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MUTAGENICITY FOLLOWING ADMINISTRATION OF DIMETHYL MERCURY IN SWISS MALE MICE[1]

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ABSTRACT

Over the past decade the consumption of mercury by industry has increased rapidly. In the environmental system, by the process of biotransformation, mercury is converted to more toxic alkyl forms, which are accumulated and magnified in higher organisms through food chain.

Following a preliminary toxicity study 20 random bred Swiss male mice, 56 days old, were injected 50 mg/Kg of dimethyl mercury intraperitoneally. Ten males served as concurrent controls. After a 24 hour recovery period each test and control male was then individually caged for one week with three untreated virgin 8 week old females. Females were replaced weekly and consecutively with fresh animals for a total of six weeks. During all mating periods, mice were fed laboratory chow and had access to tap water *ad libitum*. All females were autopsied on day 13 of exposure to males. They were scored for pregnancy and implants comprising of normal implants, late fetal deaths, and early fetal deaths; the latter appeared as black deciduomata.

DMM resulted in reduced fertility and a decrease in mean litter size. Genetic damage occurred in spermatozoa and spermatids, postmeiotic stages of spermatogenesis.

Introduction

Over the past decade the consumption of mercury by industry has increased rapidly. The U.S.A. alone uses 3000 tons/year, or 1/3 of the total world consumption [1]. Occupational hazards manifested by mercury were the concern of earlier studies [2-3]. However, more recently attention is being

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focused on the potentially fatal effects of organomercurials entering the environment from industrial pollution [1]. In the process of biotransformation, mercury is converted to more toxic alkyl forms, which are accumulated and amplified successively in higher organisms through food chains [1]. It is estimated that the concentration of mercury in pike may be 3,000 times greater than in water [4].

Jenne [5] observed that 30% of U.S. industrial wastes contained 10 μg/l mercury and 5% contained 1000 μg/l. Feick, *et al.* [6] found that mercury could be released in considerable quantities from bottom sediments in fresh water by the addition of NaCl through run off, because NaCl and $CaCl_2$ increase mercury solubility by 2-5 times.

The normal mercury content of air varies from $2\text{-}50 \times 10^{-9}$ g/cu.m with higher levels occurring over ore deposits [7]. The acceptable mercury content in food is 0.5 ppm, and in water 0.5 μg/l [8].

Two serious episodes of mercurial pollution occurred in Japan where fish and shellfish are a major portion of the human diet. Between 1953 and 1970, 121 cases of neurological disorders were reported in Minamata, where a vinylchloride plant was discharging 600 kg/yr of a mercury catalyst into the stream [9]. In 1965, 47 official cases of mercury poisoning were reported in Niigata, Japan; an acetaldehyde plant had been discharging methylmercury (MM) into the waters [10].

Use of organomercurials as seed fungicides in Sweden during the 40's and 50's increased deaths among seed-eating birds, rodents, and their predators [4]. By 1965, high levels of MM were found in Swedish chicken eggs, meats, and fish [11-13].

In 1969, the Huckleby family of New Mexico suffered mercurialism after eating pork which was fed on mercury-treated grain [9].

Hens produce eggs containing mercury when fed on mercury-treated grains. The albumin contained 75% of the mercury in the egg. Hen's liver and kidney showed higher concentrations of mercury than muscle [14]. At higher doses, egg production and hatchability decreased [15].

Pigs fed on grains containing 6 ppm mercury suffered renal necrosis and had 50 ppm mercury in liver and 64 ppm in kidney tissue after 10-21 days [16]. Pheasants and seed-eating birds consuming treated grain seeds have been found to contain up to 140 ppm mercury in liver and kidney, and 35-70 ppm in muscle.

Little is known about the genetic damage caused by mercury. Organomercurials cause polyploidy in plant cells by disturbing the spindle mechanism in mitosis [17]. Treatment of sea-urchin eggs with mercurials results in polyspermy [18]. When treated in larval or adult stages with MM or phenylmercury, female *Drosophila melanogaster* produced daughters with abnormal sex chromosomes. This suggests autosomal nondisjunction in the female parent; however, treating male *Drosophila* had no effect on the sex

chromosomes of the progeny [17]. Chromosomal breakage was observed in leukocytes of humans who had histories of consuming fish containing 1-7 ppm mercury and had high blood mercury levels [19]. Pregnant women inadvertantly exposed to MM in Minamata, Japan, showed no symptoms of mercury poisoning but their offspring suffered mental and physical defects [20]. In similar cases, Joselow, *et al.* [21] reported mercury levels 28% higher than normal in infants at birth.

