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# Chemical induction of sperm abnormalities in mice

**(25 chemicals/mutagens/teratogens/carcinogens)**

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**ABSTRACT** **The sperm of (C57BL × C3H)$F_1$ mice were examined 1, 4, and 10 weeks after a subacute treatment with one of 25 chemicals at two or more dose levels. The fraction of sperm that were abnormal in shape was elevated above control values of 1.2–3.4% for methyl methanesulfonate, ethyl methanesulfonate, griseofulvin, benzo[*a*]pyrene, METEPA [tris(2-methyl-1-aziridinyl)phosphine oxide], THIOTEPA [tris(1-aziridinyl)phosphine sulfide], mitomycin C, myleran, vinblastine sulphate, hydroxyurea, 3-methylcholanthrene, colchicine, actinomycin D, imuran, cyclophosphamide, 5-iododeoxyuridine, dichlorvos, aminopterin, and trimethylphosphate. Dimethylnitrosamine, urethane, DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], 1,1-dimethylhydrazine, caffeine, and calcium cyclamate did not induce elevated levels of sperm abnormalities. The results suggest that sperm abnormalities might provide a rapid inexpensive mammalian screen for agents that lead to errors in the differentiation of spermatogenic stem cells *in vivo* and thus indicate agents which might prove to be mutagenic, teratogenic, or carcinogenic.**

There is a need for rapid methods for screening chemicals for mutagenic, teratogenic, and carcinogenic properties *in vivo* in mammals. Present methods require large numbers of test animals, maintenance of these animals for many months, and painstaking evaluation of autopsies (1–4). Rapid bacterial methods are available for testing chemicals and body fluids for mutagenic activity (5) but many assumptions are required to extrapolate the results obtained from these assays to the *in vivo* mammalian system.

The number of abnormal sperm in mice is increased measurably with doses of x-rays of as low as 30 rads (6–8). It is probable that the increase in the number of abnormalities is a consequence of genetic damage to spermatogenic cells and we have proposed that this simple assay could be used as a measure of damage which spermatogenic cells have suffered from any physical or chemical agent.

In this paper we report on measurements of sperm abnormalities in mice following the administration of 25 diverse chemical agents thought to represent a range of mutagenic, teratogenic, and carcinogenic activities. The results show that sperm abnormalities are induced by many of these agents, that dose effect curves are readily obtained, and that the measurement of sperm abnormality frequency may indeed provide a simple assay for deleterious chemical agents.

## MATERIALS AND METHODS

**Mice.** Young hybrid male mice of genotype (C57BL × C3H/Anf)$F_1$ from Cumberland View Farms, Clinton, Tenn., or (C57BL/6 × C3H/He)$F_1$ from Bio Breeding Laboratories of Canada Ltd., Ottawa, Canada, were obtained and kept until they reached an age of 11–14 weeks before being used. They were housed in suspension, wire bottom cages in air-conditioned rooms with automated light and darkness cycles and were allowed food (Purina chow) and water ad lib.

**Cell Suspension and Scoring.** Four mice were usually used for the assays for sperm abnormalities. Mice from each such group were killed by cervical dislocation and their cauda epididymides were removed. Two sperm suspensions were prepared, each from four cauda of two mice by mincing the cauda in 2 ml of phosphate buffered physiological saline, pipetting the resulting suspension and filtering it through an 80-$\mu$m stainless steel mesh to remove tissue fragments. A fraction of each suspension was then mixed (10:1) with 1% Eosin Y ($H_2O$) and 30 min later smears were made, allowed to dry in air, and were mounted under a coverslip with Permount mounting medium. For each suspension 1000 sperm were examined at 400-fold magnifications with blue-green filters; a total of 2000 sperm were thus examined for each group. The sperm normally appeared in outline as shown in Fig. 1A. Abnormal sperm had forms readily recognizable as different as shown in Fig. 1B–F.

