Bull. Environm. Contam. Toxicol. 23,703-710 (1979)

Spermatogenesis in Agricultural Workers Exposed to Dibromochloropropane (DBCP)

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DBCP (1,2-dibromo-3-chloropropane), a soil fumigant nematocide has been manufactured in the United States for over 15 years and is used primarily on soybeans, grapes, citrus, pineapples, and peaches about 12-15 million pounds annually.

Torkelson (1961) found the compound to be moderate to highly toxic from single respiratory exposure and highly toxic on repeated exposure, producing damage even at 5 ppm. Excessive exposure resulted in injury to the liver, kidneys, and various tissues including sperm cells and seminiferous tubules, dermis, bronchioles, renal collecting tubules, lens, cornea, and alimentary canal. In 1977, a number of workers at a chemical plant in California where DBCP, ethylene dibronide (EDB), and other chemicals were formulated found that they were unable to father children. Whorton et al. (1977) conducted a study of 39 persons in this plant and found, after excluding 11 vasectomies and three women, nine of 25 exposed workers (36%) to have azoospermia and five others (20%) to have definite oligospermia. The effect appeared to be dose related. Potashnik et al. (1978) found azoospermia in six factory workers who had been chronically exposed to DBCP. Hormone studies revealed elevated FSH levels and normal LH and testosterone concentrations with testicular biopsy in all participants showing selective atrophy of the germinal epithelium. Marshall et al. (1978) demonstrated progressive decrease in sperm count in men with longer exposure to DBCP in their group of 38 male and 3 female workers exposed to the chemical. In addition, the results of endocrine evaluation and testicular biopsies showed a positive correlation between the length of exposure and impairment of testicular function. Nine of the 27 non-vasectomized men whose semen were analyzed revealed complete absence of sperm, and FSH and LH levels were increased in workers with significant spermatogenic damage while testosterone levels were not changed appreciably. Whorton et al. (1979) produced even more conclusive evidence for the deleterious effect of DBCP on the seminiferous tubules by evaluating semen samples of 142 non-vasectomized men, 107 of which had been exposed to DBCP and 35 of which had not been exposed. There was a clear-cut difference in both the distribution of sperm counts and the median counts between the exposed men and the not-exposed men. 13.1% of the exposed were azoospermic, none were severely oligospermic, and 5.7% were mildly oligospermic. FSH was discovered to be a reliable predictor of spermatogenic dysfunction as long as there was a large percentage of azoospermic males but was greatly reduced in effectiveness in a population of oligospermic men.

0007-4861/79/0023-0703 $01.60

The purpose of this study was to examine individuals who were potentially exposed to DBCP due to their occupations in agriculture or agriculture-related industry and to determine if the problem of low sperm counts occurred in them as it apparently did among workers who formulated the compound.

METHODS

Seventy-three subjects from six different states participated in the study. In establishing the study group of potentially exposed agricultural workers, a concerted attempt was made to look at all occupational groups including formulators, commercial applicators, farmers and farm workers, researchers, and sales personnel. In selecting candidates for the study, manufacturer representatives furnished names of primary distributors or direct applicators who were then contacted by project staff for identification of individual workers. Once located, the sole criterion for inclusion in the study was that a worker must have used or have been otherwise potentially exposed to DBCP. All such individuals willing to participate were included in the study. While it was neither feasible nor was it intended to obtain a random sample of all DBCP users, the above selection process- identification of as many DBCP users as possible without selective screening- would tend to produce a study group which adequately represented the target population.

Physical examinations were completed by a physician, and each individual was asked to complete a medical history questionnaire in addition to providing specific information as to his reproductive and pesticide use history. Semen samples were collected from all non-vasectomized men. Contribution of sample was accomplished through masturbation. Samples were examined immediately and/or prepared for shipment on dry ice to participating laboratories for analysis where recounts were made by the same experienced technician. Semen was examined for volume and sperm density[1] (millions of spermatozoa per mL) and morphology. Laboratory tests on all participants included measurements of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH). Serum FSH, LH, and testosterone levels were measured by senstive and specific radioimmunoassays.[2]

Kruskal-Wallis one-way analyses of variance were performed to determine if there were statistical differences among occupational groups. This nonparametric test avoids the assumption of the normal distribution which is likely violated by these data. To identify statistical differences between groups (excluding formulators), the Mann-Whitney U-tests[3] was followed. The association be-

[1] According to Cannon, the sperm count of normal semen will usually fall in the range of 60 to 150 million per mL with a mean of about 100 million per mL. Counts of less than 20 million per mL are usually considered to be distinctly abnormal. Clinical Diagnosis by Laboratory Methods: edited by I. Davidson and I. Henry, W. B. Saunders Co., Philadelphia, Penn., 1974.

