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Short–Term Toxicity of Nine Industrial Chemicals

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There are a number of industrial chemicals currently used in Canada in sufficiently large quantities that warrant a careful environmental and human health hazard assessment by the regulatory agencies. A review of the existing toxicity data for these chemicals indicated that most of the studies were inadequate due to study design, small group size, inadequate procedures or insufficient parameters being monitored. In order to determine if further studies were warranted it was decided to screen 9 of these chemicals in a short-term study using male and female rats. The chemicals were chosen based on considerations such as quantity, availability of toxicological data, chemical and structural properties and commercial availability. The chemical selected were: tri(butoxyethyl) phosphate, dimethylol urea, 2-butyne-1,4-diol, triallyl-s-triazine-trione, cyclohexanone oxime, p-toluene sulphonhydrazide, 2-nitroaniline, propargyl alcohol and 5-methyl-1H-benzotriazole. The assay consisted of a 14-day oral dosing regime followed by a comprehensive evaluation of biochemical, hematological and histopathological changes. The highest dose level was arbitrarily chosen at 100 mg/kg b.w./day for this screening study and only those chemicals which produced overt toxicity would be investigated further.

MATERIALS AND METHODS

The chemicals were obtained from Aldrich Chemical Company (St. Louis, Mo.) and the stated purity of these compounds ranged from 97-99%. Young, adult male and female Sprague-Dawley rats weighing 200-300 g were purchased from Charles-River Laboratories (St. Constante, Quebec), randomized and assigned to their treatment groups (10 animals per sex/per group). The animals were housed individually in suspended stainless steel mesh cages and acclimatized to laboratory conditions (temperature 20 ± 2°C; humidity 40-60%; 12 hr alternate light/dark cycle) for 1 week before treatment was initiated. A commercial certified laboratory rodent chow (Purina Chow, Ralston Purina) and tap water were available to the animals ad libitum. The animals were dosed orally using a flexible intragastric cannula for 14 consecutive days. The dose

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levels were 1, 10, or 100 mg/kg body weight except for two chemicals - propargyl alcohol (0.1, 1.0 or 10 mg/kg b.w.) and p-toluene sulphonhydrazide (0.5, 5.0 or 50 mg/kg b.w.). With these last two chemicals preliminary studies indicated that the animals would not tolerate 100 mg/kg b.w. The vehicle and dose levels for each chemical administered are listed below: tri(butoxyethyl)phosphate (corn oil) 1.0, 10, 100 mg/kg; dimethylol urea (1% methyl cellulose) 1.0, 10, 100 mg/kg; 2-Butyne-1,4-diol (water) 1.0, 10, 100 mg/kg; triallyl-s-triazine-trione (corn oil) 1.0, 10, 100 mg/kg; cyclohexanone oxime (corn oil) 1.0, 10, 100 mg/kg; p-toluene sulphonhydrazide (corn oil) 0.5, 5.0, 50 mg/kg; 2-Nitroaniline (corn oil) 1.0, 10, 100 mg/kg; propargyl alcohol (corn oil) 0.1, 1.0, 10; 5-methyl-1H-benzotriazole (corn oil) 1.0, 10, 100 mg/kg. Each chemical was tested individually, but males and females were treated concurrently. The control animals received an equal volume of corn oil, 1.0% methyl cellulose or tap water depending on the solubility of the test substance. All animals were examined daily for clinical signs and body weight was recorded daily. On day 15 all surviving rats were lightly anesthetized with ether and exsanguinated from the abdominal aorta. All animals were examined at the time of necropsy for gross changes. The brain, heart, liver, kidney and spleen were excised and weighed. Serum biochemical and hematological determinations were made according to the procedures described in the literature (Villeneuve et al., 1979). A sample of fresh liver was excised for the determination of microsomal aniline hydroxylase (AH, Fouts, 1963) aminopyrine demethylase (APDM, Cochin and Axelrod, 1959) and ethoxyresorufin deethylase activities (ER, Burke and Mayer, 1974). The following tissues were taken and fixed in 10% buffered formalin (pH 7.4) for routine histological examination: brain, pituitary, liver, kidneys, spleen, heart, thyroid, parathyroid, thymus, lungs, trachea, bronchi, duodenum, colon, bone marrow, heart, skin, skeletal muscle, adrenals, pancreas, small and large intestines, salivary gland, esophagus and gastric fundas and cardia, testis and ovary. Bone marrow was aspirated from the femur in order to prepare smears for differential counts (Villeneuve et al., 1979).

