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Sperm Shape Abnormalities in Carbaryl-Exposed Employees

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Semen was collected from 50 men occupationally exposed to carbaryl (1-naphthyl methyl carbamate) in a production plant for durations of 1 to 18 years and compared to semen from a control group of 34 unexposed, newly-hired workers. Employment, fertility, health, personal data, and blood samples were collected for each individual. Semen samples were analyzed for changes in sperm count, morphology, and frequency of sperm carrying double flourescent bodies (YFF). As a group, the exposed workers showed a significantly higher proportion of sperm with abnormal head shapes than did the control group ($p < 0.005$). Age, smoking habits, and medical problems did not appear to affect this result. This finding appears to be limited to men working in the carbaryl production area at the time of sampling. Sperm count and YFF did not show similar differences, which may be because they are known to be statistically less sensitive to small changes. Formerly exposed workers (away from carbaryl for an average of 6.3 years) showed a marginally significant elevation in sperm abnormalities compared to controls ($p < .05$, one-tailed statistical analyses) suggesting that the increase in abnormal morphology may not be reversible. However, the question of reversibility is sensitive to confounding factors and small sample sizes and, therefore, requires further study.

With these data a definitive link between carbaryl exposure and human seminal defects cannot be established. Although a distinct effect on sperm morphology was seen in the exposed group, the increases in sperm shape abnormalities were not related to exposure dose (estimated by number of years on the job or job classification during the year prior to semen collection). Inexplicably, the increases in sperm abnormalities were seen primarily in currently exposed men who had worked with carbaryl for less than approximately 6 years. These findings suggest the need for further study since other workplace-related factor(s) may be responsible for the elevated sperm abnormalities seen in this study.

Introduction

1-Naphthyl methyl carbamate, also known as carbaryl or Sevin, is a broad spectrum insecticide. Humans can be exposed to this agent during its manufacture and its widespread application. A clinical study of dermal exposure to carbaryl showed that carbaryl can be readily absorbed through the human skin (*1*). Although dermal absorption appears to vary with anatomical site, the scrotum generally showed high absorption for pesticides.

Though animal studies support the fact that carbaryl reaches the mammalian testes, seminal vesicles, and prostate (*2*), the reported effects of carbaryl on spermatogenesis are inconsistent. Several studies, have shown no testicular effect attributable to carbaryl (*3-6*). However, these studies generally focused on fertility and did not quantitate the effects on germ cells. As reviewed by Whorton et al. (*7*), much of the information on the effects of carbaryl on spermatogenesis comes from the Russian literature (*8-19*). Reported testicular changes include histological changes in the seminiferous epithelium. Semen evaluations showed diminished sperm counts and/or sperm motility. Chronic feeding of carbaryl produces atrophy of the seminiferous tubules, cellular degeneration, necrotic foci, and inflammation of blood vessels in the testes of

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rats (*20*). Chronic oral administration of 3 mg of carbaryl/kg of body weight in rats reduced numbers of spermatogonia and testicular spermatozoa (*21*).

Carbaryl exposure has also been shown to produce abnormal sperm. In rats, Vashakidze et al. (*10*) linked carbaryl ingestion to the production of "malformed" sperm. In mice, the proportion of abnormal and acrosomeless sperm increased almost 10-fold at doses showing no other apparent testicular effects (*22*).

In a study of the effects of carbaryl exposure on human spermatogenesis, Whorton et al. (*7*) measured sperm counts as well as blood levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in men working in the carbaryl-manufacturing process. When compared to a historical control group of unexposed men from three other studies of workplace exposure, this cohort showed no seminal or blood abnormalities that could be related to carbaryl, although a small increase of possible statistical significance was observed in the proportion of oligospermic men in the exposed group.

The mutagenicity of carbaryl remains ambiguous. It does not seem to be mutagenic in the Salmonella/ microsome assay even with metabolic activation (*23*), nor does it inhibit testicular DNA synthesis in mice (*24*). However, it is reported to be weakly positive in the induction of ouabain resistance in Chinese hamster cells (*25*), to induce unscheduled DNA synthesis in cultured human fibroblasts (*26*), and to be weakly mutagenic in the Drosophila sex-linked recessive lethal test (*27*).

A number of human semen assays have been developed for the study of chemically exposed workers (*28,29*). The approach of using a battery of semen assays increases the sensitivity of detecting chemically induced testicular abnormalities as seen in numerous human studies of exposure including cancer chemotherapeutic drugs (*30*), occupational exposure to dibromochloropropane (*31-33*), occupational exposure to lead (*34*), and antifertility agents (*35*). These studies suggest that changes in sperm count, motility, morphology, and sperm with two fluorescent bodies (YFF test-thought to represent sperm with two Y chromosomes due to meiotic nondisjunction) are all sensitive indicators of exposure of the human testes to chemical agents.

Here we report our findings of seminal analyses in a cohort of carbaryl production workers. We used the same cohort of exposed workers studied by Whorton et al. (*7*). However, we used newly hired workers in the plant as the control group instead of the historical controls used by Whorton. Plant controls were not available at the time of Whorton's study.

Method of Procedure

Description of the Cohort

As shown in Table 1, the cohort of exposed men used in this study is virtually the same as that of Whorton et al. (*7*), and where possible used the same semen sample collected by Whorton. All of these men were either current employees at the carbaryl production area or were past workers with at least one year's experience in carbaryl production at the time when Whorton et al. collected the semen samples in July 1978. Our cohort consisted of 101 males and included 52 baggers, 24 operators, and 25 other employees (supervisors, maintenance, etc.) identified from employment records. Of the 101 men, 26 declined to provide a specimen, and 25 had vasectomies. The remaining 50 men provided semen samples for analyses. For a control group, we studied 34 men who gave semen samples as part of their pre-employment medical examinations before assignment to the chemical plant.

