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Reproductive and Developmental Toxicity of *N,N*-Diethyl-*m*-toluamide in Rats

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N,N-Diethyl-*m*-toluamide (*m*DET, DEET) is widely used as a topical insect repellent. It is the active ingredient in many consumer formulations, which usually contain 10–25% *m*DET in an alcohol base. More concentrated consumer products are also available, including some that are pure technical grade *m*DET. Persons living or employed in mosquito-infested areas may have very high seasonal exposures to *m*DET. Because contradictory reports had been published on the reproductive and developmental toxicity of *m*DET, a series of studies was conducted in male and female Sprague–Dawley rats. All treatments were administered by daily subcutaneous injections of undiluted *m*DET. A dose finding study was done using 12 time-mated females per group treated on Gestational Days (GD) 6–15 with 0.50, 0.62, 0.78, 0.92, or 1.2 ml *m*DET/kg/day. No females survived 10 days of *m*DET dosing with 1.2 ml/kg/day. Deaths occurred in all other groups except the low dose (0.50 ml/kg/day). Pregnant females treated on GD 6–15 with 0 or 0.30 ml/kg/day were used for the teratology study. Half of each group was euthanized on GD 20: the second half was singly housed in nesting boxes and allowed to deliver litters. Live pups were counted and weighed soon after birth on Postnatal Day (PD) 0 and again on PD 3, 9, and 14. Proven fertile males were treated 5 days/week for 9 weeks with 0, 0.30, 0.73, 1.15, or 1.80 ml *m*DET/kg/day for a male dose-finding study. Each group consisted of 20 males. No males survived the 1.80 ml/kg/day. Deaths occurred in all remaining dose groups except the 0.30 ml/kg/day and control group. Immediately following the final treatment of the male dose study, 11 males were randomly selected from the 0.30 and 0.73 ml/kg/day groups. They were cohabited for 7 days with 4 females per male during post-treatment Weeks 1 and 2. Half of the females were euthanized 12–14 days after the last day of cohabitation for a dominant lethal study; the remaining females were singly housed in nesting boxes and allowed to deliver litters. Live pups were counted and weighed on PD 0 and 3. There was no evidence of reproductive or developmental toxicity in any of these assays, but there were signs of neurotoxicity in treated adult male and female rats, which may relate to reports of neurotoxicity in humans heavily exposed to *m*DET-containing insect repellents.

N,N-Diethyl-*m*-toluamide (*m*DET, DEET, CAS number 134-62-3) is a clear liquid widely used in an alcohol vehicle as a topical insect (mosquito) repellent. It produces a prickling or numbing sensation on lips, tongue, and mucous membranes and can produce mild to moderate dermal irritation, erythema, desquamation, and drying. *m*DET appears not to be a sensitizing agent (Ambrose *et al.*, 1959). Following cutaneous administration of the *ortho-*, *meta-*, and *para*-DET isomers, no marked differences in toxicity were exhibited (Ambrose and Yost, 1965).

Robbins and Cherniack (1986) recently reviewed the toxicity and pharmacokinetics of *m*DET. Absorption of topically applied *m*DET into the circulation has been variously reported as 8–13% of the applied dose in the hairless dog (Reifenrath *et al.*, 1980, 1981), 59% in beagle dogs (Snodgrass *et al.*, 1982), 35–48% in rats and rabbits (Snodgrass *et al.*, 1982), and 17% in humans (Feldmann and Maibach, 1970). Once absorbed, *m*DET is excreted primarily in the urine. A number of metabolites were identified in the urine of a dermally exposed human; the *N*-ethyl glucuronide was the primary product, and unmetabolized *m*DET also was present (Wu *et al.*, 1979). The rate of excretion is generally reported to be relatively rapid, with nearly complete elimination of a single dose in 2–4 days in the mouse (Blomquist and Thorsell, 1977), the hairless dog (Reifenrath *et al.*, 1980, 1981), and the beagle dog (Snodgrass *et al.*, 1982). Contrasting observations were reported by Gleiberman and Voronkina (1972) and Glieberman *et al.* (1975, 1976), who reported that DET (unspecified isomer) was detected in the tissues of treated rats for as long as 300 days after a 6-month treatment, and that DET accumulated in neonatal rats nursed by DET-treated dams. *m*DET is reported to cross the placenta, reaching the fetus at a low ratio relative to maternal plasma levels in the mouse (Blomquist *et al.*, 1975), the rat (Gleiberman *et al.*, 1976), and the rabbit (Snodgrass *et al.*, 1982).

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TABLE 1

Effects of Subcutaneous mDET Injection on Maternal and Litter Endpoints in Time-Mated Female Rats[a] Treated on Gestational Days 6–15-Dose-Finding Study

	mDET Dose (ml/kg/day)					
	Control	0.50	0.62	0.78	0.96	1.2
Pregnant survivors (N)	12	10	4	6	5	0
Body weights (BW) (g)						
Initial BW	234 ± 16[b]	235 ± 17	232 ± 21	232 ± 20	232 ± 22	234 ± 17
BW on gestation day: 6	234 ± 16	234 ± 18	233 ± 6	228 ± 29	238 ± 12	
10	251 ± 14	244 ± 16	246 ± 19	240 ± 21	249 ± 18	
15	283 ± 16	268 ± 20	274 ± 16	263 ± 30	275 ± 20	
20	342 ± 20	329 ± 29	337 ± 21	312 ± 46	327 ± 37	
Gravid Uterus	63.5 ± 13.4	65.3 ± 15.6	62.4 ± 15.2	47.8 ± 25.6	66.5 ± 14.6	
Adjusted BW[c]	278 ± 20	264 ± 18	274 ± 20	265 ± 30	261 ± 23	
Liver weight						
Absolute (g)	13.7 ± 1.4	14.4 ± 1.6	16.0 ± 2.2	15.4 ± 2.8	15.1 ± 2.0	
Relative (g/kg ADJ BW)**	49.3 ± 4.1[d]	54.5 ± 4.9[d,e]	58.4 ± 5.2[e]	58.1 ± 7.1[e]	57.8 ± 3.5[e]	
Kidney weight						
Absolute (g)	1.8 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	
Relative (g/kg ADJ BW)*	6.6 ± 0.6[d]	7.3 ± 0.7[d,e]	7.5 ± 0.6[e]	7.6 ± 0.4[e]	7.3 ± 0.6[d,e]	
Litter data						
Total implants	12.0 ± 2.4	12.4 ± 2.9	12.2 ± 3.3	11.6 ± 1.3[f]	13.6 ± 0.9	
Live implants	11.2 ± 2.8	11.4 ± 3.1	12.0 ± 2.9	11.4 ± 1.5[f]	13.2 ± 1.3	
Mean fetal weight (g)**	3.78 ± 0.35[d]	3.73 ± 0.21[d]	3.30 ± 0.28[d,e]	3.10 ± 0.50[e,f]	3.24 ± 0.66[e]	