[2] Standard method radioimmunoassays were conducted at the Medical University of South Carolina.

[3] Described in Nonparametric Statistical Methods by M. Hollander and D. Wolfe, published by John Wiley and Sons, 1973.

tween variables was determined by the Spearman rank correlation coefficient for untransformed variables and the Pearson correlation coefficient for variables on the log scale. All statistical tests were done using a per comparison error rate of 0.01.

RESULTS AND DISCUSSION

Frequency distributions of counts among the six sperm density categories used in the study (i.e., <-10, 10.1 - 20, 20.1 - 40, 40.1 - 60, 60.1 - 100, >100 million/mL) indicated highly significant differences between DBCP formulators and/or users and 9,000 males (Table 1) with no known occupational exposure to toxic chemicals studied by MacLeod (1979). Six of eight (75%) formulators and 13 of 43 (33%) users, excluding two azoospermics, had counts below 20 million/mL, whereas on 15% of the 9,000 MacLeod subjects had counts below 20 million. These distributions were significantly lower than MacLeod data. Azoospermics were excluded to be consistent with the MacLeod data. When the various classes of users were considered separately (Table 2), there were significant differences among the groups on sperm count, FSH, and LH but not testosterone. This was true whether formulators were included or excluded. Sperm counts were statistically lower for formulators, custom applicators, farmers, and farm workers than for research workers. Counts for salesmen were intermediate and were not shown to differ from those of any other group. All were statistically lower than the MacLeod data except for research workers and salesmen.

Custom applicators and farmers each had higher levels of FSH than did salesmen or research workers. Highly significant negative correlations were demonstrated between sperm counts and both serum FSH (Fig. 1) and LH levels but not testosterone levels. High FSH and LH levels occurring together were quite indicative of low sperm counts. However, low sperm counts were observed in some individuals with normal FSH and LH levels. FSH values showed a definite trend toward abnormality with 17 of 52 non-vasectomized men (33%) in the study having FSH levels above the accepted high of 17 mIU/mL. The median sperm count for this group was 3.8 million/ml. (range = 0.0-45.0 million). Conversely, 14 men with sperm counts of <-10 million had a median FSH level of 23.3 mIU/mL (range = 3.9 - 85.6). However, high FSH was an excellent prognosticator of impaired spermatogenesis since only 3 of the 17 men with FSH over 17 mIU/mL had sperm counts of 30 million or more.

A significant negative correlation was demonstrated between sperm count and DBCP use-index (average pounds used per day of use) showing that greater increments in exposure were associated with a diminution in sperm production (Table 3). The use-index was calculated by dividing the total pounds used in the lifetime by the total number of individual days on which it was used.

Sperm morphology was examined in all participants and nothing unusual was found. Historically, no persons were found who desired to father children but had been unable to do so. In most instances, their families were considered complete.

CONCLUSIONS

The data showed significant differences among occupational groups in median sperm counts with the lowest counts being found

among formulators, custom applicators, and farmers. Intermediate counts were found among sales personnel and the highest sperm counts occurred among researchers. Since the potential for exposure was undoubtedly greater among the first three groups, this indicates a greater potential for interference with spermatogenesis. This finding is further corroborated by the relation of sperm production and DBCP use-index where greater per day use was associated with lower sperm counts. Comparing frequency distributions of counts among the six sperm density categories clearly shows that users of the chemical had higher frequencies of counts below 20 million than did participants in the MacLeod study. Also, the median sperm counts for formulators and all users except salesmen and researchers were statistically lower than the median count of the MacLeod data.

High FSH and LH levels were associated with low sperm counts and, predictably, custom applicators and farmers each had higher levels of FSH than did salesmen or research workers. It is felt that serum hormone levels would have served as reliable prognostic indicator of testicular function in these participants, but in the positive sense only. That is, elevated FSH almost surely indicated low sperm counts, but additional low counts were observed in men with very normal hormone profiles. Therefore, serum hormone determinations should be useful as an initial screening procedure in which abnormal results would lead to further investigation. However, the presence of normal hormone levels does not preclude the possibility of impaired spermatogenesis or fertility.

