Bull. Environm. Contam. Toxicol. 26, 42-45 (1981)

Subchronic Study of a Mixture of Inorganic Substances Present in the Great Lakes Ecosystem in Male and Female Rats

I. Chu[1], D. C. Villeneuve[1], G. C. Becking[1] and R. Lough[2]

[1]*Environmental and Occupational Toxicology Division, Bureau of Chemical Hazards, Environmental Health Directorate, Ottawa, Onatrio K1A 0L2, Canada;* [2]*Bio Research Laboratories, Senneville, Quebec H9X 3R3, Canada*

Low levels of some heavy metals and inorganic substances have been detected in the Great Lakes ecosystem; these include arsenic, cadmium, chromium, copper, iron, mercury, nickel, selenium, zinc and fluoride (GREAT LAKES WATER QUALITY AGREEMENT, 1978). While trace quantities of some heavy metals are essential to life, large amounts are known to be toxic. The levels of individual elements may be below those which could cause toxic effects, but the potential toxicity of simultaneous exposure to a combination of these elements remains unknown. The present study was therefore designed to investigate the toxic effects of a combination of inorganic substances in the rat when administered at levels equal or greater than the objectives established in the GREAT LAKES WATER QUALITY AGREEMENT (1978).

METHODS

Sodium arsenate, cadmium acetate, chromic sulfate, cupric sulfate, ferric ammonium sulfate, lead acetate, mercuric chloride, nickel sulfate, sodium selenate, zinc sulfate and sodium fluoride were obtained commercially and all had stated purities of >99%. Double distilled de-ionized water was purchased from Lab Elite Ltd. (Montreal, Canada). Weanling Sprague-Dawley rats, obtained from BioBreeding Labs., Ottawa, Canada, were acclimated for two weeks before the experiment. Five groups of 15 male and 15 female animals each were given respectively the double distilled de-ionized water (control group), tap water, or the distilled water containing 3 levels of the chemicals as the sources of their drinking water. The concentration of each chemical in the solutions is shown in Table 1. The animals were housed separately and were fed Purina Chow ad libitum. The body weights and food and water consumption were measured weekly. Animals were examined daily for clinical signs of toxicity. Blood samples were collected using the tail bleeding technique from each of a sub-group of 5 rats for the determination of hematological and serum biochemical parameters after 1 and 3 months of treatment.

0007-4861/81/0026-0042 $01.00

Table 1. Levels of Heavy Metals and Fluoride Ions in the Drinking water of the Control and Treated Rats

Elements in µg/l	Group 1[a] Double distilled de-ionized water	Group 2[a] Tap water	Group 3[b] Low level	Group 4 Medium level	Group 5 High level	Objective[c] concentration
Arsenic	<1	<1	50(31)	250(323)	1,250(1,310)	≤5
Cadmium	0.1	1.1	0.2(0.3)	1.0(1.6)	5(5.5)	≤1
Chromium	<10	<10	50(50)	250(270)	1,250(1,290)	≤0.2
Copper	<5	414	5(16)	25(32)	125(123)	≤1,000
Iron	<	330	300(330)	1,500(1,650)	7,500(7,710)	<50
Lead	<	5	25(20)	125(105)	625(510)	≤1
Mercury	<0.01	<0.01	0.2(.28)	1(1.2)	5(8)	≤0.2
Nickel	<50	<50	25(<50)	125(130)	625(630)	-
Selenium	<1	3	10	50	250	≤1
Zinc	<50	280	30(<50)	150(200)	750(720)	≤5,000
Fluoride	30	30	1,200(1,500)	6,000(6,000)	30,000(30,000)	1,200

a Values in parentheses and all values in Groups 1 and 2 are actual levels of chemicals as analyzed by Barringer Magenta Ltd. Toronto.

b Levels which are equal to those established in the Great Lakes Water Quality Agreement 1978.

c Recommended in Guidelines for Canadian Drinking Water Quality (HEALTH AND WELFARE CANADA document, 1979).

The hemograms consisted of RBC count, hemoglobin, packed cell volume, total and differential WBC counts, mean corpuscular hemoglobin (MCV) and mean corpuscular hemoglobin concentration (MCHC). The serum biochemical profiles included fasting glucose, urea nitrogen, total bilirubin, cholesterol, total protein, albumin, globulin, alkaline phosphatase, glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), sodium, potassium, calcium and phosphorus. Eyes of each rat were subjected to a complete funduscopic and biomicroscopic examination once before treatment and again before the necropsy. Twenty-four h urine samples were collected from each of a sub-group of 5 rats for the determination of specific gravity, volume, appearance, pH, ketone bodies, bile pigment, glucose, occult blood and microscopic examination of the urinary sediment.