[a] N = 12/group.
[b] Mean ± SD.
[c] Body weight − gravid uterus = adjusted body weight.
[d,e] Groups with the same letter code do not differ from each other by a posteriori Duncan's multiple range test.
[f] N = 5; sixth pregnancy revealed by sodium sulfide stain.
* $p < 0.05$ by analysis of variance.
** $p < 0.01$ by analysis of variance.

Gleiberman *et al.* (1975, 1976) reported that DET (unspecified isomer) applied topically at doses of 100 and 1000 mg/kg/day on Days 1–19 of gestation in the rat, caused an increase in intrauterine death, which was statistically significant at 1000 mg/kg/day. No malformations were noted. When females were allowed to deliver litters after this treatment, neonatal mortality was increased at both doses (Glieberman *et al.,* 1975). In a later study (Glieberman *et al.,* 1976), the same doses applied for up to 6 months reduced the fertility of treated male and female rats. Males were more sensitive to DET than females, and treated males were reported to have sperm with reduced motility and increased numbers of abnormal forms. In addition, the offspring of treated rats of both sexes were reported to exhibit reduced neonatal vigor and impaired postnatal survival. Fetuses were not evaluated for morphologic defects.

Reduced sperm motility and abnormal morphology were not confirmed in a more recent report by Lebowitz *et al.* (1983). These investigators reported that daily topical treatment of rats with undiluted *m*DET at up to 1000 mg/kg/day for 9 weeks did not reduce sperm counts or ATP content of sperm and did not induce increased frequencies of abnormal sperm head morphology. However, no mating trials were conducted, so it is not known whether the treated rats were of normal fertility or whether their offspring might have had impaired neonatal survival, as suggested by Gleiberman *et al.* (1976).

The only published evaluation of *m*DET for teratogenicity reported a high frequency of malformations when *m*DET, in a mineral oil vehicle, was applied to the chorioallantoic membrane of chicken embryos (Kuhlmann *et al.,* 1981).

In response to a request for a Health Hazard Evaluation, NIOSH conducted a preliminary field study of National Park Service employees at the Everglades National Park (McConnel *et al.,* 1985). According to information developed in that survey, male and female park employees applied *m*DET-containing mosquito repellent to 50–70% of their body surface, with possible repellent renewal hourly. On the basis of the observed usage of *m*DET, exposure was estimated as potentially reaching a cumulative total of 100 g per week,

TABLE 2
Body Weights of Pregnant Female Rats Subcutaneously Injected with mDET on Days 6–15 of Gestation

	Replicate 1[a]		Replicate 2	
	Control	0.3 ml/kg/d	Control	0.3 ml/kg/d
All females on study (*N*)	37	37	35	36
Initial body weights	220 ± 17[b]	216 ± 26	229 ± 18	229 ± 17
Pregnant females only (*N*)	26	30	31	34
GD 6 body weight*	224 ± 15	219 ± 28	231 ± 18	230 ± 17
Body weight change				
GD 6 to GD 10	20 ± 7	23 ± 14	20 ± 5	20 ± 7
GD 10 to GD 15**	32 ± 9	24 ± 7	24 ± 8	18 ± 10
GD 15 to GD 20	56 ± 15	57 ± 11	59 ± 13	59 ± 12
GD 6 to GD 20	108 ± 22	104 ± 21	104 ± 17	96 ± 26

[a] Replicates separated by 4 weeks.
[b] Mean ± SD (g).
* Replicates differ significantly ($p < 0.05$), but treatments do not differ (2-Way ANOVA).
** Significant differences by replicates ($p < 0.01$) and treatments ($p < 0.01$) (2-Way ANOVA).

or 2 kg in a 7-month mosquito season. This would amount to maximum daily doses (assuming exposure for 5 days per week) of 286 mg/kg/day for a 70-kg man, or 400 mg/kg/day for a 50-kg woman. In view of these very high potential human exposures and the published suggestions of a possible reproductive health hazard, the present research was conducted to evaluate the reproductive and developmental toxicity of *m*DET in male and female rats. Treatments were administered by subcutaneous injection to permit accurate dose quantification.

METHODS AND MATERIALS

Chemical

N,N-Diethyl-*m*-toluamide was procured by the Midwest Research Institute (MRI) under Contract No. NO1-ES-95615 (MRI task designation BS/CV-971) with the National Institute for Environmental Health Sciences and provided to NIOSH for testing as part of the NIOSH activities under the National Toxicology Program. Analyses by MRI indicated a chemical composition of 97–98% *m*DET, 0.3% combined *o*- and *p*-DET, 1.1% *N,N*-diethylbenzamide, and 0.35% water. Specific gravity was determined to be 0.9965.