## RESULTS

The compounds chosen for study are shown in Table 1 together with their sources and a summary of their activities as mutagens, teratogens, and carcinogens in mammals treated *in vivo*. Each of the compounds was given to the mice by a subacute schedule (5 consecutive, daily intraperitoneal injections) at daily dosages shown in Fig. 2, delivered in 0.5 ml of appropriate vehicle, prepared fresh daily. Distilled water was used as the vehicle from all chemicals except benzo[*a*]pyrene, dichlorvos, DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], 3-methylcholanthrene, and myleran which were delivered in tricaprylin (Nutritional Biochemical Corp., Cleveland, Ohio) (4, 9). Measurements of sperm abnormalities were made at 1, 4, and 10 weeks following the end of the exposure. Sperm observed at these times were presumably exposed to the chemicals while they were spermatids, early primary spermatocytes, and spermatogonia respectively. The results of these experiments are shown in Fig. 2.

It is convenient to consider first the frequency of abnormalities in all groups of control animals. A total of 29 measurements (of 2000 sperm each) were made on sperm of (C57BL × C3H/Anf)$F_1$ and (C57BL/6 × C3H/He)$F_1$ mice which had received either water or tricaprylin as vehicle. There was no significant difference in frequency of abnormalities in the four groups. For this reason we pooled the results from all control groups and plotted the resultant cumulative frequency distribution in Fig. 3. The mean frequency of abnormalities was 1.8% with the 90% interval for the population extending from 1.2 to 3.4%. The expected Poisson distribution for an abnormality frequency of 1.8% is shown by the solid curve. Since there is more dispersion in the counts than can be accounted for by counting statistics

Abbreviations: DDT, 1,1.1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; METEPA, tris(2-methyl-1-aziridinyl)phosphine oxide; THIOTEPA, tris(1-aziridinyl)phosphine sulfide.

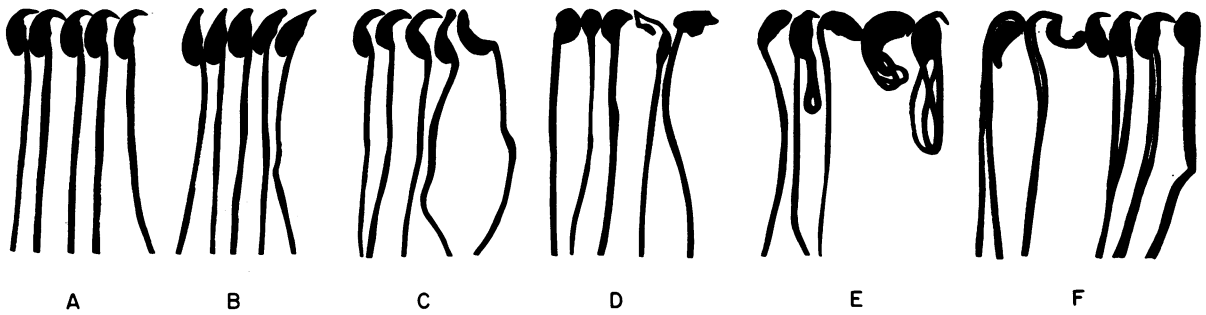


FIG. 1. The shape of A is normal whereas B to F are abnormal murine sperm. Sperm in B lack the usual hook, C have a banana-like form, D are amorphous, E are folded on themselves, and F possess two tails (×1,000).

alone, some of the dispersion must be due to differences between animals in the groups. The 90% interval for all the control groups is represented in Fig. 2 as a hatched area for each graph.

The dose-effect curves for each of the 25 agents are plotted in Fig. 2. At the lower doses tested, most agents produced no measurable increase in abnormalities above that seen in the control animals. At higher dosages, 19 of the agents showed significant elevations. The highest level (72% abnormalities) was seen 4 weeks following the administration of methyl methanesulfonate. Ten of the agents showed an elevation at 1 week, 18 at 4, and 11 at 10 weeks following treatment.

In the course of scoring the abnormalities it was noted that there were significant differences in the types of abnormalities seen with the different agents and at the different times. Abnormalities in which the hooks were up (Type B in Fig. 1) made up 25–30% of the abnormalities 1 week following methyl methanesulfonate or ethyl methanesulfonate administration but did not make up more than 5% of the abnormalities with any of the other drugs at any time. The banana-like head, (C), was prominent with hydroxyurea (46% of the abnormalities) and mitomycin C (35%) at 4 weeks, but was less frequent with the other agents such as methyl methanesulfonate (4% at 1 and 4 weeks). The amorphous type, (D), was prominent with methyl methanesulfonate (58% at 4

