (1976) Tumours of the pituitary gland. In "Pathology of Tumours in Laboratory Animals, ed. S. Turusov; IARC Sci. Publ. No. 5., Vol. 1, Part 2, IARC, Lyon.

Grant, D.L., Iverson, F., Hatina, G.V. and Villeneuve, D.C. (1974) Effects of hexachlorobenzene on liver porphyrin levels and microsomal enzymes in the rat. Environ. Physiol. Biochem. 4, 159-165.

Holme, J.A. and Dybing, E. (1983) Increased cytochrome P-450 independent drug metabolism and mutagen activation in rat liver by octachlorostyrene. Acta. Pharmacol et Toxicol., 53, 325-332.

Kuehl, D.W., Kopperman, H.L., Veith, G.D. and Glass, G.E. (1976) Isolation and identification of polychlorinated styrenes in Great Lakes fish. Bull. Environ. Contam. Toxicol. 16, 127-132.

Kuehl, D.W., Johnson, K.L., Butterworth, B.C., Leonard, E.N. and Veith, G.D. (1981) Quantification of octachlorostyrene and related compounds in Great Lakes fish by gas chromatography-mass spectrometry. J. Great Lakes Res. 7, 330-335.

Kuntzman, R. (1969) Drug and enzyme induction. Annu. Rev. Pharmacol. 9, 21-36.

Nie, N.H., Hull, C.H., Jenkins, J.G., Steinbrenner, K., and Bent, D.H. (1977) Statistical Programs for the Social Sciences, SPSS, Inc., Chicago.

Ofstad, E.B., Lunde, G., and Martinsen, K. (1978) Chlorinated aromatic hydrocarbons in fish from an area polluted by industrial effluents. Sci. Total Environ. 10, 219-230.

Reichel, W.L., Prouty, R.M., and Gay, M.L. (1977) Identification of polychlorinated sytrene compounds in heron tissues by gas-liquid chromatogrpahy-mass spectrometry. J. Assoc. Off. Anal. Chem. 60, 60-62.

Ruetman, S.H. (1973) Synthesis of perchlorinated vinyl compounds. Synthesis 11, 680-681.

Schwartz, S. and Wikoff, H.M. (1952) The relation of erythrocyte coproporphyrin and protoporphyrin to erythropoiesis. J. Biol. Chem. 194, 563-573.

Statistics Canada Report No. 32-230 (1983). Apparent percapita food consumption in Canada.

Strik, J.J.T.W.A., and Koeman, J.H. (1975) Prophyrinogenic action of hexachlorobenzene and octachlorostyrene. In Porphyrins in Human Diseases M. Doss and M. a. d. Lahn, eds., pp. 418-423, Karger, Basel.

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

Yagminas, A.P. and Villeneuve, D.C. (1977) An automated continuous flow assay for serum sorbitol dehydrogenase activity and its use in experimental liver damage. Biochem. Med. 18, 117-125.

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 b.w./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 ± S.D. obtained from 18-20 animals.
[b] Different from controls at the $P < 0.05$ 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 b.w./day is calcualted by the formula

$$\frac{1000}{\frac{\text{initial wt + final wt.}}{2 \times 1000}} \times \text{ppm} \times \text{food consumption.}$$

000019

Table 2. Biochemical Changes of Rats Fed Octachlorostyrene[a]

Treatment Octachlorostyrene (ppm)	Wet liver weight (g)	Liver weight % body weight	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 ± S.D. obtained from 18-20 animals.
[b] Significantly different from control at the $P < 0.05$ level.

000020

Table 3. Incidence of Histological Changes in Rats Treated With Octachlorostyrene for 12 Months[a,b]

Treatment Octachlorostyrene (ppm)	LIVER Architecture	Hepatocellular Nuclei	Hepatocellular Cytoplasm	Bile duct	Interstitium	THYROID Architecture	Epithelium	Colloid	Interstitium	KIDNEY 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 $\frac{\text{number of animals showing changes}}{\text{number of animals examined}}$

[b] Specific changes

Liver: Architecture - accentuated zonation. Hepatocellular nuclei-anisokaryosis. Hepatocellular cytoplasm - increased cytoplasmic volume and eosinophilic aggregated basophilic, 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.

00002

Table 4. Incidence of Tumors in Rats Fed Octachlorostyrene

	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 $\frac{\text{number of animals showing tumors}}{\text{number of animals examined}}$

Table 5. Residues of Octachlorostyrene in Rat Tissues[a]

Treatment	Tissues					
	Male			Female		
	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 ± 5.6[c]		679 ± 203[c]

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

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

Table 6. Severity of Histological Changes in Thyroids of Rats fed OCS for (A) 12 months and (B) 10 Days

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:	Mild reduction in follicle size to 75% of the normal size in at least 25% glandular area	2
	Moderate reduction in follicle size to 50% of the normal size in 50% of area	3
	Marked reduction in follicle size to 25% or less of normal size in 75% of area	4
Epithelium:	Mild increase in height to moderate cuboidal in 50% of glandular area	2
	Moderate increase in height to high cuboidal in 75% of the area	3
	Marked increase in height to columnar in 75% of the area	4
Colloid:	Mild reduction in colloidal density in 50% of glandular area	2
	Moderate reduction in the density in 75% of the area	3
	Marked reduction in the density in 75% of the area	4

Results represent mean ± S.D. obtained from 19-20 animals from Group A's and 14-15 animlas from Group B's.

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

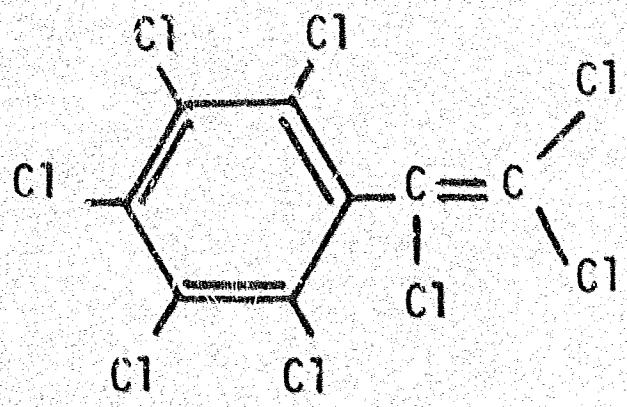


Figure 1. Structure of Octachlorostyrene

OFFICE OF TOXIC SUBSTANCES

CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

Microfiche No. (7) *	Ø421	1	No. of Pages 2
Doc I.D.	FYI-OTS-0785-0421 FW	3	Old Doc I.D. 4
Case No.(s)			5
Date Produced (6)	010184	6	Date Rec'd (6) 071885 7
Conf. Code *	N	8	

Check One: ☒ Publication ☐ Internally Generated ☐ Externally Generated

Pub/Journal Name 9

FUNDAM APPL TOXICOL

9

Author(s) 10

CHU I; VILLENEUVE D C; SECOURS

Organ. Name 11

Dept/Div 12

P.O. Box 13 Street No./Name 14

City 15 State 16 Zip 17 Country 18

MID No. (7) 19 D & B NO. (11) 20

Contractor 21

Doc Type 22

* X.G * P.U * F.Y.I V.S F.W D.

Doc Title 23

OCTACHLOROSTYRENE: A 90-DAY TOXICITY STUDY IN THE RAT VOL 4 P 547

Chemical Name (300 per name) 25	CAS No. (10) 24
OCTACHLOROSTYRENE	29082-74-4

REV 8/17/8

FUNDAMENTAL AND APPLIED TOXICOLOGY 4, 547–557 (1984)

Octachlorostyrene: A 90-Day Toxicity Study in the Rat

I. CHU,* D. C. VILLENEUVE,* V. E. SECOURS,* A. YAGMINAS,*
B. REED,* AND V. E. VALLI†

*Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Environmental Health Directorate, Ottawa, Ontario K1A 0L2, and †Biopath Analysts Ltd., Guelph, Ontario N1E 2X7, Canada

Octachlorostyrene: A 90-Day Toxicity Study in the Rat. CHU, I., VILLENEUVE, D. C., SECOURS, V. E., YAGMINAS, A., REED, B., AND VALLI, V. E. (1984). *Fundam. Appl. Toxicol.* 4, 547–557. This study was designed to provide information on the subchronic toxicity of octachlorostyrene (OCS), a demonstrated environmental pollutant in fish from the Great Lakes of North America and the Norwegian coast in Europe. Groups of 15 male and 15 female rats were administered OCS mixed in the diet at 0.05, 0.5, 5.0, 50, or 500 ppm for 13 weeks. Increased liver weights were observed in male and female rats fed 50 ppm OCS and higher, while enlarged kidney and spleen were noted in the highest dose groups. Hepatic microsomal enzyme induction occurred at 5.0 ppm OCS and higher for the males and 50 ppm and higher for the females. The chemical produced serum biochemical changes at concentrations as low as 5.0 ppm. OCS treatment resulted in hematological disturbances starting with the 0.5-ppm dose group. Dose-dependent histological changes were observed in the thyroid, kidneys, and liver of the treated animals. OCS residues accumulated in a dose-related fashion in the liver and fat of treated animals. These results indicate that OCS produced toxic effects at low levels of exposure and accumulated in the tissues of rats.

Octachlorostyrene (OCS, see Fig. 1) is an environmental contaminant 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 caught off the Norwegian coast (Ofstad *et al.*, 1978). 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 presence as an environmental pollutant, concern has been raised regarding possible effects upon man or animals from its toxicity and bioaccumulation. Previous work has shown that OCS is porphyrinogenic in Japanese quail (Strik and Koeman, 1975) and can produce serum biochemical changes, hepatic microsomal enzyme induction (*P*-450 type), and histological changes in the liver and thyroid of rats (Chu *et al.*, 1982a). In a metabolism study in rats it was found that most of the administered OCS was stored in the fatty tissues while only a small fraction was excreted as pentachlorophenyldichloroacetic acid in feces (Chu *et al.*, 1982b). The purpose of the present study was to determine the effects of prolonged feeding of OCS in the rat.

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. mp 99–100°C) (Huetman, 1973). The identity of the compound was confirmed by GC/MS (M^+ at m/e 376, 378, 380, 382) (Finnigan Instrument, Model 4000, data system 6000). All other chemicals used were of commonly available reagent grade.

Groups of 15 male and 15 female weanling Sprague–Dawley rats were fed diets (Master Fox, Purina–Ralston)

0272-0590/84 $3.00
Copyright © 1984 by the Society of Toxicology.
All rights of reproduction in any form reserved.

0090-9556/82/1006-0632$02.00/0
DRUG METABOLISM AND DISPOSITION
Copyright © 1982 by The American Society for Pharmacology and Experimental Therapeutics
Vol. 10, No. 6
Printed in U.S.A.

THE TISSUE DISTRIBUTION, METABOLISM, AND EXCRETION OF OCTACHLOROSTYRENE IN THE RAT

I. CHU, D. C. VILLENEUVE, V. SECOURS, F. M. BENOIT,[1] AND A. VIAU

Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Environmental Health Directorate, Canada

(Received April 21, 1982, and in revised form August 12, 1982)

ABSTRACT:

The tissue distribution, metabolism, and elimination of ^{14}C-octachlorostyrene (OCS) were studied in the rat. OCS was absorbed in the gastrointestinal tract after oral administration and distributed in all tissues examined. The highest concentrations were found in fat followed by adrenal glands, skin, and lungs. Decay of radioactivity in the tissues followed first-order kinetics. Approximately 8% of an iv dose was excreted in feces during 7 days after administration, while negligible amounts were found in the urine. More than 90% of the radioactivity in feces was due to the unchanged compound, while pentachlorophenyldichloroacetic acid and heptachlorostyrene in equal proportions accounted for the remaining 10%. A small amount (1%) of the dose was detected in the expired air as carbon dioxide.

Octachlorostyrene is an environmental contaminant which has been identified in Great Lakes fish (1) and herons at levels of 0.01–0.43 ppm (2). This chemical has also been found in fish caught off the Norwegian coast (3). The source of contamination is not clear but it is believed to be a byproduct in industrial processes involving carbon and chlorine in high temperature reactions (3).

Because of its presence as an environmental pollutant, concern has been raised over its toxicity and bioaccumulation. Strik and Koeman have reported that OCS is a potent porphyrinogen in the rat and Japanese quail (4). Recent acute and subacute studies have shown that OCS can produce serum biochemical changes, hepatic microsomal enzyme induction (cytochrome P-450 type), and histological changes in the liver and thyroid of rats (5). In view of its presence in the environment and the potential for human exposure, the present study was carried out to investigate the metabolism of OCS in the rat.

Materials and Methods

Syntheses. *2,6-Dichlorophenyl[^{14}C]methylcarbinol.* ^{14}C-Methyl iodide (5 mg, specific activity 5.7 mCi/mmol) with a radiochemical purity of 98% (New England Nuclear, Boston, Mass.) was diluted with unlabeled methyl iodide (0.9 g) in anhydrous ether (5 ml) and allowed to react with dry magnesium turnings (160 mg) to give methylmagnesium iodide. The Grignard reagent was added to an ethereal solution of 2,6-dichlorobenzaldehyde (1.0 g) to yield the desired alcohol (1.0 g). PMR (Brucker 80 MHz, DMSO-d_6): δ1.47 ppm (d, 3, J = 7.0 Hz, CH_3), 5.5 ppm (q, 1, J = 7.0 Hz, CH), 7.2–7.8 ppm (5 aromatic protons).

2,6-Dichloro[β-^{14}C]styrene. A mixture of 2,6-dichlorophenyl[^{14}C]methylcarbinol (1.0 g) and polyphosphoric acid (10 g) was heated with vigorous stirring for 5 min. The reaction was stopped by addition of crushed ice (100 g) followed by extraction with two 50-ml portions of ether. The combined extracts were evaporated to dryness and the oily residue which remained was purified by column chromatography using Florisil and hexanes to give 0.7 g of 2,6-dichlorostyrene.

