UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

JAN 12 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: December 21, 1981

SUBJECT: Review of a Reproduction Study with Dibromochloropropane (DBCP). #5481-88. Accession No. 246388. Caswell No. 287.

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Action Requested

Review of new data.

Background

DBCP has been evaluated under a Rebuttable Presumption Against Registration (RPAR), and on October 29, 1979 the Agency's Administrator issued the final decision to suspend all uses of DBCP. Its use on pineapples in Hawaii was not included in the final decision. In the suspension proceedings one of the hazards considered was DBCP's potential to interfere with testicular function in humans (44 FR, November 9, 1981).

The Amvac Chemical Corporation has submitted a reproduction effects study of DBCP and its contaminants (epichlorhydrin and allylchloride). The stated purpose was to investigate the possibility that the two contaminants could be the cause of testicular toxicity associated with DBCP uses and manufacture.

Discussion

The study submitted by the registrant shows that DBCP is toxic to testes of rats given subcutaneous injections of 5 or 25 mg/kg/day for 6 months. Histopathology and serum hormone levels were noted. Fertility studies with treated male rats were inconclusive at the 1 mg/kg/day dose (lowest dose tested), but the 5 and 25 mg/kg/day dosages prevented males of those groups from siring litters. Results with the contaminants show no gross or histopathological effects. Although the substances did change serum hormone concentrations (see attached review), they do not have as great an effect on reproduction parameters as does DBCP.

DATA EVALUATION RECORD

(1) CHEMICAL: DBCP

(2) CITATION: Warren, D. W., N. Ahmad, and J. W. Wisner, Jr. 1981. The effects of 1, 2-dibromo-3-chloropropane (DBCP), epichlorohydrin, and allylchloride on male reproduction in the rat. Final Report of a research contract performed for Amvac Chemical Corporation by members of the Departments of Physiology and Biophysics and Anatomy, University of Southern California, Los Angeles, California. Unpublished and Proprietary.

(3) REVIEWED BY: Signature: Roger Gardiner
Date: 1-8-82

(4) APPROVED BY: Signature: C. J. Chaisson
Date: 1-8-82

(5) FORMULATION: Technical grade and purified.

(6) CONCLUSIONS: Results of these experiments indicate that DBCP directly affects the testes in the germinal epithelium of seminiferous tubules (cells that produce spermatozoa) and the androgenic interstital tissues (Leydig cells which produce testosterone). These effects are manifested histologically (See (8) below, and are also indicated by reduced testosterone concentrations and elevated luteinizing and folicle stimulating hormone levels in serum of treated rats. These effects occur in a dose-related manner in groups given 5 and 25 mg/kg daily doses administered for up to 6 months. Testes, prostate gland, and seminal vesicle weights were also decreased with increased doses of DBCP. No effects on histological, hormonal, or reproductive organ weight observations were found in rats given 1 mg/kg/day doses given for up to 6 months.

Fertility tests with groups 5 rats of showed that DBCP caused sterility when administered for 6 months. Animals treated with 5 or 25 mg/kg doses could not sire litters after mating with untreated females. Results for the 1 mg/kg/day dose group were described by the investigators as inconclusive because 2 of 5 treated rats failed to sire litters after mating with female rat. Females were not proven fertile so that the two males failing to sire litters were not clearly shown to be sterile.

Two additional substances, epichlorohydrin and allyl chloride, known to contaminate DBCP and also known to cause sterility according to the authors, were also tested. These substances did not alter histology or weights of reproductive organs in treated rats.

However, epichlorohydrin decreased testosterone and FSH at the 1 and 5 mg/kg/day levels, while the testosterone level was significantly higher in rats given the 25 mg/kg/day doses. FSH in the males receiving the highest dose was decreased. The only hormone level apparently changed by allyl chloride treatment was for FSH which was decreased (See Table 2 Below).

In view of the results of the fertility experiment, and the unreliable nature of single measurements of serum testosterone (the authors described serum testosterone levels as episodic and widely variable without describing the time of day or other circumstances of sampling), results of this study cannot be used to support a 1 mg/kg/day no-effect level for DBCP.

A comparison of DBCP's effects and those of the two contaminants cannot be done because the contaminants were not evaluated for effects on fertility. The uncertainty expressed by the authors with regard to hormone measurements also complicates a comparison of the three substances. Generally, DBCP affected testicular histology and hormone levels, while the contaminants only affected hormones. No further assessment can be made when these limitations are considered.