Questionnaire and Medical Examination

A physician interviewed all of the participating men using a standard questionnaire emphasizing employee's work, reproductive, and medical histories and gave each a medical examination focused on the urogenital tract. The purposes of the study and the need to collect blood and semen samples were explained to each man.

Estimation of Exposure Dose

Available air sampling data were insufficient to provide precise estimates of personnel exposure to carbaryl in the work environments. However, as reviewed by Whorton et al. (*7*), data collected for the company's ongoing industrial hygiene program were available and provided valuable insight on the general range of airborne carbaryl concentrations in the workplace. Both area sampling and personal sampling revealed a wide range of airborne carbaryl concentrations. For example, in the operations area, three samples ranged from 0.36 to 14.21 mg/m^3 with a mean of 4.9 mg/m^3. In the distribution area, however, the calculated mean of 0.347 mg/m^3 seemed much more representative of true conditions, because 22 samples were taken, ranging from 0.03 to 1.8 mg/m^3. In this same area, 36 personal samples were taken with a mean of 0.439 mg/m^3 and

Table 1. Participants in semen study.

Job[a]	Numbers of men identified from company records	Declined participation	Vasectomies	Numbers of men providing semen samples	Number of men for each semen assay		
					Counts	Morphology	YFF
Exposed men							
Bagger	52[b]	16	14	22	22	22	6
Operator	24	2	5	17[d]	16[e]	16[f]	11
Other	25[g]	8[h]	6	11[i]	10[e]	11	–
Total exposed	101	26[h]	25	50	48	49	17
New hires	34	–	–	34	34	34	17

[a]Job classification in the year immediately prior to semen collection (July 1978) or during the last year of working in the carbaryl production area.

[b]Not 53 as in the study of Whorton et al. (7) because one bagger was a foreman in the year prior to semen analyses and was therefore included in the "other" category.

[c]Not 23 as in Whorton's study because of 1 man reclassified as foreman.

[d]Not 16 as in Whorton's study because one sample that was excluded from sperm counts for technical reasons was adequate for our studies on sperm morphology.

[e]One man each excluded for technical reasons as in Whorton's study.

[f]One azoospermic man excluded, but includes another man exlcuded in Whorton's study for technical reasons.

[g]Not 24 as in Whorton's study (7) because this number includes the one bagger we reclassed as a foreman.

[h]Not 9 and 27 as in Whorton's study, because one man who had originally declined participation provided a sample used in our study.

[i]Not 8 as in Whorton's study, because one man was reclassified as foreman (see b), one sample was excluded from Whorton's study for technical reasons but was adequate for our studies on sperm morphology, and one man who had previously declined participation provided a sample used in our study (see h).

a range of 0.0 to 1.8 mg/m^3. These doses are well within the ranges that might be expected to cause biological effects. For example, Best and Murray (*36*) saw significant changes in serum cholinesterase activity and urinary excretion of free 1-naphthol (a metabolite of carbaryl) in their carbaryl population exposed to similar concentrations.

We developed an ordinal ranking of exposure groups based on the type of job held during the past year. One year was chosen because semen studies in animals and humans suggest that exposures for a period from approximately 1-2 months up to 1 year before semen sampling are likely to show the maximum effect on seminal abnormalities (*28*). Men were assigned to the following exposure groups: control (new hires), low dose (supervisors, foremen, vacation and sick fill-ins for bagging or operating positions, maintenance personnel and other support staff), and high dose (full-time baggers and operators). We also grouped these men by the number of years they had worked with carbaryl.

Laboratory Analyses

Semen. Each participant was asked to provide a semen sample by masturbation after observing three days of sexual continence. Morning samples were collected at home in provided glass jars and were delivered to the plant's clinical laboratory. Because the interval between specimen production and delivery could not in every case be held to 2 hr or less, sperm motility, a time-dependent variable, was not recorded. Only one semen sample was collected from each individual. Sperm counts were measured in the plant's clinical laboratory with a hemocytometer and were recorded as number of sperm per milliliter of ejaculate. Ejaculate volumes were also recorded. Smears of sperm were prepared for subsequent microscopic analyses of morphological defects and frequencies of fluorescent bodies. For morphological analyses, air-dried smears were fixed in 95% ethanol and stained by a modified Papanicoloau method (*37*). Five hundred sperm were scored for each individual and classed as shown in Figure 1. All slides were scored blind and compared to a set of standard reference slides at regular scoring intervals. Although interlaboratory comparisons of sperm morphology scores typically show high variability (*38*), within laboratory comparisons can show remarkable reproducibility when the scoring criteria are standardized. For the analyses of the frequency of sperm carrying double fluorescent bodies (YFF), air-dried smears were stained with a quinacrine dihydrochloride method adapted from Pearson et al. (*39*). Smears were fixed for 10 min in Carnoy's, air-dried again, then allowed to soak for 5 min in pH 6.0 (mono- and dibasic sodium phosphate) buffer. The slides were stained for 6 min in 0.2% quinacrine dihydrochloride (in pH 6.0 buffer), rinsed in pH 6.0 buffer, then mounted

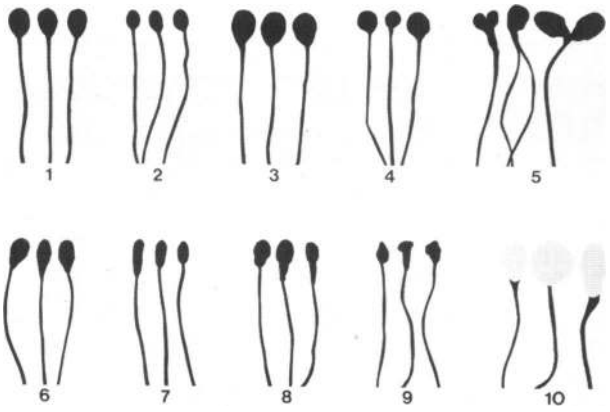

FIGURE 1. Shape variations in human sperm. The head shapes of the sperm in (*1*) are oval, which we consider normal; sperm (*2*) to (*10*) are scored abnormal: (*2*) small, (*3*) large, (*4*) rounded, (*5*) doubles, (*6*) narrow at the base of the head, (*7*) narrow, (*8*) pear-shaped, (*9*) amorphous, and (*10*) ghost-like.

