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Single-Generation Drinking Water Reproduction Study of 1,2-Dibromo-3-Chloropropane in Sprague-Dawley Rats

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1,2-Dibromo-3-chloropropane (DBCP) was formerly used in agriculture to control plant nematodes. Animal studies have shown that the oral administration or inhalation of DCBP inhibits spermatogenesis and induces testicular atrophy (Torkelson et al. 1961; Faidysh et al. 1970; Rakhmatullaev 1971; Reznik and Sprinchan 1975; Rao et al. 1983). Similar changes have been reported in humans occupationally exposed to DBCP (Whorton et al. 1979). Since DBCP residues have been detected in ground water samples from 5 U.S. states (Cohen et al. 1984), the purpose of this study was to evaluate the reproductive toxicity of DBCP in rats using drinking water as a route of administration.

MATERIALS AND METHODS

Production grade DBCP was provided by Dow Chemical U.S.A., Midland, MI. Prestudy analysis by gas-liquid gas chromatography (GC) indicated a purity of 97.30%. Male and female Sprague-Dawley rats approximately 60 days old were purchased from Charles River Breeding Laboratories (Wilmington, MA) and acclimated for at least 2 weeks prior to use. The animals were randomly allocated to 5 groups of 10 rats/sex and supplied ad libitum with drinking water solutions formulated to provide target dose levels of 0, 0.02, 0.20, 2.00 or 20.00 mg DBCP/kg/day. Food (Purina Certified Rodent Chow #5002, Ralston Purina Co., Richmond, IN) was also available ad libitum. Dose solutions were prepared biweekly with DBCP concentrations adjusted weekly based on mean group water consumption and body weight values. Aliquots were analyzed periodically for DBCP concentration using electron capture GC. Following a premating exposure period of 60 consecutive days, each male was housed with a single female of the same dose level for a cohabitation period of 5 consecutive days; the male was then returned to its cage for a 2-day rest period before being paired with another female of the same dose level for a 2nd mating period of 5 days. Following the 2nd cohabitation period, each female was transferred to a reproduction cage provided with nesting material (Beta-Chips, Hardwood Shaving, Northeastern Products, Warrensburg, NY).

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The animals were observed daily for signs of toxicity and changes in appearance and demeanor. Water consumption was recorded twice weekly, body weights were recorded weekly, and food consumption (except during cohabitation periods) was also recorded weekly. All litters were examined as soon as possible after parturition and daily thereafter. The following data were recorded for each litter: parturition date; gestation days (i.e., the number of days from 1st day of cohabitation to parturition); litter size at parturition and the number of live and dead pups at parturition. Additional parameters noted over 4 days postpartum included the number of live pups, litter weights, and dam weights. Pups were sexed at gross necropsy. The following reproductive indices were calculated: fertility (percentage of bred females that were pregnant); gestation (percentage of pregnant females that delivered a live litter); gestation survival (percentage of pups born alive) and pup viability (percentage of live born pups that survived to days 1 or 4 postpartum).

All adults, as well as pups that survived through day 4 postpartum, were sacrificed by decapitation and examined for gross pathological alterations (adults were fasted overnight prior to sacrifice). Adult males were sacrificed 2 days after concluding the 2nd cohabitation period while adult females were sacrificed 3-5 days postparturition. The uterine horns of any females that did not deliver a litter were stained with sodium sulfide to detect resorbed fetal implantation sites (Kopf et al. 1964). Weights of adult brain, heart, liver, kidneys, adrenal glands, stomach, testes or ovaries, uterus, epididymides, and male accessory sex organs (prostate, seminal vesicles, and coagulation glands weighed as a unit) were recorded. From control and top-dose animals, paraffin-embedded sections of brain, kidneys, stomach, adrenal glands, liver, and heart (adult males and females); of testes, coagulating glands, seminal vesicles, epididymis, and prostate (adult males); of ovaries, oviduct, uterus, and cervix (adult females); and of testes and epididymides (male pups) were stained with hematoxylin and eosin and examined by light microscopy.