Octachloro[β-^{14}C]styrene. This compound was synthesized from 2,6-dichlorostyrene (0.7 g) according to a method reported previously (5). The yield was 0.75 g. The radiochemical purity was >99% as analyzed by TLC with use of radiochromatogram scanner (Actigraph III, Nuclear-Chicago). The specific activity was 0.54 μCi/mg. M.p. 100.5–101°C; lit. 99–100° (6).

Procedure. Groups of four male Sprague-Dawley rats (Biobreeding Labs., Montreal, Que.) weighing 300–350 g were administered single doses of ^{14}C-OCS (40 mg/kg, 21.6 μCi) dissolved in Emulphor[2]/ethanol/physiological saline (1:1:8, v/v) *via* the femoral vein. The animals were kept in stainless-steel metabolism cages for the separate collection of urine and feces and were killed at various times after dosing to harvest tissues for the estimation of radioactivity.

The bile ducts of three rats were cannulated with Silastic tubing (0.031 cm i.d., 0.064 cm o.d., Dow-Corning, Toronto, Ont.). Single doses of ^{14}C-OCS (20 mg/kg) in Emulphor solution were administered *via* the femoral vein. Serial bile samples were taken hourly for 8 hr and then at 24 hr for the estimation of radioactivity. To determine if radioactivity was excreted in the expired air, two rats were given by gavage ^{14}C-OCS, 20 mg/kg in corn oil, and were kept individually in Jencons model Mark IV metabolism cages (Hemel Hempstead, Herts, England). The expired air was allowed to pass through two traps. The first one contained 300 ml of toluene and the second, 300 ml of ethanolamine/2-ethoxyethanol (1:2, v/v). Procedures for the analysis of radioactivity in the tissues, bile, and trapping solutions have been described elsewhere (7). Radioactive materials in fat, liver, and feces were extracted, isolated, and identified by GC and GC/MS according to a method previously described (7). The GC and GC/MS conditions used for the analysis of the extract were as follows:

A Hewlett-Packard model 5710A gas chromatograph was equipped with a 6.1-mm i.d. × 100-cm glass column packed with 3% OV-17 on Chromosorb WHP, and a flame-ionization detector. The oven, detector, and injection-port temperatures were set at 250, 300, and 300°C, respectively. The flow rate of carrier gas (N_2) was set at 40 ml/min. A capillary GC column (DB-5, 30 m × 0.25 mm) was connected directly to a Finnigan model 4000 mass spectrometer with data system model 6000, operated at 70 eV. The oven temperature was initially held at 40°C for 0.1 min and then increased at 15°C/min to 225°C. Helium was the carrier gas at a head pressure of 14 psi. The injection-port temperature was 225°C.

Results

Radioactivity was distributed in all tissues examined after administration of OCS (table 1). The fat retained the highest levels

[1] Monitoring and Criteria Division, Environmental Health Directorate.

Send reprint requests to: I. Chu, Ph.D., Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Environmental Health Directorate, Tunney's Pasture, Ottawa, Ont. K1A 0L2, Canada.

[2] Emulphor (Domtar, Canada) is a surfactant used to help dissolve lipophilic drugs in aqueous media.

000002

ACUTE AND SUBACUTE TOXICITY OF OCTACHLOROSTYRENE IN THE RAT

I. Chu, V. E. Secours, D. C. Villeneuve

Environmental Health Directorate, Health Protection Branch, Ottawa, Canada

V. E. Valli

Biopath Analysts, Guelph, Ontario, Canada

This study was designed to investigate the acute and subacute toxicity of octachlorostyrene (OCS), a demonstrated environmental pollutant in fish from the Great Lakes and off the Norwegian coast in Europe. In an acute study, groups of 10 male rats were given by gavage single doses of OCS at 1300, 1690, 2190, 2850, or 3710 mg/kg and killed 14 d later. Octachlorostyrene at a dose of 1690 mg/kg and higher caused increased liver weight, increased hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities, and increased serum cholesterol and uric acid levels. Only mild histological changes were seen in the thyroid of the treated animals. In a subacute study, groups of 10 male and female rats were fed diets containing 0.5, 5.0, 50 or 500 ppm OCS for 28 d. Growth rate and food consumption were not affected by treatment. Liver hypertrophy and hepatic microsomal enzyme induction were observed in animals fed 50 ppm OCS or higher. Elevations in serum cholesterol, total protein, potassium, and sorbitol dehydrogenase (SDH) occurred in rats fed the 500 ppm diet. Histological changes occurred in the liver and thyroid of rats exposed to as low as 5.0 ppm OCS. The OCS residues accumulated in the fat and liver in a dose-dependent manner. These data suggest that OCS can produce biochemical and histological changes in rats after administration of a single oral dose and/or when fed in the diet.

INTRODUCTION

Octachlorostyrene (OCS) is an environmental chemical that has been identified in Great Lakes fish (Kuehl et al., 1976) and heron at levels of 0.01–0.43 ppm (Reichel et al., 1977). This chemical has also been found in fish caught in the Norwegian coast (Ofstad et al., 1978). The sources of contamination have not been identified but OCS is believed to be a by-product in the manufacture of magnesium.

Because of its presence as an environmental pollutant, concern has been raised over its toxicity and possible bioaccumulation. Strik and

The authors wish to thank Dr. I. A. Marino, N. Beament, J. Kelly, B. Reed, A. Viau, and A. Yagminas for technical assistance; M. Beaudette for data handling, and J. Ireland for typing the manuscript.

Requests for reprints should be sent to I. Chu, Environmental Health Directorate, Health Protection Branch, Ottawa, Canada.

Journal of Toxicology and Environmental Health, 10:285–296, 1982
Copyright © 1982 by Hemisphere Publishing Corporation
0098-4108/82/070285-12$2.25

000002

OFFICE OF TOXIC SUBSTANCES

CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

51

Microfiche No. (7) •	0421 1	No. of Pages	2
Doc I.D.	FYI-OTS-0785-0421 FW 3	Old Doc I.D.	4
Case No. (s)			5
Date Produced (6)	6	Date Rec'd (6)	7
120184		071885	
Conf. Code •	8		
N			

Check One: ☐ Publication ☐ Internally Generated ☒ Externally Generated

Pub/Journal Name 9

9

Author(s) 10

Organ. Name 11

CANADA DEPT OF NATL HEALTH &

Dept/Div 12

P.O. Box 13 Street No./Name 14

City 15 State 16 Zip 17 Country 18

CA

MID No. (7) 19 D & B NO. (11) 20

Contractor 21

Doc Type 22

• X.G • U.P • F.Y.I.V.S F.W D.

Doc Title 23

POLYCHLORINATED STYRENES: AN ENVIRONMENTAL HEALTH PERSPECTIVE

Chemical Name (300 per name) 25	CAS No. (10) 24
OCTACHLOROSTYRENE	29082-74-4

1A

DRAFT

POLYCHLORINATED STYRENES:
AN ENVIRONMENTAL HEALTH PERSPECTIVE

Environmental Health Directorate

Health Protection Branch

December 1984

ACKNOWLEDGEMENT

This report was prepared by staff of the Bureau of Chemical Hazards, Environmental Health Directorate, Health Protection Branch, Department of National Health and Welfare. The principal author was Mrs. J. Sitwell. The assistance of Dr. V.C. Armstrong, Dr. I. Chu and Dr. J.A. Buccini by way of helpful discussions and comments during the preparation of this report and in reviewing some or all of the sections is acknowledged.

PREFACE

The chlorinated styrenes had been listed as candidate chemicals under the Environmental Contaminants Act of Canada, in 1980, but were removed from the list in 1981 owing to the lack of commercial activity involving these chemicals. However, there remained uncertainty over whether these substances were inadvertent contaminants in the Canadian environment, possibly posing a hazard to human health. This report was prepared to review the occurrence of polychlorinated styrenes in the Canadian environment and to assess the environmental health implications of these chemicals. Scientific literature, available up to December 1983, was reviewed. It was intended that the report would provide a basis for deciding whether any further investigation might be required under the Environmental Contaminants Act.

CONTENTS

	Page
ACKNOWLEDGEMENT	(i)
PREFACE	(ii)
1. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	1
1.1 Introduction	1
1.2 Summary	1
1.3 Conclusions	3
1.4 Recommendations	4
2. IDENTITY, PHYSICAL PROPERTIES, SYNTHESIS, REACTIONS	6
2.1 Nomenclature	6
2.2 Structures	7
2.3 Physical Properties	8
2.4 Synthesis	8
2.4.1 Octachlorostyrene	8
2.4.2 Hepachlorostyrene	9
2.4.3 Hexachlorostyrene	10
2.5 Reactions	10
. ANALYTICAL METHODS AND EXTRACTION PROCEDURES	10
3.1 Analytical Methods	10
3.1.1 Gas chromatography	11
3.1.2 Mass spectroscopy	13
3.1.3 Combined gas chromatography - mass spectroscopy	14
3.1.4 Infrared and nuclear magnetic resonance	14
3.1.5 Thin layer chromatography	15
3.2 Extraction Procedures	15
4. SOURCES AND ENVIRONMENTAL TRANSPORT	17
4.1 Sources	17
4.2 Environmental Transport	21
5. OCCURRENCE AND BIOLOGICAL ACCUMULATION	24
5.1 Fish and Birds	24
5.1.1 Levels in wildlife	24
5.1.2 Bioconcentration	34
5.2 Human Exposure	37
5.2.1 Levels in exposed and unexposed workers	37
5.2.2 Estimated exposure from contaminated fish	40
6. EXPERIMENTAL TOXICOLOGY	41
REFERENCES	52

1. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

1.1 Introduction

Chlorinated styrenes were listed as Candidate Chemicals in 1980 under the Environmental Contaminants Act of Canada.([1]) Candidate chemicals are those substances which are being evaluated for their potential to be dangerous to human health or the environment. The chlorinated styrenes were removed from the list of Candidate Chemicals in 1981 owing to the lack of commercial activity involving these compounds. In this report the scientific literature pertaining to the potential health effects of chlorinated styrenes, available as of December 1983, is reviewed to ascertain whether any further investigation might be warranted in Canada.

1.2 Summary

Octachlorostryrene (OCS), heptachlorostyrene (HCS) and hexachlorostyrene (6CS) are highly lipophilic and persistent compounds. The isolation of OCS in wildlife was first reported in 1969 by Koeman and coworkers ([2]), who detected the substance in sandwich terns (Sterna sandvicensis) and eider ducks (Somateria mollissima) from the Wadden Sea in The Netherlands. Further investigation, using gas chromatography-mass spectrometry, confirmed that the compound was indeed OCS, and in addition, three isomers of HCS were detected([3]). Subsequently, a multicomponent mixture of polychlorinated styrenes was identified in fish from North American and European waters. The fish were collected from Lake Ontario and Lake Huron ([4]), the Detroit River([4,5]), the Ashtabula River, Ohio([5-7]), the Saginaw River, Michigan ([5]), and the Frierfjord in Norway ([8-11]). OCS also has been identified in cormorants (Phalacrocorax carbo) from the Netherlands ([3]), Great Blue Heron (Ardea herodias) from Lake St. Clair, Michigan ([12]), herring gulls from Lake

Ontario, Lake Erie and the Detroit River ([13]), Rhine River water([14]), and in surface film on water of the Frierfjord, Norway ([15]).

Polychlorinated styrenes are not produced commercially, but seem to be formed as a by-product in industrial processes involving carbon and chlorine in high temperature reactions([9]). The source of contamination in the Frierfjord in Norway has been identified as a magnesium production plant([8]). The polychlorinated styrenes usually are present along with hexachlorobenzene (HCB). Since OCS has been shown to exhibit similar toxic effects to HCB, comparisons are made in this report on the environmental behaviour of these compounds.

The bioconcentration factor, which relates the concentration of a chemical residue in fish and in the water, was found to be 33,000 for OCS, almost twice the value for hexachlorobenzene (HCB)([18]).

The degree of human exposure to OCS in Canada is uncertain. OCS has been detected in wildlife in the four Great Lakes in Canada as well as in Lake St. Clair at the United States-Canada border. Levels of OCS in edible fish in Canada have not been reported.

Laboratory studies show that high levels of ingested OCS are stored in the fat and other tissues of rats. The highest concentrations were found in fat, followed by adrenal glands, skin, lungs, gastrointestinal tract, and liver. The half-life of OCS in the rat, following a single intravenous dose, was found to be 6.6 weeks. Only unchanged OCS was found in fat and liver samples. Two metabolites were found in the faeces. However 90% of the material analyzed in the faeces was unchanged OCS.

In rats, OCS can produce increases in liver microsomal enzymes, decreases in haematological parameters, increased weights of liver, kidney and spleen, and histological changes in the liver, kidney and thryoid. OCS is

porphyrinogenic in rats and Japanese quail and this effect may be sex-linked. It has been determined to be a phenobarbital type inducer. Some of the toxic properties of OCS are similar to those of hexachlorobenzene. These include a low order of acute toxicity, hepatic effects of the same magnitude and porphyrinogenicity.

Three studies have been completed recently on the toxicity of OCS in the rat[20-22]. In acute, subacute and subchronic studies (doses: 1300-3700 mg OCS/kg body weight in the diet; 0.5-500 ppm OCS in the diet for 28 days; or 0.05-500 ppm OCS for 90 days respectively), Chu and coworkers[21,22] examined the gross pathology, organ weights, serum biochemistry and hematological profiles and hepatic enzyme activities. OCS residues in the liver and fat were analyzed. The maximum no observable adverse effect level in the diet appeared to be 0.5 ppm (~ 0.04 mg/kg/day) for 90 days.

1.3 Conclusions

The principal route into the environment for OCS and HCS is industrial effluent. OCS has been detected in all stages of the aquatic ecosystem; biomagnification through the aquatic food chain was observed. It has been analyzed in tissues and eggs of fish-eating birds in Canada. If OCS and HCS were to be eliminated from industrial effluent, they would continue to enter the aquatic ecosystem for some time, since sediments in contaminated areas contain OCS and HCS.