in pH 6.0 buffer. The slides were read under darkfield illumination (*33*) and 500 sperm per sample were scored as OF, (those sperm carrying no fluorescent bodies), 1F, (sperm with one fluorescent body, presumably carrying a single Y chromosome) and 2F, (YFF test, i.e., those sperm with two fluorescent bodies, presumably carrying two Y chromosomes due to errors in meiotic disjunction).

Blood. Blood samples were collected by early morning venipuncture. Only the exposed cohort was studied and only 36 men agreed to venipuncture. The serum was separated, frozen, and sent to the Endocrinology Laboratory, Alta Bates Hospital, Berkeley, California, for analyses of testosterone, FSH, and LH by radioimmunoassay using the methods referenced by Whorton et al. (*7*).

Statistical Analyses

The data for sperm counts, morphology, and fluorescent bodies (YFF) was analyzed in several ways to determine whether the distribution of data for the carbaryl-exposed men was significantly different from the control cohort of new hires, and whether there was any relationship to intensity or duration of exposure to carbaryl. To determine for each parameter whether the mean value in the carbaryl-exposed men was significantly different from the control cohort we used Cochran's *t*-test for unequal variance (*40*); cumulative frequency plots were compared by using the Kolmogorov-Smirnov two-sample test (*41*). Since we expected seminal changes in one direction only, one-tailed statistical analyses were generally used (*40,42*). The contribution of certain confounding factors such as age (*43*), smoking (*44*), recent illnesses and medical drugs (*35*) were also tested by using multiple linear regression analyses (*40*). Correlation among semen parameters, blood parameters, and other data from the questionnaires were also investigated using correlation analyses (*40*).

Results

Comparison of Examination and Interview Data

Exposed workers and controls were compared for differences based on the questionnaire and the physical examination. Table 2 shows that the groups are comparable with respect to smoking, medical illness, and previous exposure to hazardous agents other than carbaryl. Because of the small sample sizes and the small number of children born to the control group, the groups were not compared for differences in reproductive capacity and outcome. The results of the physician's examination did not link any urogenital abnormalities to exposure. The control group was younger than the exposed workers. The mean age (± standard deviation, range)

Table 2. Characteristics of exposed and control populations.

	Exposed (50 men)	Unexposed (34 men)
Men with confounding factors[a]		
Smokers, more than 1 pack per day for at least 1 year	11/50 = 22%	8/34 = 24%
Men with positive medical histories	6/50 = 12%	5/34 = 15%
Smokers with positive medical histories	3/50 = 6%	2/34 = 6%
Men with previous exposure to other agents	1/50 = 2%	3/34 = 9%
Total	17/50 = 34%	12/34 = 35%
Men without confounding factors		
Total	33/50 = 66%	22/34 = 65%

[a]Confounding factors: smoker (smoked more than one pack of cigarettes/day in the last year), positive medical history (unilateral testicular atrophy, presence of epdidymal nodule, recent urinary tract infection, varicocoele, diabetes) or previous exposure to other hazardous agents.

Table 3. Effects of confounding factors and age on sperm counts in control and carbaryl-exposed men.

	Control		Exposed	
	Number	Sperm count	Number	Sperm count
Cohort	34	128.7 ± 23.6[a]	48	140.7 ± 20.3
Men without factors[b]	22	145.8 ± 34.7	35	130.1 ± 18.6
Men with factors[b]	12	97.4 ± 19.1	13	146.1 ± 50.6
Men, 18-40 yr old	33	124.7 ± 23.9	26	120.3 ± 24.0
Men, older than 40	1	258	22	151.1 ± 30.5
Men without factors, 18-40 yr old	22	145.8 ± 34.7	19	143.3 ± 30.6
Proportion of oligospermic males[c]		2/34 = 5.9%		7/48 = 14.6%

[a]Average sperm counts in millions per milliliter ± standard error of the mean.
[b]Confounding factors: smoked more than one pack of cigarettes/day in the last year, had a significant medical problem, or was previously exposed to other hazardous agents.
[c]Males with less than 20×10^6 sperm/ml ejaculate.

for the controls was 26.6 yr (± 6.0, 18-44) compared to 40.7 yr (± 10.0, 22-61) for the exposed workers. There was only one (out of 34) control male over 40 years of age, while nearly half the exposed (24 out of 50) were over 40 years of age.

Assay for Sperm Count

In Figure 2*a* we plot the sperm counts of 34 new hires and 48 carbaryl workers as cumulative distributions. As noted in Table 1, 2 of the 50 semen samples from the exposed group were not included in the calculations of sperm counts because incomplete semen specimens were obtained. Both the Kolmogorov-Smirnov two sample test and the Cochran's *t*-test showed no statistically significant differences between the two distributions or their means. As shown in Table 3, the control and exposed groups were also compared for confounding factors i.e., heavy smoking, previous exposure to other agents, and medical problems, as well as age. Although men with such factors in the control group had a slightly reduced average sperm count when compared to men without these factors, this difference was not statistically significant and is not seen in the exposed group. Comparisons of total sperm per ejaculate also showed no significant differences. Linear regression analyses suggests that age was not significantly correlated to sperm counts in these groups.