FDA guidelines for allowable body burden for human consumption of fish containing MM are based mainly on neurological dysfunction, and do not consider teratogenicity, carcinogenicity, or the possibility of genetic damage [1]. Genetic effects are important since they may be latent for several generations, and the damage may become permanent.

This study was undertaken to evaluate the mutagenicity and/or infertility caused by the intraperitoneal administration of a single acute dose of dimethylmercury in Swiss male mice using dominant lethal assay. Dominant lethal mutations are convenient indicators of genetic damage for measuring the effects of chemical mutagens [22].

Biotransformation

In bottom sediments of aquaria, decaying fish, and fish homogenates, inorganic mercury is converted to MM and DMM [23]. Jernelov [24] proposed a scheme dependent on biological processes for the breakdown of various mercurials in sediments to ionic mercury and methylation to alkyl forms (Figure 1).

Phenylmercuric acetate, ethylmercuric phosphate, and methylmercuric chloride are decomposed to metallic mercury and associated gases by a mercury-resistant strain of pseudomonas [25, 26]. Methanogenic bacteria convert ionic mercury to MM and DMM; the process is enhanced under

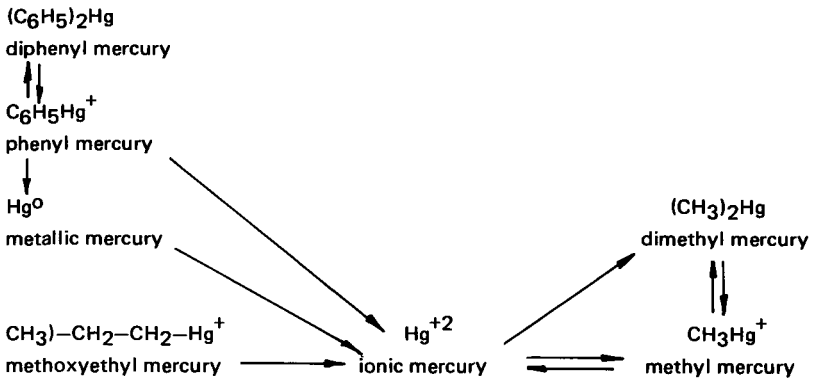

Figure 1. Breakdown and methylation of mercurials in sediments.

anaerobic conditions, especially in the presence of alkylcobalamine-synthesizing bacteria. Sewage pollution increases the formation of alkylmercurials in water [27].

The biological half-life of MM is 70-74 days in man [28] and 400-700 days in fish [29]. Almost all the mercury found in fish is in an alkyl form, even though the pollutant is not [1].

Aryl- and alkoxyalkyl-mercurials are rapidly transformed to inorganic mercury in man. Alkylmercurials are slowly degraded and excreted [4, 21].

After exposure to methylmercuric chloride, 40% of the excreted mercury is in an inorganic form; however, in the feces, 50% of mercury is inorganic, while in urine 5-20% is inorganic. The kidney shows a relatively high excretion of intact MM [30]. The main route of MM excretion is through feces [28].

Methodology

PRELIMINARY TOXICITY STUDY

Random bred Swiss male mice,[2] 56 days old, were administered DMM by a single intraperitoneal (ip) injection to 5 groups of 7 males each, in doses of 5, 10, 20, 40 and 80 mg DMM/Kg; a group of 7 males was given the solvent and served as concurrent controls. DMM was dissolved in petroleum. The mortality was recorded daily for one week. The corresponding mortalities were 0, 0, 14%, 57%, and 85%. The calculated LD_{50} was 37.5 mg DMM/Kg.

MUTAGENICITY STUDY

In a previous mutagenicity study [31], the incidences of mating in the control group were lower in the first week than in the subsequent weeks because of sexual inexperience of the males. To prepare for the mutagenicity study, 30 untreated males were mated with three virgin females each per week for two consecutive weeks to provide sexual experience.

