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A Teratogenicity and Tissue Distribution Study on Dibromochloropropane in the Rat

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Fumazone (Nemagon, 1,2-dibromo-3-chloropropane, DBCP) is a soil fumigant used to control nematode infestation in crops. It was reported to be mutagenic with Salmonella typhimurium TA1530 but not with TA1538 (ROSENKRANZ 1975) and carcinogenic in both the rat and mouse (OLSON et al. 1973). A 90 day feeding study with rats showed a retarded weight gain in females at 150 ppm and in males at 450 ppm (TORKELSON et al. 1961). Gonadotropic effects are attributable to DBCP in both male and female rats (TORKELSON et al. 1961, RAKHMATULLAEV 1971). Radishes and carrots have been reported to absorb DBCP from the soil while cooking reduced the residues of DBCP by two-thirds of the initial concentration (NEWSOME et al. 1977).

MATERIALS AND METHODS

Primiparous Wistar rats (Woodlyn Farms, Guelph, Ontario), 175-200 g were paired overnight with proven sires. Presence of sperm in the vagina on the following morning was indicative of day 1 of pregnancy in the female. The pregnant animals were randomized into four groups each of 15 rats; 3 groups for the chosen dosages and the fourth a control group. Females were housed individually and had free access to food (Master rat cubes) and water. Dosages of 0, 12.5, 25.0 or 50.0 mg of DBCP (Fairfield Chemical Co.) per kg body weight were prepared in corn oil and given daily by gavage at 1.0 mL corn oil/200 g rat on days 6 through to 15 of gestation. The purity of DBCP was 97.5% (NEWSOME et al. 1977). Necropsies were carried out on day 22 of gestation to provide the following information: litter size, individual litter weights, number of deciduomas and maternal weight gain. The maternal weight gain was determined by subtracting from the female's weight on day 1 of gestation her weight at necropsies after removing the pups and uterine horns. Approximately two-thirds of the fetuses from each litter were processed for skeleton examination while the remainder was fixed in Bouin's fluid for visceral investigation.

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Another twenty-five pregnant females were treated on days 6-15 of gestation with 25 mg DBCP per kg body weight while 5 other pregnant females received the placebo treatment. At 1, 3, 6, 12 and 24 h after their last dose on day 15 of gestation, 5 females were sacrificed by mild ether anaesthesia and exsanguination. One female of the control group was sacrificed at each of the specified times. The following samples were removed and analyzed for DBCP: pooled fetuses, spleen, brain, heart, lung, kidney, liver, fat and blood.

Samples of the tissues were weighed (0.5-1.5 g) and homogenized for 1 min at high speed with 25 mL of absolute ethanol in a Sorvall Omni-Mixer. The homogenate was filtered through Whatman No. 1 filter paper by gravity and 2 mL of the filtrate were added to 8 mL of a 1M NaCl in a 15-mL centrifuge tube. Hexane (1.0 mL) was added to the centrifuge tube, shaken and 3 µL of the hexane phase injected into a Hewlett-Packard 5700A gas chromatography (GC) apparatus. The GC unit was fitted with a ^{63}Ni electron-capture detector and a 1.8 m x 4 mm i.d. glass column packed with 6% QF-1 and 4% SE-30 on 80-100 mesh Supelcoport. Operating parameters were: column temperature 130°C, injection port 150°C, detector 300°C and carrier flow of argon-methane (95:5) 26 mL/min. With these conditions and routine working attenuation, approximately 50% full scale deflection was given by 0.015 ng of standard DBCP.

Recoveries of DBCP added to heart, lung, kidney, spleen, liver or fat at a level of 0.10 ppm then homogenized averaged 90.5% for all tissues with a standard error of ± 1.0. The recovery of DBCP added to fat at levels of 1, 5 and 10 ppm had a mean ± SE of 91.6 ± 2.3%.

RESULTS AND DISCUSSION

DBCP was not teratogenic at the dosages investigated but there was a fetotoxic effect. No skeleton or visceral anomalies of significance were observed above those noted in the control fetuses. There was a statistical difference ($P < 0.05$) between the mean fetal weight for fetuses from the 50.0 mg/kg dosed mothers and the control fetuses (Table 1). The 10 litters at the highest dose were less when compared to 13-14 litters for all other treatments but there is no significance ($P > 0.05$) with the X^2 test. At necropsy, 4 females in this group were recorded as not pregnant but in 3/4 cases the uteri were adematous and contained a pinkish fluid. It would appear that treatment with 50 mg/kg DBCP was lethal in a few cases to the developing embryo.