There are insufficient toxicological and environmental data to assess the hazard posed by OCS to the general public in Canada. The results of one 90

day rat feeding study indicate that intake levels for humans should not exceed 0.4 μg/kg/day (i.e. 1% of the maximum no-observable adverse effect level administered to rats daily over a period of 90 days). Further long term studies (subchronic and chronic) are required to confirm this figure.

There are no data from which Canadian exposure to OCS can be estimated. However, results of some Norwegian studies indicate that, for a small segment of the population which consumes fish on a regular basis, significant exposure is possible. Thus consumption by a 70 kg person of a meal containing fish fillets contaminated with OCS (0.25 mg OCS/kg fish x 0.25 kg fish/meal) would result in an exposure of 0.9 μg/kg body weight.

1.4 Recommendations

Further research should be directed towards determining the potential for human exposure to OCS in the Canadian environment. The following approach is recommended:

i. Industrial processes, in which polychlorinated styrenes may be formed as byproducts, should be identified and located. These processes will involve the use of organic material and chlorine at high temperatures, for example, carbon employed as a reducing agent in the presence of Cl_2, or electrolysis of chlorine containing compounds using graphite electrodes.

ii. Effluent from identified chemical plants should be monitored.

iii. Fish and sediments near these plants should be analyzed for polychlorinated styrenes.

iv. Edible fish in Canadian waters, which have a potential for contamination by polychlorinated styrenes, should be analyzed for polychlorinated styrene residues. Fillet and liver samples from these fish should be analyzed separately.

v. Because polychlorinated styrenes are highly persistent, there is a possibility that polychlorinated styrenes in Canadian waters may originate in the United States. Thus, it is recommended that appropriate agencies in the United States be urged to identify and monitor industrial processes which may lead to polychlorinated styrene by-products, and to take action where necessary to reduce the amounts of these compounds entering the environment.

2. IDENTITY, PHYSICAL PROPERTIES, SYNTHESIS, REACTIONS

2.1 Nomenclature

Common name	Abbreviation	IUPAC Name
octachlorostyrene perchlorostyrene	OCS	pentachloro(trichloroethenyl)benzene
heptachlorostyrene	HCS	heptachloro ethenylbenzene
cis-α,β,2,3,4,5,6-heptachlorostyrene	cis-α,β-HCS	(Z)-pentachloro(1,2-dichloroethenyl)benzene
trans-α,β,2,3,4,5,6-heptachlorostyrene	trans-α,β-HCS	(E)-pentachloro(1,2-dichloroethenyl)benzene
β,β,2,3,4,5,6-heptachlorostyrene	β,β-HCS	pentachloro(2,2-dichloroethenyl)benzene
hexachlorostyrene	6CS	hexachloro ethenylbenzene
cis-β,2,3,4,5,6-hexachlorostyrene	cis-β-6CS	(Z)-pentachloro(2-chloroethenyl)benzene
trans-β,2,3,4,5,6-hexachlorostyrene	trans-β-6CS	(E)-pentachloro(2-chloroethenyl)benzene
α,2,3,4,5,6-hexachlorostyrene	α-6CS	pentachloro(1-chloroethenyl)benzene

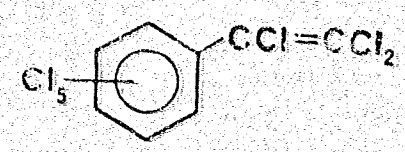
000011

C.A.S. Registry numbers:

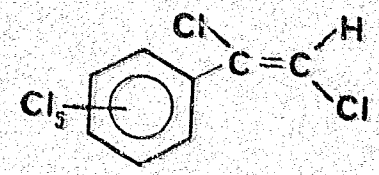
OCS	29082-74-4
heptachloroethenylbenzene	61255-81-0
pentachloro(dichloroethenyl)benzene	61593-44-0
β,β-HCS	29082-75-5
trans-α,β-HCS	29086-38-2
cis-α,β-HCS	29086-39-3
hexachloroethenylbenzene	61128-00-5

2.2 Structures

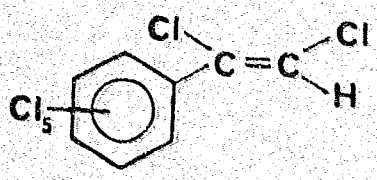
The structures of OCS and the three isomers each of HCS and 6CS are given in Figure 2.1.



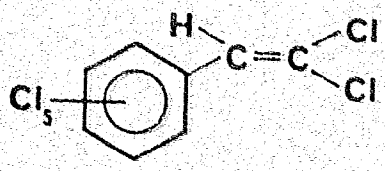
OCS



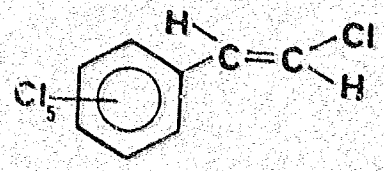
trans-α,β,2,3,4,5,6-HCS



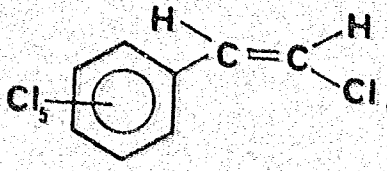
cis-α,β,2,3,4,5,6-HCS



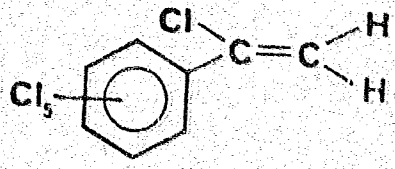
β,β,2,3,4,5,6-HCS



trans-β,2,3,4,5,6-6CS



cis-β,2,3,4,5,6-6CS



α,2,3,4,5,6-6CS

Figure 2.1 Stuctures of the Polychlorinated Styrenes

2.3 Physical Properties

Compound	Formula	M.Wt.	Melting point (°C)	Appearance
OCS	C_8Cl_8	379.7	100-5-101[a]) 99-100[b])	pale needles
cis-α,β-HCS	C_8HCl_7	345.3		colourless oil
trans-α,β-HCS	C_8HCl_7	345.3	109-110[c])	tan crystals
β,β-HCS	C_8HCl_7	345.3	108-109[c])	
6CS	$C_8H_2Cl_6$	310.8		

a) Crystallized from 95% ethanol, ref. 21.

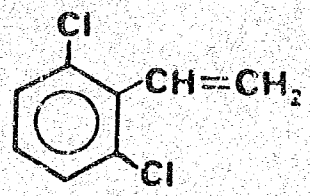
b) Crystallized from acetone/ethanol, 1:1, ref. 24.

c) Ref. 25.

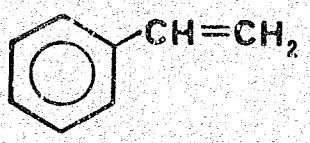
The solubility of OCS in water has been measured as 2.5 μg/L([10]), which may be compared to 6.2-7.5 μg/L for hexachlorobenzene.

2.4 Synthesis

2.4.1 Octachlorostyrene. OCS can be synthesized by several routes, which are described in equations [1] - [4].

[1] 

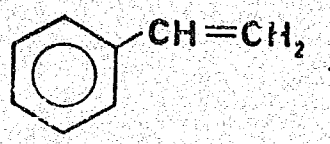
$$\xrightarrow[2.\,SbCl_5,\,190°C]{1.\,Cl_2/CCl_4} C_6Cl_5CCl{=}CCl_2 \quad \text{60\% yield Ref. 26}$$

[2] 

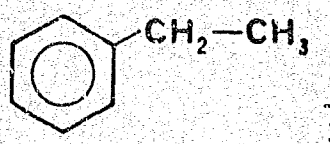
$$\xrightarrow{Cl_2/CHCl_3/FeCl_3} C_6H_5CHCl{-}CH_2Cl + C_6H_5CHCl{-}CHCl_2$$

$$\downarrow SbCl_5,\ 175°C$$

$$C_6Cl_5CCl{=}CCl_2 \quad \text{Ref. 4}$$

[3] 

$$\xrightarrow[2.\ h\nu\ (254\ nm)]{1.\ Cl_2/CCl_4} C_6Cl_5CCl{=}CCl_2 + C_6Cl_5CCl_2{-}CCl_3 \quad \text{Ref. 4}$$

[4] 

$$\xrightarrow[3.\ 605°C,\ 12.5\ sec.]{1.\ \text{vaporization at } 185°C;\ 2.\ Cl_2(Cl_2/C_8H_{10},\ 19:1)} C_6Cl_5CCl{=}CC_2 \quad \text{84\% yield Ref. 24}$$

2.4.2 Heptachlorostyrene. Three isomers of HCS can be prepared using OCS as starting material, as shown in equations [5] and [6][25].

[5]

$$C_6Cl_5CCl{=}CCl_2 + 2e \xrightarrow[\text{by method a) or b)}]{\text{electrolytic reduction}} [C_6Cl_5\bar{C}{=}CCl_2] + Cl$$

$$\swarrow \qquad \searrow$$

$$C_6Cl_5CH{=}CCl_2 \qquad C_6Cl_5C{\equiv}CCl + Cl$$

a) moving Hg cathode, 1:1 methanol/dimethoxyethane solvent containing 5% water and NH_4OAc electrolye. 23 mol% yield of crude produce (yield corrected for ~ 20% unreacted OCS).

b) Pb cathode, 1:1 methanol/dimethoxyethane solvent and HCl electrolyte (slightly acidic conditions). 24 mol% yield of crude product (corrected).

[6] $$C_6Cl_5C{=}CCl + 2e + H^+ \longrightarrow C_6Cl_5C{\equiv}CH{+}Cl$$

$$\Big\downarrow Cl_2/CCl_4$$

$$\underline{trans}\text{-}C_6Cl_5CCl{=}CHCl + \underline{cis}\text{-}C_6Cl_5CCl{=}CHCl$$

ratio 2.5:1

2.4.3 Hexachlorostyrene. No reported synthesis was found.

2.5 Reactions

Few reactions of the higher chlorinated styrenes are known. OCS, HCS and 6CS are resistant to concentrated sulphuric acid[8].

Chlorinated ethenylbenzenes can be produced by electrochemical reduction of octachlorostyrene (equations [5] and [6] above) or by reaction of OCS with zinc in refluxing dioxane[27].

OCS undergoes nucleophilic substitutions when treated with sodium methoxide in methanol, giving a mixture of 4-methoxyheptachlorostyrene and α,4-dimethoxyhexachlorostyrene[28].

3. ANALYTICAL METHODS AND EXTRACTION PROCEDURES

3.1 Analytical Methods

Gas chromatography is the most frequently used method of detecting and quantifying polychlorinated styrenes. Mass spectroscopy is rarely used alone to

detect these compounds in extracts of environmental samples. The most powerful method of analysis of the polychlorinated sytrenes is combined gas chromatography-mass spectroscopy (GC-MS). The mass spectrum of each compound emerging from the gas chromatograph can be obtained and the fragmentation data matched with retention times.

Detection limits of electron capture-gas chromatography (EC-GC) depend on the equipment used and have been observed to be 0.5 ppb OCS with an error of ± 2%([6]), or 1 ppb OCS([11]). The lowest concentrations of OCS and HCS that have been quantified in samples are given below.

Compound	Lowest amount quantified	Analytical method	Sample type	Reference
OCS	0.011 ppm	GC	heron carcass	12
	0.02 ppb	EC-GC	human blood	16
HCS	0.02 ppm	GC	sprat oil	8
	0.02 ppb	EC-GC	human blood	16

3.1.1 Gas chromatography

The relative retention times of OCS, HCS isomers and 6CS isomers depend on several characteristics of the GC column, such as polarity of the packing material, temperature programme to heat the column, flow rate of carrier gas and length of column. Table 3.1 gives relative retention times for the polychlorinated styrenes. In these reports, no attempt has been made to relate the retention times of HCS or 6CS isomers with specific structures owing to the lack of reference samples. Data have been recalculated in this report to give a relative retention time of 1.00 for OCS in order to compare the results using different apparatus.

Table 3.1 Relative retention times of the polychlorinated styrenes and GC conditions used. OCS=1.00

HCB	6CS(1)	6CS(2)	6CS(3)	HCS(1)	HCS(2)	HCS(3)	OCS	DDE	Column (column dimensions; stationary phase; column temperature programme; carrier gas flow rate)[a]	Reference
0.65	0.62	0.67	0.71 0.78	0.81	0.87	0.92 0.94	1.00		2m x 0.125 in.; 3% SE-30 on Supelcoport; 130-230°C, 8°C/min; 20mL/min	9
0.62				0.79	0.86	0.92	1.00			
	0.54	0.62	0.6[illegible]	0.73	0.83	0.91	1.00		2m x 2mm; 3% OV-101 on Gas Chrom Q; 5 min. at 125°C, 4°C/min. to 235°C.	4
0.78		0.80	0.83	0.88			1.00		As for ref. 4 except 100-225°C, 4°C/min; 25mL/min.	6
0.22			0.30	0.53	0.70	0.83	1.00		1.52m x 4mm; 1% OV-1 on Supelcoport; 140-220°C, 2°C/min; 20 mL/min.	12
				0.81	0.87	0.92	1.00		5ft x 0.125 in. (1.5m x 3mm); 10% DC 200 on Gas Chrom Q; 160-200°C, 2°C/min.	3
0.67 0.69				0.81 0.83	0.87 0.88	0.93	1.00 1.00		See ref.9	8
0.65				0.79	0.87		1.00			29
0.35							1.00	1.42	As for ref. 3 except 200°C; 50mL/min.	2
0.64							1.00		50m x 0.35mm glass capillary column; SE-54; 100-250°C, 3°C/min.; 50mL/min.	15
0.36							1.00		2m x 3mm; 1.5% SP-2250-15.9% SP 2401 on Supelcon AW DMCS; 200°C; 55mL/min.	11
							1.00	1.17	See ref. 4	4
							1.00	1.49	As for ref. 4 except isothermal column, 180°C	4

a) All columns packed with 80-100 mesh material, except ref. 11, which contained 100-120 mesh material.

3.1.2 Mass spectroscopy

The polychlorinated styrenes possess a distinctive mass spectral pattern due to the high relative abundances of the two naturally occurring isotopes of chlorine.