TABLE 1

FREQUENCY DISTRIBUTION (%) OF SPERM COUNTS (10^6/mL)

	Formulators (n=8)	Users** (n=43)	MacLeod Data (n=9,000)
≤10*	37.5	20.9	8.9
10.1-20	37.5	11.6	6.1
20.1-40	0	14.0	11.4
40.1-60	12.5	20.9	11.8
60.1-100	0	9.3	25.3
>100	12.5	23.5	36.5

* Does not include the two azoospermics found among the users

** Includes custom applicators, farmers, farmer workers, researchers, and salesmen from Table 1

Comparison	X^2 (5df)	p
Users vs. MacLeod	19.0	<0.005
Formulators vs. MacLeod	24.0	<0.001
Total vs. MacLeod	32.1	<0.001

TABLE 2

SUMMARY STATISTICS BY OCCUPATION

Occupation		Age	Sperm Count (10^6/mL)	Use Index (lb/day)	FSH	LH
Formulators (n=8)	Median	25	12.1	5,000	10.7	14.2
	Range	20-54	0.8-317	-	5.1-29.1	6.5-28.9
Custom Applicators (n=2)	Median	34.5	2.7	345	45.7	16.3
	Range	30-39	1.6-3.8	30-660	29.4-62.1	10.7-21.9
Farmers (n-18)	Median	36	17.8	710	23.3	12.0
	Range	25-58	1.4-171	30-13,500	2.0-60.5	6.0-20.3
Farm Workers (n=12)	Median	30	37.8	240	12.9	12.6
	Range	24-39	0.0-159	90-600	5.4-85.6	6.5-59.8
Researchers (n=7)	Median	36	101.5	10	4.9	6.7
	Range	33-51	41.0-188	0-40	3.3-13.2	4.1-29.5
Salesmen (n=6)	Median	35	73.0	450	7.2	6.8
	Range	26-48	32.0-166	360-720	2.2-11.8	5.0-16.7

TABLE 3

RELATIONSHIP (PARTICIPANTS PER CATEGORY)
BETWEEN SPERM COUNT AND USE INDEX

Sperm Count	U S E I N D E X ≤400	400.1-800	<800	Sperm Count Totals
<10	5	4	5	14
10.1-20	1	2	5	8
20.1-40	3	2	1	6
40.1-60	2	6	1	9
60.1-100	3	0	0	3
<100	8	2	0	10
Use Index Totals	22	16	12	50

Spearman Rank Correlation Coefficient = -0.372, $p<0.01$

Using the index of pounds used in the lifetime standardized by the number of days in the lifetime over which the usage took place yielded a good "dose-response" relationship. The higher the index, the lower the sperm count.

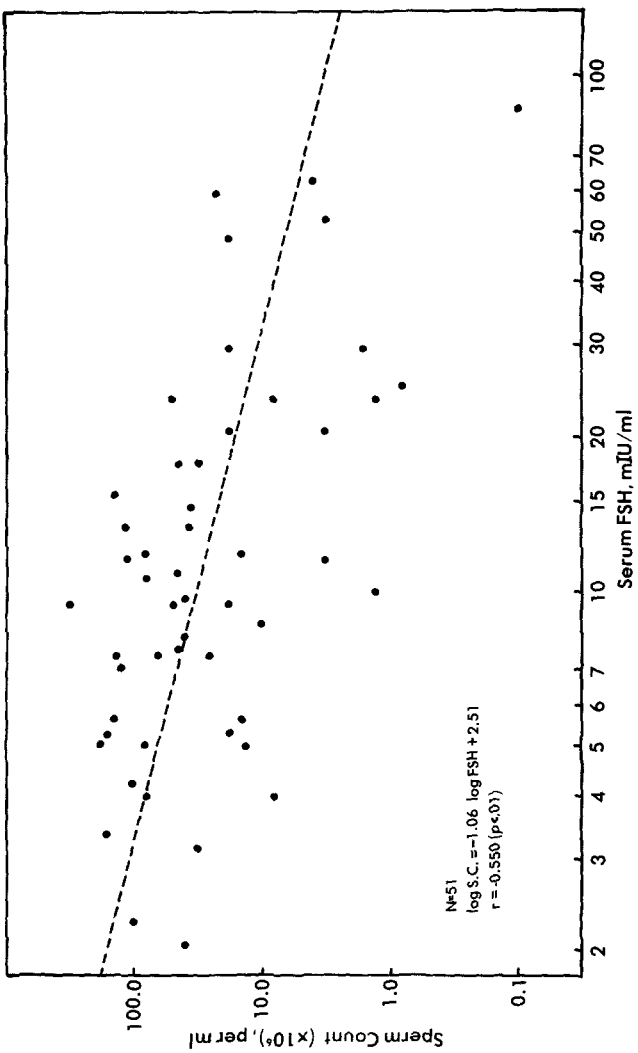

Fig. 1 Relationship of Serum FSH to Sperm Count

In conclusion, the results are quite consistent with an occurrence of primary disruption of spermatogenesis at the testicular level for all users who had extensive exposure to the compound.

ACKNOWLEDGEMENT

This work was supported by Contract No. 68-01-1950, Human Effects Monitoring Branch, Technical Services Division, Office of Pesticide Programs Environmental Protection Agency. We gratefully acknowledge the assistance of M. T. Watson, B. Brackett, K. Bunch and A. Harper. We were most appreciative to J. MacLeod for assistance and guidance.

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