The fetotoxic effect of DBCP at 50.0 mg/kg, however, is related to its maternal toxicity (Table 1). Two dosages, 25.0 and 50.0 mg/kg, affect significantly, ($P < 0.05$ and $P < 0.001$, respectively) the mean maternal weight gain when compared to the weight gain for the control group. One female in the 50.0 mg/kg group died two days after receiving her last dose. The mean ± SE maternal weight gain for the 0, 25.0 and 50.0 mg/kg treatments were 53.0 ± 4.0, 35.3 ± 4.2 and 16.3 ± 3.7 g, respectively. Where there was a reduction of the mean maternal weight gain there was an affect upon the fetal weight. Although the mean fetal weight at the 25 mg/kg dose was not statistically significant at $P < 0.05$ when compared to the control fetal weight, it was significant at $P < 0.1$. There was no maternal toxicity with the 12.5 mg/kg treatment nor any effect upon the mean fetal weight.

TABLE 1

Effects of 1,2-dibromopropane given by gavage on the 6th through to the 15th day of gestation in the rat

Dose (mg/kg)	Litters	Litter Size (mean S.E.)	Fetal Weight (g, mean S.E.)	Maternal Weight Gain (g, mean S.E.)
0	13	11.5 ± 0.7	5.0 ± 0.08	53.0 ± 4.0
12.5	13	13.2 ± 0.4	4.9 ± 0.06	43.2 ± 5.0
25.0	14	12.0 ± 0.9	4.7 ± 0.1	35.3 ± 4.2*
50.0	10	14.2 ± 0.4	4.4 ± 0.4*	16.3 ± 3.7**

* $P < 0.05$ ** $P < 0.001$

Table 2 lists the amounts of DBCP (ppm) found in the tissues examined at the specified times after the last of 10 daily oral treatments. Chromatograms of tissue samples from the control group contained peaks corresponding to an apparent DBCP concentration of 0.003 ppm in the heart, 0.002 ppm - kidney and 0.021

TABLE 2

Amount of 1,2-dibromo-3-chloropropane (ppm) in tissues at specified times after last of 10 consecutive daily doses (25 mg/kg) to pregnant rats

Sample	1 hour	3 hour	6 hour	12 hour	24 hour
Fetus	0.008 ± 0.002	0.030 ± 0.015	0.030 ± 0.013	0	NA
Spleen	0.011 ± 0.001	0.027 ± 0.009	0.023 ± 0.008	0	NA
Brain	0.101 ± 0.028	0.164 ± 0.048	0.211 ± 0.081	0.008 ± 0.001	NA
Heart	0.123 ± 0.027	0.249 ± 0.069	0.260 ± 0.116	0.013 ± 0.004	NA
Lung	0.088 ± 0.014	1.12 ± 0.602	0.646 ± 0.351	0	NA
Kidney	0.016 ± 0.003	0.041 ± 0.014	0.047 ± 0.009	0	NA
Liver	0.009 ± 0.002	0.027 ± 0.012	0.021 ± 0.007	0	NA
Fat	2.53 ± 0.381	3.87 ± 0.480	5.38 ± 0.410	1.37 ± 0.275	0.170 ± 0.071
Blood	0.082 ± 0.022	0.104 ± 0.030	0.100 ± 0.045	0.011 ± 0.001	0

Values are mean ± standard error of 5 samples NA: not analyzed

ppm - fat, and did not contain peaks in the other tissue extracts. Peaks detected in the control tissues were probably co-extractives because of the sensitivity of the method. Twice the amount found in the control tissues was set as the detectable limit.

All tissues from the experimental animals had varying amounts of DBCP and all samples except fat reached their highest values 3 h post gavage. Abdominal fat reached its highest level 6 h after treatment. Fat in comparison to all other tissues always contained the highest amount of DBCP. Heart, brain, fat and blood contained detectable amounts of DBCP at 12 h and by 24 h only fat (0.17 ppm) had DBCP. It would appear that there was no accumulation of DBCP in the tissues examined. All tissues except fat were found not to contain DBCP 24 h after the last of 10 consecutive daily doses.

In summary, dosages of 25.0 and 50.0 mg/kg DBCP were toxic for both dams and fetuses. Except for fat samples, there was no apparent accumulation in the fetus, spleen, brain, heart, lung, kidney, liver and blood following 10 consecutive daily doses of 25.0 mg/kg DBCP.

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