A recovery experiment demonstrated that most of the histological effects observed at the end of a 10 week regimen of DBCP treatment (15 mg/kg/day for 3 weeks and 6 mg/kg/day for 7 weeks) were repaired during a 10 week period without treatment. However, the authors did not measure hormone levels or test the animals for fertility by mating. Organ weights were not distinguishable from controls at the end of the recovery period.

Core Classification: Supplementary. This study was designed to evaluate whether effects on the male reproductive system of rats treated with DBCP could be attributed to the contaminants allyl chloride and epichlorohydrin. The standard multi-generation study for evaluation of reproduction toxicity was not used. The experiments described here provide specific information about DBCP's effect on the male rat reproductive system, and they provide preliminary fertility data.

(7) MATERIALS AND METHODS: Test Substances. Pure DBCP (99.7%) with no further descriptions. Technical grade DBCP (96.8) with major contaminant consisting of allyl chloride. The investigators stated that a gas chromatographic analysis was done to confirm the composition of the test material provided by the manufacturer. No lot numbers or results of the chemical analyses were reported.

Other test substances. Allyl chloride and epichlorohydrin were also tested, but these substances were not described in detail.

Animals. Male Long-Evans rats were obtained from Simonson Laboratories (Gilray, California) when they were 52 days old. These animals were housed five per cage in a room having a 12 hour light/dark cycle. They received Purina lab Chow and water ad libitum. Female Sprague-Dawley rats were obtained from Mision Laboratories (Los Angles, California). Their age, reproductive history, and husbandry were not described.

Experimental procedures: A preliminary acute LD_{50} study was conducted with groups of 10 male rats. Six doses of each chemical were used (50, 100, 150, 200, 400, and 1000 mg/kg), and the animals were observed for 14 days following dosage administration. Doses were administered in corn oil by subcutaneous injection behind the neck of each animal. Acute LD_{50} values were determined graphically by plotting the proportion dying against the natural logarithum of the dose.

Allyl chloride was given at doses of 25, 50, or 100 mg/kg/day, while epichlorohydrin was administered at 1, 5, or 25 mg/kg/day. Subgroups of 5 rats were selected from each test group for interim sacrifices at 1 and 3 months, while the remaining survivors in each group were sacrificed at 6 months.

In a recovery study, two groups of rats were given pure or technical grade DBCP at doses of 15 mg/kg/day. These animals received 6 mg/kg/day inadvertantly beginning 17 days after treatment started. This dosage was continued for 49 days until the error was discovered. Then 15 mg/kg/day dosage was given for one additional week. Fifteen or fourteen animals were sacrificed from the groups treated with pure or technical grade DBCP, respectively, at the end of this treatment regimen, and 5 others were left untreated for 10 more weeks. At that time they were sacrificed and examined. A third group of 10 rats received the 15 mg/kg dose daily for 17 days followed by 11 daily doses of 6 mg/kg. Twenty-four hours after the last dose was administered, the animals were sacrificed and examined.

Two weeks prior to termination of treatment 5 male rats from each group were individually mated with an unspecified number of female rats to investigate effects on fertility. The authors stated that after two weeks the males were sacrificed with the other animals at the end of the study. Female rats were examined daily for sperm plugs and palpated for the presence of fetuses. Laporotomies were performed on pregnant females on day 21 of gestation and fetuses were counted and grossly examined. Non-pregnant females were observed for 3 weeks after mating. This experiment involved only animals treated with technical grade DBCP.

Necropsy. Animals were sacrificed by decapitation. Trunk blood was collected and the serum was stored for future study. External lesions were fixed and saved for examination. Organs were examined grossly and lesions were fixed for examination. Sections of lung, liver, kidney, stomach, and duodenum were taken for examination. The testes were weighed; one was placed in saline and frozen for use in hormone evaluations, while the other was fixed for microscopic examination. Prostate glands and seminal vessicles were weighed separately and fixed for microscopic examinations. The epididymis was fixed for examination. Reproductive tissues were stained with hematoxylin and periodic acid Schiff's reagent.

Observations Body weight (obtained weekly and at termination) and testis, prostate gland and seminal vessicle weights as well as serum concentrations of testosterone, luteinizing hormone, follicle stimulating hormone prolactin were measured. The male reproductive organs were examined microscopically. Hormone concentrations were measured by radioimmunoassay with and without column chromatography to remove dihydrotestosterone.

Statistical methods. Student's and Ducan's New Multiple Range tests were used for comparisons of each treated group with the control group or multiple comparisons of dose groups or groups treated with the three test chemicals. Results of p less than 0.05 were considered to indicate significant differences.