Octachlorostyrene: The theoretical intensities of the parent ion peaks of OCS can be calculated using the abundance of the ^{35}Cl and ^{37}Cl isotopes. Table 3.2 shows the close agreement of measured with calculated values of OCS peak intensities. In the mass spectral fragmentation of OCS, the base group is C_8Cl_8, and there are seven groups of metastable peaks corresponding to successive loss of chlorine atoms ([30]). The compound decachloroethylbenzene (DCEB),

Table 3.2 Measured and calculated intensities of the OCS parent ion $C_8Cl_8^+$([4])

Ion Mass	376	378	380	382	384	386
Calculated intensity	33.55	87.58	100.00	65.24	26.62	6.93
Measured intensity	39.39	87.12	100.00	62.12	26.51	7.57

$C_6Cl_5.CCl_2.CCl_3$, also has C_8Cl_8 as base group, but its mass spectrum can be distinguished from that of OCS by the presence of a group of peaks due to the fragment C_7Cl_7, $(DCEB-CCl_3)^+$. This fragment, at m/e 329, is not observed in the mass spectrum of OCS. Furthermore, confusion between OCS and DCEB can be eliminated by use of GC-MS, since DCEB has a retention time 1.68 times greater than OCS([4]).

Heptachlorostyrene and hexachlorostyrene: The mass spectra of HCS and 6CS show the same fragmentation pattern as OCS([4]), with parent ions at m/e 342 and 308 respectively.

3.1.3 Combined gas chromatography-mass spectroscopy

Effluent emerging from the GC column is scanned continuously by a mass spectrometer. The time at which a specific chlorinated styrene emerges from the column can be determined from a selected mass plot (mass chromatogram) for its most characteristic masses. Mass number of M+, $(M-Cl)^+$ and (M-2Cl)+, are 376, 341 and 306 for OCS, 342, 307 and 272 for HCS and 308, 273 and 238 for 6CS. The presence of up to three isomers each of HCS and 6CS can be confirmed since the isomers have identical mass spectra but different retention times.

The polychlorinated styrenes can be detected in complex mixtures of persistent chlorinated compounds by the use of the selected ion summation technique. An ion current profile of selected ions is generated only if all the selected ions are observed above a set threshold([6]). Groups of ions which may be used in selected ion summation searches are displayed in Table 3.3.

Table 3.3 Ions of polychlorinated styrenes used in selected ion summation searches ([6])

Compound	Ions
6CS	308, 310, 312, 273, 275, 277
HCS	342, 344, 346, 307, 309, 311
OCS	376, 378, 380, 341, 343, 345

3.1.4 Infrared and nuclear magnetic resonance

Infrared and nuclear magnetic resonance (ir and nmr) data are summarized in Table 3.4. Although the β,β- and trans-α,β- isomers of HCS have very similar melting points and ir and nmr absorptions, they can be distinguished by their GC retention times. Ir and nmr methods generally are not used owing to their inability to detect the very low concentrations of substances usually found in environmental samples.

3.1.5 Thin layer chromatography

Octachlorostyrene has an R_f value of 0.86 (solvent:hexane; stationary phase:silica gel 60 F_{254} (Merck) [26].

3.2 Extraction procedures

In general, the method chosen to extract OCS does not depend on the type of sample (e.g., animal tissue, human blood, or water) but rather on the instrumentation to be used. Electron capture-gas chromatography has a high degree of selectivity and sensitivity for highly chlorinated chemicals and, unlike the less selective electron impact-mass spectrometry, it is not subject to interference from natural lipids.

Table 3.4 IR and NMR data for HCS isomers and OCS

Compound	IR(cm^{-1})		NMR δ (ppm)	Reference
cis-α,β,2,3,4,5,6-HCS	1590 (C=C)	^1H	6.50	25
trans-α,β,2,3,4,5,6-HCS	1595 (C=C)	^1H	6.67	25
β,β,2,3,4,5,6-HCS	1600 (C=C)	^1H	6.66	25
OCS		^{13}C	124.41 (C-7)	26
			125.29 (C-8)	
			132.66 (C-2,6)	
			132.60 (C-3.5)	
			134.13 (C-1)	
			135.74 (C-4)	

In most cases, extraction was performed as follows:

- sample dried with sodium sulphate (often omitted);
- extraction using Soxhlet apparatus or mechanical shaker, with chosen solvent system;
- organic phase washed with distilled water, dried with sodium sulphate or magnesium sulphate;
- treatment with concentrated sulphuric acid.

At this stage most samples are ready for GC analyses, but further clean-up on alumina or florisil chromatographic columns may be necessary. Analysis by MS requires special clean-up by gel permeation chromatography, alumina or florisil columns, which absorb the bulk of the lipids.

Extraction of cod flesh samples affords results which are not particularly reproducible owing to their low fat content (about 0.3%). A procedure for this type of sample has been developed by Norheim and Okland[11]. The method involves solubilizing a small amount of sample (0.5 g) with concentrated H_2SO_4 (6 mL) in a Soveril glass tube fitted with a screw cap. The mixture is maintained at 60°C for 4 hours, followed by cooling and addition of 1.0 mL of heptane. The tube is shaken and centrifuged after which the sample is ready for gas chromatography. The level of OCS in eight parallel samples was determined to be 0.18 ± 0.017 ppm, a 9.5% coefficient of variation.

The recovery of OCS and HCB from homogenized cod flesh using the above method was compared with two other methods, namely, direct extraction with hexane/isopropanol followed by partition with water, and column extraction with dry diethyl ether. The results are presented in Table 3.5.

Table 3.5 Recovery of OCS and HCB from homogenized cod flesh by different methods[11]

Method	Recovery (%)	
	OCS	HCB
H_2SO_4, heptane	95	95
hexane/isopropanol	86	87
dry diethyl ether	58	32

The accuracy of Norheim and Okland's procedure was tested in a collaborative study involving four other laboratories using alternative methods. Fair agreement (coefficient of variation, 27%) was obtained for the concentration of HCB in the homogenized cod flesh, whilst that for OCS was poor (coefficient of variation, 45%). Two laboratories found the OCS levels to be one third of the value of the other three laboratories. Better agreement was obtained for the levels of HCB and especially of OCS found in homogenized herring containing about 10% fat.

Norheim and Okland's method is not suitable for samples with very high fat content because of emulsion formation after the extraction with heptane.

4. SOURCES AND ENVIRONMENTAL TRANSPORT

4.1 Sources

In the Canada Gazette of August 1980 ([1]), notice was given under the Environmental Contaminants Act to determine whether polychlorinated styrenes were or would be manufactured in, or imported into Canada in 1979, 1980 or 1981. The response identified neither manufacturers nor importers of OCS, HCS or 6CS in Canada ([31]).

Veith and coworkers were unable to find any industry in the United States in 1979 which produced polychlorinated styrenes ([5]). Reichel et al. have analyzed about 1500 samples of heron and other estuarine birds from the United States ([12]). Only specimens collected from the Lake St. Clair area were found to contain polychlorinated styrenes. Although the source of these residues was not known, it should be noted that Lake St. Clair is down river from a major site of chemical industry.

The source of contamination in the Frierfjord in Norway has been identified as the Norsk Hydro magnesium production plant ([16]). Magnesium is produced in two stages in the Norsk Hydro Process. First, magnesium oxide is mixed with carbon reducing agent and hydrated magnesium chloride([32]). The mixture is formed into pellets, dried and fed into a heated furnace with Cl_2 to produce anhydrous magnesium chloride. This is followed by electrolysis of $MgCl_2$ using graphite electrodes which yields magnesium. A recent modification of the process uses only $MgCl_2$ brine in the first stage and involves drying hydrated $MgCl_2$ pellets in an HCl environment, producing anhydrous $MgCl_2$. This procedure was to be started in 1977 and was expected to result in a 40% reduction in chlorinated furnace wastes and a similar decrease in the effluent([33]). Both the chlorinating furnace and the electrolysis processes involve high temperatures, carbon and chlorine and as a result, small amounts of chlorinated hydrocarbons are formed([16]). These byproducts were discovered originally in the waste water from the plant and later in the water and sediments of the surrounding area.

The use of anhydrous $MgCl_2$, during the electrolysis process, allows for minimal consumption of graphite electrodes. The Dow Chemical Seawater Process in Freeport, Texas, however, uses anhydrous salt ($MgCl_2.2H_2O$) as a precursor which results in a high graphite electrode consumption rate of 100 g per kg of magnesium produced. It is possible that the Dow Process may be a major source of chlorinated hydrocarbons.

Magnesium production in 1979 and capacity in 1978 are displayed in Tables 4.1 and 4.2([32]). Norway, the United States and the USSR are the only countries in which an electrolytical process is used, the remaining production of magnesium occurs via the thermal reduction of MgO.

Table 4.1 World Production of Magnesium for 1979[32]

Canada	9,100 metric tons
China[a)]	2,000
France	8,700
Italy	9,100
Japan	11,000
Norway	45,350
USSR[a)]	72,500
USA	145,150

a) Estimate.

Table 4.2 Installed Magnesium Production Capacity, 1978 (metric tons/yr)[32]

Country	Producer	Production Capacity	Type Process	Magnesium Source
US	The Dow Chemical Co., Freeport, Texas	115,000	E	seawater, dolomite[b)]
	NL Industries, Rowley, Utah	22,000	E	brine
	American Magnesium, Snyder, Texas	5,000	E	brine
	Northwest Alloys, Addy, Washington	22,000	T	dolomite
Norway	Norsk Hydro, Porsgrunn	50,000	E	brine or seawater and dolomite
Canada	Chromasco, Haley, Ontario	9,000	T	dolomite

a) E = electrolysis, T = thermal reduction.

b) dolomite = $CaMg(CO_3)_2$.

OCS and HCS were identified and confirmed amongst 60 chlorinated compounds in Rhine River water[14].

Incineration of poly(vinyl chloride) (PVC) produces measurable quantities of sulphuric acid-resistant chlorinated hydrocarbons. Combustion experiments, in which the combustion temperature, transit time and oxygen level were varied, indicated that higher temperatures (up to 900°C) and shorter transit times increased the formation of chlorinated hydrocarbons[29]. The amounts of OCS, HCB and total organic chlorine emitted are presented in Table 4.3. The value of 2.3 mg OCS/kg PVC was nine times that of the next highest figure for amount of OCS emitted/kg PVC burned, and occurred at the lowest level of oxygen in the flue gas (0.5%).

Table 4.3 Levels of chlorinated hydrocarbons emitted from combustion of PVC[29]

	Emitted amounts (mg/kg PVC)		
	Mean	Median	Range
OCS	0.28	0.07	0 - 2.3
HCB	2.31	0.97	0.08 - 14.4
Total organic Cl	23.1	24.3	2.2 - 60

4.2 Environmental Transport

Although the polychlorinated styrenes are extremely hydrophobic, they can be transported in water by adhering to particulates[10]. OCS has been detected both in sediments and in the fatty surface film of water of the Frierfjord in Norway. OCS accumulates in the sediments through contact with the effluent from an identified industrial source. OCS from the water is concentrated in sediments to a higher degree than is HCB or pentachlorobenzene (PCB). Table 4.4 shows relative levels of these residues in water and in bottom sediments[9] and gives concentrations found in samples taken from different depths of sediments[10].

Transport of the polychlorinated styrenes in Norwegian waters has been studied by measuring the levels of OCS and HCS in fish[8,9]. Samples of sprat (Clupea sprattus) were collected from the Frierfjord, three neighbouring fjords, and the west coast of Norway (see Figure 4.1). Concentration levels were highest in samples collected near the industrial effluent outlet in the Frierfjord, and diminished with increasing distance from the outlet, Table 4.5. The same reduction in residue levels was found for several fish species from the Eidangerfjord compared to matching samples from the Frierfjord.

Koeman and coworkers believe that the source of OCS residues in birds from the Wadden Sea in The Netherlands is the Rhine River[2]. This conclusion is based on the close similarity of the polychlorinated biphenyls (PCB's) identified in samples from the Rhine River, the west coast and the Wadden Sea, coupled with the fact that the coastal water mainly flows northwards and chemicals in the littoral current waters are diluted to a low

Table 4.4 Levels of chemical residues in water and sediments of the Frierfjord

	OCS	HCB	5CB	Reference
Relative concentrations: water	1.00	1.68	1.81	9
sediment	1.00	1.25	0.87	9
Concentrations in bottom sediments, ppm wet wt.				
0-5 cm, 43% water	0.062	0.227	0.037	10
5-10 cm, 35% water	0.034	0.112	0.023	10
Concentrations in bottom sediments, ppm dry wt.				
0-5 cm	0.145	0.528	0.087	10
5-10 cm	0.095	0.317	0.064	10

Table 4.5 Residue levels in sprat oil (ppm)[8]

Sampling site	Date	% Oil (whole fish)	OCS	HCS	HCB
Frierfjord	Sept. 75	18	11.2	4.0	13.7
Eidangerfjord	Sept. 75	13	3.8	1.1	5.9
Ormefjord	Sept. 75	20	2.0	0.6	4.2
Sandefjord	Nov. 75	16	< 0.1	< 0.02	0.13
Torvik, Romsdal	July 75	12	-	-	0.04

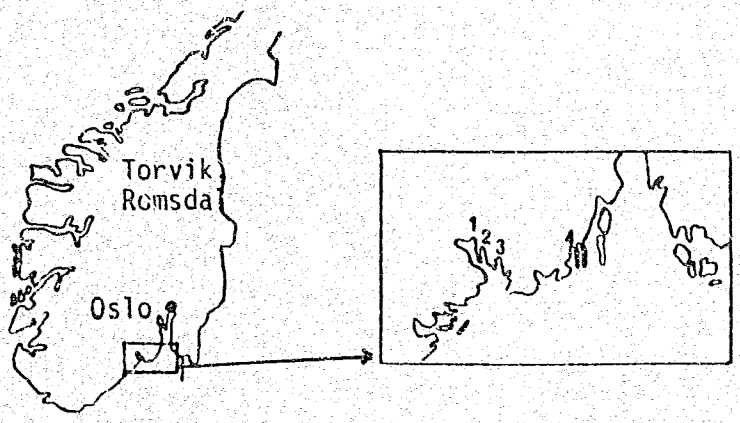


Figure. 4.1 Locations of sampling areas in Norway: 1,Frierfjord; 2,Eidangerfjord; 3,Ormefjord; 4,Sandefjord.

