Bull. Environ. Contam. Toxicol. (1988) 41:515–522
© 1988 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Results of a Short–Term Toxicity Study for Three Organic Chemicals Found in Niagara River Drinking Water

E. Komsta,[1] I. Chu,[1] V. E. Secours,[1] V. E. Valli,[2] and D. C. Villeneuve[1]

[1]Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Environmental Health Directorate, Ottawa, Ontario, KIA OL2, Canada and [2]Biopath Analysts Limited, Guelph, Ontario, N1E 2X7, Canada

During the past decade concern has been raised over the quality of drinking water in the communities that draw their supply directly from the Niagara River, which is known to be contaminated with a number of chemical pollutants. Early pollution programs dealt with conventional parameters, such as bacteria, phenols, oil, iron, phosphorus and chloride as well as general discoloration. Most of these conventional problems have been reduced due to improved treatment of municipal and industrial waste waters. At the present, attention is focused on trace organic chemicals in the Niagara River and in Lake Ontario. These substances orginate directly from municipal and industrial discharges and indirectly from major waste disposal sites along the river. Dibromomethane, hexanal and tetrahydrofuran among other chemicals were detected in treated drinking water at levels ranging from 0.2-0.8 μg/L in the Niagara River area (Canada-Ontario Review Board, 1985). Many toxicity studies on these chemicals including inhalation (Elovaara et al., 1984), neurotoxicity (Altenkirch, 1985), and mutagenicity (Van Bladeren et al. 1980, Osterman-Golkar et al. 1981) have been reported from various laboratories, and dibromomethane has been reported to be mutagenic. However, there are no toxicity data available on these chemicals when they are administered to laboratory animals in drinking water. Since the mode of administration may affect the pharmacokinetic behaviour and hence the toxic effects of these compounds, it was considered that further animal studies were needed for the regulatory agencies to assess the potential adverse effects in humans associated with the ingestion of the organic chemicals via drinking water. To this end short-term toxicity studies (4 weeks) on dibromomethane, hexanal, and tetrahydrofuran in the rat were carried out.

MATERIALS AND METHODS

Dibromomethane and hexanal were purchased from Aldrich Chemical Co. (Milwaukee, Wis.) with a stated purity of 99.6% and 99% respectively. Tetrahydrofuran was procured from BDH Chemicals (Toronto, Ontario) with a purity of 99.5%. The purity and

Send reprint requests to I. Chu at the above address.

chemical identification of these compounds were confirmed by thin-layer chromatographic, gas chromatographic (Hewlett Packard, model 5820A), and gas chromatography-mass spectrometric techniques (Finnigan mass spectrometer, model 4000, data system 6000).

Young adult Sprague-Dawley rats of either sex weighing approximately 200 g (female) and 300 g (male) were purchased from Charles River Laboratories and were acclimatized to the laboratory conditions (temperature: 21 ± 2°C; relative humidity: 40 - 60%) for 1 week before treatment. The animals, randomly divided into 10 per sex per group, were given the test chemicals in their drinking water at concentrations of 1.0, 10.0, 100.0, or 1000.0 mg/L for a period of 4 weeks. Tetrahydrofuran is freely soluble in water; its solutions were prepared by dissolving appropriate amounts of the chemical directly in tap water. Dibromomethane and hexanal, being slightly soluble in water, were initially solubilized with 0.5% w/v Emulphor (Domtar, Montreal, Canada) followed by dilution with tap water to appropriate concentrations. Control animals received tap water and the vehicle control group received 0.5% Emulphor. 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 and water consumption were measured weekly. At the termination of chemical exposure 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. Blood samples were analyzed for the following hematological parameters: hemoglobin, packed cell volume, erythrocyte count (Baker 7000), total and differential leukocyte counts and platelet count. Serum biochemical profiles were determined in a Technicon microanalyzer (Model 12/60 micro) and included sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase (AP), aspartate aminotransferase (AST), total protein, calcium, cholesterol, glucose, uric acid and lactic dehydrogenase (LDH). Hepatic microsomal aniline hydroxylase (AH; Fouts, 1963), aminopyrine demethylase (APDM; Cochin and Axelrod, 1959), and ethoxyresorufin deethylase (ER; Burke and Mayer, 1974) activities were determined based on published methods and adapted to automated instruments. A selection of tissues from the control and the highest dose groups 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, pancreas, colon, kidney, spleen, bone marrow, mesenteric and mediastinal lymph nodes, testes and epididymis in the male, ovaries and uterus in the female, skeletal muscle and heart. Potential fatty change in the liver was determined in frozen sections as previously described (Villeneuve et al., 1979).

The data were treated statistically with one-way analysis of variance. When a significant difference ($p<0.05$) was noted among the groups, the data were further analyzed by Duncan's multiple

range test in order to determine which groups were different (Nie et al., 1977).

RESULTS AND DISCUSSION

A comparison of data from the control (water) and vehicle control (0.5% Emulphor) revealed that the 2 groups were not significantly different. Thus, only those data derived from the water control were used as baseline values for comparison purposes.

No clinical signs of toxicity were observed. All animals survived the entire chemical exposure period.

