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THE MUTAGENIC POTENTIAL OF: TRIETHYLENE GLYCOL MONOHEXYL ETHER --ETC(U)
SEP 81 L J SAUERS, F R PULLIAM, J T FRUIN
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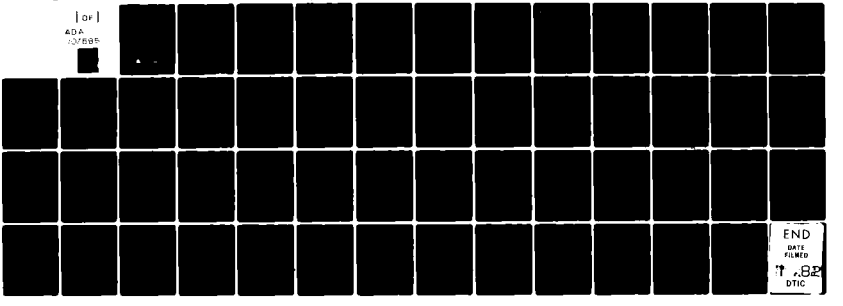

LEVEL

12

INSTITUTE REPORT NO. 107

THE MUTAGENIC POTENTIAL OF:

triethylene glycol monohexyl ether
3-(N-n-butyl-N-acetyl) aminopropionic acid ethyl ester
proprietary compound RH-398
N,N-diethyl-m-toluamide
N(n-hexyl) glutarimide

LEONARD J. SAUERS, BA, SP5
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and
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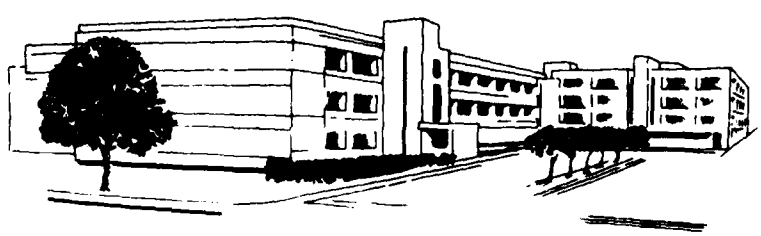
TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1981 **Toxicology Series 5**



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Toxicology Series 5

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Fred Marshall 23 Sept 81

(Signature and date)

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19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Mutagenicity, Toxicology, Ames Assay, triethylene glycol monohexyl ether; 3-(N-n-butyl-N-acetyl)aminopropionic acid ethyl ester; proprietary compound RH-398; N,N-diethyl-m-toluamide; N(n-hexyl)glutarimide.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)* The mutagenic potential of triethylene glycol monohexyl ether (SR16*); 3-(N-n-butyl-N-acetyl)aminopropionic acid ethyl ester (M3535*); proprietary compound (RH-398*); N,N-diethyl-m-toluamide (DEET*); N(n-hexyl)glutarimide (CHR3*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100 TA 1535, TA 1537, TA 1538 were exposed to doses ranging from 1 ul/plate to 3.2x 10^{-4} ul/plate for DEET, RH-398, and CHR3; and 10 ul/plate to 3.2x10^{-3} ul/plate for all other test compounds. It was determined that none of the test substances had mutagenic behavior. *Code number for compound.

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ABSTRACT

The mutagenic potential of triethylene monohexyl ether (SR16*); 3-(N-n-butyl-N-acetyl)aminopropionic acid ethyl ester (M3534*); proprietary compound (RH-398*); N,N-diethyl-m-toluamide (DEET*); N-(n-hexyl) glutarimide (CHR3*) was assessed by using the Ames Salmonella/mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 ul/plate to 3.2×10^{-4} ul/plate for DEET, RH-398, and CHR3; and 10 µl/plate to 3.2×10^{-3} ul/plate for all other test compounds. It was determined that none of the test substances had mutagenic behavior at the levels tested.

* Code number for compound.

PREFACE

AMES ASSAY REPORT:

SUBSTANCE	CODE NO.
triethylene glycol monohexyl ether	SRI6
3-(N-n-butyl-N-acetyl)aminopropionic acid ethyl ester	M3535
proprietary compound	RH-398
N,N-diethyl-m-toluamide	DEET
N-(n-hexyl)glutarimide	CHR3

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards
Letterman Army Institute of Research

PROJECT: More Effective Topical Repellents Against Disease Bearing Mosquitoes 3M62272A810

GLP STUDY NUMBER: 81004

STUDY DIRECTOR: LTC John T. Fruin, D.V.M., PhD
PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, BS
SP5 Leonard J. Sauers, BA

RAW DATA: A copy of the final report, study protocol, and retired SOPs will be retained in the LAIR Archives. Test compounds were provided by the sponsor. Chemical, analytical, stability, purity, etc. data are available from sponsor.

PURPOSE: To determine the mutagenic potential of triethylene glycol monohexyl ether; 3-(N-n-butyl-N-acetyl)aminopropionic acid ethyl ester; proprietary compound RH-398; N,N-diethyl-m-toluamide; N-(n-hexyl)glutarimide, by using the Ames Salmonella/ Mammalian Microsome Mutagenicity Test. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

ACKNOWLEDGMENTS

The authors wish to thank Carolyn Lewis MS, Mr. John Dacey, and SP4 Larry Mullen, BS, for their assistance in performing the research.

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study, GLP number 81004, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

Freddica R. Pulliam 8 Jul 81
FREDDICA R. PULLIAM, BS Date
SSG
Co-Investigator

John T. Fruin 7 July 81
JOHN T. FRUIN, DVM, PhD Date
LTC, VC
Study Director

Leonard J. Sauers 9 July 81
LEONARD J. SAUERS, BA Date
SP5
Co-Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

19 August 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81004 the following inspection was made:

19 August 1981

Inspection findings were reported to the study director on 19 Aug 81. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 81 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occcur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria is adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred.

We compare the spontaneous reversion values with our own historical values and these cited by Ames, et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we have validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance are added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistially. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial

Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on conrol plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, all the test substances will be referred to by the respective code number:

Substance	Code No
Triethylene glycol monohexyl ether	SRI6
3-(N-n-butyl-N-acetyl)aminopropionic acid-ethyl ester	M3535
Proprietary compound	RH-398
N,N-diethyl-m-toluamide	DEET
N(n-hexyl)glutarimide	CHR3

A series of assays was run to conclusively determine the mutagenic activity of the five substances. On 6 April 1981, the toxicity level was confirmed on test chemicals SRI6, M3535, DEET, and RH-398. CHR3 was assessed on 10 April 1981. The repellents were

assayed using dosages ranging from 10 ul/plate to 10^{-6} ul/plate. All sterility, positive, and negative controls were normal (Table 1). Quality control data for the assay with CHR3 is contained in that for the Ames Assay of 10 April 1981 (Table 3A). Toxicity was observed only in the initial doses for SRI6 and CHR3 (Tables 2A-2E).