Animals

Specific pathogen free Sprague–Dawley rats (Charles River Laboratories: CD (SD)BR) were used in all phases of these studies. Time-mated females weighing 201–225 g were used for initial range-finding and subsequent definitive studies in females treated during pregnancy. Females were shipped on Gestation Day (GD) 3 and received on GD 4 (day of sperm = GD 0). Reproductive performance was evaluated in males weighing 226–250 g on receipt. Virgin females (126–150 g body wt) were used to prove the pretreatment fertility of males and for mating trials in post-treatment Weeks 1 and 2. Upon receipt, 5 to 10 rats from all shipments were arbitrarily selected for pathogen screening. In all cases, no internal or external parasites were detected and serum tested negative for *Mycoplasma* and a panel of 10 rat viruses.

Experimental Design

In all phases of the study, undiluted *m*DET or distilled water (control) was administered by subcutaneous injection. Injection sites were rotated (left hip, right hip, right shoulder, left shoulder, dorsal midline) each day to avoid using the same site on consecutive days. Animals were weighed initially and ranked in order of decreasing weight. They were then blocked into the required number of groups needed for the specific phase of the experiment. Treatment groups were formed by the random selection of an animal from each block group. The study was carried out in four phases: pregnant female dose finding, teratology and postnatal viability, male dose finding, and dominant lethal and male fertility studies.

Pregnant female dose-finding study. An initial dose-finding study was performed using 12 time-mated females per group treated on GD 6–15 with 0.50, 0.62, 0.78, 0.96, or 1.2 ml (2.6, 3.2, 4.1, 5.0, or 6.3 mmol) *m*DET/kg/day or 1.0 ml water/kg/day (control). Females were weighed and randomly assigned to treatment groups by weight. Daily pretreatment body weights were used to establish the dose for that day. On GD 20, all survivors were terminated and weighed, and uterine contents were examined. Fetuses were examined for gross external malformations, weighed, then euthanized. Maternal liver and kidney weights and gravid uterus weights were also recorded.

Maternal Exposure

Teratology and postnatal viability studies. By probit analysis of the dose-finding results, 0.30 ml (1.6 mmol) *m*DET/kg/day was the projected maternal LD10 and was selected as the dose to be used in the subsequent teratogenic and neonatal survival studies. These studies were conducted in two identical replicates, separated in time by 4 weeks. In each replicate, time-mated females (35 to 37 per group) were randomly assigned by weight to the control or treatment group. Pregnant females were treated on GD 6–15, with doses calculated on the basis of daily body weight. Following the GD 15 treatments, control and treated groups each were subdivided by weight into two subgroups.

Teratology group. For the teratology study, the rats were euthanized on GD 20 and maternal body weight and liver, kidney, and gravid uterus weights were recorded. Live and dead implantations were enumerated, and live fetuses were sexed, examined for gross malformations, weighed, euthanized, and preserved in Bouin's fluid or 70% ethanol for visceral and skeletal examination with one-half of the fetuses undergoing each examination. The uteri of apparently nonpregnant females were placed in 10% sodium sulfide to identify females in which there had been an early termination of pregnancy.

Postnatal viability group. For the postnatal study, females were housed singly in solid bottom cages with absorbent bedding and allowed to deliver litters. Body weights were recorded on GD 20. Rats were then observed twice daily (before 8:00 AM and after 5:00 PM) for evidence of parturition. When delivery was observed to be complete (Postnatal Day 0), females were weighed, live and dead pups were counted, and live pups were weighed as a litter. Duration of gestation was recorded to the nearest half day. Maternal and fetal observations were repeated on Postnatal Days (PD) 3, 9, and 14, after which females and litters were euthanized.

Dose-finding study in males. Following a 2-week quarantine, each male rat was cohabited with two arbitrarily selected virgin females for 7 consecutive days. After cohabitation, females were group housed and terminated 12–14 days after removal from males to establish pregnancy status. Immediately following the 1-week cohabitation, males were individually housed and randomly assigned by weight to control or 0.30, 0.46, 0.73, 1.15, or 1.80 ml (1.6, 2.4, 3.8, 6.0, or 9.4 mmol) *m*DET/kg/day treatment groups. Each group consisted of 20 males. Treatments were administered 5 days/week for 9 weeks using Monday body weights to calculate the daily doses each week.

TABLE 3

Day 20 Necropsy Observations in Pregnant Female Rats Subcutaneously Injected with mDET on Days 6–15 of Gestation

	Replicate 1[a]		Replicate 2	
	Control	0.3 ml/kg/d	Control	0.3 ml/kg/d
Pregnant females (N)	13	14	15	17
Body weight (BW)				
Gestational day 20 (g)	336 ± 38[b]	329 ± 24	335 ± 25	331 ± 28
Gravid uterus (g)	60.3 ± 23.3	63.8 ± 15.8	50.8 ± 13.3	57.0 ± 16.6
Adjusted BW (g)[c]*	276 ± 22	265 ± 17	284 ± 18	274 ± 17
Liver weight				
Absolute (g)	13.1 ± 2.7	13.8 ± 1.6	13.4 ± 1.1	13.9 ± 1.4
Relative (g/kg ADJ BW)**	47.3 ± 3.7	52.1 ± 4.5	47.2 ± 2.8	50.9 ± 4.0
Kidney weight				
Absolute (g)	1.9 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	2.0 ± 0.2
Relative (g/kg ADJ BW)**	6.7 ± 0.5	6.9 ± 0.5	6.5 ± 0.6	7.2 ± 0.6
Litter data				
Total implants	10.8 ± 4.5	11.9 ± 1.7	11.3 ± 2.4	12.1 ± 2.6
Live implants	10.5 ± 4.3	10.9 ± 2.9	10.2 ± 2.1	11.2 ± 2.7
Fetal weight (g)				
Males***	3.97 ± 0.35	4.05 ± 0.20	3.11 ± 0.84	3.29 ± 0.86
Females***	3.79 ± 0.38	3.92 ± 0.33	2.93 ± 0.79	3.08 ± 0.82
Combined sexes***	3.89 ± 0.37	3.98 ± 0.21	3.04 ± 0.82	3.20 ± 0.84
Gross malformations			1 (Omphalocele)	1 (Craniorachischisis)

[a] Replicates separated by 4 weeks.
[b] Mean ± SD.
[c] Body weight Gestational Day 20 − gravid uterus = adjusted body weight.
* Significant treatment effect ($p < 0.05$) without significant replicate difference (2-Way ANOVA).
** Significant treatment effect ($p < 0.01$) without significant replicate difference (2-Way ANOVA).
*** Significant replicate difference ($p < 0.01$) without significant treatment effect (2-Way ANOVA).

