Lack of Toluene-Induced Dominant Lethals in Rats[1]

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ABSTRACT. The mutagenic potential of toluene was investigated with the dominant lethal mutation assay. Male Sprague Dawley rats (8-10 wk old) were injected intraperitoneally for 5 consecutive days with 346 and 692 mg per kg body weight of toluene in corn oil. To analyze for the effect of toluene on several germ cell stages, each male was mated with one untreated, virgin female per week for up to 7 weeks. Females were sacrificed 14 to 17 d after insemination for analysis of their uterine contents. The total number of implantations and the number of dead and living embryos per pregnant female were determined. From these data the dominant lethal mutation index was calculated. There was no significant effect of toluene on the number of implantations (total, dead, or alive) per pregnant female per week. The different stages of spermatogenesis from late primary spermatocyte to fully mature sperm were not affected by the action of toluene as measured by the dominant lethal mutation assay. The dominant lethal mutation indices were small positive and negative percentages, suggesting that toluene did not induce dominant lethal mutations in the germ cells of male Sprague Dawley rats under the conditions tested.

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INTRODUCTION

A dominant lethal mutation is a genetic change in a gamete that results in the death of the conceptus that inherits it. It may arise from the loss of whole chromosomes (Binkert and Schmid 1977), or fragments that result from their breakage (Matter and Jaeger 1975, 1977, Brewen et al. 1975, Generoso et al. 1979, Burki and Sheridan 1978) and aneuploidy (Bateman and Epstein 1971).

Both physical and chemical agents are known to effect changes in the genome of an organism, which could lead to dominant lethal mutations. Toluene or methyl benzene is potentially such an agent. It is a common constituent in a wide variety of industrial products and is commonly used as a solvent. Consequently, there is great opportunity for exposure. The potential clastogenic activity of toluene has been investigated in somatic cells of rodents (Dobrokhotov 1972, Lyapkalo 1973, Donner et al. 1981, Gad-El-Karim et al. 1984, Mohtashamipur et al. 1985, Schmid and Bauchinger 1985) and humans (Forni et al. 1971, Funes-Cravioto et al. 1977, Gerner-Smidt and Fredrich 1978, Maki-Paakkanen et al. 1980) and in germ cells of rodents (Feldt and Zhurkov 1985). It has also been studied for mutagenicity in bacteria with the *Salmonella*/microsomal assay (Bos et al. 1981, Haworth et al. 1983).

There is a high degree of discordance in the results reported on the clastogenicity of toluene. Mohtashamipur et al. (1985) demonstrated a dose-dependent genotoxicity of toluene in bone marrow cells of mice. They reported that the number of micronuclei per 1000 polychromatic erythrocytes increased almost linearly with intraperitoneal doses between 0.24 and 0.74 mL per kg, but decreased to the level observed at 0.24 mL per kg when a dosage of 1.0 mL per kg was administered. Dobrokhotov (1972) and Lyapkalo (1973) reported the induction of chromosome aberrations in the bone marrow of rats after exposure to toluene by injection or inhalation. Lyapkalo (1973) administered 1 g per kg per day for 12 d; Dobrokhotov (1972) used an inhalation exposure of 0.8 g per kg per day. Donner et al. (1981), on the other hand, found no evidence of chromosome aberrations in the bone marrow cells of rats after they had inhaled 300 ppm of toluene for 6 h per day 5 d per week for 15 weeks. There was, however, an increase in the frequency of sister chromatid exhanges (SCEs) in bone marrow cells cultured from animals after 11 and 13 wk of exposure. Feldt and Zhurkov (1985) found no indication of chromosome aberrations in bone marrow cells, nor dominant-lethal mutations in random-bred spontaneous hypertensive (SHR) male mice following exposure to 200 mg per kg toluene given by gavage during a 5-wk period.

Forni et al. (1971), Maki-Paakkanen et al. (1980) and Gerner-Smidt and Friedrich (1978) reported negative findings for chromosomal aberrations and sister chromatid exchanges in lymphocyte cultures from workers exposed to toluene. Funes-Cravioto et al. (1977) and Schmid and Bauchinger (1985) observed a significant increase in chromosomal aberrations among toluene-exposed workers. They also found that structural chromosomal aberrations can persist up to 2 yr after exposure.