Weight gain and food and water consumption of all treated groups were not significantly different from those of control rats and vehicle controls. Based on the water intake data the amount of chemicals ingested by the rats ranged from 0.1 to 124.65 mg/kg body wt./day for the males and 0.10 to 95.66 mg/kg body wt./day for the females (Table 1).

Dibromomethane: one male rat treated with 1.0 mg/L had an irregular splenic capsule and one male in 10 mg/L group had a pale liver.

Hexanal: one male animal treated with 10 mg/L and one female in the 100 mg/L group had dilated kidney pelves while one female in the 1000 mg/L group had unilateral hydronephrosis.

Tetrahydrofuran: a dilated renal pelvis was observed in one male dosed with 100 mg/L.

These gross changes were of a sporadic nature and could not be related to any specific chemical treatment.

Dibromomethane administered in the doses of 100 and 1000 mg/L significantly decreased lactate dehydrogenase (LDH) activities in female rats (Table 2). Significantly decreased LDH activities were also observed in female rats administered drinking water containing 10, 100 or 1000 mg/L hexanal (Table 2). These changes were not considered to be biologically significant and could not be related to any microscopic changes in the target organs.

No biochemical changes were observed in rats treated with tetrahydrofuran. The hepatic mixed function oxidases activities were not affected by treatments.

Hematological parameters determined in this study were within

TABLE 1 Body weights and water consumption of rats fed 3 chemicals via drinking water for 28 days.[a)]

	MALE				FEMALE			
Treatment mg/L in drinking	Initial body weight (g)	Weight gain (g)	Water Consumption (g/rat/day)	Approximate amount of chemical ingested (mg/kg/day)	Initial body weight (g)	Weight gain (g)	Water Consumption (g/rat/day)	Approximate amount of chemical ingested (mg/kg/day)
Control - Water	133 ± 15	214 ± 14	26 ± 3	0	116 ± 10	88 ± 12	18.8 ± 3	0
Vehicle Control 5% Emulphor	134 ± 17	210 ± 14	25 ± 2	0	116 ± 9	102 ± 18	20.9 ± 4	0
Dibromomethane								
1.0	137 ± 11	213 ± 15	25 ± 3	0.1	119 ± 12	99 ± 17	23 ± 3	0.1
10.0	137 ± 18	215 ± 14	25 ± 3	1.2	119 ± 16	96 ± 15	21 ± 4	0.9
100.0	135 ± 16	207 ± 20	24 ± 1	11.9	122 ± 11	95 ± 15	20 ± 3	8.6
1000.0	133 ± 12	209 ± 11	25 ± 2	123.8	118 ± 11	100 ± 21	19 ± 3	90 ± 8
Hexanal								
1.0	136 ± 15	200 ± 21	24 ± 2	0.1	116 ± 11	95 ± 13	21 ± 5	0.1
10.0	134 ± 18	197 ± 21	24 ± 2	1.2	116 ± 11	98 ± 15	19 ± 3	0.9
100.0	137 ± 16	203 ± 23	25 ± 3	12.6	117 ± 12	102 ± 18	19 ± 1	8.6
1000.0	136 ± 19	210 ± 18	25 ± 2	124.7	119 ± 8	104 ± 23	22 ± 4	95.7
Tetrahydrofuran								
1.0	133 ± 12	217 ± 24	23 ± 2	0.1	117 ± 11	87 ± 26	19 ± 3	0.1
10.0	131 ± 18	212 ± 17	23 ± 2	0.8	116 ± 12	88 ± 14	19 ± 3	0.1
100.0	132 ± 18	216 ± 22	26 ± 3	10.2	120 ± 15	90 ± 20	19 ± 2	10.7
1000.0	134 ± 16	215 ± 18	23 ± 2	95.5	122 ± 12	92 ± 22	20 ± 3	111.3

a) Values represent mean ± S.D. obtained from 10 animals

Table 2 Serum Lactate Dehydrogenase activity (mU/mL) of rats fed dibromomethane and hexanal via drinking water for 28 days.[a)]

Treatment mg/L in drinking water	MALE	FEMALE
Control - Water	892 ± 151	1276 ± 248
Vehicle Control 0.5% Emulphor	1089 ± 275	1262 ± 401
Dibromomethane		
1.0	958 ± 299	1000 ± 188
10.0	891 ± 309	1057 ± 224
100.0	902 ± 193	926 ± 364[b]
1000.0	910 ± 309	873 ± 240[b]
Hexanal		
1.0	820 ± 202	1038 ± 205
10.0	882 ± 208	893 ± 208[b]
100.0	840 ± 296	836 ± 324[b]
1000.0	702 ± 301	915 ± 197[b]

a) Values represent mean ± SD obtained from 10 animals
b) Statistically different from control at $p \leq 0.05$

normal ranges established for the Sprague-Dawley rats in our laboratories.