On 10 April 1981, the Ames Assay was run on test substances SRI6, M3535, DEET, and RH-398. Several instances of toxicity and scattered mutagenicity were observed in response to DEET and RH-398, so these test substances were reassayed on 28 Apr 81 using 1 ul/plate as the initial dose. CHR3 was tested on 14 April 1981. The assay of 10 Apr 81 showed an expected response to all sterility and positive control. The spontaneous reversion rates were normal when compared to our historical data (Table 3A). On 14 April 1981, unexpected results were obtained in response to positive control chemical benz(α)pyrene (BP) for strain TA 1538. TA 1538 did respond normally to aminoflourene (AF) and dimethyl-benzaanthracene (DMBA). The spontaneous reversion rates were normal for all strains when compared to our historical data (Table 3B). On 28 April 1981, all positive, negative, and sterility controls were as expected. The spontaneous reversion rate were also normal (Table 3C). On 19 August 1981, all controls were normal except for the response of TA 1538 to DMBA (Table 3D).

The mutagenic potential of SRI6 and M3535 were assessed on 10 April 1981. No evidence of such activity was observed (Table 4A-4B).

Test chemical DEET was assayed on 10 April 1981. The 10 ul/plate dose showed toxicity in all nonactivated strains. Mutagenicity was observed for activated TA 1535 and the 2 ul/plate level and for nonactivated TA 1535 at the 0.4 ul/plate dose (Table 4C). Due to these observations the repellent was retested on 28 April 1981. In that assay, only a numerical suggestion of mutagenicity was seen for activated TA 1537 at the 0.2 ul/plate dose and for activated TA 1538 at the 0.0016 ul/plate level. No dose response was observed (Table 4D).

RH-398 was assayed on 10 April 1981. At that time, mutagenicity was observed for nonactivated TA 98 at the 10 ul/plate dose, and at the 2.0 and 0.08 ul/plate for activated TA98. Due to the observed sparse background lawn at the 10 ul/plate level, it was assumed that the colonies formed were survivors rather than revertant. Activated TA 100 at the 2.0 ul/ plate dose also showed mutagenic behavior. A more than twice the spontaneous reversion rate was seen for activated TA 1535 at the 10 ul/ plate through the 0.0032 ul/plate doses and nonactivated from the 2.0 ul/ plate to 0.016 ul/plate levels (Table 4E). RH-398 was retested on 28 April 1981. At that time only activated TA 1535 at the 1.0 ul/plate level showed any mutagenic activity (Table 4F). Due to the fact that in the initial assay,

TA100, the more sensitive strain, did not react in the same way as TA 1535, and due to the atypical dose response our conclusions will be drawn from the second assay. To be assured of repeatable results, RH-398 was assayed again on 19 August 1981. At that time no evidence of mutagenicity was observed (Table 4G).

CRH3 was tested on 14 April 1981. A doubling of the spontaneous reversion rate occurred at the 0.008 ul/plate level for activated TA 98, TA 1535 and TA 1538. No dose response was seen (Table 4H).

The MUTAR values were calculated and are listed in Tables 5A-5H). Values over the 1.5 threshold occurred on 28 April 1981 for test substance DEET at 0.2 ul/plate level for activated TA 1537. The same occurred for RH-398 on 10 April 1981. Here nonactivated TA 1535 from 2.0 ul/plate through 0.016 ul/plate, activated TA 1535 at all doses, and activated TA 98 at the 2.0 ul/plate level were above the 1.5 value.

CONCLUSIONS

For a substance to be mutagenic, several criteria must be met. A more than doubling of the spontaneous rate and an obvious dose response must be observed. None of the five test substances showed these characteristics. Therefore, on the basis of the Ames Test, SRI6, M3535, DEET, RH-398, and CHR3 are not mutagenic at the levels tested.

RECOMMENDATIONS

We recommend that compounds SR16, M3535, RH-398, DEET, and CHR3 be tested using other toxicological testing systems if efficacy tests show those chemials to be promising repellents.

REFERENCES

1. McCANN, J., E. CHOI, E. YAMASAKI, and B. N. AMES. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 72:5135-5139, 1975

2. AMES, B. N., J. McCANN and E. YAMASAKI. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 31: 347-364, 1975

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 1 March 1981

4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 218: 97-106, 1956

5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976

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TABLE

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Strain No.	Histidine Requirements	Ampicillin Resistance	uvr-B Deletion	rfa Crystal Violet	Sterility Control	Respons (a)
TA 100	NG	G	NG	15.80mm	NG	+
TA 1537	NA	23.21mm	NA	NA	NG	+
WT	G	NA	G	NA	NA	+
Diluent	NA	NA	NA	NA	ETOH/NG DMSO/NG	+
Positive Control: MNNG - Test Compound (s)		Average 5884				
(a) M3535	NA	NA	NA	NA	NG	+
(b) DEET	NA	NA	NA	NA	NG	+
(c) SRI6	NA	NA	NA	NA	NG	+
(d) RH398	NA	NA	NA	NA	NG	+
(e) NA	NA	NA	NA	NA	NA	NA

G = Growth; NG = No Growth; NT = Not Tested; NA = Not Applicable;
WT = Wild Type; (a) + = Expected Response; - = Unexpected Response

Spontaneous Revertants

Strain	Time				Average
100	Beginning NO S9	87	75	72	78

Test Inculated By: Sauers, Pulliam, Lewis Date 6 April 1981

Test Read By: Sauers, Pulliam Date 8 April 1981

TABLE 2A

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) SRI-6 (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 April 1981 Performed by: Sauers, Pulliam, Lewis

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
10 ul/plate	48	72	68	63	ST
1 ul/plate	61	42	72	58	NL
0.1 ul/plate	58	59	68	62	NL
0.01 ul/plate	38	53	57	49	NL
0.001 ul/plate	70	60	56	62	NL
0.0001 ul/plate	47	53	58	53	NL
0.00001 ul/plate	56	68	49	58	NL
0.000001 ul/plate	60	58	63	60	NL

TABLE 2F

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) M-3535 (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 April 1981 Performed by: Sauers, Pulliam, Lewis

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
10 ul/plate	52	58	72	61	NL
1 ul/plate	56	68	44	56	NL
0.1 ul/plate	60	56	60	59	NL
0.01 ul/plate	82	72	66	73	NL
0.001 ul/plate	36	54	48	46	NL
0.0001 ul/plate	54	52	58	55	NL
0.00001 ul/plate	48	56	44	49	NL
0.000001 ul/plate	46	43	52	47	NL

TABLE 2C

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) RH-398 (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 April 1981 Performed by: Sauers, Pulliam, Lewis

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
10 ul/plate	60	66	68	65	NL
1 ul/plate	58	59	59	59	NL
0.1 ul/plate	54	58	53	55	NL
0.01 ul/plate	62	72	48	61	NL
0.001 ul/plate	20	41	52	38	NL
0.0001 ul/plate	57	51	48	52	NL
0.00001 ul/plate	60	69	66	65	NL
0.000001 ul/plate	74	72	74	73	NL

