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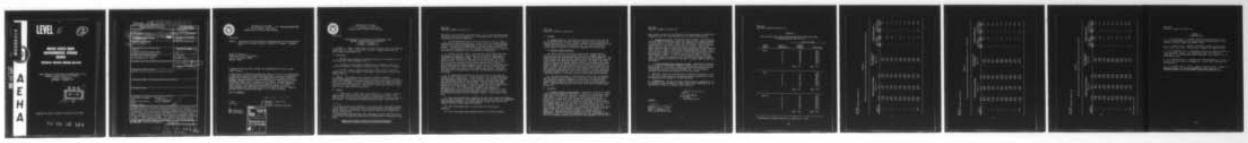


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## UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

### ABERDEEN PROVING GROUND, MD 21010

INVESTIGATION OF N,N-DIETHYL-M-TOLUAMIDE(M-DET) FOR
DOMINANT LETHAL EFFECTS IN THE MOUSE
STUDY NO. 51-0034-78
SEPTEMBER - NOVEMBER 1977



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| N,N-diethyl-m-toluamide | mutagenicity |
| m-Det | in vitro mutagenicity |
| dominant lethal effects | triethylenemelamine |
| mouse | |
| systemic toxicity | |

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
A single dose of N,N-diethyl-m-toluamide (m-Det) in corn oil was administered to male mice by gavage in a dominant lethal assay to evaluate its potential mutagenicity in male germ cells. The compound did not induce a positive mutagenic response during an 8-week mating period in which each of 10 males was cohoused weekly with 3 untreated virgin females, although there was a slight, but statistically insignificant reduction in the total number of fetal implants. This reduction was probably not genetic in origin. Triethylenemelamine (TEM), which served as the positive control, induced a dominant lethal response during weeks 1, 2, 3 and 6 of mating.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Swentzel/bbb/584-3980

HSE-LT/WP

SUBJECT: Investigation of N,N-Diethyl-m-Toluamide(m-Det) for Dominant Lethal Effects in the Mouse, Study No. 51-0034-78, September - November 1977

Executive Secretary
Armed Forces Pest Control Board
Forest Glen Section, WRAMC
WASH DC 20012

A summary of the pertinent findings and recommendations of the inclosed report follows:

A single dose of N,N-diethyl-m-toluamide (m-Det) in corn oil was administered to male mice by gavage in a dominant lethal assay to evaluate its potential mutagenicity in male germ cells. The compound did not induce a positive mutagenic response during an 8-week mating period in which each of 10 males was cohoused weekly with 3 untreated virgin females, although there was a slight, but statistically insignificant reduction in the total number of fetal implants. This reduction was probably not genetic in origin. Triethylenemelamine (TEM), which served as the positive control, induced a dominant lethal response during weeks 1, 2, 3, and 6 of mating.

FOR THE COMMANDER:

for BRENDAN E. JOYCE, Ph.D.
LTC, MSC
Director, Laboratory Services

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HQDA (DASG-PSP)
Cdr, HSC (HSPA-P)

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

# INVESTIGATION OF N,N-DIETHYL-M-TOLUAMIDE(M-DET) FOR DOMINANT LETHAL EFFECTS IN THE MOUSE*†

STUDY NO. 51-0034-78
SEPTEMBER - NOVEMBER 1977

1. AUTHORITY. Letter, Armed Forces Pest Control Board, Forest Glen Section, WRAMC, WASH, DC 20012, 17 March 1977, subject: Reregistration Data for N,N-Diethyltoluamide Repellent.

2. REFERENCES.

a. Mutagenicity Evaluation of Diethyltoluamide, Litton Biometics, Inc., LBI Project No. 20838 (September 1977).

b. Criteria for Evaluating the Mutagenicity of Chemicals, Office of Pesticide Programs, Environmental Protection Agency, 12 July 1977 (draft).

c. Title 40, Code of Federal Regulations (CFR), 1977 ed., Part 162, Regulations for the Enforcement of the Federal Insecticide, Fungicide, and Rodenticide Act.