Paternal Exposure

Dominant lethal and male fertility studies. Immediately following the final treatment of Week 9, 11 males were randomly selected from the 0.30 and 0.73 ml/kg/day treatment groups for breeding studies. Only 8 controls were available because 12 were withdrawn from the study in Week 4, having been inadvertently dosed once with 1.0 ml *m*DET/kg. Each male selected for breeding was cohabited with 4 virgin females for 7 consecutive days, after which a second set of 4 virgin females were introduced for 7 days. At the end of the cohabitation period, two females from each male were arbitrarily assigned to each of two subgroups: dominant lethal, and male fertility group.

Dominant lethal group. For the dominant lethal study, the female rats were euthanized 12–14 days after removal from the males. Uterine contents were evaluated by enumerating live and dead implants and the number of corpora lutea. Uteri from apparently nonpregnant females were placed in 10% sodium sulfide to identify females in which there had been an early termination of pregnancy.

Male fertility group. For the postnatal study, females were housed singly in solid bottom cages with absorbent bedding. They were observed twice daily (before 8:00 AM and after 5:00 PM) beginning 20 days after the first day of cohabitation. When delivery was observed to be complete (PD 0), females were weighed, live and dead pups were counted, and live pups were weighed as a litter. Maternal and litter observations were repeated on PD 3, after which females and litters were terminated.

Testicular histopathology. Three of the rats misdosed with 1.0 ml *m*DET/kg, control rats, plus seven other males not selected for breeding (three from 0.30, two from 0.73, and two from 1.15 ml/kg/day groups) were used for unscheduled histopathologic evaluation of testes following the final treatment (Friday of Week 9). Following carbon dioxide asphyxiation, rats were systemically perfused with Ringer's saline containing 0.1% procaine followed by systemic perfusion with Karnovsky's fixative (5% glutaraldehyde, 4% paraformaldehyde, 0.2 M cacodylate). Testes and epididymides were subsequently imbedded in glycol methacrylate and stained with PAS-hematoxylin.

Tests of neuromuscular coordination. Neuromuscular evaluations were initiated due to observations suggestive of neurotoxicity, such as curled feet and abnormal gait. Forty male rats, 10 each from the control, 0.30, 0.73, and 1.80 ml *m*DET/kg/day groups, were arbitrarily selected for rotorod testing. The rotorod consisted of a 9-cm (diameter) × 10-cm (length) drum, initially rotating at 6 rpm. For each 15 sec the animal successfully remained on the drum, the speed was increased by 3 rpm. When the animal fell from the drum, the highest speed maintained for 15 sec was recorded. After 30 sec, the animal was replaced on the drum, turning at the same rotational speed as when it fell. Retesting was continued in this manner for a total of five trials per rat. The order of testing was randomized for each test session, conducted once weekly. Baseline performance was recorded the week before treatments began, and rats were reevaluated in Weeks 1 through 4 of treatment. Selected rats from the control, low, and intermediate *m*DET groups were reevaluated in Week 16.

TABLE 4

Visceral Observations in Fetuses from Litters of Pregnant Female Rats Subcutaneously Injected with 0.3 ml/kg/day mDET on Days 6–15 of Gestation

	Replicate 1[a]		Replicate 2		Combined Replicates	
	Control	Treated	Control	Treated	Control	Treated
Litters (fetuses) examined	12 (67)	14 (76)	15 (77)	17 (94)	27 (144)	31 (171)
Malformations						
Hydrocephaly	0					
Hydronephrosis	0		0	1 (1)	0	1 (1)
Hydroureter	0	1 (1)	0	1 (1)	0	2 (2)
Total urinary	0	0	0	1 (1)	0	1 (1)
Unilateral anophthalmia/	0	1 (1)	0	1 (1)	0	2 (2)
microphthalmia	0	0	0	1 (1)	0	1 (1)
Total malformations	0	1 (1)	0	1 (1)	0	2 (2)
Variations						
Dilated renal pelvis	2 (2)	1 (1)	0	0	2 (2)	1 (2)
Dilated ureter(s)	0	2 (4)	2 (2)	0	2 (2)	2 (4)
Total urinary	2 (2)	2 (4)	2 (2)	0	4 (4)	2 (4)
Abdominal hemorrhage	1 (1)	0	0	1 (1)	1 (1)	1 (1)
Total variations	3 (3)	2 (4)	2 (2)	1 (1)	5 (5)	3 (5)
Ectopic testes[b]	0	0	0	2 (2)	0	2 (2)

[a] Replicates separated by 4 weeks.

[b] Based on a total of 73 control and 90 exposed male fetuses examined.

Statistical analysis. Body weights and organ weights (absolute and relative) were evaluated by analysis of variance (ANOVA). Single-classification or double-classification (dose × replicate) models were used as appropriate. When analysis of variance indicated significant differences, Duncan's multiple range test was applied to clarify group differences. Nonparametric ranking tests (Mann–Whitney and Kruskal–Wallis), with corrections for ties, were applied to counts (e.g., numbers of implants, corpora lutea, fetuses) and proportions (e.g., percentage survival, percentage of implants alive). Probit analysis of mortality data and all other statistical procedures were conducted using the Statistical Analysis System (SAS) (SAS Institute, Cary, NC).