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) DEET (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 April 1981 Performed by: Sauers, Lewis, Pulliam

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
10 ul/plate	63	60	52	58	NL
1 ul/plate	84	77	101	87	NL
0.1 ul/plate	76	48	46	57	NL
0.01 ul/plate	43	39	44	42	NL
0.001 ul/plate	50	39	38	42	NL
0.0001 ul/plate	59	63	67	63	NL
0.00001 ul/plate	69	48	54	57	NL
0.000001 ul/plate	59	59	68	62	NL

TABLE 2E

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) CHR3 (2) _____

(3) _____ (4) _____ (5) _____

Date: 10 April 1981 Performed by: Sauers, Pulliam, Mullen, Kellner

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
10 ul/plate	TOXIC	TOXIC	TOXIC	TOXIC	NG
1 ul/plate	22	32	28	27	ST
0.1 ul/plate	62	58	46	55	NL
0.01 ul/plate	36	43	48	42	NL
0.001 ul/plate	72	82	52	69	NL
0.0001 ul/plate	58	67	72	66	NL
0.00001 ul/plate	74	78	68	73	NL
0.000001 ul/plate	64	68	76	69	NL

TABLE 3A

10 April 1981

Strain Verification Control

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response
93	NG	G	NG	14.15 mm	NG	+
100	NG	G	NG	14.58 mm	NG	+
1535	NG	NT	NG	14.54 mm	NG	+
1537	NG	23.04	NG	14.55 mm	NG	+
1538	NG	NT	NG	15 mm	NG	+
WT	G	NT	G		NG	+

Sterility Control

His-Bio Mix Initial: NG End: NG Diluent: NG Nutrient Broth: NG
Top Agar Initial: NG End: NG MGA Plate: NG
S-9 Mix Initial: NG End: NG MGA Plate w/bacteria: G
Test Compound (a) SR16-NG : (b) M3535-NG : (c) DEET-NG : (d) RH-398-NG: (e) CHR3 -NG : (f) NA
G=Growth; NG=No Growth; NT=Not Tested; NA=Not applicable; WT=Wild Type

Spontaneous Revertant Rate and Positive Control Revertant Rate

Compound	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
AF		yes	(452, 386, 384) (407)	(298, 276, 248) (274)			(248, 212, 198) (219)
BP		yes	(76, 98, 84) (86)	(172, 198, 196) (189)		(210, 136, 152) (166)	(132, 86, 98) (105)
DMBA		yes	(68, 62, 56) (62)	(212, 172, 156) (180)		(22. 19, 23) (21)	(86, 94, 102) (94)
MMNG		no		(5286,6112,6348) (5915)	(4312,4820,5168) (4767)		
Strain Performance							
Beginning	Spontaneous	yes	(18, 19, 25)	(88, 76, 68)	(8, 16, 18)	(8, 8, 6)	(16, 12, 14)
End	Revertants		(24, 23, 30) (23)	(77, 74, 78) (77)	(16, 14,21) (16)	(6, 9, 8) (8)	(28, 12, 15) (16)
Beginning		no	(15, 20, 18)	(60, 78, 94)	(8, 20, 8)	(7, 8, 7)	(15, 9, 12)
End			(16, 15, 17) (17)	(78, 97, 84) (82)	(18, 20, 21) (16)	(6, 6, 9) (7)	(22, 11, 8) (13)
Aminoanthracene		yes	(3080,3612,3410) (3367)	(4112,3716,3914) (3914)		(87,106,112) (102)	(146,204,134) (178)

TABLE 3B 14 April 1981

Strain Verification Control

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response
93	NG	G	NG	14.09 mm	NG	+
100	NG	G	NG	14.85 mm	NG	+
1535	NG	NA	NG	16.70 mm	NG	+
1537	NG	20.35 mm	NG	17.09 mm	NG	+
1538	NG	NA	G	16.40 mm	NG	- *
WT	G	NA	G	NA	NG	+

Sterility Control

His-Bio Mix Initial: NG End: NG Diluent: NG Nutrient Broth: NG
Top Agar Initial: NG End: NG MGA Plate: NG
S-9 Mix Initial: NG End: NG MGA Plate w/bacteria: G
Test Compound (a) CHR3-NG : (b) NA : (c) NA : (d) NA : (e) NA : (f) NA
G=Growth; NG=No Growth; NT=Not Tested; NA=Not applicable; WT=Wild Type * couple isolated colonies

Spontaneous Revertant Rate and Positive Control Revertant Rate

Compound	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
AF		yes	(556,568,612) (579)	(428,360,246) (345)			(424,564,460) (483)
BP		yes	(288,336,252) (292)	(432,420,388) (413)		(62,75,82) (73)	(9,12,13) (11)
DMBA		yes	(58, 47, 51) (52)	(162,270,202) (211)		(29,18,24) (24)	(27,35,30) (31)
MMNG		no		(5040,6212,7416) (6223)	(6116,6938,8212) (7089)		
Strain Performance							
Beginning	Spontaneous	yes	(12,19,14)	(65,66,51)	(10,12,6)	(5,6,6)	(7,14,contam)
End	Revertants		(20,27,23) (19)	(82,54,65) (64)	(8,8,12) (9)	(4,5,6) (5)	(15,20,11) (13)
Beginning		no	(25,30,29)	(68,92,85)	(10,15,6)	(8,5,5)	(26,28,20)
End			(20,17,13) (22)	(33,45,48) (62)	(5, 9,10) (9)	(3,2,2) (4)	(8, 9,10) (17)

TABLE 3C

28 April 1981

Strain Verification Control

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response
93	NG	G	NG	14.15 mm	NG	+
100	NG	G	NG	16.76 mm	NG	+
1535	NG	NA	NG	16.45 mm	NG	+
1537	NG	19.57	NG	17.13 mm	NG	+
1538	NG	NA	NG	15.10 mm	5	-*
WT	G	NA	G	NA	NG	+

Sterility Control

His-Bio Mix Initial: NG End: NG Diluent: NG Nutrient Broth: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG MGA Plate w/bacteria: G

Test Compound (a) DEET-NG : (b) RH-398-NG : (c) NA : (d) NA : (e) NA : (f) NA

G=Growth; NG=No Growth; NT=Not Tested; NA=Not applicable; WT=Wild Type *= isolated colonies sterility control of 7A1538