3. PURPOSE. The purpose of this test was to determine whether N,N-diethyltoluamide (m-Det) can produce a mutagenic reaction in male germ cells. This compound is presently being evaluated by this Agency to satisify the safety requirements of the US Environmental Protection Agency for human usage of this insect repellent. The dominant lethal assay with mice is one part of this safety evaluation program.

4. GENERAL.

a. M-Det, which a widely used insect repellent, is a clear to slightly amber colored liquid with a boiling point of approximately 290°C. The material used in this study was supplied by the McLaughlin, Gormley, King Co. (Lot #7141). The formulation contained 95 percent meta- isomer with the

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* In conducting the study described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education, and Welfare, Publication No. (NIH) 74-23, Revised 1972, second printing 1974.

† The experiments reported herein were performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

remainder a mixture of ortho-and para-isomers. Corn oil was selected as the solvent for test solutions because of its widespread usage, lack of toxicity and nonreaction with test chemicals.

b. Triethylenemelamine [2,4,6-tris-(1-aziridinyl)-s-triazine] (TEM), a compound which exhibits pharmacological actions similar to nitrogen mustard,[1] was used to evaluate the responsiveness of the test system to an established mutagen. The compound (lot number 9558, supplied by Lederle Laboratories Division, American Cyanamid Co.) was prepared for administration in distilled water which was adjusted to pH 7.1 to prevent acid hydrolysis.

c. The test, positive control, and concurrent control groups were each comprised of 10 male mice. Each of these mice received a single dose of the appropriate compound by gavage. The test animals were administered a dosage of m-Det (600 mg/kg of body weight) which approximates 1/5 of the acute oral $LD_{50}$. The positive controls were administered 10 mg/kg of TEM. Both the test and concurrent control males received 5 ml/kg of corn oil. Each treated male was cohoused sequentially with groups of 3 untreated virgin females 5 days/week for eight successive weeks. All tests were performed with ICR/Ha Swiss mice (Charles River Breeding Laboratories). Males were 8-10 weeks old at the beginning of the test and females were 8-10 weeks old when mated.

d. All females were killed by cervical dislocation 13 days after the midweek of their cohabitation. The mean pregnancy rate was determined weekly for each group of mated females. Total implants, living implants, and early fetal deaths were counted for each pregnant female and weekly means were computed from each group for each parameter. Pregnant females with fewer than 8 total implants and/or 1 or more early fetal deaths were expressed as a percentage of the total number of females pregnant that week in that particular group. All of these weekly parameters were compared to corresponding data from the concurrent control group by Student's "t" test. Any weekly mean value not within established control limits for this strain of mice[2] was considered a positive mutagenic response. These control limits were:

(1) One or more weekly means exceeding 1.00 early fetal deaths per pregnancy, with at least 55 percent of the pregnant females having early deaths; by considering these parameters together, any instance where an elevated mean was due to an atypical individual female would be eliminated.

(2) One or more weekly means of less than 8 total implants per pregnancy.

(3) One or more weekly mean pregnancy rates of less than 30 percent.

5. FINDINGS.

a. Systemic Toxicity. The dosages of compounds used in this test did not produce mortality. One male that was treated with m-Det convulsed and became prostrate within 1 hour of dosing, however, he regained mobility, and a normal appearance within an additional hour. All males in each group gained weight and appeared to be healthy throughout the mating period.

b. Dominant Lethal Evaluation.

(1) None of the males in the test were sterile, although reduced fertility, indicated by a significantly lower total pregnancy rate among cohoused females was observed among males treated with TEM (Appendix A). The total pregnancy rate among females cohoused with males treated with m-Det was slightly lower than among the concurrent controls. The means of the total number of implants per pregnant female during the entire mating period were equivalent between the TEM (Appendix B) and concurrent control (Appendix C) groups. However, the 8-week mean for implants per pregnant female from the m-Det group was lower than the other groups (Appendix D) and fell below the mean previously determined with this strain of mice.[2] The total percentage of pregnant females with less than 8 total implants each was significantly higher among females in the m-Det group than among corresponding controls.