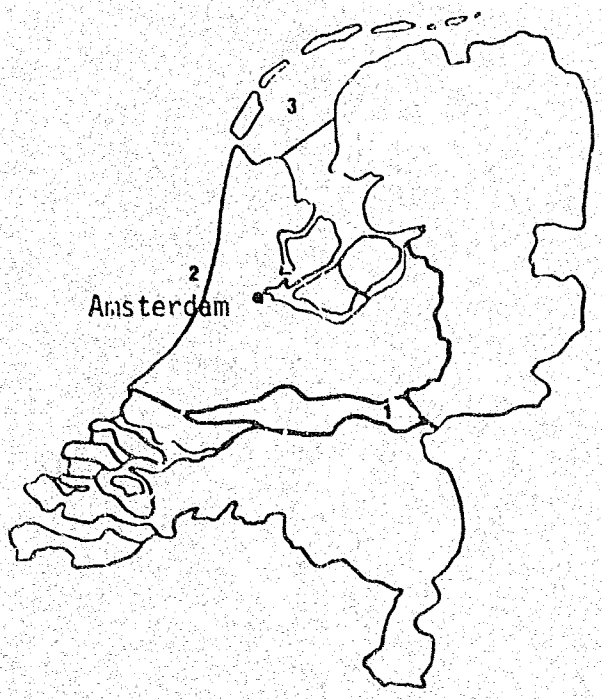


Figure 4.2 Sampling areas in the Netherlands: 1,River Rhine; 2,West coast; 3,The Wadden Sea.

degree[2], Figure 4.2. Furthermore, OCS has been identified in Rhine River water[14].

The fatty surface film and water from polluted and unpolluted fjords and lakes in Norway were analyzed for persistent chlorinated compounds[15]. Extracts of water samples from the Frierfjord were composed of PCB's (4-6 ng/L) and "substantial amounts" of OCS and 5CB, whilst surface samples contained mainly PCB's (5-40 μg/g lipid) and only small amounts of OCS and 5CB. The ratio of the concentration in surface film to the concentration in water is 5-100 times lower for OCS and 5CB than for PCB's. The pollutants found in the surface film are believed, by Ofstad et al[15] to be supplied principally from the atmosphere. Since the typical effluent chemicals, OCS and 5CB, are not concentrated in the surface film to the same extent as PCB's, it is less likely that they are airborne in origin. Hence their source is probably via transport from the underlying water, which is a less important route to the surface film than is the atmosphere[15].

5. OCCURRENCE AND BIOLOGICAL ACCUMULATION

5.1 Fish and Birds

5.1.1 Levels in wildlife

OCS, HCS and 6CS, and HCB coexist in biotic systems and have been detected and quantified in numerous fish and bird species. Quantitative data obtained from these species can be used to compare the extent of environmental contamination by the chlorinated styrenes with respect to the well-documented pollutant, HCB.

Table 5.1 Levels of polychlorinated styrenes and HCB detected in fish and birds

Species	Location	Date	Number analyzed	Fat %	Weight basis:	Average levels (ppm)[a], mean (range or error) OCS	HCSb)	6CS	HCB	Reference
FISH										
cod (Gadus morrhua) whole fish	Frierfjord	1975	7		fat wt.:	94(10-361)	30(6.1-92)		55.6(8.3-141)	9
whole fish	"	1975	1	11	fat wt.:	50.8	28.9		48.4	34
fillet	"	1976	3		fat wt.:	150(131-169)	36(26-46)		31(21-39)	9
fillet	"	1976	8		fat wt.:	78(3.2-164)	9.2(0.3-24.1)		26(5.2-44.8)	9
fillet	"	~1980		~ 0.3	wet wt.: calc. in fat:	0.18±0.017 60			0.25±0.014 83	11
f	"	~1979	6	0.7±0.4	wet wt.: fat wt.:	0.331±0.182 60±32.3			0.105±0.043 19.9±11.8	10 10
	Eidangerfjord	1976	3		fat wt.:	3.1(2.9-3.3)	1.2(0.7-2.5)		1.8(1.5-3.3)	9
liver	Frierfjord	1975	3		fat wt.:	431(223-675)	87(44-121)		170(79-208)	9
liver	"	1976	2		fat wt.:	22(12-42)	4.9(2.3-8.2)		14(8.4-23)	9
liver	"	1976	6		fat wt.:	196(16-585)	21(1.4-61)	3 isomers detected	43(16.5-109)	9
liver	"	~1979	6	44.1 ± 19.2	wet wt.: fat wt.:	49.7±44.7 88.5±60.7			16.8±15.8 30.9±20.9	10 10
liver	Eidangerfjord	1976	3		fat wt.:	11.2(6.2-21)	2.6(2.0-3.8)		5.3(4.2-6.4)	9
skin	Frierfjord	~1979	6	0.4±0.1	wet wt.: fat wt.:	0.437±0.173 115.1±88.1			0.194±0.079 54.4±35.9	10 10
whiting (Gadus merlangus) whole fish	Frierfjord	1975	2		fat wt.:	67(47-87)	20(16-23)		56(20-92)	9
fillet	"	1976	3		fat wt.:	87(12-165)	8.7(0.9-15)		13(8.6-16)	9
liver	"	1976	3		fat wt.:	144(28-256)	14(2.7-24)		26(17-41)	9
polleck (Gadus pollachius) fillet	Frierfjord	1976	2		fat wt.:	24(7-50)	14(13-14)		44(18-91)	9
fillet	Eidangerfjord	1975/76	3		fat wt.:	3.9(3.1-5.2)	1.0(0.8-1.1)		7.1(3.5-11)	9
liver	Frierfjord	1976	2		fat wt.:	8.3(4-11)	2.7(1.4-3.7)		22(11-38)	9
liver	Eidangerfjord	1975/76	2		fat wt.:	9.6(5.1-14)	2.8(1.4-4.1)		8.2(5.3-11)	9
saithe (Pollachius virens) fillet	Frierfjord	~1979	13	0.9±0.5	wet wt.: fat wt.:	0.227±0.166 39.5±46.1			0.140±0.058 21.8±15.8	10 10

0000030

Table 5.1 Levels of polychlorinated styrenes and HCB detected in fish and birds (Cont'd)

Species	Location	Date	Number analyzed	Fat %	Weight basis:	Residue levels (ppm)[c], mean (range or error) OCS	HCSb)	6CS	HCB	Reference
liver	"	~1979	13	66.3±16.3	wet wt.:	19.3±13			11.6±8.4	10
					fat wt.:	29.7±18.5			17.8±11.5	10
skin	"	~1979	13	0.8±0.6	wet wt.:	0.583±0.780			0.322±0.310	10
						66.7±88.7			63.5±80.2	10
plaice (Pleuronectes platessa)										
whole fish	Frierfjord	1975	2		fat wt.:	17(15-18)	15(11-18)		28(18-38)	9
whole fish	"	1976	3		fat wt.:	53(21-91)	10(1.7-19)		13(10-19)	9
sprat (Clupea sprattus)										
whole fish	Frierfjord	1975	4		fat wt.:	19 (10-40)	6.9(3.9-12)		29(13-64)	9
whole fish	"	1974		13	fat wt.:	8.8	2.9		16.0	8
whole fish	"	1975		18	fat wt.:	11.2	4.0		13.7	8
whole fish	Eidangerfjord	1975		13	fat wt.:	3.8	1.1		5.9	8
whole fish	"	1975/76	3		fat wt.:	5.0 (3.6-6.4)	1.3 (0.6-2.3)		5.2 (1.7-8)	9
whole fish	Ormefjord	1975		20	fat wt.:	2.0	0.6		4.2	8
whole fish	Sandefjord	1975		16	fat wt.:	< 0.1	< 0.02		0.13	8
herring (Clupea harengus)										
whole fish	Frierfjord	~1980		10		detected[c)]			detected[c)]	11
walleye (Stizostedion vitreum vitreum)										
whole fish	Detroit R.	1972/73				detected	3 isomers detected	3 isomers detected		4
whole fish	Lake Huron	1972/73				detected	3 isomers detected	3 isomers detected		4
alewife (Alosa pseudoharengus)										
whole fish	Lake Ontario	1972/73				detected	3 isomers detected	3 isomers detected		4
3 alewives, 9 gizzard shad, 3 white bass, 1 yellow perch, 3 bullhead composite sample	Ashtabula R. Ohio	1976			wet wt.:	0.50d)	2 isomers detected	2 isomers detected	3.14d)	5

000031

BLANK PAGE(S)

Table 5.1 Levels of polychlorinated styrenes and HCB detected in fish and birds (Cont'd)

Species	Location	Date	Number analyzed	Fat %	Weight basis	Residue levels (ppm)[a], mean (range or error)				Reference
						DCS	HCSb)	6CS	HCB	
1 perch 1 drum 1 sucker composite sample	Saginaw R, MI.	1976			wet wt.:	detected	1 isomer detected		0.490	5
13 catfish 1 drum composite sample	Saginaw R, MI.	1976			wet wt.:	detected	1 isomer detected		0.131	5
3 perch 1 catfish 1 drum composite sample	Saginaw R, MI.	1976			wet wt.:	detected	1 isomer detected		0.237	5
2 carp composite sample	Detroit R, MI.	1976			wet wt.:	detected			0.167	5
1 white sucker 1 hog sucker 2 stone rollers 1 carp composite sample	Ashtabula R, Ohio	1978			wet wt.:	detected	detected	detected	0.447	7
eel (Anguilla vulgaris)										
whole body	Frierfjord	1975	2	31	fat wt.:	5.8(5.0-6.5)	2.6(1.9-3.2)		21(20-22)	9,34
whole body	"	1976	3		fat wt.:	17(8-25)	3.4(1.4-4.7)		13(7-20)	9
hermit crab (Pagurus sp.)										
soft tissues, composite	Frierfjord	~1979	3	2.9	wet wt.: fat wt.:	0.06 5.3			0.05 4.3	10 10
crab (Carcinus maenas)										
soft tissues, composite	Frierfjord	~1979	3		wet wt.:	0.311			0.35	10
snail (Littorina littorea)										
soft tissues, composite	Frierfjord	~1979	3	3.7	wet wt.: fat wt.:	0.145 10.3			0.188 13.9	10 10
sea star (Asteroidea)										
soft tissues	Frierfjord	~1979	12	5.7±1.6	wet wt.: fat wt.:	0.011±0.003 0.21±0.08			0.057±0.029 1.03 ±0.44	10 10
brittle star (Ophiura albida)										
soft tissues	Frierfjord	~1979	15	0.9±0.7	wet wt.: fat wt.:	0.471±0.160 58.8± 23.3			0.182±0.098 21.2±6.9	10 10

000033

Table 5.1 Levels of polychlorinated styrenes and HCB detected in fish and birds (Cont'd)

Species	Location	Date	Number analyzed	Fat %	Weight basis:	Residue levels (ppm)[a], mean (range or error) OCS	HCSb)	6CS	HCB	Reference
BIRDS										
sandwich tern (Sterna sandvicensis) tissue and eggs	Wadden Sea	1965/66				detected			detected	2
eider duck (Somateria mollissima) tissue and eggs	Wadden Sea	1966/67				detected			detected	2
cormorant (Phalacrocorax carbo) whole body	The Netherlands	~1972				detected	3 isomers detected			3
Great blue heron (Ardea herodius) whole body	Lake St. Clair, MI.	1970			wet wt.:	0.39 0.43	3 isomers detected	detected	detected	12
egg	Lake St. Clair, MI.	1973			wet wt.:	0.01 0.06 0.23	-- detected detected			12
herring gull (Larus argentatus) egg	Hamilton Harbour, L. Ontario	up to 1979				detected	3 isomers		detected	38
egg	Agawa Rocks, L. Superior	up to 1979				detected	3 isomers		detected	38
egg	Fighting Island, L. Erie	up to 1979				detected	3 isomers		detected	38
egg	Chantry Island, L. Huron	up to 1979				detected	3 isomers		detected	38

a) Limits of dectection were not stated

b) Sum of two isomers (ref. 8) or of three isomers (ref. 9)

c) Detection limit, 0.001 ppm.

d) EC-GC, detection limit 0.5 ppb.

000034

Residue levels of OCS, HCS, 6CS and HCB found in fish and birds are given in Table 5.1.

Fish: on a wet weight basis, the concentrations in fish flesh and in fish liver differ by a factor of 100. This difference, in part at least, is a reflection of the different proportions of fat in flesh and in liver. However, it may be observed that residue levels on a fat weight basis are of the same order of magnitude in the flesh and liver of cod and saithe (Table 5.2).

Table 5.2 Fillet and liver OCS concentrations (fat and wet weight) in two species of fish

Sample	% Fat	OCS, ppm wet wt.	OCS, ppm fat wt.	Reference
cod fillet	0.7	0.331	60	10
cod liver	44.1	49.7	88.5	10
saithe fillet	0.9	0.227	39.5	10
saithe liver	66.3	19.3	29.7	10

The ratios of liver to fillet concentrations for OCS, HCS and HCB were calculated from the levels (on a fat weight basis) given in Table 5.3. These figures indicated that the internal distribution of OCS and HCS is very similar, whilst no conclusion could be drawn about the relationship between the distribution of OCS and HCB.