No significant effects of carbaryl exposure on sperm counts were seen even when men 18 to 40 yr old without confounding factors were compared with similar controls. However, a slightly higher proportion of oligospermic men (men with less than 20×10^6 sperm/ml) was found in the exposed group (7/48 = 14.6%) than in the control group (2/34 = 5.9%), but this difference was not statistically significant ($p = 0.1$, one-tailed analyses).

Assay for Sperm Morphology

In Figure 2*b* the cumulative distributions of sperm shape abnormalities in 34 controls and 49 carbaryl workers are plotted. One of the 50 men from the exposed group was not analyzed because

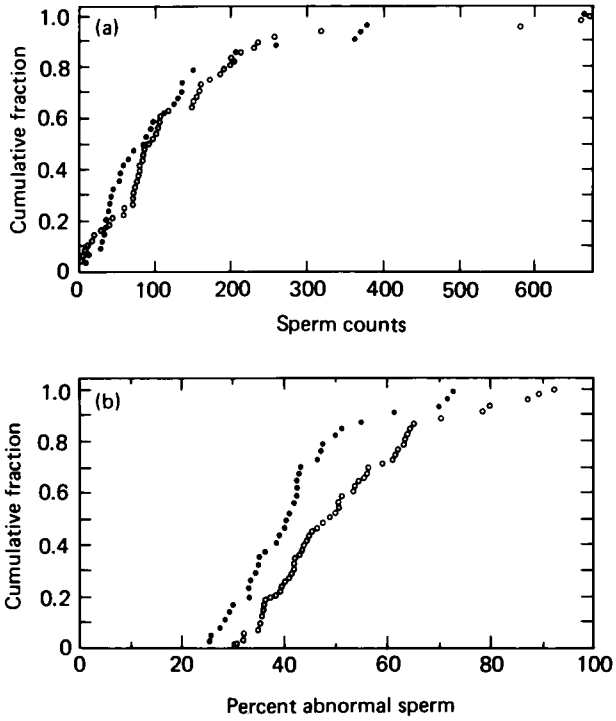

FIGURE 2. Sperm counts (*a*) and sperm morphology (*b*) in control and carbaryl-exposed men. Each point in both panels represents the value for (•) a single control or (o) exposed individual. Sperm counts (*a*) are given in millions of sperm per milliliter in a single ejaculate of each man. Sperm morphology (*b*) represents the frequency of abnormally shaped sperm per individual based on the analyses of 500 sperm per ejaculate. The data for the men in each group are ranked and plotted as cumulative distributions.

of azoospermia (see Table 1) yet the two samples excluded from the analyses of sperm counts were accepted for the analyses of morphology. The distribution of abnormal sperm morphology in the exposed workers is significantly elevated above controls ($p < 0.005$, Cochran's t-test). For each semen sample, sperm were assigned to shape categories as shown in Figure 1. The difference in the proportion of abnormally shaped sperm between exposed and control men was not due to an elevation in a specific type of morphological abnormality in sperm.

In Table 4 this difference was analyzed by exposure status (current vs previous), confounding factors, age, and proportion of teratospermic men. None of the confounding factors, including age, significantly affected the proportion of sperm abnormalities in the control or exposed males.

Previously Exposed Workers. Of the 49 exposed men analyzed for sperm morphology, 19 were previously employed in the carbaryl area and presumably were currently working with different chemicals in other areas of the plant. The average time (± standard deviation, range) since employment in the carbaryl area was 6.3 years (± 3.9, 1-12). Previously exposed workers as a group showed a marginally significant ($p< 0.05$, one-tailed) elevation in mean (± SEM) sperm abnormalities (50.0 ± 4.1%) when compared to controls (41.9 ± 2.1%). When men without factors were compared, the sample sizes were reduced and the difference was no longer statistically significant.

Proportion of Teratospermics. As shown in Table 4, when currently and previously exposed men were grouped together and compared to controls, the elevation in the proportion of teratospermic men (14/49 = 28.6% vs. 4/34 = 11.8%) approaches accepted values of statistical significance ($p = 0.06$, one-tailed). We define teratospermic men as those with more than 60% abnormal sperm forms.

Dose Response. To determine whether the elevation in sperm abnormalities was related to exposure dose during the past year, all new hires and currently exposed men were grouped as control, low, or high exposure as described in Methods. The results, plotted in Figure 3, indicate that low and high exposure groups did not differ significantly from each other but were both significantly elevated over the controls.

Time Response. To determine the relationship between sperm abnormalities and years working with carbaryl, we plotted percent abnormal sperm against number of years exposed for the 30 currently exposed workers (Fig. 4). Among the currently exposed there is a significant negative correlation ($r = -0.42$, $p < 0.025$). This compares with the significant negative correlation found between age and percent abnormal sperm ($r = -0.55$, $p < 0.005$) in the same group of currently exposed workers, suggesting that the higher sperm abnormalities are

Table 4. Effects of exposure status, confounding factors, and age on the percent abnormal sperm in control and carbaryl-exposed men.

	Controls		Currently exposed		Previously exposed	
	Number	Abnormal sperm, %	Number	Abnormal sperm, %	Number	Abnormal sperm, %
Cohort	34	41.9 ± 2.1[a]	30	52.0 ± 2.6 $p < 0.005$[b]	19	50.0 ± 4.1 $p < 0.05$
Men without factors[c]	22	42.0 ± 2.7	21	52.8 ± 3.2 $p < 0.01$	15	48.2 ± 4.0 N.S.
Men with factors	12	41.7 ± 3.7	9	50.0 ± 4.8 N.S.	4	57.1 ± 13.4 N.S.
Men 18-40 yr old	33	41.8 ± 2.2	18	57.9 ± 3.4 $p < 0.001$	7	50.9 ± 6.3 N.S.
Men older than 40	1	47.0	12	43.1 ± 2.4 N.T.	12	49.5 ± 4.7 N.T.
Men without factors and 18-40 yr old	22	42.0 ± 2.7	14	56.2 ± 4.3 $p < 0.01$	5	46.6 ± 5.8 N.S.
Teratospermic males, %[d]		4/34 = 11.8%		9/30 = 30%		5/19 = 26.3% $p = 0.06$[e]

[a]Average percent of abnormally shaped sperm ± standard error of the mean.