Only the tissues from the control and the highest dose groups were examined histopathologically, and even at this dose level the changes were generally mild in nature. The thyroid, liver and kidney were the target organs affected by treatment (Table 3). All 3 chemicals produced mild thyroid changes which consisted of increased epithelial height, reduced colloid density and angular collapse of follicles. Hepatic changes were characterized by mild increases in perivenous cytoplasmic homogeneity and periportal cytoplasmic density. Animals treated with hexanal and tetrahydrofuran also appeared to have more incidences of anisokaryosis than did dibromomethane. Morphological alterations in the kidney were confined to the proximal tubules and glomeruli. Tubular changes consisted of eosinophilic inclusions, pyknosis and central displacement of nuclei. In some cases the inclusions protruded into the lumen. Glomerular changes were characterized by adhesions of the visceral and parietal layers of Bowman's capsules, and were observed in both control and treated animals but the changes appeared to be more prevalent in the treated groups. In general

TABLE 3 Prevalence of histological changes in rats fed drinking water containing dibromomethane, hexanal or tetrahydrofuran at 1000 mg/L[a]

Tissues	Control Water	Vehicle Control 0.5% Emulphor	Treatment[c]			Control Water	Vehicle Control 0.5% Emulphor	Treatment		
			1	2	3			1	2	3
			MALE					FEMALE		
THYROID										
- Reduced follicular size	5/0	1/0[b]	5/0	6/0	3/0	2/0	3/0	4/0	4/0	3/0
- Increased epithelial height	1/0	4/0	8/0	5/1	1/0	0/0	0/0	2/0	1/0	0/0
- Reduced colloid density	0/0	0/0	2/0	3/0	2/0	0/0	0/0	2/0	0/0	1/0
LIVER										
- Anisokaryosis	0/0	0/0	0/10	3/0	3/0	0/0	2/0	1/0	2/0	7/1
- Increased cytoplasmic homogeneity	3/0	5/1	8/0	10/0	7/0	1/0	3/2	0/0	0/0	0/0
KIDNEY										
- Glomerular adhesions	6/0	3/1	7/3	6/0	6/1	8/0	9/0	8/0	5/1	7/0
- Tubular cytoplasmic inclusions	2/0	1/1	4/0	3/2	1/0	0/0	0/0	3/0	4/1	3/0

a) Number examined was 10

b) Values denote $\frac{\text{number of animals with minimal-mild changes}}{\text{number of animals with moderate to severe changes}}$

c) 1- Dibromomethane
2- Hexanal
3- Tetrahydrofuran

the changes in the male rats were more severe than in the females.

Data presented above indicated that administration of the 3 chemicals via drinking water up to 1000 mg/L produced no overt toxic effects. Although treatment-related morphological changes were observed in the highest dose groups; these were considered to be mild and adaptative in nature, and could not be related to any functional changes. The only biochemical parameter affected by treatment was the reduced LDH activity in hexanal and dibromomethane treated female rats. However, the biological significance of this change is uncertain. The growth rate and hematological parameter were not affected. Since the levels of the 3 chemicals in Niagara drinking water are reported to be 0.2 - 0.8 μg/L (Canada-Ontario Review Board, 1985), which is $1/10^3$ of the concentration of the lowest dosing solution (1 mg/L) given to the test animals, our data indicate that there exists at least a 10^3 fold concentration factor between the levels of chemicals found in the Niagara drinking water and the levels that did not produce significant biological effects in the rat.

ACKNOWLEDGMENTS. The authors are indebted to Drs. S. Magwood and B. MacDonald for useful discussions, Messrs A. Viau, J. Kelly, and A. Yagminas, Mrs. N. Beament and Mrs. B. Reed for technical ssistance Mrs. M. Beaudette for data handling and Mrs. J. Ireland for the preparation of the manuscript.

REFERENCES

Altenkirch H, (1985) Neurotoxic effects of organic solvent mixtures, clinical and experimental aspects. J Neurol 232 (suppl):211

Burke MD, Mayer RT (1974) Ethoxyresorufin: direct fluorometric assays of microsomal o-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab Dispos 2: 583-588

Canada-Ontario Review Board (1985) Trace organics in drinking water along the Niagara river.

Cochin J, 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

Elovaara E, Pfäffli P, Savolainen H (1984) Burden and biochemical effects of extended tetrahydrofuran vapour inhalation of three concentration levels. Acta Pharmacol. et Toxicol. 54: 221-226

Fouts JR, (1963) Factors influencing the metabolism of drugs in liver microsomes. Ann NY Acad Sci 104: 875-880

Osterman-Golkar S, Hussain S, Walles S, Anderstam B, Sigvardsson K (1981) Chemical reactivity and mutagenecity of some dihalomethanes. Chem Biol Interact 46: 121-130

Nie NH, Hull CH, Jenkins JG, Steinbrinner K, Bent DH, (1977) Statistical Programs for the Social Sciences, pp. 53-56 SPSS, Inc. Chicago

Van Bladeren PJ, Breimer DD, Rotteveel-Smijs GMT, Mohn GR, (1980) Mutagenic activation of dibromomethane and diiodomethane by mammalian microsomes and glutathione S-transferases. Mut Res 74: 341-346

Villeneuve DC, Valli VE, Chu I, Secours V, Ritter L, Becking GC, (1979) Ninety-day toxicity of photomirex in male rat: Toxicology 12: 235-250

Received January 28, 1988; accepted March 18, 1988.