(2) The data from females in the TEM group indicate that the pregnancy rate was below 30 percent (Appendix A) during week 1 of mating and early fetal deaths per pregnancy exceeded 1.0 with at least 55 percent of the pregnant females having 1 or more early fetal deaths each during weeks 2, 3, and 6. These were the only data in this test indicative of dominant lethal mutagenicity.

6. DISCUSSION.

a. Effects of M-Det on Implantations. Eventhough m-Det did not induce a dominant lethal response in this test, it caused a slight decrease in the total number of implants per pregnancy. Examination of approximately 7500 pregnant mice of this strain over a 2 year period indicated that the distribution of weekly mean total implants per pregnancy was symmetrical around a peak of 11.5 - 11.9.[2] Although the 8-week mean (11.4) for implants per pregnant female from the m-Det group was only slightly below this range, 4 of 8 weekly means were less than 10.7. This effect, which was also indicated by a significantly higher rate of pregnant females with less than 8 total implants each, was probably not genetic in origin. Reduction in the number of implants may be caused by aspermia or reduced motility of spermatozoa. Reduced motility and changes in functional morphology of spermatozoa, as well as inhibited spermatogenesis, have been observed among male rats treated by repeated applications of m-Det dermally.[3] One or all of

these anomalies might have contributed to the reduced number of implants per pregnancy as well as to the decreased fertility observed among males.

b. Mutagenicity of TEM. The dominant lethal response, indicated by an increased number of early fetal deaths per pregnancy and a reduced pregnancy rate among females in the TEM groups, was unequivocal and demonstrated the responsiveness of the animals in this test to a mutagen. Previous dominant lethal mutation tests with TEM administered to mice by a single gavage demonstrated that week 2 of mating was the most sensitive period for dominant lethal effects.[4] However, these effects were demonstrated in weeks 1 and 3 after increased dosage. This expansion is within the post meiotic period of spermatogenesis, however, pre-meiotic as well as post meiotic stages of spermatogenesis (weeks 1, 2, 3 and 6) were affected by TEM in the present study. This might be attributed to the relatively high dosage (10 mg/kg) used since differential sensitivity can occur in various stages of germ cell development.[5]

c. In Vitro Mutagenicity Evaluation of M-Det. N,N-Diethyl-m-toluamide was evaluated for its mutagenic potential in the histidine reverse mutation system of Ames in five strains of Salmonella typhimurium and one strain of Saccharomyces cerevisiae (reference paragraph 2a). The investigators concluded that m-Det was not mutagenic in this test system.

7. CONCLUSION. M-Det has been evaluated for potential mutagenicity in the in vitro test system of Ames and the present dominant lethal assay in mice. The compound was not mutagenic in either evaluation.

8. RECOMMENDATION. The data from these studies should be submitted to the Environmental Protection Agency to support the US Army's application for re-registration of M-Det as a topical insect repellent.

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HSE-LT/WP
Study No. 51-0034-78, Sep-Nov 1977

APPENDIX A

Fertility Data from 8 Weeks of Mating Male Mice Treated with M-Det, Triethylenemelamine, and Corn Oil.

| Dosage Group | Week of Cohabitation | Fertility Index: Number Pregnant | Fertility Index: Percentage |
|---|---|---|---|
| M-Det | 1 | 14 | 46.7 |
| | 2 | 19 | 63.3 |
| | 3 | 20 | 66.7 |
| | 4 | 15 | 50.0 |
| | 5 | 15 | 50.0 |
| | 6 | 19 | 63.3 |
| | 7 | 19 | 63.3 |
| | 8 | 16 | 53.3 |
| | Total | 137 | Mean 57.1 |
| TEM | 1 | 8 | 29.6 |
| | 2 | 12 | 40.0 |
| | 3 | 16 | 53.3 |
| | 4 | 14 | 46.7 |
| | 5 | 17 | 56.7 |
| | 6 | 9 | 30.0 |
| | 7 | 15 | 50.0 |
| | 8 | 22 | 73.3 |
| | Total | 113 | Mean 47.5* |
| Corn Oil | 1 | 21 | 70.0 |
| | 2 | 18 | 60.0 |
| | 3 | 17 | 56.7 |
| | 4 | 21 | 70.0 |
| | 5 | 21 | 70.0 |
| | 6 | 19 | 63.3 |
| | 7 | 20 | 66.7 |
| | 8 | 16 | 53.3 |
| | Total | 153 | Mean 63.8 |

* Significantly different from corn oil control at $p < 0.02$.