Table 1. Mutagenic, teratogenic, and carcinogenic activities of 25 chemical agents in mammals compared to their sperm abnormality inducing action†

| Chemical Agent | Source* | Dominant Lethal Mutations | Cytogenetic Aberrations: Somatic Cells | Cytogenetic Aberrations: Germ Cells | Cytogenetic Aberrations: F1 Translocations | Specific Locus Mutations | Host-Mediated Mutations | Teratogenicity | Carcinogenicity | Sperm Abnormality at 1 & 4 wks |
|---|---|---|---|---|---|---|---|---|---|---|
| Actinomycin D | a | - 9 | | + 10 | | | | + 11 | ± 12 | + |
| Aminopterin | b | - 9 | | | | | | + 13 | ± 12 | + |
| Benzo(a)pyrene | c | + 9 | | | | | | ± 14,15 | + 12 | + |
| Caffeine | d | - 16 | - 17 | - 18 | - 19 | - 20 | - 21 | ± 22,23 | - 12 | - |
| Calcium cyclamate | b | - 9 | ± 24,25 | - 26 | | | - 27 | - 28 | + 29 | - |
| Colchicine | c | - 9 | + 30 | | | | | ± 31,32 | - 12 | + |
| Cyclophosphamide | e | + 33 | + 34 | ± 35,36 | + 35 | | + 37 | + 38 | ± 4,39 | + |
| Dichlorvos | f | ± 9,40 | - 36 | - 36 | | | - 27,86 | ± 41,42 | - 43 | + |
| 1,1-Dimethyl-hydrazine | g | - 9 | | | | | | | + 12 | - |
| Dimethyl-nitrosamine | b | - 9 | | | | | + 21 | - 44 | + 12 | - |
| DDT | g | - 9 | ± 45,46 | | | | - 27 | - 47 | ± 48,49 | - |
| Ethyl methane-sulfonate | c | + 9 | | - 50 | + 50 | ± 51 | + 52 | + 53 | + 12 | + |
| Griseofulvin | c | - 9 | | | | | | + 54 | ± 55,56 | + |
| Hydroxyurea | h | - 9 | ± 57,58 | | | | | + 58 | - 12 | + |
| Imuran | i | - 9 | ± 57,59 | | | | | ± 60,61 | ± 12 | + |
| 5-Iododeoxyuridine | c | - 9 | | | | | | + 62 | ± 12,63 | + |
| Methylcholanthrene | j | - 9 | | | | + 64 | | ± 65,66 | + 12 | + |
| Methyl methane-sulfonate | k | + 9,67 | | ± 68,69 | + 70 | ± 51 | | + 53 | + 12 | + |
| METEPA | b | + 9 | | | | | | + 71 | + 71 | + |
| Mitomycin C | c | + 9 | + 72 | ± 73,74 | | + 75 | - 21 | + 76 | + 12 | + |
| Myleran | l | + 9 | + 77 | ± 78,69 | | - 79 | | ± 80,81 | ± 12, 4 | + |
| THIOTEPA | m | + 9,82 | + 82 | | | | | ± 83,84 | + 4,12 | + |
| Trimethylphosphate | l | + 9,40 | + 85 | | | | + 86 | | | + |
| Urethane | n | - 9 | | - 87 | | | | ± 88,89 | + 12 | - |
| Vinblastine sulfate | o | - 9 | + 90 | | | | | ± 90,91 | - 12 | + |

+, Positive results; −, negative results; ±, results vary with species, cell type studied, or are inconsistent.

* a, Merck and Co. Inc., Montreal, Canada; b, K & K Laboratories, Plainview, N.Y.; c, Sigma Chemical Co., St. Louis, Mo.; d, Eastman Organic Chemicals, Rochester, N.Y.; e, Frank W. Horner Ltd., Toronto, Canada; f, Vapona, technical (93%) Shell Chemical, Toronto, Canada; g, Matheson, Coleman and Bell, Norwood, Ohio; h, Calbiochem, San Diego, Calif.; i, Burroughs Wellcome and Co., Canada Ltd., Montreal, Canada; j, J. T. Baker Chemical Co., Phillipsburg, N. J.; k, Aldrich Chemical Co., Milwaukee, Wisc.; l, Chemical Procurement Laboratories Inc., College Point, N.Y.; m, Lederle Products Dept., Cyanamid of Canada Ltd., Toronto, Canada; n, British Drug House, Toronto, Canada; o, Eli Lilly and Co., Toronto, Canada.