Spontaneous Revertant Rate and Positive Control Revertant Rate

Compound	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
AF	2 ug	yes	(444,403,289) (379)	(187,229,159) (192)			(488,705,420) (540)
BP	2 ug	yes	(108,137,159) (135)	(570,706,617) (631)		(175,238,148) (187)	(206,155,170) (177)
DMBA	20 ug	yes	(50,60,92) (67)	(209,200,197) (202)		27,28,25) (27)	(39,27,38) (35)
MMNG	2 ug	no		(1445,2031,1535) (1670)			
	20 ug				(1730,1910,2148) (1929)		
Strain Performance							
Beginning	Spontaneous	yes	(42,18,26)	(78,57,59)	(17,8,16)	(2,9,9)	(13,15,10)
End	Revertants		(38,38,38) (33)	(113,116,99) (87)	(11,18,14) (14)	(14,11,6) (8)	(26,12,23) (16)
Beginning		no	(23,28,12)	(55,65,75)	(21,26,21)	(6,3,4)	(18,25,16)
End			(18,19,21) (20)	(60,17,56) (55)	(7,11,2) (16)	(4,5,4) (4)	(11,4,3) (13)

TABLE 3C

28 April 1981

Strain Verification Control

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response
93	NG	G	NG	14.15 mm	NG	+
100	NG	G	NG	16.76 mm	NG	+
1535	NG	NA	NG	16.45 mm	NG	+
1537	NG	19.57	NG	17.13 mm	NG	+
1538	NG	NA	NG	15.10 mm	5	-*
WT	G	NA	G	NA	NG	+

Sterility Control

His-Bio Mix Initial: NG End: NG Diluent: NG Nutrient Broth: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG MGA Plate w/bacteria: G

Test Compound (a) DEET-NG : (b) RH-398-NG : (c) NA : (d) NA : (e) NA : (f) NA

G=Growth; NG=No Growth; NT=Not Tested; NA=Not applicable; WT=Wild Type *= isolated colonies sterility control of 7A1538

Spontaneous Revertant Rate and Positive Control Revertant Rate

Compound	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
AF	2 ug	yes	(444,403,289) (379)	(187,229,159) (192)			(488,705,4[illegible] (540)
BP	2 ug	yes	(103,137,159) (135)	(570,706,617) (631)		(175,238,148) (187)	(206,155,170) (177)
DMBA	20 ug	yes	(50,60,92) (67)	(209,200,197) (202)		27,28,25) (27)	(39,27,38) (35)
MMNG	2 ug	no		(1445,2031,1535) (1670)			
	20 ug				(1730,1910,2148) (1929)		
Strain Performance							
Beginning End	Spontaneous Revertants	yes	(42,18,26) (38,38,38) (33)	(78,57,59) (113,116,99) (87)	(17,8,16) (11,18,14) (14)	(2,9,9) (14,11,6) (8)	(13,15,10) (26,12,23) (16)
Beginning End		no	(23,28,12) (18,19,21) (20)	(55,65,75) (60,17,56) (55)	(21,26,21) (7,11,2) (16)	(6,3,4) (4,5,4) (4)	(18,25,16) (11,4,3) (13)

TABLE 3D

Strain Verification Control

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response
98	NG	G	NG	14.59	NG	+
100	NG	G	NG	15.11	NG	+
1535	NG	NA	NG	13.89	NG	+
1537	NG	16.13	NG	14.25	NG	+
1538	NG	NA	NG	15.07	NG	+
WT	G	NA	G	NA	NG	+

Sterility Control

His-Bio Mix Initial: NG End: NG Diluent: NG Nutrient Broth: NG
Top Agar Initial: NG End: NG MGA Plate: NG
S-9 Mix Initial: NG End: NG MGA Plate w/bacteria: G
Test Compound (a)oct glut-NG (b)RH398-NG : (c)NA : (d) NA : (e) NA : (f) NA
G=Growth; NG=No Growth; NT=Not Tested; NA=Not applicable; WT=Wild Type

Spontaneous Revertant Rate and Positive Control Revertant Rate

Compound	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
AF		yes	(262,341,548) (384)	(258,272,278) (269)			(400,388,378) (389)
BP		yes	(50,193,111) (118)	(312,363,378) (351)		(61,49,63) (58)	(147,97,162) (135)
DMBA		yes	(59,59,51) (56)	(259,273,209) (247)		(23,34,25) (27)	(24,19,51) (31)
MMNG		no		(650,712,668) (677)	(441,432,392) (422)		
Strain Performance			(27,20,23)	(81,75,84)	(12,10,10)	(5,6,7)	(26,29,26)
Beginning	Spontaneous	yes	(18,20,17)	(77,77,72)	(12,11,13)	(12,12,7)	(18,20.19)
End	Revertants		(21)	(78)	(11)	(8)	(23)
Beginning		no	(19,23,19)	(78,70,107)	(12,14,15)	(4,3,8)	(11,11,24)
End			(19,9,15) (17)	(62,82,60) (77)	(7,12,10) (12)	(4,6,7) (5)	(17,15,12) (15)

TABLE 4A

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
* SRI6	10 ul/plate	no	(12,14,9) (12)	(28,36,24) (29)	(5,2,5) (4)	(2,2,3) (2)	(Toxic, Toxic,2) (Toxic)
*		yes	(20,16,19) (18)	(68,54,52) (58)	(11,13,16) (13)	(2,5,6) (4)	(Toxic, 4,Toxic) (Toxic)
SRI6	2 ul/plate	no	(18,18,12) (16)	(56,76,64) (65)	(9,10,12) (10)	(3,3,7) (4)	(8,9,12) (10)
		yes	(25,26,29) (27)	(68,56,56) (60)	(27,24,15) (22)	(5,4,4) (4)	(23,21,14) (19)
SRI6	0.4 ul/plate	no	(26,20,17) (21)	(76,63,94) (78)	(20,15,14) (16)	(10,4,4) (6)	(12,10,9) (10)
		yes	(24,23,36) (28)	(92,66,54) (71)	(18,21,13) (17)	(5,9,8) (7)	(9,18,18) (15)

-continued

TABLE 4A, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
SRI6	0.08 ul/plate	no	(28,32,28) (29)	(56,66,54) (59)	(25,16,23) (21)	(2,6,3) (4)	(9,7,8) (8)
		yes	(22,25,26) (24)	(68,52,67) (62)	(16,21,15) (17)	(8,7,6) (7)	(18,14,16) (16)
SRI6	0.016 ul/plate	no	(21,19,17) (19)	(47,60,58) (55)	(18,19,16) (18)	(4,7,5) (5)	(11,10,12) (11)
		yes	(12,21,23) (19)	(68,42,54) (55)	(2,4,6) (4)	(2,5,3) (3)	(15,6,contam) (10)
SRI6	0.0032 ul/plate	no	(12,15,16) (14)	(52,54,76) (61)	(6,1,5) (4)	(3,3,3) (3)	(2,6,8) (5)
		yes	(26,28,22) (25)	(58,64,58) (60)	(8,9,7) (8)	(1,4,2) (2)	(9,5,4) (6)