The data were subjected to a one-way analysis of variance. When significant differences ($p < 0.05$) were indicated the data were subjected to Duncan's Multiple Range test to determine which groups were significantly different.

RESULTS AND DISCUSSION

Two rats died during the dosing period. One male in the highest dose group and one female in the lowest dose group died after receiving 8 doses of 2-butyne-1,4 diol. Neither of these deaths were judged to be treatment-related. Some of the animals in the high dose group had blood-tinged nasal discharges, piloerection and diarrhea. Three males and two females dosed with 100 mg/kg b.w. triallyl-s-triazine-trione were killed after 10, 11, 11, 10 and 11 doses respectively. These animals had lost considerable weight and were sacrificed for humane reasons. All rats (male and

female) treated with 100.0 mg/kg body weight of p-toluene sulphonhydrazide showed hind leg weakness and some had alopecia in the lower abdominal region. No clinical signs were observed with the other chemicals tested.

Two chemicals produced an effect on body weight gain (Table 1). A significant growth depression was observed in male rats dosed with 100 mg/kg b.w. 2-butyne-1,4-diol and in male and female rats dosed with 100 mg/kg b.w. triallyl-s-triazine-trione. The remaining chemicals showed no effect on body weight gain in either sex at the dose levels reported here. p-Toluene sulphonhydrazide when administered at 100 mg/kg b.w. in a preliminary study caused a weight loss in both males and females; however no such effect was noted here when the dose administered was reduced to 50 mg/kg b.w.

The effects of treatment on organ weights are summarized in Table 2. A significant increase in liver weight expressed as percent body weight was observed in both males and females dosed with 100 mg/kg b.w. 2-butyne-1,4-diol. Male rats dosed with 100 mg/kg b.w. triallyl-s-triazine trione showed increased liver and kidney weights and a decreased spleen weight. Cyclohexanone oxime caused enlarged spleens in both males and females at 100 mg/kg b.w. Toluene sulphonhydrazide caused an increase in kidney weight in males dosed with 50 mg/kg b.w. and a decreased spleen weight in females dosed at 0.5 mg/kg b.w.

Both males and females treated with 100 mg/kg b.w. 2-butyne 1,4-diol showed an increase in serum cholesterol levels (control males, 60.0 mg/100 ml; treated males, 77.1; control females, 65.3; treated females 112.1). Female rats treated with this same chemical at 100 mg/kg showed an increase in serum calcium levels (controls, 10.6 mg/100 ml; treated, 11.2) and decreased GOT

Table 1. Summary of effects of body weight gain

Treatment	Initial Body Weight (g)	Body Weight Gain (g) (or loss)
Control (male)	305 ± 9 (10)	86 ± 16
2-Butyne-1,4-diol (100 mg/kg male)	300 ± 9 (9)	51 ± 13
Control (male)	298 ± 5 (10)	64 ± 19
Triallyl-s-triazine-trione (100 mg/kg (male)	295 ± 5 (7)	-41 ± 32
Control (female)	223 ± 15 (10)	15 ± 10
Triallyl-s-triazine-trione (100 mg/kg (female)	212 ± 13 (8)	- 4 ± 11

Values represent mean ± S.D. Treated group values significantly different from controls ($p \leq 0.05$). Values in parenthesis are number of animals per group.

Table 2. Summary of effects on organ weights

Treatment	Liver Weight (% b.w.)	Spleen Weight (% b.w.)	Kidney Weight (% b.w.)
Control (male)	3.6 ± 0.3 (10)	-	-
2-Butyne-1,4-diol (100 mg/kg female)	5.5 ± 0.5 (9)	-	-
Control (female)	3.5 ± 0.4 (10)	-	-
2-Butyne-1,4-diol (100 mg/kg female)	5.3 ± 0.5 (10)	-	-
Control (male)	4.0 ± 0.3 (10)	0.19 ± 0.03 (10)	0.35 ± 0.09 (10)
Triallyl-s-triazine-trione (100 mg/kg male)	6.0 ± 0.6 (7)	0.14 ± 0.03 (7)	0.52 ± 0.06 (7)
Control (male)	-	0.20 ± 0.03 (10)	-
Cyclohexane Oxime (100 mg/kg male)	-	0.30 ± 0.04 (10)	-
Control (female)	-	0.21 ± 0.03 (10)	-
Cyclohexane Oxime (100 mg/kg female)	-	0.41 ± 0.04 (10)	
Control (male)	-	-	0.37 ± 0.03 (10)
P-Toluene Sulphonhydrazide (50 mg/kg male)	-	-	0.41 ± 0.03 (10)
Control (female)	-	0.26 ± 0.04 (10)	-
P-Toluene Sulphonhydrazide (0.5 mg/kg female)	-	0.21 ± 0.02 (10)	-

Values represent mean ± S.D. Treated group values significantly different from control group values ($p \leq 0.05$).