[b]The probability of significance when compared to the control grouping using Cochran's t-test, one-tailed (*40, 42*), N.S. = not statistically significant, N.T. = not tested.

[c]Confounding factors: smoked more than one pack of cigarettes per day in the last year, had a significant medical problem, or was previously exposed to other hazardous agents.

[d]Males who by our scoring criteria show more than 60% abnormal sperm forms.

[e]Probability of significance when values for currently and previously exposed men are combined using test for comparison of proportions, one-tailed (*40, 42*).

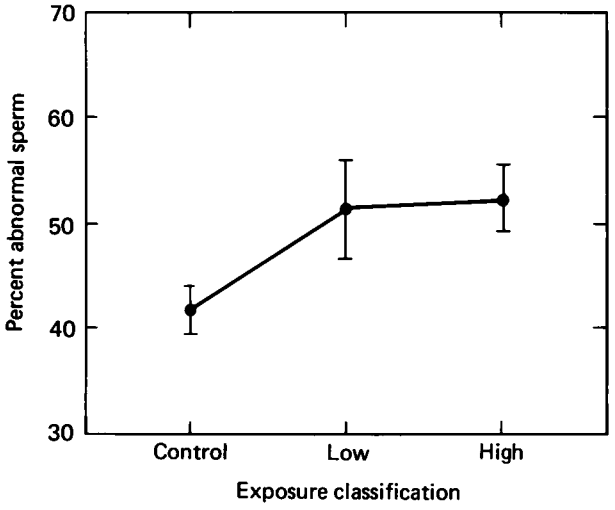

FIGURE 3. Sperm morphology in men currently working with carbaryl grouped by job classification. Each point and error bar represents the mean and standard error of the mean of 34 control, 11 low-dose males and 19 high-dose males. Males were grouped as low or high dose according to the job they held during the year immediately before to semen collection. Only those men working with carbaryl at the time of semen collection were included.

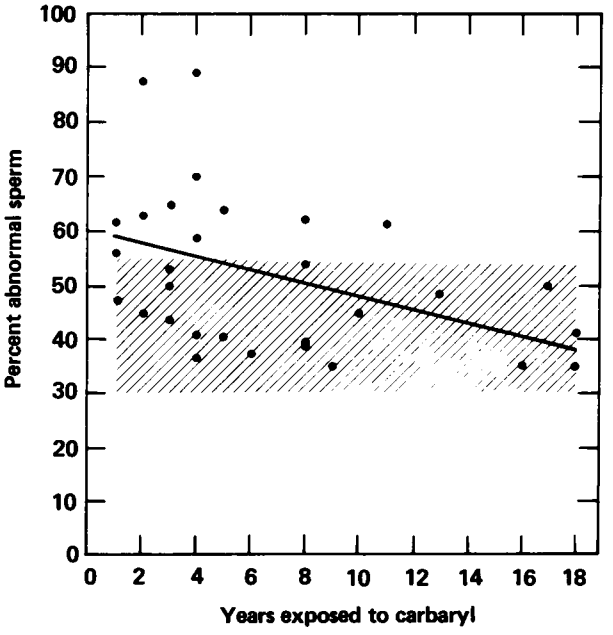

FIGURE 4. Sperm morphology in men vs. number of years working with carbaryl. Each point represents the percent abnormal sperm for one man. Only those men working with carbaryl at the time of semen collection are included. The solid line represents the linear regression line through these data. The shaded region represents the control mean ± one standard deviation.

found in the younger members of the exposed group, mainly those that have worked with carbaryl for less than approximately 6 years.

In previously exposed men, no significant correlations were found among levels of sperm abnormalities, years working with carbaryl, and years since working with carbaryl. These analyses were hampered by small sample sizes.

Assay for Fluorescent Bodies

A sample of 17 men in the high exposure classification (Fig. 3), i.e., currently exposed baggers and operators, showed 1.0 ± 0.3% of sperm with double fluorescent bodies (2F, Table 5) in comparison to 17 control males who showed 0.8 ± 0.2%. This difference was not statistically significant. The control and exposed men also did not differ in the frequency of sperm with single fluorescent bodies (1F). However, these two groups did differ as expected in sperm morphology ($p < 0.01$).

Correlations Among Semen and Blood Parameters

No relationships were found among sperm abnormalities, sperm with double fluorescent bodies, FSH, LH, and testosterone. However, a significant correlation ($r = -0.42, p < 0.005$) was found between sperm abnormalities and sperm count in the carbaryl exposed workers. A plot of sperm abnormalities vs. sperm counts of these males suggests that the men with sperm concentrations of less than 80 times 10^6/ml of semen as a group had more sperm abnormalities than those with over 80 × 10^6 sperm/ml (18 men, 64.0 ± 3.8% of abnormal sperm vs. 29 men,

Table 5. Semen characteristics of men scored for sperm with fluorescent bodies, YFF test.

	n	Sperm counts	Abnormal sperm, %	Sperm with 1F, %	Sperm with 2F, %
Controls	17	131.9 ± 39.6[a]	41.2 ± 2.5	44.7 ± 0.9	0.8 ± 0.2
Exposed	17	130.8 ± 36.5	52.6 ± 3.6	44.3 ± 1.0	1.0 ± 0.3
Significance[b]		N.S.	$p < 0.01$	N.S.	N.S.

[a]Mean ± standard error of the mean.
[b]Cochran's t-test one-tailed, N.S. = not significant (*40, 42*).

Table 6. Sample size required to detect a 25% change in mean value over control value.