RESULTS

Dose-finding in pregnant females. Data are summarized in Table 1. No females survived 10 days of *m*DET dosing with 1.2 ml/kg/day. Deaths occurred in all other groups except the low dose (0.50 ml/kg/day). Probit analysis of maternal mortality data indicated an LD50 of 0.71 and an LD10 of 0.30 ml/kg/day. Despite the mortality observed, the body weights of pregnant survivors were not significantly affected at any dose. Maternal liver and kidney weights were elevated in *m*DET-treated groups, but only relative organ weights differed significantly from control values. The only fetal characteristic affected by *m*DET treatment was fetal weight, which was significantly reduced at 0.78 and 0.96 ml/kg/day. Fetal body weights at 0.62 ml/kg/day were intermediate, not differing significantly by post hoc analysis from either the two higher dose levels (0.78 and 0.96 mg/kg/day) or the control and 0.50 ml/kg/day groups. No gross external malformations were noted. On the basis of the projected maternal LD10, 0.30 ml/kg/day was selected for the teratology and neonatal survival studies.

Toxic Signs in mDET-Treated Females

Body weight data for replicates 1 and 2 are presented in Table 2. No mortality occurred in either replicate. Rats in replicate 2 consistently weighed slightly more than those in replicate 1, but replicates differed significantly only on three occasions (GD 10, 12, and 13). There was no significant dose × replicate interaction. *m*DET toxicity was reflected in a significant dose-related reduction of maternal body weight on GD 14 and 15, but this difference was resolved by GD 20 (Table 2).

Beginning on GD 9 in replicate 1, a gait disturbance of the hind limbs was incidentally noted in pregnant *m*DET-treated rats. This was seen first as a rotation of the foot with the fifth digit lacking strength and being turned under. As treatment continued the condition worsened, with the development of a high-stepping gait in the hindlimbs and progression from fifth to first digits of the apparent disturbance. These observations were confirmed in the second replicate, when the condition was first noted in two rats on GD 7. Expression of the condition was variable, being obvious in some rats and mild or intermittently observed in others.

TABLE 5
Skeletal Observations in Fetuses from Litters of Pregnant Female Rats Subcutaneously Injected with mDET on Days 6–15 of Gestation

	Replicate 1[a]		Replicate 2		Combined Replicates	
	Control	Treated	Control	Treated	Control	Treated
Litters (fetuses) examined	13 (70)	14 (76)	15 (76)	17 (95)	28 (146)	31 (171)
Malformations						
Cervical Rib	1 (1)	0	0	0	1 (1)	0
Variations						
Reduced ossification:						
Skull	2 (3)	1 (1)	1 (1)	4 (8)	3 (4)	5 (9)
Sternebrae	1 (1)	0	8 (21)	7 (27)	9 (22)	7 (27)
Vertebrae	1 (1)	0	2 (5)	3 (5)	3 (6)	3 (5)
Appendicular skeleton	1 (2)	0	5 (11)	7 (15)	6 (13)	7 (15)
Total	2 (3)	1 (1)	9 (25)	8 (33)	11 (28)	9 (34)
Misaligned:						
Sternebrae	4 (6)	4 (4)	7 (9)	6 (9)	11 (15)	10 (13)
Vertebrae	2 (3)	1 (1)	2 (3)	1 (1)	4 (6)	(2)
Total	6 (9)	5 (5)	9 (12)	6 (9)	15 (21)	11 (14)
Extra thoraco-lumbar rib(s)	1 (1)	2 (2)	2 (2)	4 (8)	3 (3)	6 (10)
Total variations	7 (10)	7 (7)	12 (33)	14 (48)	19 (43)	21 (55)

[a] Replicates separated by 4 weeks.

Maternal Exposure

Teratology study. Observations from necropsy of pregnant females in replicates 1 and 2 are presented in Table 3. There were no significant differences by replicate and no dose-replicate interactions. *m*DET toxicity was suggested by a significant treatment-related reduction in adjusted maternal body weight and increased relative liver and kidney weights. Two grossly malformed fetuses were observed, both in replicate 2: one control fetus with omphalocele and one *m*DET-treated fetus with craniorachischisis and open eye. In contrast to the dose-finding study, *m*DET-treated fetuses tended to be heavier than control fetuses, but not significantly so. The numbers of implants and live fetuses per litter were not affected by *m*DET treatment. Results of visceral and skeletal examinations of fetuses are summarized in Tables 4 and 5. Sporadic malformations and variations were observed, but there were no significant treatment-related differences.

Postnatal growth and survival. Results are summarized in Table 6, in which it is evident that there were no adverse effects on reproductive success. Through Postnatal Day 9, treated females had lower body weights than controls, but the difference was not statistically significant at any time. This suggests a gradual recovery from the treatment-related reduction in late-gestational body weight previously noted (Table 2).

Dose-finding study in males. Mortality was 100% at 1.80 ml/kg/day, 18 of 20 died at the next lower dose (1.15 ml/kg/day), 1 of 20 died in the 0.73 ml/kg/day group, and 4 of 19 died at 0.46 ml/kg/day (one infertile male was removed from study). There were no deaths in the remaining two groups (control and 0.30 ml/kg/day). In the 0.46 and 0.73 ml/kg/day groups, all five deaths occurred immediately following treatment without preliminary signs of morbidity. Necropsy revealed markedly hemorrhagic lungs in these five rats.

Toxic Signs in mDET-Treated Males.

Analysis of variance indicated significant differences in body weights in Week 2 and all subsequent weeks (Table 7, Fig. 1). In Week 2, groups treated with 0.30, 0.73, and 1.80 ml/kg/day weighed significantly less than controls. In Weeks 3–5, the 0.73, 1.15, and 1.80 ml/kg/day groups differed from controls, and in Weeks 7 through 9, all treated groups were significantly lighter than controls, but did not differ from each other.