If toluene is clastogenic in somatic cells, there is no apparent reason for it not to be clastogenic in germ cells. However, Feldt and Zhurkov (1985) reported a lack of clastogenicity in germ cells of mice. Because of the possible species differences in germ cell response, as well as the potential for human exposure to toluene and the discordant conclusions reported, we conducted a study to determine if subchronic exposure to relatively low levels of toluene could induce transmissible chromosomal aberrations in germ cells of male Sprague Dawley rats, which would be expressed as dominant lethal mutations.

MATERIALS AND METHODS

Male (8-10 wk old) and female (6-8 wk old) Sprague Dawley rats were purchased from Harlans (Indianapolis, IN). Males were housed individually; females were housed in groups of four in stainless steel wire mesh cages and given food and water *ad libitum*. The mean room temperature was 24 ±4°C. A 12-h light and dark cycle was used.

Animals were dosed with toluene in corn oil for five consecutive days. Two dose levels of toluene were used in this investigation:

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0.4 mL per kg (346 mg/kg) and 0.8 mL per kg (692 mg/kg) body weight. One group of 39 male rats received 346 mg per kg, and a second group of 18 male rats received 692 mg per kg body weight of toluene by intraperitoneal injection in a total volume of 1 mL. A comparable number of animals received 1 mL of corn oil, the vehicle for toluene.

Several post-treatment mating intervals were used to determine germ cell sensitivity to toluene. Each male was mated with one untreated (8-10 wk old) virgin female per week. In the first low dose group each male was mated with one female per week for 7 weeks. In the second high dose group four mating periods were used: weeks 1, 3, 5, and 7. Males and females remained together up to 5 days. Females were checked daily for copulatory plugs or the presence of sperm in the vaginal canal. Smears were made daily before 1000 h, until sperm were observed or until the mating period ended. The day that sperm were observed was considered day 1 of mating. Females and males were numbered for identification. Females mated to different males were caged in groups of four until they were sacrificed, between day 14 and 17 after mating, for an analysis of their uterine contents. By number identification, we could match males with females as well as maintain a record of the day of insemination.

Males were the treated subjects and females were used to analyze sperm from each male on a weekly basis. Each week of mating was considered independently to determine if certain stages of spermatogenesis were more sensitive than others to the putative mutagenic action of toluene.

Data collected for each female included pregnancy, implantation sites, live fetuses, dead fetuses, and deciduomata. The variable for the proportion of mated females which became pregnant was analyzed y a standard X^2-test. The variables for the number of implantation sites, living implants, and dead implants per pregnant females were analyzed with the Mann-Whitney, non-parametric analysis of variance. Dominant lethality is comprised of the pre- and post-implantation losses. One way to express these losses is through calculation of indices reflecting the relative differences in the number of live implants for treated and control groups. This was done with the formula of Ehling and Malling (1968) for the Dominant Lethal Index (DLI):

$$DLI = 1 - \frac{\text{live implants/pregnant female (experimental group)}}{\text{live implants/pregnant female (control group)}} \times 100$$

These indices can not be tested statistically, but results may be used to supplement other tests.

RESULTS AND DISCUSSION

Data in Table 1 summarize the results of the dominant lethal mutation assay with toluene. No toluene treatment-related effects were observed relative to the reproductive fecundity of the treated male rats, the number of implantations, and live fetuses per female.

A significant increase in the number of dead implants per pregnant female as well as an increase in the percentage of females with one or more dead implants are indications of dominant lethality (Generoso 1984). Our data indicated that the percentage of females with