	Sperm parameter		
	Count	Morphology	YFF
Assumed distribution	Log-normal	Normal	Poisson[a]
Mean	132×10^6/ml	41.9%	0.8%
Standard deviation	160×10^6	12.4%	0.7%
25% increase in standard deviation units	0.2	0.8	0.3
Sample size for 5% level test with 90% power[b]	214	26	41

[a]Normal with square root transform.
[b]See Owen (*45*).

43.6 ± 1.8% abnormal sperm with a $p < 0.001$, Cochran's t-test). These correlations suggest that sperm counts as well as sperm morphology may be affected in the carbaryl workers. The negative findings with the assay for sperm counts may be due to the relative statistical sensitivities of these two assays (see Table 6). Comparisons of controls did not show similar correlations.

Discussion

The results of this study show that workers in a carbaryl-production area had increased levels of morphologically abnormal sperm when compared to controls. Although the elevations in sperm abnormalities were common to currently exposed workers grouped by job classification as low dose and high dose, dose dependence was not found. Neither sperm count nor presence of double-fluorescent bodies were significantly affected in the exposed groups.

We observed a negative correlation between number of years working in the carbaryl area and percent abnormal sperm. We conjecture three possible explanations for this curious relationship. First, the men working longer may be currently exposed less because of seniority; second, some form of biological or pharmacological adaption to exposure may occur (although we don't know of any precedent for this); and third, there may be selection for nonaffected males (for example, older workers who are particularly sensitive to carbaryl and who might have high levels of sperm abnormalities may be switching to other jobs). These conjectures can only be tested by further study of carbaryl workers.

Reversibility of the Sperm Morphology Effect

The previously and currently exposed workers were compared to controls to determine whether the elevated proportion of sperm abnormalities in the currently exposed might be reversible with time after exposure. The distribution of sperm abnormalities in previously exposed men was marginally elevated above controls ($p < 0.05$). The mean of the distribution of previously exposed men (50.0%) was high and very similar to that of the currently exposed men (52.0%). However, when men with confounding factors were excluded from comparison, the mean of the previously exposed group (46.6%) more closely approached the control mean of 42.0%, the sample sizes were reduced, and the difference is no longer statistically significant. A similar comparison of controls and currently exposed men still showed a statistically significant elevation in the exposed. Both a larger sample size and repeated, periodic semen evaluations in a group of men who have left their jobs in the carbaryl area are needed to properly evaluate the possible reversibility of the sperm morphology effect.

Effects of Age on Sperm Morphology

The average age (± SD) of the carbaryl exposed group (40.7 ± 10.0 yr) was significantly higher than the control group (26.6 ± 5.6 yr), $p < 0.001$. The possibility arises that the increase in percent abnormal sperm seen in the exposed group is due simply to the increased ages of the men in that group. Schirren et al. (*43*) detected a slight increase with patient age of abnormal forms of sperm, but this was based on men with andrological disorders. In an independent study of 24 healthy men, 18 to 73 years of age, we found no statistically significant correlation between age and percent abnormal sperm, $r = 0.03$ (unpublished data). Also, in the present study there was no statistically significant correlation between age and percent abnormal sperm in the control group ($r = 0.07$). Furthermore, among the exposed carbaryl workers, there was a

statistically significant inverse correlation between age and percent abnormal sperm, ($r = -0.30$, $p < 0.05$), indicating that the younger men had the higher percentage of sperm abnormalities. This is further seen by examining Table 4. The 18 currently exposed men under 40 years of age show 57.9% ± 3.4 abnormal sperm, while those older than 40 years show 43.1% ± 2.4, almost control levels. This difference is highly significant ($p < 0.005$, Cochran's one tailed t-test). We conclude that the statistically significant increase in abnormal sperm among the carbaryl exposed group is not due to semen defects resulting from advancing age.

Statistical Characteristics of the Semen Assays

The different results obtained with assays for sperm counts, morphology and and fluorescent bodies (YFF) may be due to intrinsic differences in their statistical sensitivities. Table 6 shows the sample sizes required to detect a 25% change in the mean value of the controls in our sample (*45*). The sample sizes required depend on the spread of the measurements in the controls (measured by the standard deviations). Thus, if (a) all three sperm assays are equally affected by the perturbing agent(s) and (b) the effect is small, we would have predicted the sperm morphology assay to be most likely to show a statistically significant response.

Genetic Implication of Findings in Human Semen

Decades of studies on human and animal semen have yielded compelling evidence that sperm can be used to assess testicular function and to diagnose pathology. Males with reduced sperm counts, reduced sperm motility, or increased abnormal sperm shapes are usually less fertile. However, the extent of heritable genetic abnormalities associated with increased sperm anomalies remains unclear. Several lines of indirect evidence on mice and humans support the link between induced sperm abnormalities and heritable genetic effects (*28*, *46*). First, a high correlation exists between agents that induce germ-cell mutations in mice such as dominant lethal mutations and those that induce sperm-shape abnormalities. Second, when male mice exposed to agents that induce sperm abnormalities (such as lead acetate or ionizing radiation) were mated to normal females, an increased proportion of their offspring showed sperm defects. Subsequent studies have shown that sperm changes in these abnormal offspring behave like dominant mutations and that recessive and X-linked genes are also known to be involved in sperm shaping (*28*).

In humans, several studies have shown a link between abnormal embryos and seminal abnormalities in the male parent. In a study by Furuhjelm et al. (*47*) of the Karolinska Institute in Sweden, fathers of 201 spontaneous abortions had reduced sperm counts and marked increases in sperm abnormalities when compared to fathers of normal pregnancies (see Fig. 5). Similar results were obtained in a study by Czeizel et al. (*48*) in 50 husbands of women with two or more spontaneous abortions and 50 men who had fathered normal healthy children. These animal and human studies suggest a genetic link between seminal defects (especially increases in sperm shape abnormalities) and genetic abnormalities in offspring.