Most males developed skin lesions at one or several injection sites. These appeared to be attributable to scratching, which was often observed after dosing. A gait disturbance involving the hindlimbs, and rarely of forelimbs, was observed which developed in the same way and was indistinguishable from that described for females. In some males,

TABLE 6

Postnatal Observations in Female Rats and Their Offspring following Subcutaneous Injection of mDET on Days 6–15 of Gestation

	Replicate 1[a]		Replicate 2	
	Control	0.3 ml/kg/d	Control	0.3 ml/kg/d
Viable litters[b]/pregnant	13/13	16/16	16/16	17/17
Duration of gestation (days)	21.2 ± 0.3[c]	21.4 ± 0.5	21.6 ± 0.5	21.6 ± 0.7
Maternal body weight (g)				
Gestation Day 20	327 ± 28	318 ± 30	335 ± 30	323 ± 25
Postnatal Day 0	265 ± 22	255 ± 23	269 ± 19	264 ± 19
Postnatal Day 3	266 ± 25	259 ± 26	267 ± 19	264 ± 16
Postnatal Day 9	286 ± 19	276 ± 21	290 ± 17	286 ± 18
Postnatal Day 14	285 ± 15	290 ± 31	289 ± 15	283 ± 16
Live pups per litter				
Postnatal Day 0	10.9 ± 1.8	10.6 ± 2.8	11.6 ± 2.4	11.6 ± 2.3
Postnatal Day 3	10.8 ± 1.9	10.6 ± 2.8	11.4 ± 2.4	11.6 ± 2.3
Postnatal Day 9	10.8 ± 1.9	10.6 ± 2.8	11.4 ± 2.4	11.6 ± 2.2
Postnatal Day 14	10.8 ± 1.9	10.6 ± 2.8	11.4 ± 2.4	11.5 ± 2.2
Average pup birth weight (g)	6.0 ± 0.5	6.0 ± 0.6	6.0 ± 0.4	6.0 ± 0.4
Average weight gain (g) per pup, Day 0 to				
Day 3	2.5 ± 0.4	2.6 ± 0.6	2.8 ± 0.7	2.6 ± 0.6
Day 9	12.1 ± 1.1	12.1 ± 2.3	12.1 ± 2.0	11.6 ± 2.1
Day 14	21.7 ± 1.9	21.6 ± 3.4	23.1 ± 3.1	22.4 ± 2.7

[a] Replicates separated by 4 weeks.
[b] A litter with at least one liveborn pup.
[c] Mean ± SD.

this disturbance was accompanied by cannibalization of the toes on affected feet.

The baseline rotorod performance for each group was similar (Fig. 2), with the exception of group 2 (0.30 ml/kg/day) which showed an elevated proficiency level, probably attributable to random error. Subsequently, a sharp dose-related decline in rotorod performance was apparent in the *m*DET-treated groups. By Week 2 (after 7 days of dosing), an apparent dose–effect relationship emerged, but the data for Week 3 suggest some recovery in all the dosed groups. In Week 4 only treated animals were tested because some of the control animals were accidently given a high dose of *m*DET on Monday of that week. At that time, only one high dose animal remained alive, and no further rotorod tests were conducted during *m*DET exposure. In Week 16 (the seventh post-treatment week), a follow-up rotorod test was conducted using four control animals and six each from the low and intermediate (0.30 and 0.73 ml/kg/day) dose groups (Fig. 2). Few of the rats tested in this final session had been previously tested, so data cannot be directly related to the baseline values. Furthermore, in the exposed animals there was selection by virtue of survival, and the animals had increased considerably in size and weight, making the rotorod task more difficult. Nevertheless, there does not appear to be residual impairment of rotorod performance in this post-treatment evaluation.

During Week 6 of dosing, it was noted that one treated male had a palpable abdominal mass. When that male died later that week, necropsy revealed a grossly distended, urine-filled bladder. No stones were found, but a small, cream-colored amorphous deposit was found at the neck of the bladder. As subsequent males died, necropsies were performed and abnormally large, urine-filled bladders were a common finding. These bladders had translucent walls and the urine was generally clear, but in several instances was deep green in color. In two cases, a tulip-shaped blockage was found in the bladder with the "stem" extending into the neck of the urethra. What appeared to be copulatory plugs were often observed in the excreta trays, and on several occasions during dosing a coagulum was found on the penis. X-ray dispersive analysis of these plugs showed low levels of zinc. Blood samples were collected from four moribund males and clinical chemistry showed 0.8 to 1.1 mg/dl creatinine (0.5–1.7 mg/dl normal range), less than 1.0 to 5.2 mg/dl uric acid (2.5–8.5 normal), and 42 to 90 mg/dl BUN (6.0–25.0 normal).

Paternal Exposure

Dominant lethal and male fertility study. Table 8 summarizes the overall fertility of males combining the data from dominant lethal and postnatal studies. All males impregnated at least one of four females in first post-treatment week. Over half of the males in both treatment groups impregnated ⩾seven of the eight cohabited females. Microscopic examination of testes did not reveal signs of altered spermatogenesis, even in the testes of the two surviving rats treated with 1.15 ml/kg/day.

Dominant lethal study. Results of matings in the first two post-treatment weeks are summarized in Table 9. There was no evidence of induced dominant lethal mutations or other adverse effects on intrauterine survival of fetuses sired by *m*DET-treated males.

Male fertility study. Indices on numbers of live pups and their growth and survival are summarized in Table 10. In these rats, there clearly was no adverse effect of paternal *m*DET exposure on intrauterine survival or neonatal growth and survival.