Values in parenthesis are number of animals per group.

(controls, 144.8 mU/100 ml; treated, 106.5) and glucose levels (controls, 177.5 mg/100 ml; treated, 154.6). Males and females dosed with 100 mg/kg triallyl-s-triazine trione had increased cholesterol levels (control males, 72.4 mg/100 ml; treated, 153.8; control females, 73.4; treated, 98.0), decreased alkaline phosphatase levels (control males, 442 mU/ml; treated males, 194; control females, 341; treated females, 251) and decreased potassium levels (control males, 4.9 mg/100 ml; treated, 3.2; control females, 5.0; treated, 4.3). Males dosed with 100 mg/kg showed increased bilirubin (controls, 0.13 mg/100 ml; treated, 0.32) and sorbitol dehydrogenase activity (controls, 7.9; treated, 14.7). Males of this dose group also showed decreased calcium (controls, 11.1 mg/100 ml; treated, 9.4), inorganic phosphorus (controls, 8.9 mg/100 ml; treated, 6.5) and uric acid (controls, 1.59 mg/100 ml; treated, 1.25). Females treated with 100 mg/kg showed decreased glucose levels (control, 143 mg/100 ml; treated, 107). Males treated with 100 mg/kg cyclohexanone oxime had increased serum potassium levels (control, 4.5 mg/100 ml; treated, 4.9). Males treated with 50 mg/kg p-toluene sulphonhydrazide had increased potassium levels (controls, 4.8 mg/100 ml; treated, 5.1) and both males and females from this same dose group showed decreased GOT levels (control males, 223 mU/ml; treated males, 142; control females, 174; treated females, 85).

Females treated with 100 mg/kg 2-butyne-1,4-diol showed a slight increase in APDM activity (controls, 11.0 nmol HCHO/hr/mg protein; treated 14.8). Triallyl-s-triazine trione did not cause any induction of the mixed function oxidases monitored. Cyclohexanone oxime given at 100.0 mg/kg b.w. produced a significant increase in AH activity in both males and females (control males, 15.7 nmol PAP/hr/mg protein; treated males, 24.9; control females, 19.6; treated, 44.2). Females dosed with 100 mg/kg of this chemical also showed increased APDM activity (controls, 25.4; treated, 47.1). p-Toluene sulphonhydrazide at 50 mg/kg b.w. caused increased AH activity in both males and females (control males, 21.2 nmol PAP/hr/mg protein; treated males, 28.1; control females, 18.4; treated females, 27.6). The remaining chemicals showed no effects on hepatic microsomal enzyme activities.

2-Butyne-1,4-diol when administered to females at 100 mg/kg caused decreased red cell counts (controls, 7.5 x $10^6/\mu L$ treated 6.8), decreased hemoglobin content (controls, 14.4 g/dL; treated, 13.1) and hematocrit (controls, 40.9%; treated, 37.5). None of the hematological parameters were affected in males.

Triallyl-s-triazine trione caused no alteration of hematological parameters in either males or females.

Both males and females treated with 100 mg/kg b.w. cyclohexanone oxime showed decreased RBC (control males, 6.9 x $10^6/\mu L$; treated males 5.6; control females 6.9; treated females, 5.1), decreased hemoglobin (control males, 13.3 g/dL; treated males, 11.6; control females, 13.5; treated females, 1.2), decreased hematocrit (control male, 37.5%; treated males, 32.7; control females, 38.0;

treated, 32.1), increased MCH (control males, 19.2 pg; treated males, 20.9; control females, 19.3; treated females, 21.9) and increased MCV (control males, 54.3 fL; treated males, 59.0; control females, 54.3; treated females, 62.8) In addition males dosed 100 mg/kg b.w. showed increased platelet values (controls, 11.4; treated, 13.2).

Examination of bone marrow smears indicated that cyclohexanone oxime administered at 100.0 mg/kg b.w. caused a significant decrease in myeloid counts, increased number of erythroids and decreased lymphocytes and monocytes in both male and female rats. p-Toluene sulphonhydrazide did not produce any hematological alterations at the dose levels used in this study, i.e. maximum 50 mg/kg b.w. (In a preliminary study where the chemical was administered to male rats at 100.0 mg/kg b.w. there was a significant increase in RBC, HCT, HGB and MCH and decrease in WBC counts).