TABLE 1

Dominant lethal mutation data for male Sprague Dawley rats treated with toluene

Mating interval (days post-treatment)	Toluene dosage (mg/kg)	Pregnant females*	Implants per female ($\bar{x}$ ±SE)	Live fetuses per female ($\bar{x}$ ±SE)	Dead implants (%)	Females with two or more dead implants (%)	Dominant Lethal Index**
1							
(Days 1-5)	0	19	8.2 ±1.1	7.8 ±1.1	15	36	
	346	27	9.7 ±0.9	9.0 ±0.9	11	26	−15.4
	0	7	14.6 ±0.4	12.9 ±1.4	12	14	
	692	7	13.6 ±0.6	13 ±0.7	4	14	−0.8
2							
(Days 8-12)	0	24	10.7 ±0.8	9.7 ±0.8	9	17	
	346	31	12.3 ±0.5	10.3 ±0.7	16	42	−6.2
3							
(Days 15-19)	0	27	12.6 ±0.5	12.3 ±0.5	6	15	
	346	32	12.6 ±0.6	12.2 ±0.5	6	13	0.8
	0	12	14.0 ±0.7	12.7 ±1.0	9	33	
	692	15	12.3 ±0.8	12.1 ±0.8	2	7	4.7
4							
(Days 22-26)	0	34	12.5 ±0.6	11.2 ±0.7	11	29	
	346	35	13.2 ±0.3	11.9 ±0.5	11	31	−6.2
5							
(Days 29-33)	0	30	13.2 ±0.5	12.4 ±0.6	6	20	
	346	33	13.6 ±0.3	12.8 ±0.4	9	21	−3.2
	0	13	14.7 ±0.3	12.7 ±1.2	14	23	
	692	16	15.3 ±0.5	14.9 ±0.5	2	6	−17.3
6							
(Days 36-40)	0	29	12.5 ±0	10.6 ±0.5	12	38	
	346	27	13.3 ±0.4	11.6 ±0.5	16	48	−9.4
7							
(Days 43-47)	0	33	12.5 ±0.5	10.6 ±0.6	16	30	
	346	32	13.2 ±0.5	10.8 ±0.8	21	38	−1.9
	0	9	13.3 ±1.7	12.8 ±1.6	4	11	
	692	11	13.9 ±0.8	13.4 ±0.8	4	9	−4.7

*Of possible 18 females for 692 mg/kg and 39 for 346 mg/kg

**$\left(1 - \frac{\text{live implants/pregnant female (experimental group)}}{\text{live implants/pregnant female (control group)}} \times 100\right)$

two or more dead implants and the number of dead implants per pregnant female were no greater in the treatment group than in the control group. This demonstrates that toluene at the dose levels used did not induce dominant lethal mutations in germ cells of male Sprague Dawley rats.

The dominant lethal mutation index for each posttreatment mating interval was a small positive or negative number, with the largest value being observed during the spermatid stage. This confirms the observations of other investigators (e.g., Ehling and Malling 1968, Generoso et al. 1974, Wardhaugh 1981, Teaf et al. 1985) that the spermatid stage (week 3 posttreatment) has the greatest sensitivity to the action of many mutagenic agents that induce dominant lethals. Even though the DLI was positive for the spermatid stage, the value which is less than 5 is not at a level which indicates sensitivity with respect to the induction of dominant lethal mutations.

The significance of the dominant lethal test is that it gives some indication of heritable chromosomal damage. The endpoint, which is dead fetuses in females inseminated by treated males, though fatal in itself, is an indication that viable types of chromosomal damage may also be induced in the germ cells of treated individuals. Data from this investigation are consistent with previously published data (Feldt and Zhurkov 1985) that indicate a lack of dominant lethal mutations in male rodents treated with toluene. This suggests a lack of induction of viable heritable chromosomal damage in germ cells, including those which lead to dominant lethal mutations.

There are reports of chromosome aberrations in bone marrow cells following exposure to toluene (Dobrokhotov 1972, Lyapkalo 1973). If chromosome aberrations occur in somatic cells but not in germ cells following exposure to toluene, one possible explanation could be that there is a testicular barrier that prevents contact between toluene, its metabolites, and the germ cells. If, on the other hand, chromosome aberrations are induced in meiotic or postmeiotic germ cells, the absence of dominant lethal effect may be due to: 1) the inability of the affected sperm to complete the maturation process, thus preventing their involvement in the fertilization process, or 2) the inability of the affected mature sperm to fertilize eggs.

In conclusion, dominant lethal mutations measured to assess possible genetic damage to germ cells of rats from *in vivo* exposure to toluene were not increased in the present study. This suggests that toluene is nonmutagenic relative to inducing lesions that are expressed as dominant lethal mutations.

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