DISCUSSION

In the studies reported here, there was no evidence of teratogenicity, embryotoxicity, or impaired perinatal survival when pregnant rats were injected subcutaneously with 0.30 ml (1.6 mmol) *m*DET/kg/day on GD 6–15. Male rats injected subcutaneously with 0.30 or 0.73 ml (1.6 or 3.8 mmol) *m*DET/kg/day, 5 days per week for 9 weeks showed no evidence of induced dominant lethal mutations, reduced fertility, or impaired perinatal survival of their offspring. If percutaneous absorption of *m*DET is assumed to be 25% or less

TABLE 7
Weekly Body Weights in Male Rats Injected Subcutaneously with mDET, 5 Days/Week, for 9 Weeks

	mDET dose in ml/kg/day					
	Control	0.30	0.46	0.73	1.15	1.80
Week 1	391 ± 24[1]	379 ± 34	391 ± 20	385 ± 22	391 ± 22	383 ± 18
(*N*)	20	20	20	20	20	20
Week 2*[2]	421 ± 25[a]	401 ± 28[b]	411 ± 20[a]	401 ± 25[b]	402 ± 24[a,b]	393 ± 19[b]
(*N*)	20	20	20	20	18	12
Week 3***	441 ± 28[a]	422 ± 32[a,b,c]	416 ± 23[a,b]	411 ± 27[b,c,d]	400 ± 25[c,d]	395 ± 15[d]
(*N*)	20	20	20	20	10	4
Week 4***	464 ± 28[a]	439 ± 33[a,b]	443 ± 21[a,b]	426 ± 33[b]	417 ± 28[b,c]	398 ± 16[c]
(*N*)	20	20	18	20	10	3
Week 5***	490 ± 17[a]	455 ± 35[a,b]	457 ± 26[a,b]	438 ± 34[b]	421 ± 31[b,c]	382[c]
(*N*)	8[3]	20	18	20	8	1
Week 6***	511 ± 17[a]	473 ± 38[b]	472 ± 30[b]	455 ± 35[b]	427 ± 33[c]	
(*N*)	8	20	18	20	7	0
Week 7***	530 ± 18[a]	480 ± 39[b]	481 ± 33[b]	459 ± 34[b]	461 ± 36[b]	
(*N*)	8	20	17	20	3	0
Week 8***	546 ± 18[a]	492 ± 45[b]	489 ± 36[b]	472 ± 35[b]	471 ± 41[b]	
(*N*)	8	20	16	20	2	0
Week 9**	561 ± 13[a]	495 ± 58[b]	499 ± 41[b]	486 ± 37[b]	482 ± 33[b]	
(*N*)	8	20	16	19	2	0

[1] Mean ± SD (g).
[2] Statistically significant differences between groups: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[3] 12 controls were misdosed in week 4 and were removed from the study.
[a,b,c] Within a week, groups that share an alphabetic superscript do not differ from each other.

(Reifenrath *et al.*, 1980, 1981), then doses employed here are equivalent to or greater than the effective systemic doses reported by Gleiberman *et al.* (1975, 1976) as adversely affecting male and female reproductive function in the rat.

Glieberman and Voronkina (1972) studied the absorption of *m*DET and suggested that *m*DET toxicity is expressed first through the deterioration of the nervous system, with progressive accumulation in the organs and tissues. Neuro-

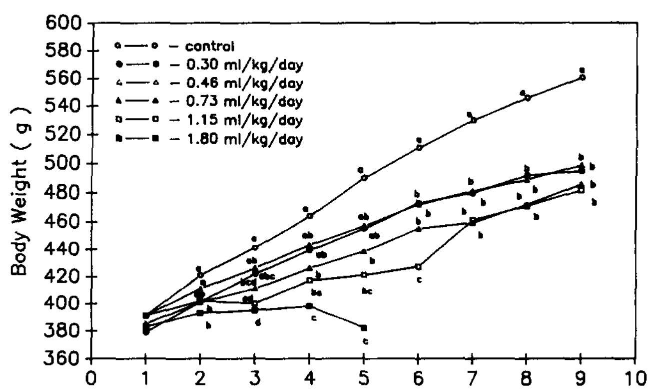

FIG. 1. Male body weights throughout 9-week treatment period. Analysis of variance indicated significant group differences ($p < 0.05$) in all weeks after the first. Groups sharing the same letter superscript do not differ from one another by post hoc Duncan's multiple range test ($p < 0.05$).

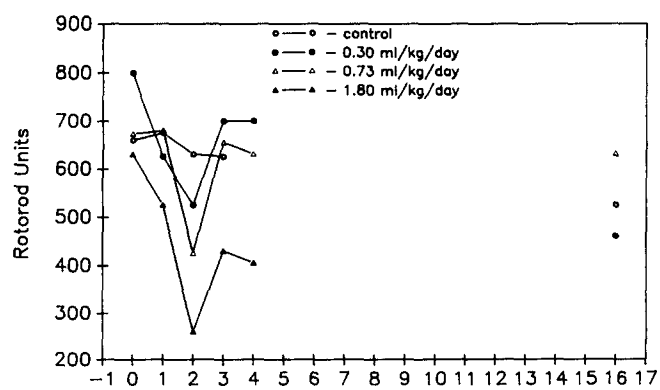

FIG. 2. Rotorod performance by adult male rats expressed in arbitrary units (rate of rotation/minute by 33). Regular testing was suspended after Week 4, but a single follow-up assessment was made in Week 16, treatments having ended after Week 9.

TABLE 8

Number of Male Rats Treated with mDET by Subcutaneous Injection for 5 Days/Week, for 9 Weeks, that Impregnated Cohabited Untreated Virgin Females[a] during Post-Treatment Weeks 1 and 2

Treatment Group	No. of Males	No. impregnated females/male 0	1	2	3	4	5	6	7	8
Control	8	0	0	0	0	3[b]	2	0	1	2
0.30 ml/kg	11	0	0	1[c]	0	2	1	0	5	2
0.73 ml/kg	11	0	0	0	0	2	2	1	5	1

[a] Four females/male/week for 2 weeks.
[b] Three of four females escaped from the cage of one of these males in Week 1, and those three are excluded from the totals.
[c] This male died during Breeding Week 2.