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APPENDIX B

Implantations and Fetal Deaths Per Pregnant Female Mated with Triethylenemelamine Treated Males

| Week of Cohabitation | Implantations Per Pregnancy Mean ± SD Living | Dead | Total | Early Fetal Deaths Per Pregnancy Mean ± SD | Pregnant Females with Less than 8 total implants Number Total | Percent Mean | One or more early fetal deaths Number Total | Percent Mean |
|---|---|---|---|---|---|---|---|---|
| 1 | 10.63 ±2.13 | 1.25 ±0.71 | 11.88 ±2.10 | 0.75 ±0.89 | 0 | 0 | 4 | 50.0 |
| 2 | 7.83 ±2.52 | 1.92 ±1.51 | 9.75 ±2.90 | 1.08 ±1.16 | 3 | 25.0 | 7 | 58.3 |
| 3 | 8.69 ±4.03 | 2.06 ±2.52 | 10.75 ±2.77 | 1.81 ±2.59 | 3 | 18.8 | 9 | 56.3 |
| 4 | 11.14 ±2.41 | 0.93 ±0.73 | 12.07 ±2.30 | 0.71 ±0.73 | 0 | 0 | 8 | 57.1 |
| 5 | 11.35 ±3.46 | 0.53 ±0.62 | 11.88 ±3.60 | 0.53 ±0.62 | 2 | 11.8 | 8 | 47.1 |
| 6 | 11.22 ±1.92 | 1.56 ±0.88 | 12.78 ±2.33 | 1.11 ±0.78 | 0 | 0 | 7 | 77.8 |
| 7 | 11.87 ±3.11 | 1.07 ±1.33 | 12.93 ±3.01 | 0.47 ±0.74 | 1 | 6.7 | 5 | 33.3 |
| 8 | 11.95 ±2.08 | 0.95 ±0.90 | 12.91 ±1.72 | 0.55 ±0.74 | 0 | 0 | 9 | 40.9 |
| Total | 10.59 ±1.51 | 1.28 ±0.53 | 11.87 ±1.12 | 0.88 ±0.45 | 9 | 7.8 | 56 | 52.6 |

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APPENDIX C

Implantations and Fetal Deaths Per Pregnant Female Mated with Corn Oil Treated Males

| Week of Cohabitation | Implantations Per Pregnancy Mean ± SD Living | Dead | Total | Early Fetal Deaths Per Pregnancy Mean ± SD | Pregnant Females with Less than 8 total implants Number | Percent | One or more early fetal deaths Number | Percent |
|---|---|---|---|---|---|---|---|---|
| | | | | | Total | Mean | Total | Mean |
| 1 | 10.10 ±3.02 | 0.62 ±0.92 | 10.71 ±2.92 | 0.33 ±0.58 | 2 | 9.5 | 6 | 28.6 |
| 2 | 10.06 ±3.19 | 0.78 ±0.88 | 10.83 ±3.05 | 0.56 ±0.70 | 1 | 5.6 | 8 | 44.4 |
| 3 | 11.31 ±1.70 | 0.81 ±0.75 | 12.13 ±1.67 | 0.75 ±0.77 | 0 | 0 | 9 | 56.3 |
| 4 | 11.71 1.59 | 1.0 ±1.18 | 12.71 ±1.85 | 0.62 ±0.67 | 0 | 0 | 11 | 52.4 |
| 5 | 11.33 ±2.65 | 0.57 ±0.81 | 11.90 ±2.34 | 0.43 ±0.68 | 1 | 4.8 | 7 | 33.3 |
| 6 | 10.63 ±2.65 | 1.37 ±1.92 | 12.0 ±1.73 | 0.63 ±1.01 | 0 | 0 | 6 | 31.6 |
| 7 | 11.55 ±3.05 | 1.00 ±1.97 | 12.55 ±2.35 | 0.65 ±1.27 | 1 | 5.0 | 6 | 30.0 |
| 8 | 11.44 ±1.31 | 0.81 ±1.11 | 12.25 ±1.48 | 0.31 ±0.70 | 0 | 0 | 3 | 18.8 |
| Total | 11.02 ±0.66 | 0.87 ±0.25 | 11.89 ±0.74 | 0.54 ±0.16 | 5 | 3.11 | 56 | 36.9 |

