Testicular function among epichlorohydrin workers

T H MILBY,¹ M D WHORTON,¹ H A STUBBS,¹ C E ROSS,² R E JOYNER,² AND L I LIPSHULTZ³

From Environmental Health Associates, Inc.,¹ Berkeley, Ca, Corporate Medical Department,² Shell Oil Company, Houston, Texas, and Division of Urology, Department of Surgery,³ University of Texas Medical School at Houston, Texas, USA

ABSTRACT Epichlorohydrin (1,2-epoxy-3-chloropropane) (ECH) is a colourless liquid used in the production of insecticides, agricultural chemicals, epoxy resins, and many other products. It is highly reactive and an alkylating agent suspected of possessing carcinogenic properties in man. The results of a clinical-epidemiological investigation to ascertain whether exposure to ECH may be associated with sperm count suppression among ECH production workers at two chemical plants are presented. Medical histories and physical examinations with special emphasis on the genitourinary tract were completed on each participant. Blood samples and three semen specimens were also obtained. Since no internal control groups were available, the data arising from this effort were analysed for each plant (plant A, 44 men; plant B, 84 men) using a control group of 90 chemical plant workers unexposed to any agents known to be toxic to the testes who were included in previous studies. This study provides no evidence that exposure to ECH at the concentrations existing at the two plants studied is responsible for sperm count suppression.

Epichlorohydrin (1,2-epoxy-3-chloropropane) (ECH) is a colourless liquid used in the production of glycerine, epoxy resins, insecticides, agricultural chemicals, and many other products. The US Government estimates that in 1978 the production capacity in the United States was 470 million pounds and that thousands of workers were potentially exposed to ECH in the workplace.¹

ECH is a highly reactive chemical. It is an alkylating agent, which suggests that it may also possess carcinogenic properties. It is highly irritating to the eyes, skin, and respiratory tract; skin contact may result in delayed blistering and deep-seated pain; and allergic contact dermatitis is occasionally associated with exposure to ECH. Animals exposed repeatedly to ECH have developed lung, kidney, and liver injury.¹ In rats ECH produces spermatocoeles, causing infertility in a manner similar to alpha-chlorohydrin, the latter compound being a commercially used rat sterilising agent.² ECH has been shown to be carcinogenic in experimental animals³ and may be carcinogenic in man (P E Enterline *et al*, unpublished observations).

The recently discovered relationship between

work place exposure to 1,2-dibromo-3-chloropropane (DBCP) and sperm count suppression among male workers has alerted scientists to the possibility that other toxins present in the work environment might produce similar effects.⁴⁻⁶ These and other studies have shown that clinical-epidemiological investigations of testicular function are both technically feasible and culturally acceptable in the United States.⁷

The Shell Chemical Corporation produces ECH at two locations—the Deer Park Chemical Plant at Deer Park, Texas (plant A), where ECH has been produced since May 1948, and the Norco Chemical Plant, Norco, Louisiana (plant B), where ECH has been produced since April 1955. To evaluate the testicular effects of ECH on employees who work with it, both plants were included in an epidemiological study that focused on testicular function. Methods and results for each plant will first be described separately. All results will then be pooled and interpreted together.

Methods

One of the authors (LL) completed a medical history and physical examination on each of the participants. Special emphasis was given to the genitourinary

Received 2 September 1980

Accepted 10 December 1980

tract. Three semen samples, each obtained at home after 48 hours of sexual continence, were requested from each man. Blood was obtained from each participant for measuring serum follicle stimulating hormone (FSH), leutinising hormone (LH), and testosterone. The semen samples were analysed at a University of Texas laboratory, and the hormone assays were performed by the Baylor College of Medicine Reproductive Research Laboratory. A sample of blood specimens was also sent to the endocrinology laboratory at Alta Bates Hospital in Berkeley, California, for duplicate analysis.

Data on intensity of exposure to ECH were available from plant A but not plant B. These data were based on exposure estimates from industrial hygiene survey data, personal knowledge of exposure, and plant employment records. From these sources of information the plant A management developed a list of 122 current employees, all of whom were working in job categories associated with ECH production.