(8) REPORTED RESULTS: Acute studies. Acute subcustaneous LD_{50} values were determined as follows:

Test substance	LD_{50} (mg/kg)
Pure DBCP	275
Technical grade DBCP	300
Allyl chloride	310
Epichlorohydrin	110

Reported signs of toxicity included lethargy, weight loss, and lack of grooming.

Main study. Table 1 summarizes body weight and organ weight data for all test groups at the end of the 6 month study, and Table 2 lists serum hormone concentraions for each group at the end of the experiment. Dose-related effects were noted for body, testes prostate, and seminal vesicles weights.

Table 3 summarizes the incidence of testicular pathology in rats treated with DBCP. Epichlorohydrin and allyl chloride affected the testes of treated rats only after administration of 25 mg/kg for six months (1 rat with regression of seminiferous tubules in 15 examined). Only 1 rat in 20 treated with 50 mg allyl chloride per kg body weight for six months showed testicular effects.

The intensity of testicular effects increased with dosage and length of treatment. At low doses and after 1 to 3 months treatment the number of seminiferous tubules affected was small. At the highest dose level (25mg/kg) or after the 5 mg/kg dose had been administered for 6 months the number of tubules affected was seen to increase. Histologically, the regions affected least were lined only with Sertoli cells and contained spermatogonia or other germinal elements. In rats given 5 or 25 mg/kg/day for 3 to 6 months, the tubule effects were observed with Leydig cells which appeared to be small and had clumping or nuclear chromation. The latter effect was described by the authors as indicative of atrophy, and it was noted in the groups given 25 mg/kg/day for 6 months.

No other histopathological findings were reported.

Injection site tumors were noted in rats given 5 or 25 mg/kg for 6 months (3 of 30 and 2 of 26, respectively).

TABLE 1

Summary of organ and body weight (g) in rats given DBCP, Allyl Chloride, and Epichlorohydrin[1]/

Test Group	Body	Tests	Prostate	Seminal Vesicles
Controls	558.6± 15.9	3.32±0.07	0.58±0.03	1.63±0.04
DBCP [2]/				
1 mg/kg	535.5±13.5	[3]/	0.45±0.03 [4]/	1.65±0.06
	535.8±20.3	[3]/	0.53±0.04	1.82±0.09
5 mg/kg	475.7±17.2 [4]/	2.15±0.14 [4]/	0.49±0.03 [4]/	1.62±0.06
	490.3±24.7 [4]/	0.78±0.04 [4]/	0.50±0.03 [4]/	1.63±0.10
25 mg/kg	324.5±16.0 [4]/	0.74±0.04 [4]/	0.30±0.03 [4]/	0.98±0.11 [4]/
	342.6±17.2 [4]/		0.39±0.02 [4]/	1.15±0.08 [4]/
Allyl Chloride				
25 mg/kg	548.9±10.6	3.30=±0.10	0.56±0.03	1.69±0.09
50 mg/kg	521.8±14.6 [4]/	3.27±0.06	0.49±0.04 [4]/	1.60±0.07
Epichlorohydrin				
1 mg/kg	554.5±16.3	3.57±0.08	3.57±0.08	1.55±0.04
5 mg/kg	539.3±13.4	3.34±0.06	0.56±0.04	1.64±0.06
25 mg/kg	486.8±12.3 [4]/	3.34±0.06	0.46±0.04 [4]/	1.77±0.11

1/ Values represent group mean ± standard errors of the mean.

2/ Two values are given for each dose of DBCP. The first is the result from rats treated with pure DBCP and the second is from rats given technical grade DBCP.

3/ Results were only reported graphically.

4/ The difference between control and treated groups is statistically significant at p less than 0.05.

TABLE 2

Summary of mean serum concentrations of hormones (ng/ml) in rats treated with DBCP Allyl Chloride, or Epichlorphydrin [1].

Test Group	Testosterone	Luteinizing Hormone	Folicle Stimulating Hormone	Prolactin
Controls	3.23±0.26	48.4±3.1	606±26	5.81±0.80
DBCP [2]				
1 mg/kg	2.21±0.23 [3]	68.1±4.4	663±59	7.25±1.06
	4.13±0.44 [3]	66.4±6.2	523±26	8.40±1.97
5 mg/kg	2.79±0.25 [3]	83.2±10.1 [3]	713±49	7.87±1.79
	2.60±0.26 [3]	66.1±9.0	561±24	6.04±1.24
25 mg/kg	1.96±0.26 [3]	159.1±24.8 [3]	1147±76 [3]	6.81±2.47
	2.63±0.31	168.0±1.75 [3]	1226±47 [3]	7.10±1.90
Allyl Chloride				
25 mg/kg	2.88±0.24	45.2±4.2	516±15 [3]	12.27±2.14
50 mg/kg	3.23±0.36	41.9±5.2	473±16 [3]	9.56±1.49
Epichloro-hydrin				
1 mg/kg)	1.86±0.21 [3]	39.0±5.0	447±21 [3]	8.70±2.28
5 mg/kg	2.12±0.20 [3]	38.6±2.5	503±25 [3]	10.51±2.31
25 mg/kg	4.49±0.64 [3]	35.8±2.2	500±17 [3]	7.51±1.90 [3]