No treatment related hematological aberrations were detected in rats administered tri (butoxyethyl) phosphate, dimethylol urea, 2-nitroaniline or 5-methyl-1H benzotriazole.

All males and only one female dosed with 100.0 mg/kg b.w. triallyl-s-triazine trione had smaller spleens and occasionally dark liver and kidneys. The spleen in the animals sacrificed before term were also smaller in size and the kidneys in one of the males appeared darker. Lung congestion was observed in a few females (6/30) at all dose levels as well as in controls (1/10). Enlarged spleens were noticed in females on 100.0 mg/kg b.w. cyclohexanone oxime (10/10).

The most severe histological changes in this study were associated with the administration of triallyl-s-triazine trione, and are restricted to the 100 mg/kg dose group. This chemical caused moderate to severe thymic cortical atrophy which tended to be more severe and predominant in males. In males, this was accompanied by moderate to severe atrophy of the mesenteric node. There were also mild to moderate renal tubular degenerative changes that occurred in males only. Splenic changes consisting of a moderate degree of follicular and mantle zone atrophy were more predominant and severe in males. Females dosed with 100 mg/kg b.w. cyclohexanone oxime displayed splenic changes (sinusoidal hyperplasia) which were more prevalent and severe than in males. Histologically there were cellularity changes noted in bone marrow which were more severe in females than males dosed 100 mg/kg b.w. A mild to moderate incidence of kidney inclusions was found in males (but not females) dosed with 100 mg/kg b.w.

Of the nine chemicals tested, only four showed toxic properties in this short-term study (2-butyne-1,4-diol, triallyl-s-triazine-trione, cyclohexanone oxime, p-toluene sulphonhydrazide). Of these, triallyl-s-triazine trione was the most toxic based on the severe body weight changes and histopathological changes it produced. This chemical also caused a variety of biochemical

effects including elevated bilirubin and cholesterol levels and decreases in calcium, phosphorus and potassium levels. The loss of body weight observed in this study is not unexpected in light of the thymic cortical atrophy. The changes induced by this chemical were in general more severe in males than females. Unlike the other three chemicals, the hematopoietic system was unaffected by treatment. p-Toluene sulphonhydrazide did not have the same dramatic histological effects as triallyl-s-triazine trione, but when it was administered at 100 mg/kg b.w. in a preliminary study it did cause reduced body weight gain and hind limb paralysis. At the highest dose level used in this study (50 mg/kg b.w.) neither of these effects was observed. Similarly, hematological changes (increased hemoglobin, hematocrit, RBC and MCH) and biochemical changes (increased cholesterol levels, induction of mixed function oxidases) were observed at 100 mg/kg b.w. but not observed at 50. It would appear then that 50 mg/kg b.w. would be the no effect level for this chemical. 2-Butyne-1,4-diol affected body weight gain and produced some overt signs of toxicity as demonstrated by the nasal discharges and diarrhea. The biochemical effects included increased cholesterol and SDH levels and an induction of mixed function oxidase activity (AH and APDM). Females appeared to be less sensitive to this chemical than males in that they did not show any reduction in body weight gain, increased SDH activity of induction of AH or APDM activities. They did, however, show some hematological effects at 100 mg/kg b.w. (decreased RBC, hemoglobin and hematocrit; increased MCH and MCV) that were not evident in the males. There were no significant treatment-related histological changes observed in either males or females. The effects seen with cyclohexanone oxime were primarily associated with the hematopoietic system and agree with previous reports on the hematotoxicity of this chemical (Lomonova, 1966, Tsulaya et al., 1975, Derelanko et al., 1985). The only other significant finding was that this chemical induced mixed function oxidase activity and females were more sensitive than males. The hematopietic changes were characterized by reduced hemoglobin, hematocrit and numbers of red blood cells. The spleen was enlarged and on histological examination showed sinusoidal hyperplasia. The cellularity of the bone marrow was affected and a quantitative cytological evaluation revealed that there was a decreased number of myeloid cells and an increased production of erythroid cells. These changes were more prevalent and severe in females than in males.

Four of the 9 chemicals tested in this study produced toxic effects in 14-day repeated-dose studies. Based on the information generated, further studies are warranted on triallyl-s-triazine trione, p-toluene sulphonhydrazide, 2 butyne-1,4-diol and cyclohexanone oxime to address the potential long-term effects of these chemicals.

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