Table 5.3 Ratio of liver to fillet OSC concentrations as determined by fat weight

Species	Location	Liver/fillet ratios OCS	HCS	HCB	Reference
cod	Frierfjord	0.15	0.14	0.45	9
	Frierfjord	2.5	2.3	1.65	9
	Frierfjord	1.5		1.6	10
	Eidangerfjord	3.6	2.2	2.9	9
whiting	Frierfjord	1.66	1.61	2	9
pollack	Frierfjord	0.35	0.19	0.5	9
	Eidangerfjord	2.5	2.8	1.2	9
saithe	Frierfjord	0.75		0.82	10

Different life patterns and feeding habits will affect the amount of pollutants taken up by the various species. To ease the comparison of species, relevant data have been abstracted from Table 5.1 for fish from the Frierfjord. For each species, an average of the residue levels in fat for whole fish, fillet, liver and skin may be calculated (Table 5.4). If the presence of residues were simply a result of uptake directly from water, one would expect a small range in levels of these compounds for all species. In reality, concentrations differed by a factor of 650 for OCS, or if the sea star was omitted, by a factor of 26, and by 48 or 11 respectively for HCB. Thus, it is necessary to take feeding and living habits into account.

Table 5.4 Average residue concentration in fat (body burden) of OCS, HCS, and HCB for aquatic species in Frierfjord

Species	Feeding and living habits	Body burdens, ppm fat wt.			Ratio
		OCS	HCS	HCB	OCS/HCB
cod	fish, crustaceans	137	31	49	2.8
whiting		99	14	32	3.1
pollack		16	8	33	0.5
saithe		45		34	1.3
plaice	fish, bottom dweller	35	12.5	20.5	1.7
sprat	plankton	13	4.6	20	0.65
snail	algae; rock dweller	11	3	17	0.65
eel	bottom dweller	5.3		4.3	1.2
hermit crab	carcass scavenger	10.3		13.9	0.74
sea star	mussels (which are filter feeders); slow moving bottom dweller	0.21		1.03	0.2
brittle star	detritus; fast moving bottom dweller	59		21	2.8

Most fish, either directly or indirectly, are dependent on phytoplankton for their existence, since it forms the first stage of the aquatic food chain[35]. Zooplankton and some small fish feed on phytoplankton, and these animals in turn are eaten by larger fish. Phytoplankton from the uppermost 4 metres of the Frierfjord was found to contain OCS and HCB[36]. HCB concentrations varied from 0.02 to 0.94 ppm dry weight. From the OCS:HCB ratios (average 0.20, range 0.13-0.38) it was calculated that the OCS concentrations in phytoplankton range from 0.004 to 0.2 ppm dry weight. So, it can be seen that OCS is present at all levels in the marine food chain.

The level of chlorinated hydrocarbons in fish from a polluted area will depend on the extent to which they and their food source concentrate a given compound. On examination of the figures in the table above, several trends are apparent. The lowest levels of OCS were found in sea stars whose diet is mussels. Mussels are filter feeders and, as expected, the proportion of OCS:

HCB in mussels was found to be very similar to that in water[36]. The concentration of OCS in mussels was estimated (in this report) to be about 0.025 ppm dry weight (using the OCS:HCB ratio and the HCB concentrations given by Rygg and Bokn, ref. 36). Hence, the low level of OCS in the sea star reflects the manner in which OCS is accumulated in its source of food.

The exposure routes for sprat, snail and eel will be through plankton, algae and the respiratory system. These species had intermediate levels of OCS. Although the hermit crab is a carcass scavenger, it also had an intermediate OCS level. One may speculate that the remains on the carcass are flesh tissues rather than high fat organs. In contrast to the other shell fish, the brittle star, which is a detritus feeder, had high concentrations of OCS. The detritus is composed of unconsolidated sediments and has been shown to contain 0.062 ppm OCS, wet weight, in the first 5 cm[10]. This concentration is 25 times higher than the solubility of OCS in water, 2.5 μg/L (0.0025 ppm), and may result in higher concentrations of OCS in the brittle star compared to other bottom dwellers.

The gadoid fishes, (cod, whiting, pollack and saithe), and plaice had notably higher concentrations of OCS and HCS than other species. With the exception of pollack, their OCS/HCB ratio was greater than one, whilst the ratio for all the other species (excluding the eel) was less than one. Thus, not only do these species have significantly higher concentrations of OCS and HCS, but also they concentrate the polychlorinated styrenes preferentially. The diet of the gadoid fishes and plaice is fish. Fish accumulate polychlorinated hydrocarbons very efficiently[37], so it is reasonable to expect that fish-eating aquatic species will acquire the highest levels of

OCS, HCS and HCB. This, indeed, has been observed in the gadoid fishes and plaice and indicates the importance of biomagnification (accumulation by ingestion) for fish-eating species.

The skin of cod and saithe had higher levels of OCS and HCB on a fat weight basis than liver or flesh from the same samples. The outer cell layer of fish skin is protected by a mucous substance which could attract hydrophobic contaminants. However, it is not possible to distinguish between dermal absorption as a route to the skin or an internal pathway.

Birds: apart from the eider ducks, the bird species in Table 5.1 eat mainly or only fish. Feeding habits seem to be a significant factor in the uptake of chemical contaminants. Noticeably higher concentration of PCB's and DDE were detected in herring gull eggs than in common gull eggs for eight locations in Norway[39]. The herring gulls' diet was composed of fish and other marine organisms, whilst insects were of very little importance. In contrast, the common gulls ate mainly insects and vegetables, whereas fish, crustaceans and other marine species were less important. Herring gulls of the Great Lakes eat mainly alewife and smelt; furthermore, OCS, three isomers of HCS and three isomers of 6CS have been detected in alewives from Lake Ontario and the Ashtabula River, Ohio[4,5]. The eider ducks' diet consists of plants, insects and some small animals. The ratio of OCS to HCB levels ranged from 0.5 to 1 in herring gull eggs collected in the Great Lakes region[38].

It appears that accumulation through food chains plays an important role in the residue levels of polychlorinated styrenes in fish, shell-fish and birds.

5.1.2 Bioconcentration

Bioconcentration factors are used to estimate the residue-forming potential of chemicals. Specifically, bioconcentration is the accumulation of a chemical residue in fish via the gills or other membranes, but excludes accumulation through the diet. The bioconcentration factor (BCF) relates the concentration of the chemical in fish (C_F) and in water (C_W) as follows:

$$C_F = C_W \times BCF$$

The relationship holds if the concentration in fish is directly proportional to the concentration in water. The concentration of a chemical in fish will increase until a steady state is reached (rate of uptake equals rate of depuration). Measurements near the steady state are required, and these can be facilitated by accelerating the uptake process through the use of small fish at high temperatures. Veith and coworkers determined the BCF of octachlorostyrene in fathead minnows (*Pimephales promelas*) and related the result to the *n*-octanol/water partition coefficient, *P*, for OCS[18]. Their results for OCS and HCB are presented in Table 5.5.

BLANK PAGE(S)

Table 5.5 Bioconcentration factors for OCS and HCB determined in fathead minnows, 32-day exposures[18]

Chemical	Mean exposure C_W (μg/L)	BCF	Bioconcentration potential[a]	Log BCF	Log P
OCS	7.1	33,000	65	4.52	6.29
HCB	2.6	18,500	36	4.27	5.23

a) Bioconcentration potential calculated relative to p,p'-DDE=100.

Veith et al. established an empirical relationship between log BCF and the log of the n-octanol/water partition coefficient (log P) from the results of 30 organic chemicals. This was expressed as:

$$\log BCF = 0.85 \log P - 0.70$$

which had a correlation coefficient of 0.897. Using this expression, the calculated log BCF values for OCS and HCB are 4.65 and 3.75 respectively. Log P, which is frequently used to indicate the lipophilic nature of chemicals, gives a very good estimate of the bioconcentration potential of OCS.

The results of the laboratory study of Veith and coworkers can be compared to actual residue values found in sprat oil samples. Sprats are plankton eaters, so residues will arise from bioconcentration through the gills and biomagnification of the chemicals in food. There are insufficient data available to determine the bioconcentration potential in phytoplankton of the chemicals of interest, so the proportion of residues arising from bioconcentration and biomagnification is unknown.

The relative amounts of OCS, HCS, hexa-, penta-, tetra-, and trichlorobenzene (HCB, 5CB, 4CB and 3CB, respectively) have been calculated for samples of sprat oil and extracts from a carbon filter placed in polluted effluent[8], Table 5.6. The concentrations of each compound varied by a factor of about five between samples, nevertheless, the ratios were relatively constant. Constant ratios imply that the uptake and metabolism of a specific compound is proportional to its concentration in water and is relatively independent of other compounds[18]. Relative bioconcentration factors can be determined by comparing the ratios in sprat oil to the corresponding ratios found after analysis of the carbon filter extracts.

Table 5.6 Relative concentrations of chlorinated hydrocarbons to OCS[6]

Sample	OCS:OCS	HCS:OCS	HCB:OCS	5CB:OCS	4CB:OCS	3CB:OCS
Carbon filter extracts	1	0.28	2.5	1.7	1.2	3.5
Sprat oil: Frierfjord	1	0.38	1.4	0.23	0.017	0.030
Eidangerfjord	1	0.32	1.4	0.21	0.015	0.09
Ormefjord	1	0.30	2.1	0.25	0.015	0.030
Sprat oil ratio (av.)/carbon filter extracts ratio (Relative bioconcentration factors)	1	1.19	0.65	0.14	0.013	0.014

The relative BCF's compare favourably with the data of Veith *et al.* (relative BCF's, HCB:OCS=0.56; 1,2,4-3CB:OCS=0.08).

5.2 Human Exposure

5.2.1 Levels in exposed and unexposed workers

Humans may be exposed to polychlorinated styrenes through contaminated drinking water or food, or by occupational exposure to chlorinated hydrocarbons. Lunde and Bjorseth analyzed blood samples taken from exposed and non-exposed Norwegian workers[16,17]. The subjects were arranged into three groups: those in group A worked in the mercantile division of a magnesium production plant; group B workers were employed in a poly(vinyl chloride) plant; and those in group C worked at various stages in the magnesium production process. The results in Table 5.7 and Figure 5.1 show the mean and range of residue levels in each group. Compounds were identified and quantified by EC-GC using two columns of different polarity.

Table 5.7 Polychlorinated hydrocarbons in human blood samples[16]

Group	Number of Subjects	Residue level (ppb)[a], mean and range		
		OCS	HCS[b]	HCB
A	9	0.20(6/9)[c] 0-0.43	0.46 (4/9) 0-0.75	1.04 (9/9) 0.19-2.49
B	9	0.24 (8/9) 0-0.38	0.26 (8/9) 0-0.63	1.54 (9/9) 0.56-3.10
C	17	1.24 (16/17) 0-4.96	0.30 (13/17) 0-0.76	29.61 (17/17) 4.75-158.62

a) Detection limits were not stated, but the lowest level, both of OCS and HCS, quoted was 0.02 ppb.

b) Sum of two or more isomers.

c) Number of subjects in whom the chemical was identified.

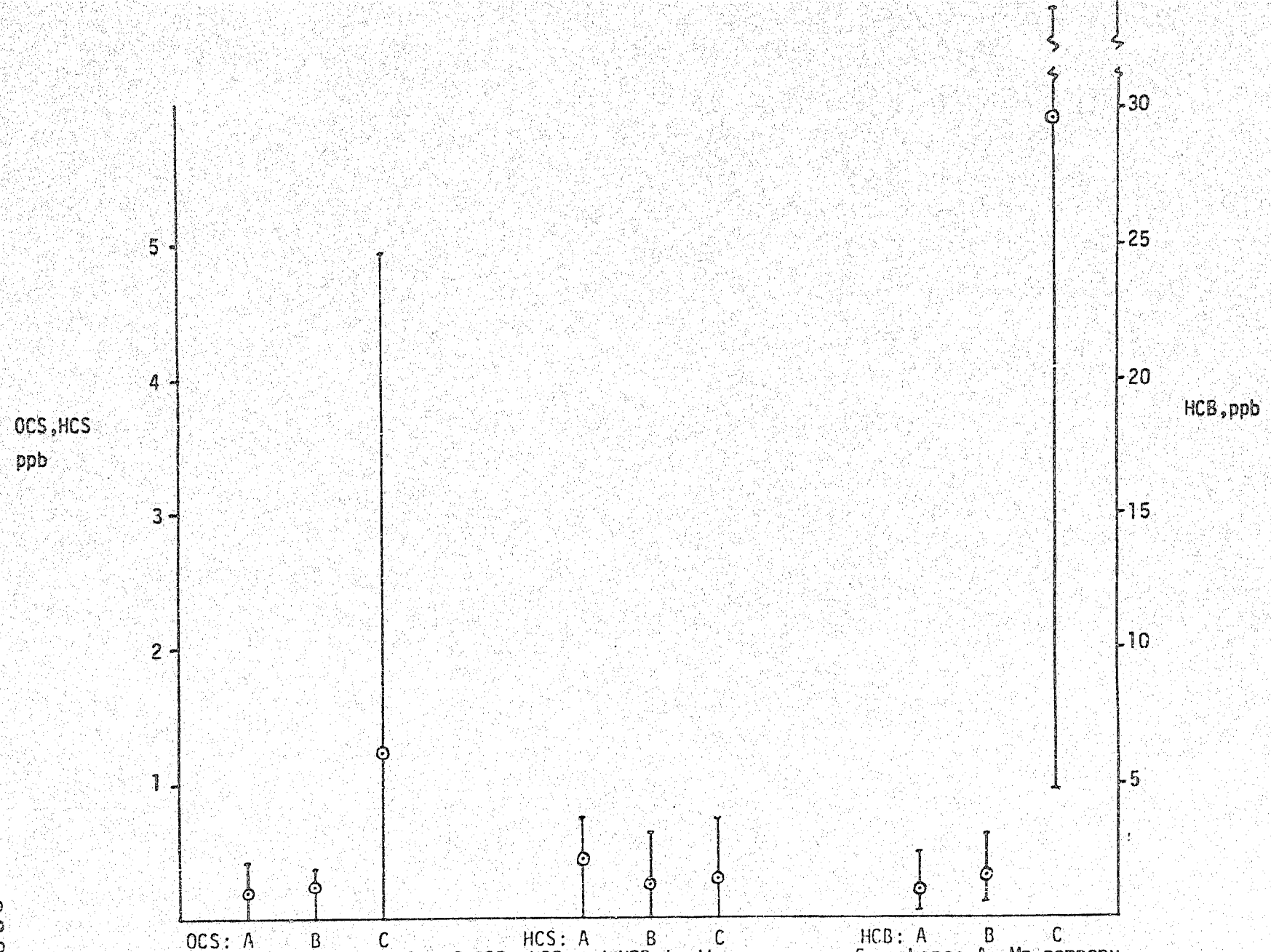


Figure 5.1 Residue levels of OCS, HCS and HCB in three groups of workers: A, Mg company mercantile division; B, PVC plant workers; C, Mg company plant workers.