† Numbers given are representative references.

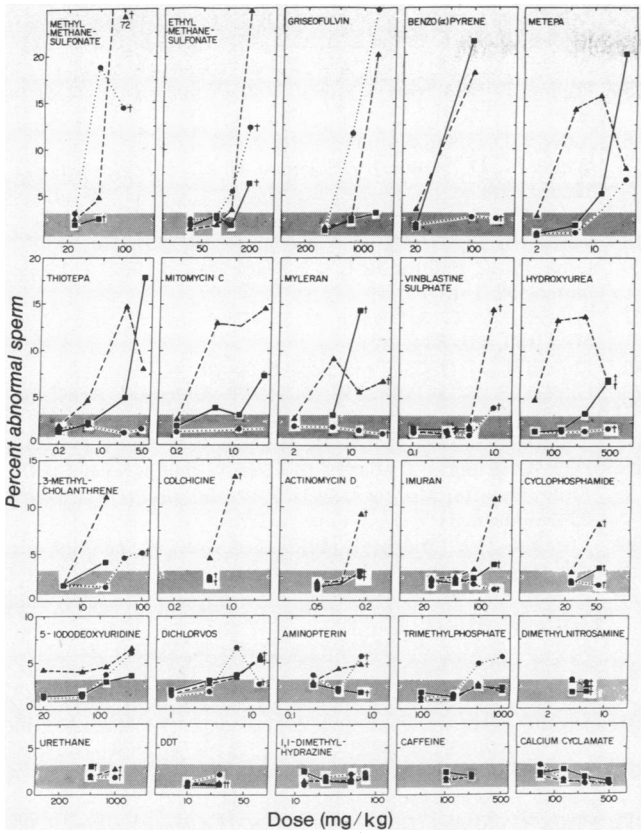


FIG. 2. Percent of sperm that are abnormal as a function of individual dose of chemical administered. Chemicals were given intraperitoneally on 5 consecutive days and the numbers of abnormally shaped sperm per 2000 in the cauda epididymis were measured 1 week (●·····●), 4 weeks (▲– – –▲), and 10 weeks (■—■) later. The hatched area is the 90% interval for all values of the control groups. Symbol † denotes groups in which some animals died. The 25 panels have been arranged by maximum percent abnormalities. Folded sperm (Fig. 1E) were not included in the percent abnormalities shown here.

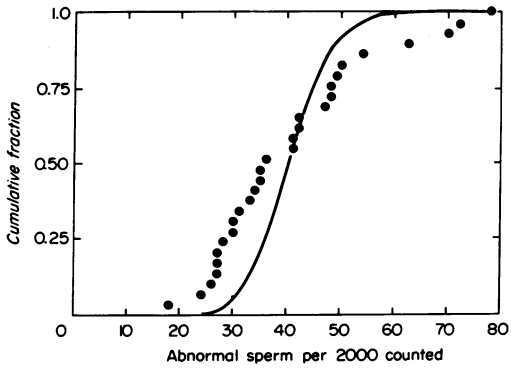


FIG. 3. The cumulative distribution function for number of abnormal sperm seen in 2000 sperm counted: A total of 29 independent control groups of four mice each were examined. The average number of abnormalities seen in all groups was 36. The solid line is the Poisson distribution for an average of 36 abnormalities in 2000 counts. Ninety per cent of the animals have between 24 and 68 shape abnormalities out of 2000 sperm counted. The range corresponds to the hatched area in Fig. 2.

weeks) but less frequent with mitomycin C (15% at 4 weeks). Folded forms (E) were most prominent with vinblastine (56 and 45% at 1 and 4 weeks, respectively) and least with hydroxyurea (3% at these times). For the results shown in Fig. 2, folded sperm were scored as abnormalities only when the head shape was also clearly abnormal. Twin-tailed forms, (F), make up 10% of the abnormalities with THIOTEPA and dichlorvos, but very few were seen with most of the other agents, including colchicine. All forms were seen at 1, 4, and 10 weeks.