* Slight background lawn

TABLE 4B

Number of Revertants/Plate

	Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
*	M3535	10 ul/plate	no	(Toxic,14,16) (15)	(27,24,36) (29)	(8,2,6) (5)	(Toxic,Toxic,2) (Toxic)	(Toxic,Toxic,4) (Toxic)
*			yes	(14, 7,18) (13)	(38,42,48) (43)	(4,5,Toxic) (4)	(Toxic,Toxic,Toxic) (Toxic)	(Toxic,Toxic2 (Toxic)
	M3535	2 ul/plate	no	(18,14,29) (20)	(58,66,64) (63)	(3,5,5) (4)	(Toxic,Toxic,Toxic) (Tixic)	(Toxic,Toxic,4) (Toxic)
			yes	(17,18,31) (22)	(58,70,56) (61)	(7,10,12) (10)	(5,3,2) (3)	(16,7,8) (10)
	M3535	0.4 ul/plate	no	(24,28,18) (23)	(54,58,62) (58)	(2,6,12) (7)	(8,3,2) (4)	(4,5,2) (4)
			yes	(21,35,34) (30)	(72,76,72) (73)	(16,11,8) (12)	(4,5,8) (7)	(11,20,2) (17)

-continued

* slight background lawn

TABLE 4B, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
M3535	0.08 ul/plate	no	(11,16,31) (19)	(48,36,44) (43)	(8,6,8) (7)	(2,3,3) (3)	(9,2,7) (6)
		yes	(25,16,18) (20)	(58,72,56) (62)	(14,16,11) (14)	(5,4,5) (5)	(13,10,6) (10)
M3535	0.016 ul/plate	no	(22,16,14) (17)	(48,58,60) (55)	(10,11,9) (10)	(7,7,5) (6)	(10,8,6) (8)
		yes	(18,35,28) (27)	(56,54,60) (57)	(4,5,9) (6)	(2,5,7) (5)	(12,8,10) (10)
M3535	0.0032 ul/plate	no	(25,28,36) (30)	(64,66,82) (71)	(6,3,5) (5)	(2,3,5) (3)	(6,5,5) (5)
		yes	(42,24,24) (30)	(62,64,72) (66)	(14,19,16) (16)	(6,3,3) (4)	(25,18,21) (21)

TABLE 4C

Number of Revertants/Plate

	Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
*	DEET	10 ul/plate	no	(Toxic,20,Contam) (Toxic)	(162,Toxic,Toxic) (Toxic)	(Tox,Tox,Tox) (Toxic)	(Tox,Tox,Tox) (Toxic)	(Tox,Tox,Tox (Toxic)
*			yes	(3,2,1) (2)	(20,24,32) (25)	(21,3,16) (13)	(1,4,3) (3)	(0,4,5) (3)
	DEET	2 ul/plate	no	(24,28,16) (23)	(68,82,56) (69)	(30,15,24) (23)	(2,3,3) (3)	(13,7,10) (10)
			yes	(29,38,41) (36)	(92,74,84) (83)	(34,49,35) (39)	(3,2,6) (4)	(12,13,8) (11)
	DEET	0.4 ul/plate	no	(13,25,23) (20)	(81,88,90) (86)	(36,25,38) (33)	(4,7,4) (5)	(9,15,13) (12)
			yes	(19,47,28) (31)	(64,57,66) (62)	(20,29,15) (21)	(2,1,1) (1)	(Contaminati.) (Contamination)

-continued

* slight background lawn

TABLE 4C, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
DEET	0.08 ul/plate	no	(25,28,12) (22)	(78,62,60) (67)	(18,32,21) (24)	(4,5,5) (5)	(9,10,4) (8)
		yes	(24,26,26) (25)	(76,82,72) (77)	(26,32,28) (29)	(5,7,7) (6)	(17,19,16) (17)
DEET	0.016 ul/plate	no	(27,20,14) (17)	(58,66,44) (56)	(24,19,16) (20)	(5,5,4) (5)	(5,10,16) (10)
		yes	(18,24,13) (18)	(61,54,44) (53)	(24,22,30) (25)	(8,3,5) (5)	(8,12,8) (9)
DEET	0.0032 ul/plate	no	(23,22,28) (24)	(48,63,57) (56)	(34,26,24) (28)	(3,6,7) (5)	(22,18,21) (20)
		yes	(19,35,28) (27)	(76,58,82) (72)	(26,32,28) (29)	(6,14,5) (9)	(14,22,14) (17)

TABLE 4D 28 April 1981

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
DEET	1 ul/plate	no	(18,16,24) (19)	(71,72,49) (64)	(7,2,11) (7)	(5,2,5) (4)	(6,9,9) (8)
		yes	(24,15,23) (21)	(68,39,85) (64)	(17,16,20) (18)	(2,5,2) (3)	(21,22,20) (21)
DEET	0.2 ul/plate	no	(15,29,23) (22)	(54,30,52) (45)	(9,11,8) (9)	(3,2,3) (3)	(5,7,11) (8)
		yes	(33,20,40) (31)	(52,59,72) (61)	(11,10,9) (10)	(12,25,23) (20)	(7,5,6) (6)
DEET	0.04 ul/plate	no	(20,4,13) (12)	(39,42,20) (34)	(5,8,3) (5)	(3,5,2) (3)	(4,5,11) (7)
		yes	(30,23,26) (26)	(84,59,64) (69)	(12,20,5) (12)	(9,8,4) (7)	(11,6,15) (11)

-continued

TABLE 4D, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
DEET	0.008 ul/plate	no	(26,18,23) (22)	(59,72,58) (63)	(14,20,6) (13)	(3,4,5) (4)	(10,7,6) (8)
		yes	(29,15,23) (22)	(65,76,55) (65)	(18,16,11) (15)	(7,5,3) (5)	(15,12,11) (13)
DEET	0.0016 ul/plate	no	(20,17,16) (18)	(41,55,18) (38)	(11,18,21) (17)	(5,6,2) (4)	(17,11,10) (13)
		yes	(27,33,36) (32)	(86,74,68) (76)	(19,21,22) (21)	(5,4,8) (6)	(33,37,28) (33)
DEET	0.00032 ul/plate	no	(19,11,21) (17)	(47,61,58) (55)	(22,9,11) (14)	(3,2,7) (4)	(11,10,17) (13)
		yes	(19,15,20) (18)	(41,76,58) (58)	(9,11,20) (13)	(9,2,8) (6)	(18,12,14) (15)

TABLE 4E

10 April 1981

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
RH-398	10 ul/plate *	no	(44,44,30) (39)	(158,140,153) (150)	(23,14,7) (15)	(4,2,1) (2)	(4,3,7) (5)
	*	yes	(27,31,28) (29)	(95,174,78) (149)	(80,55,66) (67)	(2,3,2) (2)	(20,9,8) (12)
RH-398	2 ul/plate	no	(21,28,28) (26)	(126,136,155) (139)	(40,58,52) (50)	(1,2,6) (3)	(9,9,8) (9)
		yes	(69,61,79) (70)	(171,166,165) (167)	(72,74,49) (65)	(5,10,4) (6)	(21,21,20) (21)
RH-398	0.4 ul/plate	no	(23,26,13) (21)	(130,123,89) (114)	(40,50,25) (38)	(5,2,7) (5)	(14,5,16) (12)
		yes	(32,36,37) (35)	(103,128,132) (121)	(57,50,61) (56)	(6,7,11) (8)	(22,16,12) (17)