It was not feasible to obtain a control group of non-ECH workers from either plant. Therefore, a group of 90 chemical plant workers included in previous studies by the authors were used as controls.⁷ These 90 men had been selected previously because they were judged not to have been exposed to agents known to be toxic to the testes.

All men who had past surgical interruption of the vas deferens were excluded from participation in this study. In neither the "exposed" group nor in the control group were men excluded from participation because of the presence of a varicocele, a history of postpubertal mumps, or suspected fertility problems.

Results

PLANT A

The invited cohort at plant A consisted of 122 men currently working with ECH in the crude, finishing, and resin operations. Of these 122 men, 53 (43%) agreed to participate fully in the study. Nine had had vasectomies; thus only 44 actually provided semen specimens. Their mean age was 33.5 years. There were no non-exposed individuals in the cohort. Nineteen of the 122 men volunteered to be interviewed by one of us (DW or TM) but did not agree to participate otherwise; thus 72 (59%) of the invited cohort were contacted in some manner.

Sperm count distribution: plant A ECH—workers v controls

Figure 1 shows the cumulative percentage distributions of sperm counts for two groups: the 44-member plant A ECH cohort and the 90-member control population. The plant A ECH distribution

Fig 1 Cumulative percentage distribution for sperm counts: plant A ECH workers ($n = 44$); controls ($n = 90$) —. (Source: EHA Data, 1978.)

closely resembles the control group distribution, and the medians for these distributions are essentially identical. Application of the Kolmogorov-Smirnov two-sample test fails to show a significant difference between the ECH and control distributions at $\alpha = 0.05$. In the ECH cohort there were no sperm counts of zero (azospermia); three sperm counts (6.8%) were greater than zero but less than 20 million/ml (oligospermia). The lower portion of the sperm count distribution is biologically more important than the upper portion because counts of less than 20 million/ml are considered to represent true oligospermia and may be associated with varying degrees of clinical infertility. It is important to emphasise that the sperm count distribution for plant A ECH workers closely approximates the Environmental Health Associates, Inc, control in the oligospermic range of the distribution curve.

Sperm count and duration of exposure

One of the means by which the relationship between sperm count and duration of exposure to ECH was examined is provided in table 1. The population of 44 ECH workers was dichotomised by years of work with ECH—less than one, one, or greater. Mean sperm count, standard deviation, and range were determined for each work-year group. The t -test for differences between the means of independent samples of unequal sizes was applied, and no significant difference between the means was found at $\alpha = 0.05$.

A second method used to examine the relation between sperm count and duration of exposure entailed dichotomising the sperm count distribution

Table 1 Mean, standard deviation, and range for sperm count by years of ECH work for two plants*

	Year of ECH work	No of men	Mean†	SD	Range
Plant A	< 1	14	81.4	44.6	25-159
	≥ 1	30	88.0‡	47.0	11-198
Plant B	< 1	10	69.9	37.5	26-137
	≥ 1	30	124.8§	79.1	26-326
Total	< 1	24	76.6	40.9	25-159
	≥ 1	60	106.4	64.5	11-326

*All current ECH workers.

†Sperm count values in million cells/ml.

‡NS, two-tailed $p = 0.6$.§Significant, two-tailed $p < 0.05$.

—below 20 million and equal to or greater than 20 million—and by years of work with ECH—under one year, one year, or longer. A simple four-fold table (table 2) shows no significant association between low sperm count and longer than one year's exposure to ($\chi^2_1 = 0.34$, $p = 0.56$).

Sperm count and intensity of exposure

To examine sperm counts by intensity of exposure two separate exposure categorisation schemes were devised. Firstly, jobs were placed in categories 1-4 on the basis of results from a two-year programme of personal sampling necessitating 18 job classifications.

Milby, Whorton, Stubbs, Ross, Joyner, and Lipshultz

Job classifications that were not sampled were categorised by analogy to those jobs that were. The average ECH exposure level for the four categories were: category 1: 1.0 ppm or greater; category 2: 0.5-0.9 ppm; category 3: 0.3-0.4 ppm; and category 4: 0.1-0.2 ppm. Table 3 provides the frequency and percentage distributions of sperm count for these categories of exposure.

