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Subchronic Effects of a Mixture of "Persistent" Chemicals Found in the Great Lakes

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The 1978 Great Lakes Water Quality Agreement established guidelines or objectives for a series of "persistent" toxic substances that had been identified as contaminants in the Great Lakes ecosystem. Although substantial toxicological information was available on each chemical individually, little was known on the combined effects of these substances. The present study was initiated, therefore, to delineate the combined effects of these "persistent" chemicals and to determine if the established guidelines do permit some measure of safety in a toxicological sense.

MATERIALS AND METHODS

Ground cube diet (Purina Ralston) blended with 4% corn oil was used as the control diet. The 15 chemicals studied in this experiment were dissolved in ether, poured into 4% corn oil and stirred overnight at about 40°C. They were then blended into the ground cube diet, at four different concentration levels. The chemicals employed and the water quality objectives (WQO) for their presence in water are listed in Table I.

Table 1. List of Compounds Studied and the Water Quality Objectives (WQO) for Their Presence in Water

Chemicals	ppb	Chemicals	ppb
Aldrin	0.0005	Mirex	0.005
Dieldrin	0.0005	Toxaphene	0.008
DDT	0.0015	Arochlor 1254	0.001
DDE	0.0015	DEHP	0.600
Endrin	0.002	MEHP	0.200
Heptachlor	0.001	Dibutylphthalate	4.000
Lindane	0.010	Chlordane	0.060
Methoxychlor	0.040		

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Male and female Sprague-Dawley rats (21 days old) were purchased from Charles River Canada, Montreal, acclimatized for 1 week and divided into 5 groups of 20 animals for each sex. The animals were housed individually and allowed food and water *ad libitum*. Conditions of temperature (about 24°C), relative humidity (about 50%) and illumination (12 hour light - 12 hour dark) were regulated.

The five groups received the following diets, for 91 days:

Group 1 - (control group) 4% corn oil in ground cubes;
Group 2 - 1X WQO guideline (in 4% corn oil, in ground cubes)
Group 3 - 10X WQO guideline; " "
Group 4 - 100X WQO guideline; " "
Group 5 - 1000X WQO guideline. " "

The beginning of the experiment was staggered over 4 days, starting with 50 male rats (10 in each group) on Day 1 to end with 50 female rats on Day 4.

The animals were weighed on Day 1 and every week thereafter. Food intake was measured during Weeks 1, 4, 8 and 12. Urine samples were collected during Weeks 5 and 9 to be analysed. A clinical examination of the animals was performed once a week, at weighing, and a thorough clinical observation was made every fourth week. Teeth were trimmed whenever needed. At the end of the experiment, after 13 weeks on test, the animals were exsanguinated via the abdominal aorta while under ether anesthesia. A 2-ml aliquot of blood was preserved in anticoagulant for hematological purposes (10 males and 10 females per group). The remainder was poured into a tube and centrifuged; the serum was recovered and frozen for biochemical assays (all animals). The autopsies were scheduled over four days, with 50 animals sacrificed per day.

On days 1, 2, 3 and 4 of the experiment, the food containers were weighed full and the weight recorded. Two to 4 days later, the weight was taken again and recorded. During Weeks 4, 8 and 12, the food intake was also measured over 2 or 3 days; the average daily consumption was calculated.

At autopsy all animals were examined for gross pathological changes and the following tissues excised and weighed: liver, spleen, kidney, heart and brain. A portion of the liver was used to prepare a homogenate for measurement of mixed function oxidase activity. All other tissues were collected and preserved in 4% formaldehyde solution for further histological evaluation.

At the end of the study, the following hematological parameters were determined on half the animals (10 males and 10 females per group): hemoglobin, hematocrit, erythrocyte count (RBC), total and differential counts of leucocytes (WBC). The following parameters were also calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Bone marrow smears were prepared using femoral marrow aspirated into EDTA solution immediately after the death of the animal. The smears were then fixed in absolute methanol, stained with Wright's stain and prepared with cover slips. Cells were counted in the "granule trail" to a total of 500 and the erythroid, myeloid and lymphocytic plus monocytic cells were totalled separately. A category was reserved for bare nuclei which were injured in smearing and therefore unidentifiable. The results were analysed using an analysis of variance and where significant differences occurred ($p < 0.05$), the Least Significant Difference test was performed.

Serum collected from each animal at the end of the experiment was used for biochemical determinations. The following analyses were performed using an autoanalyzer (SMA 12/60, Technicon): calcium, cholesterol, glucose, uric acid, inorganic phosphorus, sodium, potassium, total bilirubin, alkaline phosphatase, glutamic oxalacetic transaminase (GOT), total protein, and lactic dehydrogenase (LDH).

Hepatic microsomal aniline hydroxylase (AH) and aminopyrine demethylase (APDM) activities were also determined on fresh liver homogenate (12,000 xg supernatant) by an automated procedure based on the methods of Fouts (1963) and Cochin & Axelrod (1959), respectively.

The data were subjected to a one way analysis of variance. In cases where significant differences ($p < 0.05$) were found, the data were subjected to Duncan's Multiple Range test to determine the groups where a significant difference had occurred.

RESULTS AND DISCUSSION

No animals exhibited abnormalities throughout the experiment with the exception of two female rats who died in the first two weeks of treatment (100X WQO). The cause of death could not be determined but did not appear to be treatment related.

The food intake in male rats showed no significant differences between groups during Weeks 4, 8 and 12 (Fig. 1). Female rats, however, exhibited several significant differences. During Week 4, Group 3 differed from Group 1, and during Week 12, Groups 3, 4 and 5 differed from Group 1 (Fig. 2).