-continued

* sparse background lawn

TABLE 4E, concluded 10 April 1981

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
RH-398	0.08 ul/plate	no	(19,30,22) (24)	(114,102,91) (102)	(36,29,51) (39)	(11,5,4) (7)	(15,12,16) (14)
		yes	(64,47,46) (52)	(113,104,107) (108)	(52,60,66) (59)	(9,4,10) (8)	(29,15,20) (21)
RH-398	0.016 ul/plate	no	(20,28,30) (26)	(93,51,59) (68)	(34,34,51) (40)	(11,3,5) (6)	(13,13,8) (11)
		yes	(44,30,37) (37)	(101,99,84) (95)	(59,39,45) (48)	(11,11,11) (11)	(26,26,27) (26)
RH-398	0.0032 ul/plate	no	(40,8,12) (20)	(97,101,66) (88)	(25,33,31) (30)	(5,5,4) (5)	(9,15,11) (12)
		yes	(46,36,37) (40)	(98,88,80) (89)	(51,48,45) (48)	(8,1,3) (4)	(25,32,35) (31)

*Sparse background lawn

28 April 1981

TABLE 4F

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
RH-398	1 ul/plate	no	(30,12,29) (24)	(77,73,75) (75)	(28,32,18) (26)	(9,3,7) (6)	(15,10,2) (9)
		yes	(30,24,26) (27)	(72,99,87) (86)	(27,37,22) (29)	(16,2,12) (10)	(9,10,6) (8)
RH-398	0.2 ul/plate	no	(14,5,24) (14)	(70,50,53) (58)	(5,11,10) (9)	(5,2,6) (4)	(8,9,3) (7)
		yes	(18,30,33) (28)	(50,69,57) (59)	(13,20,25) (19)	(3,3,3) (3)	(21,14,17) (17)
RH-398	0.04 ul/plate	no	(15,12,19) (15)	(53,33,50) (45)	(14,11,15) (13)	(2,7,6) (5)	(11,13,7) (10)
		yes	(23,26,22) (24)	(75,52,62) (63)	(12,13,17) (14)	(4,[illegible]) (6)	(14,14,[illegible]) (15)

-continued

28 April 1981

TABLE 4F, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
RH-398	0.008 ul/plate	no	(25,28,18) (24)	(55,68,46) (56)	(19,20,20) (20)	(9,5,5) (6)	(7,6,8) (7)
		yes	(36,23,24) (28)	(57,69,76) (67)	(24,18,23) (22)	(7,5,6) (6)	(10,15,9) (11)
RH-398	0.0016 ul/plate	no	(11, 20,33) (21)	(63,68,71) (67)	(18,15,12) (15)	(6,4,2) (4)	(8,4,6) (6)
		yes	(42,42,30) (38)	(69,55,35) (53)	(20,19,24) (21)	(9,2,5) (5)	(14,13,10) (12)
RH-398	0.00032 ul/plate	no	(16,22,25) (21)	(48,55,41) (48)	(12,6,6) (8)	(3,6,6) (5)	(3,12,10) (8)
		yes	(31,48,25) (35)	(79,87,66) (77)	(14,18,18) (17)	(6,9,6) (7)	(8,22,14) (15)

TABLE 4G

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
RH-398	1 ul/plate	no	(20,15,29) (21)	(86,62,90) (79)	(14,15,13) (14)	(6,5,3) (5)	(12,17,16) (15)
		yes	(25,27,37) (30)	(107,105,90) (101)	(19,15,19) (18)	(4,3,3) (3)	(17,16,20) (18)
	0.2 ul/plate	no	(19,24,17) (20)	(86,92,66) (81)	(24,10,8) (14)	(4,4,7) (5)	(10,15,20) (14)
		yes	(31,35,22) (29)	(111,95,74) (93)	(24,13,14) (17)	(5,8,5) (6)	(11,[illegible],[illegible]) ([illegible])
	0.04 ul/plate	no	(22,20,25) (22)	(100,88,91) (93)	(8,14,12) (11)	(2,10,5) (5)	(15,[illegible],12) ([illegible])
		yes	(33,22,44) (33)	(104,124,113) (114)	(20,17,10) (16)	(9,5,6) (7)	(23,[illegible],10) ([illegible])

-continued

Study Number: 81004 Date: 19 Aug 81 By: Sauers, Kellner

TABLE 4G, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
RH-398	0.008 ul/plate	no	(12,12,15) (13)	(49,75,74) (66)	(7,10,12) (10)	(5,6,5) (5)	(10,11,13) (11)
		yes	(29,15,29) (24)	(80,107,99) (95)	(16,19,14) (16)	(7,6,4) (6)	(24,15,24) (21)
	0.0016 ul/plate	no	(21,15,9) (15)	(69,69,90) (76)	(9,19,13) (14)	(4,8,4) (5)	(14,9,6) (10)
		yes	(30,27,22) (26)	(70,62,78) (70)	(12,18,11) (14)	(4,9,3) (5)	(21,6,13) (13)
	0.00032 ul/pl.	no	(13,19,11) (14)	(61,67,55) (61)	(12,15,17) (15)	(5,6,6) (6)	(14,10,9) (11)
		yes	(15,10,23) (16)	(53,78,56) (62)	(13,9,8) (10)	(4,5,8) (6)	(24,22,11) (19)

Study Number: 81004 Date: 19 Aug 81 By: Sauers, Kellner

TABLE 4H

14 April 1981

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
CHR3	1 ul/plate	no	(24,34,26) (28)	(60,78,88) (75)	(21,19,14) (18)	(7,6,5) (6)	(25,30,26) (27)
		yes	(14,17,23) (18)	(27,47,34) (36)	(9,18,11) (13)	(5,5,Toxic) (5)	(7,14,10) (10)
CHR3	0.2 ul/plate	no	(31,25,29) (28)	(70,72,73) (72)	(9,7,10) (9)	(4,3,7) (5)	(6,11,11) (9)
		yes	(22,27,25) (25)	(70,61,68) (66)	(7,11,12) (10)	(4,4,4) (4)	(27,21,[illegible]) (21)
CHR3	0.04 ul/plate	no	(30,27,24) (27)	(51,55,83) (63)	(12,8,14) (11)	(1[illegible],0)	(12,12,[illegible]) (13)
		yes	(28,24,32) (28)	(68,69,54) (57)	(15,15,[illegible]) (17)	(5,[illegible])	(14,20,[illegible]) (17)