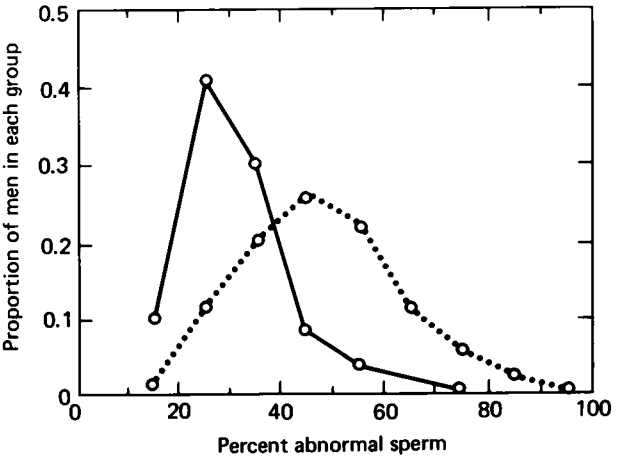

FIGURE 5. Sperm-shape abnormalities in fathers of spontaneous abortions. Dashed line represents the distribution of sperm-shape abnormalities in 201 fathers of spontaneous abortions. Data were collected in Stockholm during 1958 to 1961. Solid line represents sperm shape abnormalities in 116 fathers of pregnancies resulting in normal living children. Reproduced from Furuhjelm et al., (*46*).

Conclusions

Men working in a carbaryl production plant showed higher percentages of sperm shape abnormalities than did new-hires in the plant. This result cannot be explained by differences in smoking habits, illness, medication, or age.

Sperm density and YFF assays did not identify statistically significant differences related to exposure. These assays, however, are shown to be statistically less sensitive than the sperm morphology assay.

For sperm morphology, there was no dose dependence as judged by job classification in the

carbaryl area; however, there was a curious, inverse relationship with the number of years on the job, suggesting that the men working for less than 6 years were most affected.

A comparison of men working with carbaryl at the time of semen collection and those men no longer in the area suggests that the effect on morphology may not be reversible. Since confounding factors and small sample sizes affect this conclusion further human studies are required to fully assess reversibility.

Based on the human data presented, we strongly recommend further animal and human studies with carbaryl to determine to what extent, (a) carbaryl is responsible for the elevated sperm abnormalities seen in the production workers and (b) elevated levels of sperm abnormalities may be related to heritable genetic damage.

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REFERENCES

1. Feldmann, R. J., and Maibach, H. I. Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol. 28: 126 (1974).
2. Thomas, J. A. Actions of pesticides and other drugs on the male reproductive system. USNTIS PB Rept. No. 237381/9GA, 1974.
3. Lillie, R. J. Studies on the reproductive performance and progeny performance of caged white leghorns fed malathion and carbaryl. Poultry Sci. 52: 266 (1973).
4. Weil, C. S., Woodside, M. D., Bernard, J. B., Condra, N. I., King, J. M., and Carpenter, C. P. Comparative effect of carbaryl on rat reproduction and guinea pig teratology when fed either in the diet or by stomach intubation. Toxicol. Appl. Pharmacol. 26: 621 (1973).
5. Jordan, M., Srebro, Z., Pierscinska, E., and Wozny, L. Preliminary observations on the effects on the organs of mice of administering some carbamate pesticides. Bull. Environ. Contam. Toxicol. 14: 205 (1975).
6. Dikshith, T. S. S., Gupta, P. K., Gaur, J. S., Datta, K. K., and Mathur, A. K. Ninety-day toxicity of carbaryl in male rats. Environ. Res. 12: 161 (1976).
7. Whorton, M. D., Milby, T. H., Stubbs, H. A., Avashia, B. H., and Hull, E. Q. Testicular function among carbaryl exposed employees. J. Toxicol. Environ. Health 5: 929 (1979).
8. Vashakidze, V. I. Effect of Sevin intoxication on sexual function of experimental animals. Soobshch. Akad. Nauk. Gruz. SSR 39: 471 (1965).
9. Rybakova, M. N. Toxic effects of Sevin on animals. Gig. Sanit. 31: 42 (1966).
10. Vashakidze, V. I., Shavladze, N. S., and Gvineriya, I. S. Harmful effects of nitrogen-containing pesticides (Dinoc and Sevin) on the sexual function of male rats. Sb. Tr. Nauch.-Issled. Inst. Gig. Tr. Profzabol. Gruz. SSR 10: 205 (1966).
11. Rybakova, M. N. Comparative toxic effects of Sevin and DDT in food crops. Vop. Pitan. 26: 9 (1967).
12. Shtenberg, A. I., Orlova, N. V., Pozdynakov, A. L., Toropova, G. P., Zhalbe, E. P., Khovaeva, L. A., and Akincheva, M. Y. Functional and morphological parallels of the state of the gonads of animals under the effect of Sevin. Vop. Gig. Toksikol. Pestits. Tr. Nauch. Sess. Akad. Med. Nauk. SSSR 124 (1967).
13. Shtenberg, A. I., and Rybakova, M. N. Effect of carbaryl on the neuroendocrine system of rats. Food Cosmet. Toxicol. 6: 461 (1968).
14. Orlova, N. V., and Zhalbe, E. Maximum permissible amounts of Sevin in food products. Vop. Pitan. 27: 49 (1968).
15. Shtenberg, A. I. and Orlova, N. V. Mode of toxicity of Sevin, a carbamate pesticide. Vestn. Akad. Med. Nauk, SSR 25: 72 (1970).
16. Orlova, N. V. Adverse effect of Sevin on warm-blooded animals. Gig. Primen. Toksikol. Pestits. Klin. Otravlenii 8: 208 (1970).
17. Shtenberg, A. I., and Ozhovan, M. V. Effect of low Sevin doses on the reproductive function of animals in a number of generations. Vop. Pitan. 30: 42 (1971).
18. Krylov, D. G. Testing the action of Sevin on the bank mole population. Gig. Primen. Toksikol. Pestits. Klin. Otravelenii 8: 218 (1970).
19. Krylova, T. V., and Denisova, A. V. Action of Sevin on the Mongolian tree creeper. Biol. Nauk 16: 25 (1973).
20. Przezdziecki, Z., and Komorowska-Malewska, W. Influence of carbaryl on Wistar rat gonads. Mater. Nauk. Krajowego Zjazdn. Endokrynol. Pol. 8: 296 (1974).
21. Kitagawa, K., Wakakura, M., and Ishikawa, S. Light microscopic study of endocrine organs of rats treated by carbamate insecticide. J. Toxicol. Sci. 2: 53 (1977).
22. Degraeve, N. J., Moutschen-Damen, M., Houbrechts, N., and Colizzi, A. Apropos des risques d'un insecticide: le carbaryl utilise seul et en combination avec les nitrites. Bull. Soc. Roy. Sci. Liege 45: 46 (1976).
23. McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. (U.S.) 72: 5135 (1975).
24. Seiler, J. P. Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. Mutat. Res. 46: 305 (1977).
25. Ahmed, F. E., Lewis, N. J., and Hart, R. W. Pesticide induced ouabain resistant mutants in Chinese hamster V79 cells. Chem. Biol. Interact. 19: 369 (1977).
26. Ahmed, F. E., Hart, R. W., and Lewis, N. J. Pesticide induced DNA damage and its repair in cultured human cells. Mutat. Res. 42: 161 (1977).
27. Brzheskii, V. V. The study of the mutagenic properties of an insecticide from the carbamate group—Sevin. Sov. Genet. 8: 798 (1972).
28. Wyrobek, A. J., and Bruce, W. R. The induction of sperm-shape abnormalities in mice and huamns. In: Chemical Mutagens A. Hollaender and F. de Serres, Eds. Vol. 5, Plenum Press, New York, 1978.
29. Wyrobek, A. J., and Gledhill, B. L. Human semen assays