After 13 weeks of treatment all animals were lightly anesthetized with ether and blood was removed from the abdominal aorta. All animals were subjected to gross pathological examination. The organs which were excised and weighed were: adrenals, heart, kidneys, liver, lungs, ovaries, testes, pituitary, prostate, uterus, spleen, thyroid and brain. All tissues of the animals from Group 1 (double distilled de-ionized water) and Group 5 (high concentration) were excised and fixed in 10% buffered formalin for histopathologic evaluation using the method described by VILLENEUVE et al (1979). The tissues examined in this manner included: adrenals, aorta, central and peripheral nerves, stomach, small and large intestine, colon, rectum, urinary bladder, kidneys, heart, lungs, mesentery lymph node, mammary glands, esophagus, ovaries, prostate, salivary glands, pancreas, pituitary, testes, uterus, muscle, skin, thymus, thyroid, tongue, trachea and sternum. Smears of the femoral bone marrow from the control (Group 1) and high dose (Group 5) animals were assessed for cellularity following May-Grundwald-Giemsa staining.

Levels of elements and fluoride were determined in the drinking water prior to the start of the experiment. Arsenic and selenium (hydride-flameless atomic absorption), cadmium and lead (flame atomic absorption), chromium, copper, iron, nickel and zinc (inductively-coupled plasma emission), and fluoride (ion selective electrode) were analyzed in drinking water samples by Barringer Magenta Co., Toronto, using standard methods.

RESULTS AND DISCUSSION

No spontaneous death occurred in the control or treated animals. Clinical signs of toxicity were not observed. The food consumption and weight gain were not affected by treatment. The fluid intake showed no significant difference between groups. (Group 1 male: 39-45 ml/rat/day: female: 39-46/ml/day). The organ weights were not affected by treatment; these included: adrenals, heart, liver, lungs, ovaries, testes, pituitary, prostate, uterus, spleen, thyroid and brain. No significant differences were observed in the following serum biochemical values:

fasting glucose, BUN, total bilirubin, cholesterol, total protein, alkaline phosphatase, albumin, globulin, albumin/globulin ratio, SGOT, SGPT, sodium, potassium, calcium and phosphorus. All hematological parameters were found to be normal: RBC, Hgb, Hct, MCV, MCHC, platlets, total and differential WBC and the cellularity of the bone marrow. Urinalysis and ophthalmological examination showed no treatment-related changes. Gross pathologic examination revealed that some control and treated animals had dilated pelvis, congested thymus, reddening stomach wall and excessive mucus in the trachea. These lesions were of sporadic occurrence rather than specific in nature. Histopathologic results showed some subclinical intercurrent infections which were equally evident in the control and treated animals. These changes consisted of mild murine respiratory mycoplasmosis, mononuclear infiltrates in the liver and heart, hydronephrosis, lymphocytic thyroiditis, mild prostatitis, cystitis and necrotic gastric mucosa.

The data presented here indicate that no deleterious effects occur in rats fed a mixture of inorganic substances for 90 days. The results are not entirely unexpected for the Great Lakes Water Quality Objectives are established to protect the "most sensitive species". In most cases this species is an aquatic organism which is affected at much lower levels than would cause an effect in mammals. However the study does answer a question as to whether even at these low levels there might be some deleterious interactive effects. The results quite clearly show that even at concentrations 25 times higher than the "objective" levels no effects were observed histologically, biochemically or hematologically. Since the levels of inorganic elements (except Zn and Cu) chosen in the present study are in most cases, higher than those recommended to be safe levels for Canadian drinking water (HEALTH AND WELFARE CANADA, 1979), it would indicate that the Water Quality Objectives contained in the 1978 Agreement afford some measure of protection for humans in addition to aquatic organisms.

CONCLUSION

The oral administration of inorganic substances at levels up to 25 times greater than the objectives established in the Great Lakes Water Quality Agreement for 13 weeks did not cause any observed toxic effects in the rat.

REFERENCES

GREAT LAKES WATER QUALITY AGREEMENT OF 1978, International Joint Commission, Canada and the United States.

HEALTH AND WELFARE CANADA document: Guideline for Canadian Drinking Water Quality (1979).

VILLENEUVE, D.C., L. RITTER, G. FELSKY, R.J. NORSTROM, I.A. MARINO, V.E. VALLI, I. CHU, and G.C. BECKING: Toxicol. Appl. Pharmacol. 47, 105(1979).