Only small differences exist in the average concentration values for groups A and B, whilst the levels in group C (the exposed workers) are significantly higher for OCS and HCB. Human fat has not been analyzed for OCS or HCS, but the trends for other chlorinated hydrocarbons detected in blood and fat samples give an indication of what levels of OCS and HCS may be expected in human fat. The concentrations of these compounds, all analyzed by the same EC-GC technique, are displayed in Table 5.8.

The average of the fat/blood ratios for PCB, HCB and p,p'-DDE is 140. Using this value of 140, one can estimate the levels of OCS and HCS in human fat. The concentration of OCS would be approximately 30 ppb and 170 ppb in unexposed and exposed Norwegian workers respectively, and of HCS, 60 ppb and 40 ppb respectively.

Table 5.8 Chlorinated hydrocarbons in blood and fat samples[16,17]

	Residue levels (ppb)[a]				
	PCB	HCB	p,p'-DDE	OCS	HCS
Human fat					
non-exposed person (10[b])	1,000	80	340		
Blood samples					
non-exposed (5)	4.9	0.5	5.0		
exposed to cutting oils (8)	6.1	0.5	3.9		
non-exposed (9)	--	1.04		0.20	0.46
Mg plant workers (17)	0.15	29.6		1.24	0.30
Ratio fat/blood					
non-exposed	204	160	68		
exposed to cutting oils	164	160	87		

a) Average values for each group.
b) Number of subjects in the group.

It is evident that polychlorinated styrene by-products, from the magnesium production plant, are accumulated in plant workers at significantly higher levels than in the general population. The calculated OCS concentrations in fat of exposed and unexposed workers were 0.085 times (1/12th) and 0.015 times (1/67th) respectively, the level analyzed in the fat of male rats fed 0.05 ppm OCS in the diet for 90 days (Table 6.4, ref. 22).

5.2.2 Estimated exposure from contaminated fish

Data on concentrations of OCS in fish in North America is quite limited. One study found 0.5 ppm OCS in whole fish samples collected in Ohio (5). Since whole fish samples include high fat tissues where OCS accumulated preferentially, it is likely that fish fillets will contain somewhat lower OCS levels.

It is necessary to use Norwegian data in order to make some estimate of possible exposure to OCS from contaminated fish. The average OCS concentration in cod and saithe fillets from the Frierfjord in Norway was 0.25 ppm on a wet weight basis. Consumption of a 250 gram portion of fish fillets containing 0.25 ppm OCS would lead to ingestion of 62.5 μg of OCS. The amount ingested by a 70 kg man would be 0.9 μg/kg or 0.0009 ppm in one meal.

A reliable estimate of Canadian exposure to OCS from eating contaminated fish cannot be made until OCS levels in edible fish in Canadian waters are quantified.

6. EXPERIMENTAL TOXICOLOGY

Chu and coworkers have studied the tissue distribution, metabolism, excretion and toxicity of OCS in the rat (20-22).

The tissue distribution, metabolism and elimination of OCS in the rat was studied after oral or intravenous administration of ^{14}C-OCS (20). Radioactivity was distributed in all tissues examined after ^{14}C-OCS was administered, Table 6.1. The highest levels of radioactivity were detected in fat, followed by adrenal glands, skin, lungs, gastrointestinal tract, and liver. The high ^{14}C concentration in the adrenal glands was accounted for by the high lipid content of that organ. The elimination rate was best described by the equation:

$$\log C = \alpha + \beta t + E_i \quad (i = 1, \ldots n)$$

where C is concentration, t is time, and E_i is an error term which was assumed to be normally distributed about the mean of the n observations. The rate constant, β, was similar for all tissues examined even though there was a large variation in initial concentrations. The mean half-life of elimination of OCS from fat, adrenal, skin, lung, g.i. tract and liver was found to be 6.6 weeks. Excretion of OCS is described in Table 6.2. The major route of excretion was via the faeces.

Table 6.1 Tissue distribution of radioactivity in rats (mean ± S.D. ppm equivalents of OCS) (ref. 20)

	3 rats, i.v. dose of 20 mg/kg[a]	2 rats, oral dose of 20 mg/kg	4 rats, i.v. dose of 40 mg/kg OCS						
Tissue	24 h	48 h	1 week	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
Fat	135 ± 37	119	233 ± 53	161 ± 41	142 ± 35	93 ± 20	53 ± 4.9	44 ± 9.2
Adrenal	74 ± 49	56	58 ± 12	47 ± 11	39 ± 4.4	21 ± 6.1	12 ± 1.6	12 ± 19
Skin	29 ± 7.4	21	18 ± 13	26 ± 11	22 ± 3.8	12 ± 2.8	11 ± 3.0	9.4 ± 2.9
Lung	32 ± 1.0	6.9	44 ± 6.6	20 ± 5.3	15 ± 9.7	12 ± 6.8	6.8 ± 0.2	5.9 ± 1.7
Gastrointestinal tract	27 ± 4.3	11.4	16 ± 5.6	12 ± 5.1	12 ± 2.2	11 ± 3.1	4.0 ± 0.9	4.5 ± 1.3
Liver	18 ± 2.6	6.4	8.9 ± 2.4	6.4 ± 1.4	6.4 ± 1.4	2.3 ± 0.5	1.5 ± 0.1	1.5 ± 0.3
Bladder	7.5 ± 1.9	5.5	7.3 ± 0.8	9.7 ± 2.4	3.1 ± 1.1	2.8 ± 3.2	1.6 ± 1.4	1.0 ± 0.4
Kidney	12 ± 2.6	5.9	7.5 ± 1.4	6.8 ± 1.4	5.8 ± 1.8	2.9 ± 0.4	1.5 ± 0.2	1.5 ± 0.21
Spleen	5.9 ± 1.6	2.6	6.3 ± 1.7	4.7 ± 1.7	3.8 ± 0.4	2.5 ± 0.6	1.5 ± 0.1	1.2 ± 0.5
Brain	10 ± 1.8	3.5	4.6 ± 1.4	2.4 ± 1.0	2.0 ± 0.2	1.4 ± 0.3	1.3 ± 0.3	1.3 ± 0.4
Heart	6.9 ± 0.8	3.4	4.6 ± 0.4	3.6 ± 0.8	2.4 ± 0.6	1.5 ± 0.2	1.0 ± 0.1	1.0 ± 0.3
Muscle	4.8 ± 0.8	1.5	2.3 ± 0.2	2.6 ± 0.7	2.2 ± 0.3	1.7 ± 0.8	1.4 ± 0.2	1.9 ± 1.1
Testes	5.0 ± 0.5	1.6	2.6 ± 0.8	1.5 ± 0.3	1.1 ± 0.2	1.0 ± 0.4	0.6 ± 0.1	0.6 ± 0.1
Blood	3.4 ± 0.6	1.3	1.7 ± 0.2	1.3 ± 0.2	1.0 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2

[a] The bile ducts of the 3 rats were cannulated. Serial bile samples were taken at 24 h for the estimation of radioactivty.

000049

Table 6.2 Excretion of OCS in the rat (percentage of dose) (ref 20)

		Time after dose			
		24 h	48 h	1 week	16 weeks
oral dose:	faeces	-[a]	16%	-	-
	expired air (as CO_2)	-	1%	-	-
	urine	-	0.26%	-	-
i.v. dose:	faeces	-		8.2%	36%
	urine	-		0.4%	-
	bile	3%		-	-

[a] denotes percentage dose not determined at that time period

All of the radioactive material found in fat and liver samples, and 90% of the radioactivity in the faeces, was unchanged OCS. Two metabolites, which were tentatively identified as pentachlorophenyldichloroacetic acid and heptachlorostyrene, each accounted for about 5% of the radioactivity present in the faeces. The finding of a high ^{14}C content in the lungs of rats was a unique feature of OCS, contrasting it to other lipophilic organochlorine compounds. It was thought that the rate of elimination of OCS was not limited by the rate of metabolism since 90% of the radioactivity found in faeces of intravenous-dosed rats was unchanged OCS.

Chu and coworkers have studied the toxicity of OCS in male and female Sprague-Dawley rats (21,22).

Acute study: groups of 10 male rats were given single oral doses of OCS at 1300, 1690, 2190, 2850, or 3710 mg/kg, dissolved in corn oil. The control group was fed an equal volume of corn oil. The rats, none of which had died, were sacrificed on the fourteenth day after dosing. Hypokinesia and muscle tremors were noted in animals which were fed the two highest doses of OCS. Enlarged livers were found in groups dosed at 1690, 2850 and 3710 mg/kg OCS. Livers in the groups fed at 1300 and 2190 mg/kg were larger than those of the controls, but were not significantly different ($p > 0.05$). Aniline hydroxylase, (AH) and aminopyrine demethylase (APDM) enzyme activities were raised in all dosed groups, as were the serum uric acid levels. The serum cholesterol levels were higher in groups dosed with 1690, 2190, and 2850 mg/kg. The levels were also elevated in the 1300 and 3710 mg/kg groups, but not to a statistically significant extent. Mild to moderate histological changes were observed in the thyroid of treated rats.

Subacute study: groups of 10 male and 10 female weanling Sprague-Dawley rats were provided with food containing 0, 0.5, 5.0, 50, or 500 ppm OCS for 28 days. No deaths occurred over the 28-day period nor were any clinical signs of toxicity observed in the treated animals. Treatment did not affect food consumption or body weight gain. Changes in pathology, biochemistry and haematology are summarized in Table 6.3.

Subchronic study: groups of 15 male and 15 female weanling Sprague-Dawley rats were fed diets containing 0, 0.05, 0.5, 5.0, 50 or 500 ppm OCS for 13 weeks. No clinical signs of toxicity were observed throughout the treatment period. One male rat from the control group and one from the 0.05 ppm group

Table 6.3 Changes observed in rats fed diets containing octachlorostyrene (refs 21,22)

Effect	Dosage groups (ppm) affected at significance level $p < 0.05$			
	28 day study		90 day study	
	male	female	male	female
increased weight of:				
liver	50,500	500	50,500	50,500
kidney			500	500
spleen			500	500
increased activity of:				
APDH[a]	5,50,500	50,500	5,50,500	50, 500
AH[b]	50,500	50	50	50
serum biochemistry:				
cholesterol increased	500	500	5,50,500	500
total protein increased	5,50,500	500	500	500
SDH[c] increased	500	N.S.[d]		
potassium decreased	N.S.	500		
haematological changes, decrease in:				
haemaglobin	N.S.	500	50,500	500
haematocrit	N.S.	500	500	500
red blood cells	N.S.	500	500	N.S.
neutrophil increased			N.S.	N.S.
lymphocytes increased			N.S.	N.S.
eosinophil increased			500	N.S.
platelets increased			500	N.S.

000052

Table 6.3 Continued

Effect	Dosage groups (ppm) affected at significance level $p < 0.05$			
	28 day study		90 day study	
	male	female	male	female
histological Changes:				
liver (moderate to severe)			50,500	500
thyroid (mild)			0.5,5,50,500	0.5,5,500
kidney (mild to moderate)			0.05,0.5,5,50,500	500
bone marrow cytology:				
myeloid increased			5,50,500	50
erythroid decreased			0.05,0.5,5,50,500	0.5,50
M/E ratio increased			0.5,5,50,500	0.5,50,500
lymphocyte-monocyte increased			0.05,0.5,5,50	5

[a]Aminopyrine demethylase
[b]Aniline hydroxylase
[c]Sorbitol dehydrogenase
[d] Not significant, $p > 0.05$.

000053

died during the treatment period. Although the cause of death was unknown, it did not appear to be treatment-related. Effects on pathology, biochemistry and haematology are presented in Table 6.3.

Dose dependent increases in aminopyrine demethylase (APDM) and aniline hydroxylase (AH) enzyme activities were observed. Males were more responsive to the enzyme induction than females. Serum cholesterol levels, total protein and sorbitol dehydrogenase (SDH) were elevated. Serum potassium levels decreased in female rats fed 500 ppm OCS. Several haematological disturbances were observed in the treated animals, including reductions in haemoglobin, haematocrit levels and erythrocyte numbers. Increased numbers of neutrophils, eosinophils and lymphocytes were found. In bone marrow smears, a reduction in the ratio of cells undergoing proliferation relative to those maturing (P/M), was observed.

An increase in the weights of liver, kidney and spleen were observed. The liver, kidney and thyroid were the target organs affected by OCS treatment. The prevalence and severity of lesions were dose-dependent. The changes in the liver were moderate to severe, those in the kidney mild to moderate, whilst mild changes were seen in the thyroid.

Neurological effects such as tremor and irritability, which are common following exposure to organochlorinated compounds, were not observed in rats fed 500 ppm OCS. The metabolism study using ^{14}C-OCS demonstrated the presence of relatively high levels of OCS in the brain. Therefore, the lack of neurological signs could not be ascribed to the inability of OCS to reach the central nervous system.

Hepatic effects of OCS were found to be of the same order of magnitude as HCB. The increase in hepatic microsomal APDM but not ethoxyresorutin

dethylase (ER) activities, was thought to indicate that the enzyme induction produced by OCS was similar to a phenobarbital type. From the histological and biochemical data, it appeared that 0.5 ppm was the maximum no effect level.