APPENDIX D

Implantations and Fetal Deaths Per Pregnant Female Mated with M-Det Treated Males

| Week of Cohabitation | Implantations Per Pregnancy Mean ± SD | | | Early Fetal Deaths Per Pregnancy Mean ± SD | Pregnant Females with | | | |
|---|---|---|---|---|---|---|---|---|
| | Living | Dead | Total | | Less than 8 total implants | | One or more early fetal deaths | |
| | | | | | Number Total | Percent Mean | Number Total | Percent Mean |
| 1 | 9.93 ±3.58 | 0.64 ±0.63 | 10.57 ±3.44 | 0.57 ±0.64 | 2 | 14.3 | 7 | 50.0 |
| 2 | 9.84 ±3.10 | 0.63 ±1.01 | 10.47 ±3.26 | 0.26 ±0.56 | 3 | 15.8 | 4 | 21.1 |
| 3 | 11.50 ±1.70 | 0.80 ±0.77 | 12.30 ±1.53 | 0.70 ±0.73 | 0 | 0 | 11 | 55.0 |
| 4 | 11.0 ±3.51 | 0.53 ±0.64 | 11.53 ±3.46 | ±0.40 ±0.63 | 2 | 13.3 | 5 | 33.3 |
| 5 | 12.67 ±2.19 | 0.73 ±0.96 | 13.40 ±2.47 | ±0.67 ±0.98 | 0 | 0 | 6 | 40.0 |
| 6 | 9.84 ±3.42 | 0.84 ±0.96 | 10.68 ±3.51 | ±0.47 ±0.61 | 3 | 15.8 | 8 | 42.1 |
| 7 | 9.63 ±3.68 | 0.89 ±0.99 | 10.53 ±3.58 | 0.32 ±0.48 | 4 | 21.1 | 6 | 31.6 |
| 8 | 10.44 ±3.92 | 1.25 ±1.44 | 11.69 ±2.85 | 0.88 ±1.31 | 2 | 12.5 | 8 | 50.0 |
| Total | 10.61 ±1.06 | 0.79 ±0.22 | 11.40 ±1.05 | 0.53 ±0.21 | 16 | 11.60* | 55 | 40.39 |

* Significant of P <0.02

# APPENDIX E
# LITERATURE CITED

1. F. S. Philips and J. B. Thiersch, The nitrogen mustard-like action of 2,4,6-tris(ethylenimino)-s-triazine and other bis (ethylenimines). Journ. Pharmacol. and Exp. Therap. 100, 398-407 (1950).

2. S. S. Epstein, et al., Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23, 288-325 (1972).

3. S. Ye. Gleyberman, et al., Experimental study of the long-term effects of using repellents. Report I: An experimental study of the effect of long-term use of diethyltoluamide (DETA) repellent. Med. Parazitol. 45, (1) 65-69 (1976). Russ.

4. C. A. Schreiner and J. R. Steelman, Oral triethylenemelamine: Effects in the dominant lethal assay for mutagenicity. Toxicol. Appl. Pharmacol. 42, 487-495 (1977).

5. A. J. Bateman and S. S. Epstein, Dominant lethal mutations in mammals, in Chemical Mutagens: Principles and Methods for Their Detection, A. Hollaender, (ed.), Vol. 2, pp 541-568. Plenum Press, New York (1971).