The second job categorisation scheme was based on the results of combined industrial hygiene sampling and personal judgment. Jobs were again placed in categories 1-4 by plant personnel, but a different set of definition were devised: category 1: greatest average daily exposure; category 2: less daily exposure than category 1; category 3: less daily exposure than category 2; category 4: incidental exposure by virtue of working around or near an ECH area, but less daily exposure than category 3. The frequency and cumulative percentage distributions of sperm counts for these exposure categories are displayed in table 4.

An inherent problem in both of these exposure groupings was the limited number of men in each category. Even with consolidation of groups, however, there was no evidence of significant relationships between sperm count and either of these schemes of categorisation of exposure intensity.

Table 2 Sperm count values by years worked with ECH and by sperm count category for two plants

	Sperm count category (million/ml)	Years worked with ECH		Total	Significance χ^2, p
		< 1	≥ 1		
Plant A	< 20	0	3	3	
	≥ 20	14	27	41	NS
Total		14	30	44	0.341, 0.56
Plant B*	< 20	0	4	4	
	≥ 20	18	62	80	NS
Total		18	66	84	0.199, 0.66
Total	< 20	0	7	7	
	≥ 20	32	89	121	NS
Total		32	96	128	1.259, 0.26

*Current ECH workers.

NS = Not significant.

Table 3 Frequency and cumulative percentage distributions of sperm counts by industrial hygiene sampling categories for plant A

Sperm-count (million/ml)	Categories							
	1		2		3		4	
	No	Cum %	No	Cum %	No	Cum %	No	Cum %
0- 9	0	0.0	0	0.0	0	0.0	0	0.0
10- 19	1	16.7	1	4.3	1	12.5	0	7.4
20- 39	3	33.3	1	33.3	3	50.0	12	59.2
40- 99	1	100.0	2	100.0	4	100.0	11	100.0
≥ 100	1	100.0	2	100.0	4	100.0	11	100.0
Total	6		3		8		27	

Categories: see text.

Cum = Cumulative.

Table 4 Frequency and cumulative percentage distributions of sperm counts by category system based on industrial data and subjective judgment for plant A

Sperm-count (million/ml)	Categories							
	1		2		3		4	
	No	Cum %	No	Cum %	No	Cum %	No	Cum %
0- 9	0	0.0	0	0.0	0	0.0	0	0.0
10- 19	1	20.0	1	4.3	1	9.1	0	0.0
20- 39	1	40.0	3	17.2	0	9.1	0	0.0
40- 99	2	80.0	12	69.4	3	36.4	2	40.0
≥100	1	100.0	7	100.0	7	100.0	3	100.0
Total	5		23		11		5	

Categories: see text.
Cum = Cumulative.

Hormone data

Hormone assays (testosterone, FSH, LH) were all essentially within expected limits. Nevertheless, the insensitivity of FSH, LH, and testosterone as predictors of testicular dysfunction has been reported.⁶ There were no significant differences between the hormone results from the two laboratories participating in this study.

PLANT B

At plant B 187 men were classified as either present or past ECH workers, and of these, 84 men (44.9%) provided samples of semen. The mean age for these men was 37.6, and they had worked with ECH for an average of 4.9 years. The mean age of the 103 non-participants was 40.2, and the mean number of ECH work years was 5.9.

Seventeen of the 103 non-participants replied to a questionnaire. Two major reasons were given for not taking part: no more children were desired and reluctance to provide semen samples.

Fig 2 Cumulative percentage distribution for sperm counts: plant B ECH workers ($n = 84$); controls ($n = 90$) —.

(Source: EHA Data, 1978.)

Sperm count distribution: plant B ECH workers v controls

Figure 2 shows the cumulative percentage distributions of sperm counts for two groups: the 84-member plant B ECH cohort and the 90-member control population. These distributions are essentially identical; the median sperm count for the plant B group is 84 million, and the control group median is 81 million.