It was also noted that at the higher doses of the individual agents there was often reduced numbers of sperm in the epididymides as had been observed previously with x-rays. This measure of drug effect was found to be erratic and the effects of dosage were not as clear as for sperm abnormalities.

## DISCUSSION

In Table 1, the effects of the 25 agents on sperm abnormalities at 1 and 4 weeks after exposure are compared with the mutagenic, teratogenic, and carcinogenic activities of these agents in mammals. Those agents well known for their mutagenic activity *in vivo* such as methyl methanesulfonate, ethyl methanesulfonate, METEPA, THIOTEPA, mitomycin C, and myleran [as well as x-rays (92–94)] all produce marked elevations in sperm abnormalities. In some cases the elevations are seen at week 1, in all at week 4, and in most they persist for 10 weeks (Fig. 2). Cyclophosphamide, also known to be mutagenic, induces abnormalities but the level and duration are much less pronounced than those observed with the other alkylating agents. The antimetabolites, hydroxyurea, Imuran, IUrd, and aminopterin, produce less marked effects which seem to disappear in the case of the last of these agents, before 10 weeks. While mutagenesis studies with these four agents have either yielded negative or conflicting results, all are well known as teratogenic agents. Griseofulvin, vinblastine, colchicine, and actinomycin D are also known for their teratogenic effects, but have not been extensively studied for their mutagenic activity. Benzo[*a*]pyrene and methylcholanthrene are known for their carcinogenic effects, and have also been shown to have teratogenic and mutagenic activity. All these agents give rise to sperm abnormalities.

We are then left with a number of agents with less marked effects on sperm shape. Dichlorvos is normally considered not to be mutagenic or teratogenic. Most of the studies with this chemical however have used oral and inhalation exposure (36, 40, 42, 86). Dominant lethal and teratogenic studies in which dichlorvos was given intraperitoneally have been positive (9, 41). Trimethylphosphate gives positive results in the dominant lethal assay for cells exposed in post meiosis (50). At this period the sperm are abnormal but there is no increase in abnormalities for premeiotic cells.

Dimethylnitrosamine is particularly interesting. This agent is clearly mutagenic as assayed by the host-mediated assays, yet it leads to no sperm abnormalities and is negative in the dominant lethal assay. These apparently conflicting results may be explained by biochemical studies which have shown that DNA was alkylated much less effectively by this agent in the testis than it was in the bone marrow or liver (95). Perhaps the inactivity of urethane in inducing sperm abnormalities may be explained similarly. Like dimethylnitrosamine, this compound is inactive on the dominant lethal assay and does not lead to cytogenetic aberrations in the testis, but is teratogenic and carcinogenic. Dimethylhydrazine, caffeine, DDT, and calcium cyclamate, do not induce abnormalities in sperm and are not usually considered mutagenic. In addition, we have found glucose, hypertonic saline, adrenalin, chloral hydrate, and pentabarbitone given in acute intraperitoneal doses up to the toxic limit, do not lead

to an increase in the number of abnormal sperm produced (W. R. Bruce and J. Heddle, unpublished results).

We have suggested that the abnormalities in sperm morphology are a consequence of chromosomal aberrations (8). Recent studies with translocation bearing mice make this appear unlikely, and suggest that the late changes may be due instead to genetic changes in the genes responsible for spermatogenesis (Wyrobek *et al.*, unpublished results). It is of course also possible to induce changes in differentiation during gene expression, including late steps after transcription and translation of the genetic message. Folded head type abnormalities, seen with vinblastine, might well be an example of a non-genetic damage since this type of abnormality is not seen at longer periods after treatment and since vinblastine is known to interact with microtubules that have been shown to be present during sperm development (96, 97). Experiments are now in progress to determine the relative importance of genetic and non-genetic factors in the induction of sperm abnormalities.

Even in the absence of a complete understanding of the mechanism of abnormality induction, however, it appears that the assay of sperm abnormalities will be of value. The male gametes may be examined rapidly, reproducibly, and in large numbers. An agent which induces abnormalities is an agent which is interfering either with the integrity of the DNA itself or with the expression of this genetic material. While the sensitivity of spermatogenic cells to mutagenic, teratogenic, or carcinogenic agents does not exactly parallel that of many types of the somatic, or indeed of even the female germ cells, the male germ line is an important population which may be easily and inexpensively monitored for deleterious effects.

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