for workplace monitoring. In: Proceedings of the Workshop on Methodology for Assessing Reproductive Hazards in the Workplace, P. F. Infante and M. S. Legator, Eds., NIOSH #81-100, Cincinnati, 1980, pp. 327-355.
30. Sieber, S. M., and Adamson, R. H. Toxicity of antineoplastic agents in man: Chromosomal aberations, antifertility effects, congenital malformations, and carcinogenic potential. Adv. Cancer Res. 22: 57 (1975).
31. Whorton, M. D., Krauss, R. M., Marshall, S., and Milby, T. H. Infertility in male pesticide workers. Lancet ii: 1259 (1977).
32. Whorton, M. D., Krauss, R. M., Stubbs, H. A., and Milby, T. H. DBCP-induced infertility in male pesticide workers. J. Occup. Med. 21: 161 (1979).
33. Kapp, R. W., Picciano, D. J., and Jacobson, C. B. Y-Chromosomal nondisjunction in dibromochloropropane-exposed workmen. Mutat. Res. 64: 47 (1979).
34. Lancranjan, I., Popescu, H. I., Gavanescu, O., Klepsch, I., and Serbanescu, M. Reproductive ability of workmen occupationally exposed to lead. Arch. Environ. Health. 30: 396 (1975).
35. MacLeod, J. Human seminal cytology following the administration of certain antispermatogenic compounds. In: Symposium on Agents Affecting Fertility, C. R. Austin and J. S. Perry, Eds., Little, Brown and Co., Boston, 1965, pp. 93-123.
36. Best, E. M., Jr., and Murray, B. L. Observations on workers exposed to Sevin insecticide: a preliminary report. J. Occup. Med. 4: 507 (1962).
37. Humason, G. L. Animal Tissue Techniques. W. H. Freeman and Co., San Francisco, 1972, pp. 456-457.
38. Fredricsson, B. Morphologic evaluation of spermatozoa in different laboratories. Andrologia 11: 57 (1979).
39. Pearson, P. L., Bobrow, M., and Vosa, C. G. Technique for identifying Y. chromosomes in human interphase nucleus. Nature 226: 78 (1970).
40. Snedecor, G. W., and Cochran, W. G. Statistical Methods. The Iowa State University Press, Ames, Iowa, 1967.
41. Siegel, S. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, 1956, pp. 127-136.
42. Goldstein, A. Biostatistics: An Introductory Text. The MacMillan Co. New York, 1964, p. 55.
43. Schirren, C., Laudahn, G., Hartmann, E., Heinze, I., and Richter, E. Untersuchungen zur Korrelation morphologischer und biochemischer Messgrössen im menschlichen Ejakulat bei verschiedenen andrologischen Diagnosen. I. Mitteilung: Beziehungen zwischen Ejakulatvolumen, Zahl, Motiltät und Morphologie der Spermatozoen unter Berucksichtigung des Lebensalters. Andrologia 7: 117 (1975).
44. Viczian, H. Ergebnisse von Spermauntersuchungen bei Zigarettenrauchern. Z. Haut Geschlechtskr. 44: 183 (1969).
45. Owen, D. B. Handbook of Statistical Tables, Addison-Wesley, Reading, Mass. 1962, pp. 41-42.
46. Wyrobek, A. J., and Bruce, W. R. Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. (U.S.), 72: 4425 (1975).
47. Furuhjelm, M., Jonson, B., and Lagergren, C-.G. The quality of human semen in spontaneous abortion. Inter. J. Fertil. 7: 17 (1962).
48. Czeizel, E., Hancsok, M., and Viczian, M. Examination of the semen of husbands of habitually aborting women. Orvosi Hetilap 108: 1591 (1967).