There is no significant difference between the exposed group and the control group sperm count distributions by the Kolmogorov-Smirnov two-sample test. None of the men in the ECH cohort was azospermic and only 4.8% were oligospermic. As was emphasised earlier, the significance of the sperm count distribution at the lower end of the distribution is greater in biological terms than the distribution at the higher end of the curve. Thus it is worth noting that the sperm count distribution representing plant B ECH workers closely approximates to the control population distribution at this lower end of the distribution curve.

Sperm counts by duration of exposure

At plant B, unlike plant A, past as well as present ECH workers were available for study although, again, no non-ECH workers were available for

Table 5 Mean, standard deviation, and range of 84 sperm count values by present or past ECH work, plant B

Years since last ECH work	No of samples	Mean*	SD	Range
0 (currently working with ECH)	40	111.1	74.6	26-326
≥1	44	105.6	74.8	4-257

*Sperm count values in million cells/ml.

controls. The group of present workers (40 men) was dichotomised by categories of years worked with ECH—less than one, and one or more (table 1). The mean sperm count of the 10 men who fell into the less than one year category was 69.9 million cells/ml and for those 30 men in the one year or greater category 124.8 million. We consider this observation to be an expression of chance, to which no biological significance can be attributed.

The dichotomisations of the sperm count distribution as for plant A (<20 million/ml, ≥20 million/ml; and of years of work with ECH—<1, ≥1) yielded the results of table 2. No association was found between sperm count group and ECH work year group in this plant ($\chi^2_1 = 0.20$, $p = 0.65$).

The participating population of 84 men was also dichotomised into 40 present and 44 past ECH workers (table 5). The mean sperm count for present ECH workers was 111.1 million cells/ml; for past ECH workers, 105.6 million cells/ml. There were no azospermic men in either group. Four of the past but none of the present ECH workers had sperm counts below 20 million cells/ml. We know of no explanation for the fact that among the past ECH workers four were slightly oligospermic.

Hormone data

Hormone assays for testosterone, FSH, and LH showed no findings of significance.

Discussion

The major objective of this study was relatively straightforward, to determine whether exposure to ECH is associated with detectable impairment of testicular function among plant A and plant B employees. Previous experience⁸ has shown that determination of sperm density (sperm count) is a simple and valid measure of testicular function. This experience also convinced us of the usefulness of comparing exposed and control group sperm count distributions as a means for examining evidence that a chemical substance is capable of producing impaired testicular function.

A major finding in this study is that the distributions of sperm counts in both plant populations are practically identical to the distribution observed in the control population. In addition to comparing the sperm count distributions for the two ECH-worker cohorts with the control, the plant A and plant B data were examined in several ways in an effort to identify possible effects of intensity or duration of exposure on sperm count distributional properties. No such effects were found in this study. Furthermore, the pooled data from plants A and B presented in tables 1 and 2 provide no evidence of an

association between sperm counts and exposure to ECH.

The non-participants comprised a large portion of the total population at both plants. Two such groups are identifiable at both plants: non-participating ECH workers and non-participating individuals who did not work with ECH. For the purposes of interpreting the research findings presented here, the importance of the non-ECH workers group is probably minimal. The opposite is true, however, in the case of the two groups of non-participating ECH workers. At plant A this group numbered 69, and at plant B, 103. In view of these relatively large numbers, a legitimate question is, "How representative of the semen quality of the entire population of workers exposed to ECH were the samples provided by the participants?" An attempt was made at both plants to examine this question. At plant B a questionnaire was distributed to the non-participating ECH workers asking each why he did not volunteer; while at plant A a direct interview was conducted by one of us (DW or TM).

Answers received via questionnaire or interview suggesting already known infertility problems would have led us to fear that the subfertile portion of the population had been missed. The most commonly expressed reasons for non-participation, however, appeared to be (1) disinterest based on advancing age, (2) no wish for additional children, and (3) existence of a vasectomy. Of the four interviewees who stated that they found "this type of examination objectionable," only one thought this was a major reason for his non-participation. Thus, while few responses from non-participants were elicited, those that were in no way suggested that non-participation had anything to do with perceived fertility problems. Rather, it appeared that non-participants were simply disinterested because of the somewhat unpleasant nature of the study requirements (to produce a semen specimen) or by virtue of the fact that they had reached a point in life where fathering of children was no longer a desired or possible function.