1/ Values are presented as group mean ± standard error of the mean

2/ For each dose level of DBCP, the first value is from the group given pure DBCP and the second is from the group treated with technical grade DBCP.

3/ The difference between control and treated groups is stistically significant at p less than 0.05.

TABLE 3

Incidence of testicular lesions in rats treated with DBCP (number with lesion/number examined).

	Time of observation (months)		
Test group	1	3	6
Controls	-	-	1/17
DBCP (pure and technical) [1]			
1 mg/kg	0/10	0/10	2/29 [3]
5 mg/kg	0/4 [2]	6/10	15/30
25 mg/kg	5/9	7/7	13/15 [2]

[1] Since results were similar for both technical grade and pure DBCP, the incidences have been combined.

[2] The number of animals observed to have isolated effects on seminiferous tubules was not reported for rats given the dosage of pure DNCP.

[3] Both animals were treated with technical grade DBCP.

In the recovery study 19 of the 29 rats sacrificed immediately after the last 15 mg/kg dose showed testicular effects. These effects were characterized by a few seminiferous tubules with cellular disassociation. Germinal elements were seen in these tubules. Few tubules were lined only with Sertoli cells. In the group sacrificed ten weeks after the last 15 mg/kg dose, all ten animals showed partial cellular disociation in their seminiferous tubules. (It should be noted that these data are combined results from treatment groups given pure or technical grade DBCP).

Body weights of rats in the recovery experiment were approximately 60% of those for untreated controls at the end of treatment. By the end of the 10 week recovery period treated animals weighed approximately 85% as much as controls. The authors stated that testes weights were 50 to 60% of that for controls at the end of the treatment (although no control data were provided for animals sacrificed after 73 days on test). After recovery no differences were observed between testes weights of treated and control rats. Similar results were obtained for prostate gland weights, while seminal vesicle weights were approximately 70% of that for controls at the end of treatment and no different after the recovery period.

Results of hormone assays in the experiment were considered by the authors to be uninterpretable because of the extreme variability in the measurements and the small number of animals used.

Table 4 summarizes the results of the fertility experiment. The authors stated that the parameters and observations made on the reproductive systems of rats treated with the 1 mg/kg/day dosage could not explain the low fertility of that group. The authors also stated that no evaluation of the ability of the females to conceive and bear young was made. Those rats showing histologically altered testicular morphology in the 5 and 25 mg/kg groups could not sire litters. However, the authors emphasize the inconclusive results for animals having no histological changes in the 1 mg/kg/day dose group and the need for further study of DBCP at lower doses.

No effects on the genitalia of fetuses were found after gross examination.

Table 4

Results of the fertility experiment (expressed as number failing to sire litters per number mated).

Treatment group 1/	Fertility		% Fertility
1 mg/kg/day	2/5 3/	3/5	60
5 mg/kg/day	4/5	1/5	20
25 mg/kg/day	0/2 2/		0

1/ Technical grade DBCP was the only test substance used.

2/ Only two matings were shown by the occurrence of vaginal plugs.

3/ One of the three which failed to sire litters showed profoundly altered testicular morphology as states by the authors.

(9) DISCUSSION: The experiments reported provide specific information on the effects of DBCP on the reproductive system in male rats. However, there are aspects of the protocols which are not fully described. The most important of these is the choice of subcutaneous injection behind the neck in view of the routes of exposure most likely for humans (inhalation, and in the diet or drinking water). No rationale for the choice of the route of administration used in these experiments was provided.

Other aspects of the protocol not clearly discussed include:

Whether or not the controls were sham injected with the corn oil vehicle.

Whether or not males were mated with one or more females in the fertility experiment.

The age at the time of mating and reproduction history for the female rats which were used in the fertility experiment.

Rationale for use of Sprague-Dawley females instead of Long-Evans females (same strain as the males).

The circumstances of hormone measurements including time of day blood samples were taken.

(10) TECHNICAL REVIEW TIME: 24 Hrs.