Tissues and diet to be analyzed for OCS residues were homogenized in a mixture of water and acetonitrile, extracted with hexane, and treated with concentrated sulphuric acid. OCS residues were quantified using EC-GC apparatus and standard solutions. Based on food consumption data, the amount of OCS ingested ranged from 0.034 - 35mg/kg/day (males) and 0.043 - 42 mg/kg/day (females) in the 28-day study, and from 0.0036 - 41 mg/kg/day (males and 0.0043 - 47 mg/kg/day (females) in the 90-day feeding study.

The OCS residues in liver and fat are shown in Table 6.4. From the data in Table 6.4, it appears that the levels of OCS in the liver and fat of male rats are still increasing after 28 days of exposure. In contrast, levels of OCS in the tissues of female rats fed OCS for 28 days are approaching steady state concentrations, where the rate of excretion is approximately equal to the rate of ingestion.

Holme and Dybing studied the inducing effects of OCS on the microsomal cytochrome P-450 system in rats (23). Groups of male and female Wistar rats were given single injections of 10, 50, 100 or 200 mg/kg OCS intraperitoneally five days before being killed. Other groups were treated with 200 mg/kg OCS intraperitoneally, then killed 1, 2, 3 or 5 days later, in order to observe the time-course of the inducing effects. In a third study, a group of rats was injected with 250 mg/kg OCS weekly for eight weeks, then were killed one week later. The results were compared to duplicate experiments using HCB.

OCS and HCB were found to act very similarly. Both compounds caused increases in the following liver microsomal parameters: microsomal protein, cytochrome P-450 level, and activities of cytochrome P-450 reductase, ethylmorphine (EM) N-demethylase, 4-nitroanisole (PNA) O-demethylase and acetanilide (AcA) hydroxylase. No significant changes were observed at the 10 mg/kg OCS dose, whilst maximum effects were found at 100 and 200 mg/kg of OCS and HCB respectively. The level of cytochrome b_5 was unchanged and the aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH) activity showed a decrease only in the 200 mg/kg dosed group. In the time-course study, a dose of 200 mg/kg was observed to cause a rise in inducible monooxygenase activities one day after treatment. These activity levels increased daily, with maxima attained on the fifth day. The effects of 8 weekly injections of 250 mg/kg OCS were compared to the results from acute experiments. After the repeated doses, no increase in cytochrome P-450 was observed, whereas the cytochrome b_5 level increased. The increases in microsomal activities were similar to those in the acute experiments.

In agreement with Chu et al.[21], OCS was determined to be a phenobarbital type inducer.

Strik and Koeman studied the porphyrinogenic action of OCS and HCB in female Japanese quail and male Wistar rats [19]. The quail were orally dosed with 500 mg/kg/day OCS or HCB for 3 days. Macroscopic fluorescence of porphyrins was observed in 7 out of 10 kidneys of OCS dosed quail and in 1 out of 10 kidneys of HCB dosed animals. Macroscopic fluorescence of liver porphyrins was not detected in either group. Male Wistar rats were fed with 400 ppm OCS or 300 ppm HCB for up to 12 weeks. No fluorescence was observed in the livers of

these animals. Female rats treated with 300 ppm HCB showed fluorescence in 2 out of 6 livers after 8 weeks of feeding, in 6 out of 6 livers after 12 week of treatment, and again in all 6 livers 2 weeks after withdrawal of HCB. It should be noted that Chu et al. did not find OCS to be porphyrinogenic in male or female rats in a 28-day study [21]. The concentrations of HCB and one of its metabolites, pentachlorothiophenol, decreased slower in female than in male livers. Histological examination of livers from OCS dosed male rats revealed formation of megalocytosis, basophilic spots, whorls and hypertrophy of hepatocytes.

There was an increase in the activity of 5-aminolaevulinic acid synthase (ALAS) and the cytochrome P-450 content in livers of OCS treated animals. The AH and APDM activities increased with length of treatment. Activity levels found after 8 weeks of feeding at 400 ppm OCS were comparable, although slightly greater, than the levels found by Chu et al.[21] in Sprague-Dawley rats given a diet containing 500 ppm OCS for 4 weeks. Glutamate dehydrogenase activity was not changed by OCS treatment.

It was concluded that OCS is more porphyrinogenic than HCB and that porphyria and whorl formation, resulting from OCS treatment, may be sex-linked. However, these conclusions are not in agreement with the results from the more extensive work conducted by Chu et al. [21, 22].

Table 6.4 Octachlorostyrene residues in the liver and fat of rats, ppm (mean ± S.D.) (refs 21,22)

Level in the diet (ppm)	Study	liver		fat	
		male	female	male	female
0 (control)	28d	0.01 ± 0.0	0.03 ± 0.02	0.23 ± 0.10	0.44 ± 0.25
	90d	0.13 ± 0.05	0.07 ± 0.02	2.14 ± 0.77	1.34 ± 0.60
0.05	90d	0.12 ± 0.05	0.06 ± 0.01	2.0 ± 0.52	2.0 ± 0.37
0.5	28d	0.39 ± 0.10	0.42 ± 0.14	7.7 ± 1.2	10 ± 3.4
	90d	1.0 ± 1.02	0.36 ± 0.14	16 ± 8.9	7.5 ± 0.76
5.0	28d	3.2 ± 1.4	3.0 ± 1.1	80 ± 19	66 ± 7.5
	90d	3.6 ± 0.90	2.8 ± 1.0	79 ± 9.8	84 ± 53
50	28d	27.3 ± 1.9	26 ± 6.6	682 ± 123	679 ± 203
	90d	37 ± 9.3	26 ± 7.5	924 ± 685	1020 ± 500
500	28d	212 ± 11	253 ± 51	6890 ± 440	9490 ± 1530
	90d	296 ± 84	277 ± 61	12,000 ± 3730	11,100 ± 1530

0000058

REFERENCES

1. The Canada Gazette, Part 1, August 23, 1980, p. 5040: November 29, 1980, p. 7239.

2. J.H. Koeman, M.C. ten Noever de Brauw and R.H. de Vos, Chlorinated Biphenyls in Fish, Mussels and Birds from the River Rhine and the Netherlands Coastal Area, Nature, 221, 1126 (1969).

3. M.C. ten Noever de Brauw and J.H. Koeman, Identification of Chlorinated Styrenes in Cormorant Tissues by a Computerised GC-MS System, Sci, Total Environ., 1, 427 (1972/1973).

4. D.W. Kuehl, H.L. Kopperman, G.D. Veith and G.E. Glass, Isolation and Identification of Polychlorinated Styrenes in Great Lakes Fish, Bull. Environ. Contam. Toxicol., 16, 127 (1976).

5. G.D. Veith, D.W. Kuehl, E.N. Leonard, F.A. Puglisi and A.E. Lemke, Polychlorinated Biphenyls and Other Organic Chemical Residues in Fish from Major Watersheds of the United States, 1976, Pesticides Monitoring Journal, 13, 1 (1979).

6. D.W. Kuehl, E.N. Leonard, K.J. Welch and G. Veith, Identification of Hazardous Organic Chemicals in Fish from the Ashtabula River, Ohio, and Wabash River, Indiana, J. Assoc. Off. Anal. Chem. 63, 1238 (1980).

7. G.D. Veith, D.W. Kuehl, E.N. Leonard, K. Welch and G. Pratt, Polychlorinated Biphenyls and Other Organic Chemical Residues in Fish from Major United States Watersheds Near the Great Lakes, 1978, Pesticides Monitoring Journal, 15, 1 (1981).

8. G. Lunde and E.B. Ofstad, Determination of Fat-Soluble Chlorinated Compounds in Fish, Z. Anal. Chem., 282, 395 (1976).

9. E.B. Ofstad, G. Lunde, K. Martinsen and B. Rygg, Chlorinated Aromatic Hydrocarbons in Fish from an Area Polluted by Industrial Effluents, Sci. Total Environ., 10, 219 (1978).

10. J.E. Bjerk and E.M. Brevik, Organochlorine Compounds in Aquatic Environments, Archives of Environ. Contam. Toxicol., 9, 743 (1980).

11. G. Norheim and E.M. Okland, Rapid Extraction of Some Persistent Chlorinated Hydrocarbons from Biological Material with Low Fat Content, Analyst, 105, 990 (1980).

12. W.L. Reichel, R.M. Prouty and M.L. Gay, Identification of Polychlorinated Styrene Compounds in Heron Tissues by Gas-Liquid Chromatography-Mass Spectrometry, J. Assoc. Off. Anal. Chem., 60, 60 (1977).

13. D. Hallett, Canadian Wildlife Service, Dept. of Environment, Personal communication, 1981.

14. J. Freudenthal, The Detection and Identification of Unknown Halogenated Compounds in Environmental Samples, Int. J. Environ. Anal. Chem., 5, 311 (1978).

15. E.B. Ofstad, G. Lunde and H. Drangsholt, Chlorinated Organic Compounds in the Fatty Surface Film on Water, Int. J. Environ. Anal. Chem. 6, 119 (1979).

16. G. Lunde and A. Bjorseth, Human Blood Samples as Indicators of Occupational Exposure to Persistent Chlorinated Hydrocarbons, Sci. Total Environ., 8, 241 (1977).

17. A. Bjorseth and G. Lunde, Characterisation of Occupational Exposure to Persistent Chlorinated Hydrocarbons by Analysis of Human blood and fat Samples, Int. Symp. Control Air Pollution Work Environ. (Proc.), Publ. by Arbetarskyddsfonden, Stockholm, Sweden, Part 1, 396 (1978).

18. G.D. Veith, D.L. Defoe and B.V. Bergstedt, Measuring and Estimating the Bioconcentration Factor of Chemicals in Fish, J. Fish, Res. Board Can., 36, 1040 (1979).

19. J.J.T.W.A. Strik and J.H. Koeman, Porphyrinogenic Action of Hexachlorobenzene and Octachlorostyrene, Porphyrins in Human Diseases, Edited by Manfred Doss, Marburg a.d. Lahn, 1st. Int. Porphyrin Meeting, Freiburg/Br., 1975 (Karger, Basel 1976) p. 418-423.

20. I. Chu, D.C. Villeneuve, V. Secours, F.M. Benoit and A. Viau, The Tissue Distribution Metabolism, and Excretion of Octachlorostyrene in the Rat, Drug Metabolism and Disposition, 10, 632 (1982).

21. I. Chu, V.E. Secours, D.C. Villeneuve and V.E. Valli, Acute and Subacute Toxicity of Octachlorostyrene in the Rat, J. Toxicol. Environ. Health, 10, 285 (1982).

22. I. Chu, D.C. Villeneuve, V.E. Secours, A. Yagminas, B. Reed and V.E. Valli, Octachlorostyrene: a 90-Day Toxicity Study in the Rat, Fund. Appl. Toxicol., 4, 547 (1984).

23. J.A. Holme and E. Dybing, Induction of Liver Microsomal Cytochrome P-450 and Associated Monooxygenases by Octachlorostyrene in the Rat, Toxicol. and Appl. Pharm., In press, 1981.

24. S.H. Ruetman, Synthesis of Perchlorinated Vinyl Compounds, Synthesis, 680 (1973).

25. J.N. Seiber, Electrolytic Dechlorination of Perchlorinated Styrene and Vinylpyridines, J. Org. Chem., 36, 2000 (1971).

26. D. Bieniek and F. Korte, Einfache Synthese Des Octachlorstyrols, Chemosphere, 7, 729, (1978).

27. M. Ballester, J. Castaner, J. Riera, I. Tabernero and C. Cornet, Synthesis of the First Perchloroarylacetylenes, Tetrahedron Letters, 2353 (1977).

28. M. Ballester, J. Riera, L. Julia, J. Castaner and F. Ros, Synthesis of New Perchloroaromatic Phenols. Part I. Styrene and Phenylacetylene Derivatives, Anales de Quimica, Ser. C. 76, 255 (1980).

29. B. Ahling, A. Bjorseth and G. Lunde, Formation of Chlorinated Hydrocarbons During Combustion of Poly(vinyl chloride), Chemosphere, 7, 799 (1978).

30. J. Rivera, J. Castaner and M. Ballester, Mass Spectra of Some Perchloroaromatic Compounds, Anales de Quimica, 70, 1194 (1974).

31. T. Leah, Dept. of Environment, Private Communication, 1981.

32. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Edition, Vol. 14, Publ. by John Wiley and Sons, New York, 1981.

33. O.C. Bockman, J.A. Crowo, S.T. Falck and J.G. Johansen, Heksaklorbenzen og andre Halogenerte Organiske Forbindelser. Resultater fra Arbeidet med Eliminering av et Miljoproblem, 12th. Nordiska Symposiet om Vattenforskning, Visby, Norway, 11-13th May, 1976. Publ. by Nordforsk, Miljovardssekretariatet, Publikation 1976:2, p. 349.

34. G. Lunde, Persistente og Ikke-persistente Fettloselige Klorerte Forbindelser i Marine Organismer, 12th. Nordiska Symposiet om Vattenforskning, Visby, Norway, 11-13th May, 1976. Publ. by Nordforsk, Miljovardssekretariatet, Publikation 1976:2, p. 337.

35. Van Nostrand's Scientific Encyclopedia, 5th Edition. Edited by D.M. Considine, publ. by Van Nostrand Reinhold Company, 1976.

36. B. Rygg and T. Bokn, Klorerte Hydrokarboner i Vann, Sedimenter og Organismer i Frierfjordomradet, 12th Nordiska Symposiet om Vattenforskning, Visby, Norway, 11-13th May, 1976. Publ. by Nordforsk, Miljovardssekretariatet, Publikation 1976:2, p. 363.

37. D.W. Kuehl, Unusual Polyhalogenated Chemical Residues Identified in Fish Tissue from the Environment, Chemosphere, 10, 231 (1981).

38. R. Breacher, Canadian Wildlife Service, Dept. of Environment, Personal communication, December 1981.

39. J.E. Bjerk and G. Holt, Residues of DDE and PCB in Eggs from Herring Gull (Larus Argentatus) and Common Gull (Larus canus) in Norway, Acta Vet. Scand., 12, 429 (1971).