It is important to recognise an inherent weakness in studies of this type. The results of this study are subject to potential confounding factors related to participation. Data such as marital state, age, and number of children were not available for conducting a thorough comparison of responders and non-responders.

Conclusion

This study has provided no evidence that exposure to ECH at the levels existing at the plants included is related to sperm count suppression. This does not

necessarily indicate, however, that no association exists between sperm count suppression and exposure to ECH at higher levels of exposure.

This study was supported by Shell Oil Company, Houston, Texas.

References

- ¹ National Institute of Occupational Safety and Health. Occupational exposure to epichlorohydrin. Washington: Department of Health, Education and Welfare, 1976. (Publication No 76-206.)
- ² Cooper ERA, Jones AR, Jackson H. Effects of alpha-

chlorohydrin and related compounds on the reproductive organs and fertility of the male rat. *J Reprod Fertil* 1974;**38**:7379-86.

- ³ Van Duuran BL. Prediction of carcinogenicity based on structure, reactivity, and possible metabolic pathways. *Journal of Environmental Pathology and Toxicology* (in press).
- ⁴ Whorton D, Krauss RM, Marshal S, Milby TH. Infertility in male pesticide workers. *Lancet* 1979;ii:1259-61.
- ⁵ Whorton D, Milby TH, Krauss RM. Testicular function in DBCP-exposed pesticide workers. *JOM* 1979;**21**:161.
- ⁶ Glass RI, Lyness RN, Mengle DC, Kahn E. Sperm count depression in pesticide applicators exposed to dibromochloropane. *Am J Epidem* 1979;**109**:346-51.
- ⁷ Milby TH, Whorton D. Epidemiological assessment of occupationally related, chemically induced sperm count suppression. *JOM* 1980;**22**:77-82.

The August 1981 issue

THE AUGUST 1981 ISSUE CONTAINS THE FOLLOWING PAPERS

Determination of lead in plasma and studies on its relationship to lead in erythrocytes P E DESILVA

Nasal cancer in England and Wales: an occupational survey E D ACHESON, R H COWDELL, AND E H RANG

An epidemiological survey of eight oil refineries in Britain L RUSHTON AND M R ALDERSON

Mortality of nickel workers: experience of men working with metallic nickel J E COX, R DOLL, W A SCOTT, AND S SMITH

A case-control study of bladder cancer in the United States rubber and tyre industry H CHECKOWAY, A H SMITH, A J MCMICHAEL, F S JONES, R R MONSON, AND H A TYROLER

Mortality of United Kingdom acrylonitrile polymerisation workers J B WERNER AND J T CARTER

Classification of chest radiographs for epidemiological purposes by people not experienced in the radiology of pneumoconiosis LIZ COPLAND, J BURNS, AND M JACOBSEN

Behaviour of indicators of exposure and effect after cessation of occupational exposure to lead L ALESSIO, M R CASTOLDI, P Odone, AND I FRANCHINI

Erythrocyte factors concerned in the inhibition of ALA-D by lead T SAKAI, S YANAGIHARA, AND K USHIO

Ventilatory function in Nigerian coal miners BL JAIN AND J M PATRICK

Hand-arm vibration in the aetiology of hearing loss in lumberjacks I PYYKKÖ, J STARCK, M FÄRKKILÄ, M HOIKKALA, O KORHONEN, AND M NURMINEN

First report of byssinosis in Hong Kong P G M MORGAN AND S G ONG

Castor bean allergy in the upholstery department of a furniture factory M D TOPPING, F H TYRER, AND R K LOWING

Lead accumulation in teeth as a function of age with different exposures ANNE STEENHOUT AND M POURTOIS

Thin-layer chromatography of hippuric and m-methylhippuric acid in urine after mixed exposure to toluene and xylene GRAŻYNA BIENIEK AND T WILCZOK

Book reviews

Notice

Information section

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, price £4.25 (USA \$9.20), including postage