The body weight gains were not significantly different among the groups. The relative organ weights (percentage of body weight) for the heart, liver, spleen, kidney and brain exhibited no statistically significant differences.

The hematological changes (hemoglobin, hematocrit, RBC, WBC, MCV, MCHC, and MCH) seen in this study were considered mild and were not outside the normal range of values for Sprague-Dawley rats. The

lymphocyte count observed at 1000X WQO (6.74 ± 0.48 x $10^3/mm^3$ did not differ from those obtained in the controls (6.43 ± 0.46 x $10^3/mm^3$); the percentage of monocytes also did not differ (7.1 ± 0.5 vs 7.6 ± 0.89%).

No changes were found in the bone marrow smears in either male or female rats. In general these tissues were normal with a tendency to high cellularity (60-80%). Cell distribution was normal and there were equal proportions of erythroid predominance in females. The maturation of all cell types was synchronous and normal.

No biochemical parameter (calcium, cholesterol, glucose, uric acid, bilirubin, inorganic phosphorous, alkaline phosphatase, SGOT, LDH, protein) for either sex was altered by the treatment. Male rats in Group 2 (IX WQO) showed a slight decrease in potassium levels as compared with the other groups; female rats in Group 4 exhibited a slight decrease in sodium levels when compared with the other groups. Neither of the changes was dose-related, and was not considered to be toxicologically significant. Furthermore, the hepatic microsomal activities for AH and APDM were all within the values observed in the control group.

The compounds fed at levels of 1 to 1000 times the WQO guidelines did not cause dose-dependent histological changes in the tissues of animals of either sex. The livers were relatively normal throughout with only a few animals in each group exhibiting mild abnormalities. The renal changes were largely related to ageing changes in basement membranes. There was an inverse effect of treatment on the lung with a reduced amount of inflammatory disease in the high-dose groups (4 and 5), which may indicate the exhalation of volatiles from these compounds may have some mild antiseptic effect. In summary, the treatments caused no dose-related changes in the tissues examined.

These experiments demonstrate that mixtures of 15 different compounds, considered to be "persistent", can be well tolerated by both male and female rats when consumed for 91 days. No dose-related toxicological changes were noted. Although food consumption was significantly lower for the females during Week 12, body weight was not significantly affected. Male rats exhibited no such change. The levels of compounds fed in the study were 1 to 1000-fold multiples of the water quality objectives proposed for control of "persistent" chemicals found in the Great Lakes. The results are reassuring since they indicate, when several important contaminants were tested in combination at their water quality objectives and even at levels several orders of magnitude higher, they were devoid of toxic consequences.

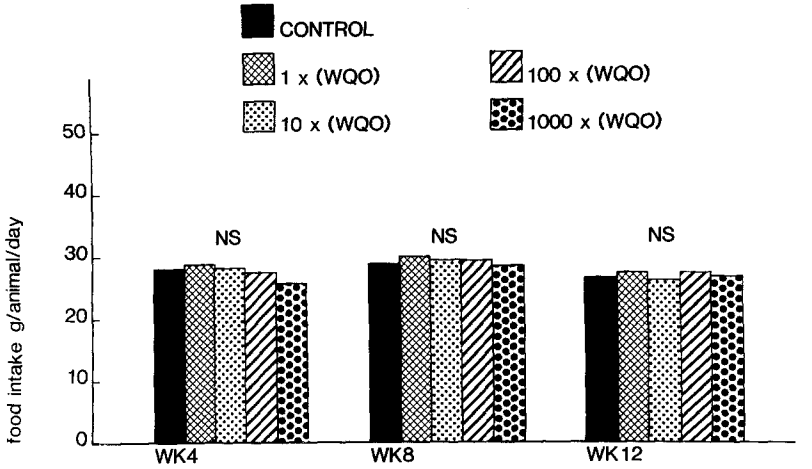

Figure 1. Food intake of male rats during subchronic feeding.

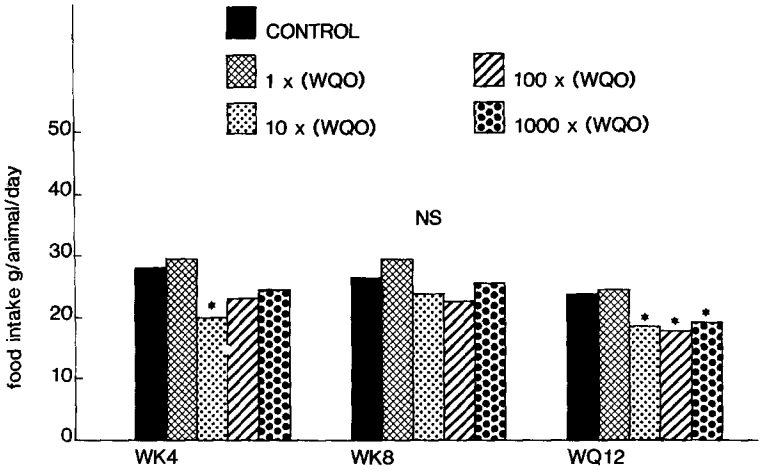

Figure 2. Food intake of female rats during subchronic feeding.

REFERENCES

Cochin J, Axelrod JJ (1959) Biochemical and pharmacological changes in the rat following chronic administration of morphine, naloprhine and normorphine. J Pharmacol Expt Therap 125, 105-110.

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

"Great Lakes Water Quality Agreement of 1978". International Joint Commission, November 22, 1978. Annex I.

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