Body weights, water and food consumption, gestation days, and organ weights were evaluated by Bartlett's test (Winer 1971) for equality of variances. Based upon the outcome of Bartlett's test, a parametric or non-parametric analysis of variance (ANOVA) was conducted. Litter summary data and the number of live pups/litter on day 1 of lactation were also analyzed by a non-parametric ANOVA. If ANOVA indicated statistical significance, the data were further analyzed by Dunnett's test (Steel and Torrie 1960) or Wilcoxon's rank-sum test (Steel and Torrie 1960) with Bonferroni's correction (Miller 1966). The fertility and gestation indices were analyzed using Fisher's exact probability test (Siegel 1956). Evaluation of the pup sex ratio was by the binomial test (Steel and Torrie 1960) and gestation and pup survival indices were analyzed using the litter as the experimental unit by the Wilcoxon test, as modified by Haseman and Hoel (1974). Levels for statistical significance were set at alpha = 0.01 (Bartlett's), alpha = 0.10 (ANOVA), and alpha = 0.05 (all others).

RESULTS AND DISCUSSION

Based upon analyses of dose solutions, water consumption and body weight values, the average dosages of DBCP imbibed by rats were 0, 0.017, 0.21, 3.25 and 14.47 mg/kg/day (males) and 0, 0.013, 0.31, 2.67 and 24.38 mg/kg/day (females). For both sexes, the overall average dose levels of DBCP consumed were 0, 0.015, 0.26, 2.96 and 19.43 mg/kg/day. These corresponded to average DBCP drinking water concentrations of approximately 0.13, 1.6, 26 and 324 µg/ml (ppm), respectively.

No mortality was noted among adult animals and no untoward clinical signs or changes in demeanor were noted throughout the study. Rats from the top dose level drank and ate considerably less than any of the other experimental groups, and they gained much less body weight (Table 1). Comparable decreases in body

Table 1. Body weight gain, water and food consumption in rats imbibing DBCP.

Average Dose Level (mg/kg/day)	Males			Females		
	Gain[a]	Water[b]	Food[c]	Gain[a]	Water[b]	Food[c]
0	168.4 (32.0)	38.7 (5.9)	28.8 (4.1)	68.3 (18.0)	35.5 (6.7)	20.3 (1.8)
0.015	165.7 (30.1)	38.4 (5.8)	28.2 (3.6)	69.4 (12.2)	32.3 (5.7)	20.7 (1.3)
0.26	138.1 (31.9)	38.3 (6.5)	26.3 (2.8)	78.9 (19.3)	34.2 (6.1)	21.2 (1.6)
2.96	144.3 (27.5)	37.2 (7.4)	27.4 (3.5)	84.3 (28.2)	33.2 (6.8)	20.9 (2.5)
19.43	61.5* (3.5)	17.6# (3.0)	21.6# (2.8)	19.9* (12.6)	14.8# (2.6)	17.9# (2.0)

[a]Mean body weight gain ± (SD) recorded from study days -1 to 69 for 10 rats/dose level.
[b]Mean of mean water consumption data, n = 18 determinations, ± (pooled SD) for 10 rats/dose level (ml/rat/day).
[c]Mean of mean food consumption data, n = 9 determinations, ± (pooled SD) for 10 rats/dose level (g/rat/day).
*Identified as statistically different from control value by Dunnett's test (alpha = 0.05).
#Indicates statistical differences from control values identified by Dunnett's test (alpha = 0.05). Water and food consumption data for the top dose level rats were statistically lower than control values at almost every time point.

weight, water and food consumption were also noted for top dose females during gestation and lactation (data not shown). Body weights, water consumption, and food consumption of rats imbibing lower dose levels of DBCP were comparable to those of controls. Similar effects have been reported by Weinstein (1979) and Kyle et al. (1982) in other drinking water toxicity studies of DBCP.

The effects of DBCP administration on reproductive parameters are shown in Table 2. Fertility, gestation indices, litter size at parturition and day 1 postpartum, and pup sex ratio were unaffected by imbibing average DBCP dose levels, up to and including 19.43 mg/kg/day. Treatment-related effects noted at this dose level were limited to a statistically significant decrease in the average litter weight of pups versus controls on days 1 and 4 postpartum. In addition, although not statistically significant, a decrease in the day 4 survival index of the top dose level pups was also noted. The marked decreases in body weight, water and food consumption noted among top dose females imbibing DBCP were probably responsible for an inability of the dams to adequately nurse their offspring thereby resulting in a decrease in pup growth and survival.