Twenty experienced males were then injected intraperitoneally with 50 mg DMM/Kg, the remaining 10 males served as concurrent controls. After a 24 hour recovery period each test and control male was then individually caged for one week with three untreated virgin, 8 week old females. Females were replaced weekly and consecutively with fresh animals for a total of six weeks. During all mating periods, mice were fed laboratory chow and had access to tap water *ad libitum*. Females were checked every morning for the presence of vaginal plugs indicative of presumptive mating. All females were autopsied on day 13 of exposure to males (day of vaginal plug = day 0). The dissected females were scored for total implants comprising of normal implants, late fetal deaths, and early fetal deaths; the latter appeared as black deciduomata.

[2] Charles River Laboratory, Wilmington, Massachusetts.

Results and Discussion

The poor physical condition of several treated males prevented them from mating, and as such they were excluded from the assay during the first week.

The overall pregnancy rate was 58.1% in the treated group, and 69.8% in the control (Table 1). The lower rate in the treated group occurred only in the first two weeks, and was more pronounced in the first week.

The number of implants per pregnancy were lower in the treated group than in the control group for all weeks except week 4 and 6; the overall means were 10.8 in the treated group, and 12.5 in the controls. The greatest differences occurred in the first two weeks. This reduction in average litter size indicates an increase in preimplantation losses. Similar losses were also observed with lead

Table 1. Incidences of Mutagenicity and Infertility Following Administration of Dimethylmercury (ip) to Swiss Male Mice

		Week of Mating						
	Group	*1*	*2*	*3*	*4*	*5*	*6*	*Overall*
No. of Females Exposed to males	T	27	21	12	9	9	8	86
	C	30	30	30	30	27	25	172
No. pregnant	T	12	10	8	7	7	6	50
	C	23	20	20	19	20	18	120
Per cent pregnant	T	44.4	47.6	66.7	77.8	77.8	75.0	58.1
	C	76.7	66.7	66.7	63.3	77.1	72.9	69.6
No. of early embryonic deaths	T	18	12	9	10	12	8	69
	C	7	12	11	14	24	18	86
No. of late fetal deaths	T	5	4	2	3	5	1	20
	C	7	6	6	6	6	5	36
Total No. of implants	T	112	96	81	83	81	85	538
	C	296	242	239	220	260	238	1495
No. of implants per pregnancy	T	9.3	9.6	10.1	11.9	11.6	14.2	10.8
	C	12.9	12.1	11.9	11.6	13.0	13.2	12.5
Mutagenicity Index	T	16.1	12.5	11.1	12.0	14.8	9.4	12.8
	C	2.4	5.0	4.6	6.2	9.2	7.6	5.8
X^2		26.5[a]	5.9[b]	4.3[c]	2.7	2.0	0.3	28.1

T = Treated Group
C = Control Group

$$\text{Mutagenicity Index} = \frac{\text{(No. of Early Embryonic Deaths)}}{\text{(Total No. of Implants)}} \times 100$$

[a] $X^2 = 10.83$ ($p \leq 0.001$)
[b] $X^2 = 5.41$ ($p \leq 0.01$)
[c] $X^2 = 2.71$ ($p \leq 0.05$)

subacetate [31], methyl mercuric chloride in rats and Swiss Webster mice [32]. However, no such losses were observed with methylmercury dicyandiamide in CBA mice [17]. Cytologically, following treatment with TEPA the preimplantation losses were due to retarded malformed embryos which failed to implant [33].

The overall mutagenicity index was 12.8 for the treated males, and 5.8 for the control males. This difference indicates that the number of postimplantation losses was more than doubled by DMM. During the last three weeks only three males survived.

The statistical analysis showed that the mutagenicity index was high in the first three weeks which represents postmeiotic stages of spermatogenesis. The index was highest in the first week, where it was approximately 7 times greater than the control group. Values [34] of χ^2 (excluding late deaths) indicated that the results were significant at 99.9% in the first week, 99% in the second week, and 95% in the third week.

Conclusion

In Swiss male mice, intraperitoneal injection of 50 mg/Kg of DMM resulted in reduced fertility and a decrease in mean implants per pregnancy. An overall increase in mutagenicity was also observed. Genetic damage occurred in spermatozoa and spermatids, in the postmeiotic stages of spermatogenesis. Further mutagenicity studies of DMM and other mercurials are needed to determine the threshold dose levels for both premeiotic and postmeiotic stages.

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