TABLE 9

Dominant Lethal Effects Observed in Untreated Virgin Females Cohabited with Male Rats for 2 Weeks following Subcutaneous Injections with mDET, 5 Days/Week, for 9 Weeks

	Control	0.30 ml/kg/Day	0.73 ml/kg/Day
Number of males (*N*)	8	11	11
Number of females pregnant/mated (%)	21/30 (70.0)	33/42 (78.6)	32/44 (72.7)
Corpora lutea	14.2 ± 2.0[a]	13.8 ± 1.8	13.8 ± 1.9
Percentage implanted	92.0 ± 13.2	92.3 ± 18.5	93.1 ± 18.4
Implantations			
Total	13.1 ± 2.9	12.7 ± 2.9	13.0 ± 3.0
Live	12.1 ± 3.3	12.1 ± 2.8	12.5 ± 3.1
Dead	1.0 ± 1.7	0.6 ± 1.0	0.5 ± 0.7
Percentage alive	92.0 ± 13.2	95.9 ± 6.6	93.4 ± 17.7

[a] Mean ± SD.

toxic effects incidentally observed here, and confirmed in rotorod tests, have not been reported in previous animal studies of *m*DET. However, there are reports in the literature of apparent neurotoxic effects in humans, specifically children, exposed to large quantities of *m*DET-containing insect repellents.

Gryboski *et al.* (1961) reported the case of a 3.5-year-old girl whose skin, bedding, and nightclothes had been sprayed with mosquito repellent. She was hospitalized with signs of encephalopathy, including tremors, slurred speech, and disorientation, but recovered with supportive therapy. deGarbino and Laborde (1983) reviewed that case and another three published cases (one of which ended in death) and reported a fifth case (also fatal) of toxic encephalopathy associated with heavy exposure to *m*DET-containing insect repellent. All five of these cases involved girls ranging in age from 17 months to 6 years.

An unexpected observation in this study was distended bladders in some *m*DET-treated male rats. Urethral blockage was initially suspected, and the mildly elevated BUN results support this diagnosis. However, upon further review of the literature, urethral deposits like those described here have been variously called "soft calculi" (Vulpe' *et al.,* 1956), "rubbery plugs" (Bruss *et al.,* 1970), and "urethral plugs" (Kunstyr *et al.,* 1982). These plugs are reported to be common in normal male rats (even being called a secondary sex characteristic) and apparently do not ordinarily interfere with urination. They have been shown to be derived from the coagulating gland and seminal vesicle secretions (explaining their low zinc content) and become the seminal plug normally seen after copulation. In this case, however, the failing health of these animals could have enhanced the effect of the innocuous seminal plug. The persistence of the plugs in exposed rats may have lead to postrenal obstruction of urine flow resulting in an elevated BUN (Tietz and Finley, 1983). Alternatively, the elevated BUN could be associated with renal toxicity, but serum creatinine levels were within normal ranges in the *m*DET-exposed animals. Kidneys of the adult animals exposed to *m*DET were not examined histopathologically. Interestingly, in the NIOSH field study of the Everglades workers having high occupational exposure to *m*DET insect repellent, workers reported bladder control problems, such as difficulty in starting and stopping the urinary stream (McConnel *et al.,* 1985).

Urethral plugs presumably were seen here only in treated rats because they were an incidental observation not system-

TABLE 10

Postnatal Observations in Litters of Untreated Virgin Females Cohabited for 2 Weeks with Male Rats Injected Subcutaneously following Subcutaneous Injections with mDET, 5 Days/Week, for 9 Weeks

	Control	0.30 ml/kg/Day	0.73 ml/kg/Day
Number of males	8	11	11
Number of females pregnant/mated (%)	24/31 (77.4)	33/42 (78.6)	35/44 (79.5)
Viable litters[a]	24	33	35
Live pups/litter			
Day 0	12.8 ± 1.8[b]	12.7 ± 2.2	13.1 ± 2.4
Day 3	12.6 ± 1.8	12.6 ± 2.1	13.0 ± 2.4
Percentage neonatal survival Days 0–3	99.0 ± 2.8	99.1 ± 2.5	99.4 ± 2.1
Average pup weight (g)			
Day 0	6.0 ± 0.4	5.9 ± 0.5	6.0 ± 0.5
Day 3	8.4 ± 0.8	8.4 ± 0.9	8.4 ± 0.8
Average pup weight gain (g) Days 0–3	2.4 ± 0.6	2.4 ± 0.5	2.4 ± 0.5
Maternal body weight (g)			
Postnatal Day 0	255 ± 16	265 ± 15	262 ± 15
Postnatal Day 3	259 ± 16	267 ± 14	265 ± 15

[a] A litter with at least one live born pup.
[b] Mean ± SD.

ically sought in all rats. Male rats normally ejaculate spontaneously but seminal plugs are not ordinarily seen because of oral grooming of the penis. Studies (Orbach, 1961; Beach, 1975) have shown that when otherwise untreated rats were restrained with a stiff plastic girdle around the thorax or by adhesive tape muzzles, 1–3 seminal plugs per day were detected. In the present study, plugs were frequently seen beneath the cages or on the penis of unrestrained rats suggesting the possibility of a treatment-induced alteration in grooming behavior.

The studies reported here do not confirm reports of potential reproductive or developmental toxicity associated with *m*DET exposure in rats. However, a number of incidental observations are suggestive of neurotoxicity under the conditions of these tests. Isolated human case reports and anecdotal comments from heavily exposed workers suggest that *m*DET may be neurotoxic in humans under extreme conditions of use. Additional evaluation of *m*DET for neurotoxicity is therefore warranted.

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