-continued

TABLE 4H, concluded

Number of Revertants/Plate

Test Compd.	Amount of Compd. Added	S-9 Mix Added	Strain No. 98	100	1535	1537	1538
CHR3	0.008 ul/plate	no	(30,25,19) (25)	(74,80,83) (79)	(13,17,19) (16)	(7,6,6) (6)	(12,15,14) (14)
		yes	(46,38,35) (40)	(77,72,102) (84)	(21,24,30) (25)	(5,12,6) (8)	(35,25,31) (30)
CHR3	0.0016 ul/plate	no	(25,18,25) (23)	(68,75,59) (67)	(11,18,16) (15)	(7,3,4) (5)	(7,7,11) (8)
		yes	(19,35,32) (29)	(90,70,83) (81)	(10,13,16) (13)	(4,8,7) (6)	(16,19,31) (22)
CHR3	0.00032 ul/plate	no	(15,20,21) (19)	(65,74,64) (68)	(11,20,9) (13)	(3,5,4) (4)	(10,15,8) (11)
		yes	(37,27,28) (31)	(74,71,76) (74)	(16,12,17) (15)	(6,6,7) (6)	(16,16,23) (18)

TABLE 5A

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: SRI6 Dissolved in: ETOH

Date: 10 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
10 ul/pl	TA98	*	*	0.08 ul/plate	TA1535	0.4	0.1
2 ul/pl	TA98	*	0.15	0.016 ul/plate	TA1535	0.16	*
0.4 ul/pl	TA98	0.18	0.19	0.0032 ul/plate	TA1535	*	*
0.08 ul/pl	TA98	0.55	0.04				
0.016 ul/pl	TA98	0.09	*	10 ul/plate	TA1537	*	*
0.0032 ul/pl	TA98	*	0.07	2 ul/plate	TA1537	*	*
				0.4 ul/plate	TA1537	*	*
10 ul/pl	TA100	*	*	0.08 ul/plate	TA1537	*	*
2 ul/pl	TA100	*	*	0.016 ul/plate	TA1537	*	*
0.4 ul/pl	TA100	*	*	0.0032 ul/plate	TA1537	*	*
0.08 ul/pl	TA100	*	*				
0.016 ul/pl	TA100	*	*	10 ul/plate	TA1538	*	*
0.0032 ul/pl	TA100	*	*	2 ul/plate	TA1538	*	0.18
				0.4 ul/plate	TA1538	*	*
10 ul/pl	TA1535	*	*	0.08 ul/plate	TA1538	*	*
2 ul/pl	TA1535	*	0.5	0.016 ul/plate	TA1538	*	*
0.4 ul/pl	TA1535	*	0.1	0.0032	TA1538	*	*

*Calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 5B

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: M3535 Dissolved in: ETOH

Date: 10 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
10 ul/pl	TA98	*	*	0.08 ul/plate	TA1535	*	*
2 ul/pl	TA98	0.14	*	0.016 ul/plate	TA1535	*	*
0.4 ul/pl	TA98	0.28	0.26	0.0032 ul/plate	TA1535	*	*
0.08 ul/pl	TA98	0.09	*				
0.016 ul/pl	TA98	*	0.15	10 ul/plate	TA1537	*	*
0.0032 ul/pl	TA98	0.6	0.26	2 ul/plate	TA1537	*	*
				0.4 ul/plate	TA1537	*	*
10 ul/pl	TA100	*	*	0.08	TA1537	*	*
2 ul/pl	TA100	*	*	0.016 ul/plate	TA1537	*	*
0.4 ul/pl	TA100	*	*	0.0032 ul/plate	TA1537	*	*
0.08 ul/pl	TA100	*	*				
0.016 ul/pl	TA100	*	*	10 ul/plate	TA1538	*	*
0.0032 ul/pl	TA100	*	*	2 ul/plate	TA1538	*	*
				0.4 ul/plate	TA1538	*	*
10 ul/pl	TA1535	*	*	0.08 ul/plate	TA1538	*	*
2 ul/pl	TA1535	*	*	0.016 ul/plate	TA1538	*	*
0.4 ul/pl	TA1535	*	*	0.0032 ul/plate	TA1538	*	0.3

* Calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 5C

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: DEET Dissolved in: ETOH

Date: 10 April 1981 Performed by Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
10 ul/pl	TA98	*	*	0.08 ul/plate	TA1535	0.63	1.3
2 ul/pl	TA98	0.23	0.48	0.016 ul/plate	TA1535	0.32	0.9
0.4 ul/pl	TA98	0.14	0.3	0.0032 ul/plate	TA1535	0.95	1.5
0.08 ul/pl	TA98	0.23	0.07				
0.016 ul/pl	TA98	*	*	10 ul/plate	TA1537	*	*
0.0032 ul/pl	TA98	0.32	0.15	2 ul/plate	TA1537	*	*
				0.4 ul/plate	TA1537	*	*
10 ul/pl	TA100	*	*	0. l/plate	TA1537	*	
2 ul/pl	TA100	*	0.05	0.016 ul/plate	TA1537	*	*
0.4 ul/pl	TA100	0.04	*	0.0032 ul/plate	TA1537	*	0.14
0.08 ul/pl	TA100	*	*				
0.016 ul/pl	TA100	*	*	10 ul/plate	TA1538	*	*
0.0032 ul/pl	TA100	*	*	2 ul/plate	TA1538	*	*
				0.4 ul/plate	TA1538	*	*
10 ul/pl	TA1535	*	*	0.08	TA1538	*	0.06
2 ul/pl	TA1535	0.56	2.3	0.016 ul/plate	TA1538	*	*
0.4 ul/pl	TA1535	1.35	0.5	0.0032 ul/plate	TA1538	0.83	0.06

*Calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 5D

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: DEET Dissolved in: ETOH

Date: 28 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 ul/pl	TA98	*	*	0.008 ul/plate	TA1535	*	0.1
0.2 ul/pl	TA98	0.09	*	0.0016 ul/plate	TA1535	0.16	0.7
0.04 ul/pl	TA98	*	*	0.00032 ul/plate	TA1535	*	*
0.008 ul/pl	TA98	0.09	*				
0.0016 ul/pl	TA98	*	*	1 ul/plate	TA1537	*	*
0.00032 ul/pl	TA98	*	*	0.2 ul/plate	TA1537	*	1.62
				0.04 ul/plate	TA1537	*	*
1 ul/pl	TA100	*	*	0.008 ul/plate	TA1537	*	*
0.2 ul/pl	TA100	*	*	0.0016 ul/plate	TA1537	*	*
0.04 ul/pl	TA100	*	*	0.00032 ul/plate	TA1537	*	*
0.008 ul/pl	TA100	0.08	*				
0.0016 ul/pl	TA100	*	*	1 ul/plate	TA1538	*	0.3
0.00032 ul/pl	TA100	*	*	0.2 ul/plate	TA1538	*	*
				0.04 ul/plate	TA1538	*	*
1 ul/pl	TA1535	*	0.4	0.008	TA1538	*	*
0.2 ul/pl	TA1535	*	*	0.0016 ul/plate	TA1538	*	1.03
0.04 ul/pl	TA1535	*	*	0.00032 ul/plate	TA1538	*	*