Consistent with observations during premating exposure, the terminal body weights of adults given an average dose of 19.43 mg/kg/day were significantly depressed (Table 3). Slight decreases in absolute (heart, kidney, adrenal glands and male accessory sex organs) and increases in relative (stomach and brain) organ weights noted among rats from this dose level only (data not shown) were attributed to a poor nutritional status and decreased body weights (Oishi et al. 1979; Schwartz et al. 1973; Feron et al. 1973). As shown in Table 3, testicular weights were unaffected by DBCP administration. Although a statistically significant increase in relative liver weight noted among top dose males was attributed to treatment (Table 3), this effect was not associated with any morphological evidence of liver injury. No lesions were noted at gross necropsy among any adults or offspring that were attributed to treatment, and light microscopic examination of all tissues collected from top dose animals, including reproductive organs, revealed no treatment-related effects.

Although several studies have shown that DBCP, if given in sufficiently high doses by either gavage or inhalation, can adversely affect reproduction in rats, the results of the present study demonstrate no adverse reproductive effects in male or female Sprague-Dawley rats administered DBCP via the drinking water at average dose levels up to 19.43 mg/kg/day. Similar observations were reported in another drinking water reproduction study of DBCP by Weinstein (1979). Differences in the pharmacokinetic behavior of DBCP as a function of the route of administration may afford an explanation for these results. Treatment-related effects noted in the present study were limited to decreases in body weights, water and food consumption, as well as slight increases in relative liver weights, in adults imbibing an average DBCP dose level of 19.43 mg/kg/day. Neonatal effects, noted among top dose litters only, were limited to decreases in

Table 2. Reproductive data for rats imbibing DBCP

	Average Dose Level (mg/kg/day)				
	0	0.015	0.26	2.96	19.43
No. of females	10	10	10	10	10
Fertility index[a]	80% (8/10)	70% (7/10)	90% (9/10)	90% (9/10)	100% (10/10)
Gestation days[b]	25±2	26±3	25±1	23±1	26±3
Gestation index[c]	100% (8/8)	100% (7/7)	90% (9/10)	100% (9/9)	100% (10/10)
Gestation survival index[d]	98% (81/83)	100% (100/100)	97% (99/102)	99% (108/109)	99% (105/106)
No. live pups, day 1[b]	10±5	14±2	11±4	12±3	10±2
Pup survival indices[e]					
day 1	99% (80/81)	99% (99/100)	100% (99/99)	100% (108/108)	95% (100/105)
day 4	99% (80/81)	95% (95/100)	97% (96/99)	100% (108/108)	75% (79/105)
Pup body weight (g)[b]					
day 1	6.8±0.3	6.2±0.6	6.7±0.7	6.6±0.6	5.2±0.6*
day 4	9.7±1.0	8.7±1.3	9.9±1.6	9.3±1.2	6.4±1.1*
Sex ratio of pups (% M:%F)	48:52	51:49	49:51	46:54	49:51

[a]Number of females that either delivered a litter expressed as a percentage of females bred (absolute values in parentheses).
[b]Mean ± SD.
[c]Number of females delivering a live litter expressed as a percentage of the females that delivered a litter (absolute values in parentheses).
[d]Percentage of newborn pups alive at birth (absolute values in parentheses).
[e]Percentage of live born pups surviving to day 1 or day 4 postpartum (absolute values in parentheses).
*Identified as statistically different from control values by Dunnett's test (alpha = 0.05).

Table 3. Organ weights among male rats imbibing DBCP[a].

Average Dose Level (mg/kg/day)	Terminal Body Weight (g)	Organ Weights[b] Testes Abs	Testes Rel	Liver Abs	Liver Rel
0	467 (47)	3.20 (0.32)	0.70 (0.09)	11.51 (1.90)	2.46 (0.24)
0.015	467 (45)	3.40 (0.32)	0.73 (0.08)	11.55 (1.75)	2.47 (0.19)
0.26	443 (42)	3.27 (0.53)	0.74 (0.14)	10.63 (1.30)	2.40 (0.17)
2.96	452 (38)	3.26 (0.27)	0.73 (0.10)	11.18 (1.49)	2.47 (0.19)
19.43	389* (42)	2.82 (0.37)	0.73 (0.11)	10.58 (1.41)	2.72* (0.17)

[a]Mean ± (SD) of 10 rats/dose level.
[b]Absolute (g) and relative (g/100 g terminal body weight).
*Identified as statistically different from control value by Dunnett's test (alpha = 0.05).

pup growth and survival. These changes were considered secondary effects resulting from maternal toxicity associated with this dose level.

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