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Subacute Toxicity and Mutagenicity of *cis*-9,10-Epoxystearic Acid

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This study is part of a continuing program to assess the toxic and mutagenic potential of chemicals present in pulp mill effluents. cis-9,10-Epoxystearic acid (abbreviated as epoxystearic acid), chlorinated guaiacols and resinous acids are examples of such contaminants which have been found in river water and have been shown to be toxic to fish (LEACH and THAKORE, 1975). A previous report has revealed that epoxystearic acid is not a carcinogen when it is applied to rodent skin (IARC monograph 1976). Results of a metabolism study carried out in our laboratories have indicated that epoxystearic acid is rapidly biotransformed to carbon dioxide (CHU et al. 1979). This communication deals with the subacute toxicity of epoxystearic acid in the rat and with bacterial mutagenicity in Salmonella. Preliminary qualitative results on the mutagenicity of epoxystearic acid have been presented previously (DOUGLAS et al. 1980; NESTMANN et al. 1980a).

METHODS

Epoxystearic acid was synthesized using a procedure as described by CHU et al. (1979). The purity (> 99%) of this chemical was established by melting point and gas chromatography. Five groups of weanling Sprague-Dawley rats (Canadian Breeding Farms, Montreal, Canada), containing ten males and ten females each were given daily oral doses of epoxystearic acid in corn oil at 0 (control group), 2, 10, 50 or 250 mg/kg body weight. The volume of the corn oil was 10 ml/kg body weight and doses were adjusted weekly according to body weight. Animals were housed in individual cages and were allowed free access to food (Purina Chow) and water. Body weight gain and food intake were monitored on a weekly basis and clinical signs observed daily. At the end of four weeks, all animals were anesthetized with ether and exsanguinated via the abdominal aorta. Organ weights (liver, brain, heart, kidney, spleen, adrenal, thyroid, gonad, and pituitary), gross pathologic changes and hematology were determined for all groups in a manner similar to that described by VILLENEUVE et al. (1979). The following hematological parameters were determined: hemoglobin concentration, hematocrit value, erythrocyte count, total and differential counts of leukocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin. Serum from

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each animal was used for biochemical profiling using a SMA 12/60 microanalyzer (Technicon). The serum biochemical parameters monitored in this fashion included sodium, potassium, inorganic phosphorus, total bilirubin, alkaline phosphatase, glutamic oxalacetic transaminase, total protein, calcium, cholesterol, glucose, uric acid and lactic dehydrogenase.

Microsopic examination of tissues was carried out on all animals in the control groups and the groups receiving the highest dose of epoxystearic acid. The tissues examined histologically included brain, pituitary, liver, adrenal, thyroid, parathyroid, thymus, lungs, trachea, bronchi, thoracic aorta, esophagus, gastric cardia, fundus and pylorus, duodenum, pancreas, colon, kidney, spleen, bone marrow, mesenteric and mediastinal lymph nodes, testes or ovary, epididymis, skeletal muscle and heart.

Salmonella strains TA1535, TA100, TA1537, TA1538, and TA98, were obtained from Dr. B.N. AMES (Berkeley, California). Procedures for bacterial testing and for preparing Aroclor 1254-induced rat liver for metabolic activation have been previously described (AMES et al. 1975). Mutagens used as positive controls were the same as in an earlier report (NESTMANN et al. 1980b).

RESULTS AND DISCUSSION

Growth rate and food consumption were not affected by treatment and no clinical signs of toxicity were observed. Several rats died during the course of the experiment but the cause was due to respiratory tract injury resulting from the dosing. Necropsy of the rats revealed the presence of oily liquid in the lungs or thoracic cavity, and/or blood in the esophagus, trachea or lungs. The organ weights, expressed as wet weight or as percentage of body weight, were not altered by treatment. Both hematological and serum biochemical values were found to be normal for the control and treated rats.

Histological studies revealed no treatment-related lesions in any tissues examined. Some alterations, such as aggregates of mononuclear cells in the liver, and lymphoid aggregates of the lung were found in both control and treated animals and were due to bacterial infection.

Based on the results, it would appear that epoxystearic acid possesses a low order of toxicity in the rat. Our findings are consistent with the results of a previous study which showed that this compound was rapidly metabolized to carbon dioxide and was not accumulated in the body (CHU et al. 1979).

All 5 strains of Salmonella showed a lethal but not a mutagenic response to epoxystearic acid (Table 1), TA1537 being far more sensitive than the others. In light of the high correlation between mutagenicity in Salmonella and cancer in mammals (AMES et al. 1975), these negative results are not surprising since epoxystearic acid is not a proven carcinogen (IARC monograph 1976).

TABLE 1

Mutagenic effect of epoxystearic acid in Salmonella

Amount per plate (mg)[a]	His$^+$ revertants per plate[b]									
	TA1535		TA100		TA1537		TA1538		TA98	
	-S9[c]	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	35	13	218	207	13	23	15	20	30	34
0.1					7	21				
0.25					4	13				
0.5					2[d]	8[d]				
1.0					1[d]	0[d]				
1.25	38	29	160	138			5	23	27	24
2.5	31	34	112	122			7	19	25	20
5.0	30	21	73	111			9	12	13	17
10.0	17[d]	7[d]	9[d]	25[d]			8[d]	7[d]	11[d]	14[d]

[a] Epoxystearic acid was dissolved in dimethyl sulfoxide. The volume added to each plate was 0.1 ml, including the solvent control.

[b] Numbers of revertants are means of 2 plate counts. These data are from 1 of 2 replicate experiments. Positive control values were similar to those reported previously (NESTMANN et al. 1980b).

[c] S9 is the abbreviation for the Aroclor 1254-induced rat liver preparation including required co-factors (AMES et al. 1975).

[d] Bacterial lethality is evident by reduced numbers of His$^+$ revertants and by a reduced background of His$^-$ cells.

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