* Calculated value resulted in negative MUTAR or zero MUTAR

TABLE 5E

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: RH-393 Dissolved in: ETOH

Date: 10 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
10 ul/pl	TA98	1.02	0.22	0.08 ul/plate	TA1535	1.82	4.3
2 ul/pl	TA98	0.42	1.75	0.016 ul/plate	TA1535	1.9	3.2
0.4 ul/pl	TA98	0.18	0.45	0.0032 ul/plate	TA1535	1.11	3.2
0.08 ul/pl	TA98	0.32	1.08				
0.016 ul/pl	TA98	0.42	0.52	10 ul/plate	TA1537	*	*
0.0032 ul/pl	TA98	0.14	0.63	2 ul/plate	TA1537	*	*
				0.4 ul/plate	TA1537	*	*
10 ul/pl	TA100	0.65	0.63	0.08 ul/plate	TA1537	*	*
2 ul/pl	TA100	0.54	0.78	0.016 ul/plate	TA1537	*	0.41
0.4 ul/pl	TA100	0.31	0.38	0.0032 ul/plate	TA1537	*	*
0.08 ul/pl	TA100	0.19	0.27				
0.016 ul/pl	TA100	*	0.16	10 ul/plate	TA1538	*	*
0.0032 ul/pl	TA100	0.06	0.10	2 ul/plate	TA1538	*	0.3
				0.4 ul/plate	TA1538	*	0.06
10 ul/pl	TA1535	*	5.1	0.08 ul/plate	TA1538	0.12	0.3
2 ul/pl	TA1535	2.7	4.9	0.016 ul/plate	TA1538	*	0.61
0.4 ul/pl	TA1535	1.75	4.0	0.0032 ul/plate	TA1538	*	0.91

* Calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 5F

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: RH-398 Dissolved in: ETOH

Date: 28 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 ul/pl	TA98	0.18	*	0.008 ul/plate	TA1535	0.4	0.8
0.2 ul/pl	TA98	*	*	0.0016 ul/plate	TA1535	*	0.7
0.04 ul/pl	TA98	*	*	0.00032 ul/plate	TA1535	*	0.3
0.008 ul/pl	TA98	0.18	*				
0.0016 ul/pl	TA98	0.05	0.19	1 ul/plate	TA1537	0.33	0.27
0.00032 ul/pl	TA98	0.05	0.07	0.2 ul/plate	TA1537	*	*
				0.04 ul/plate	TA1537	0.16	*
1 ul/pl	TA100	0.19	*	0.008 ul/plate	TA1537	0.33	*
0.2 ul/pl	TA100	0.03	*	0.0016 ul/plate	TA1537	*	*
0.04 ul/pl	TA100	*	*	0.00032 ul/plate	TA1537	0.16	*
0.008 ul/pl	TA100	0.01					
0.0016 ul/pl	TA100	0.11	*	1 ul/plate	TA1538	*	*
0.00032 ul/pl	TA100	*	*	0.2 ul/plate	TA1538	*	0.06
				0.04 ul/plate	TA1538	*	*
1 ul/pl	TA1535	0.87	1.5	0.008 ul/plate	TA1538	*	*
0.2 ul/pl	TA1535	*	0.5	0.0016 ul/plate	TA1538	*	*
0.04 ul/pl	TA1535	*	*	0.00032 ul/plate	TA1538	*	*

* Calculated value resulted in negative MUTAR or zero MUTAR

TABLE 5

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: CHR3 Dissolved in: ETOH

Date: 14 April 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 ul/pl	TA98	0.28	*	0.008 ul/plate	TA1535	0.55	1.6
0.2 ul/pl	TA98	0.28	0.22	0.0016 ul/plate	TA1535	0.48	0.4
0.04 ul/pl	TA98	0.23	0.34	0.00032 ul/plate	TA1535	0.32	0.6
0.008 ul/pl	TA98	0.14	0.78				
0.0016 ul/pl	TA98	0.05	0.37	1 ul/plate	TA1537	0.33	*
0.00032 ul/pl	TA98	*	0.45	0.2 ul/plate	TA1537	0.16	*
				0.04 ul/plate	TA1537	0.49	0.14
1 ul/pl	TA100	0.12	*	0.008 ul/plate	TA1537	0.33	0.41
0.2 ul/pl	TA100	0.10	0.02	0.0016 ul/plate	TA1537	0.16	0.14
0.04 ul/pl	TA100	0.01	*	0.00032 ul/plate	TA1537	*	0.14
0.008 ul/pl	TA100	0.16	0.17				
0.0016 ul/pl	TA100	0.05	0.15	1 ul/plate	TA1538	1.19	*
0.00032 ul/pl	TA100	0.06	0.09	0.2 ul/plate	TA1538	*	0.61
				0.04 ul/plate	TA1538	*	0.36
1 ul/pl	TA1535	0.71	0.40	0.008 ul/plate	TA1538	*	1.15
0.2 ul/pl	TA1535	*	0.10	0.0016 ul/plate	TA1538	*	0.67
0.04 ul/pl	TA1535	0.16	0.80	0.00032 ul/plate	TA1538	*	0.42

* Calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 5H

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: RH-398 Dissolved in: ETOH

Date: 19 August 1981 Performed by: Sauers

Concentration	Strain	MUTAR act	MUTAR	Concentration	Strain	MUTAR act	MUTAR
1 ul/plate	TA 98	0.36	0.19	0.008 ul/plate	TA 1535	0.45	*
0.2 ul/plate	TA 98	0.32	0.14	0.0016 ul/plate	TA 1535	0.27	0.13
0.04 ul/plate	TA 98	0.48	0.24	0.00032 ul/plate	TA 1535	*	0.19
0.008 ul/plate	TA 98	0.12	*				
0.0016 ul/pl.	TA 98	0.20	*	1 ul/plate	TA 1537	*	*
0.00032 ul/pl.	TA 98	*	*	0.2 ul/plate	TA 1537	*	*
				0.04 ul/plate	TA 1537	*	0.15
1 ul/plate	TA 100	0.21	0.02	0.008 ul/plate	TA 1537	*	*
0.2 ul/plate	TA 100	0.14	0.04	0.0016 ul/plate	TA 1537	*	*
0.04 ul/plate	TA 100	0.33	0.17	0.00032 ul/plate	TA 1537	*	0.15
0.008 ul/plate	TA 100	0.16	*				
0.0016 ul/pl.	TA 100	*	*	1 ul/plate	TA 1538	*	*
0.00032 ul/pl.	TA 100	*	*	0.2 ul/plate	TA 1538	*	*
				0.04 ul/plate	TA 1538	*	*
1 ul/plate	TA 1535	0.64	0.13	0.008 ul/plate	TA 1538	*	*
0.2 ul/plate	TA 1535	0.55	0.13	0.0016 ul/plate	TA 1538	*	*
0.04 ul/plate	TA 1535	0.45	*	0.00032 ul/plate	TA 1538	*	*

* Calculated value resulted in negative MUTAR or zero MUTAR

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