UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Registration of MYX 6121 Herbicide (Pelargonic Acid) by Mycogen Corporation (DP Barcode D218309; Submission S489350; ID # 053219-00007; Case 003622). Review of Three Mutagenicity Studies.

TO: Paul L. Zubkoff, Ph.D.
Regulatory Action Leader
Biopesticides and Pollution Prevention Division (7501W)

FROM: Sheryl K. Reilly, Ph.D. SKR
Reviewer
Biopesticides and Pollution Prevention Division (7501W)

THRU: J. Thomas McClintock, Ph.D. JTMc 11/13/95
Team Leader
Biopesticides and Pollution Prevention Division (7501W)

ACTION REQUESTED: Review of an in vivo mouse micronucleus assay, a mouse lymphoma forward mutation assay, and an Ames assay submitted for the registration of MYX 6121 Herbicide.

CONCLUSIONS: The studies are summarized as follows:

§152-17 (MRID 436037-01): Mammalian Cells In Culture Gene Mutation Assay in Mouse lymphoma cells (L5178Y TK+/-). In a mouse lymphoma forward mutation assay, target L5178Y TK+/- cells were exposed for 4 hours to nonactivated doses of 150, 200, 300, 600, 800, 1000, 1200,1400 or 1600 μg/ml and S9-activated concentrations of 37.5, 50, 75, 100, 150, 200, 300, 500, or 600 μg/ml of pelargonic acid and evaluated for mutagenic effects. In activated (+S9) cultures, the test substance doubled the number of mutant colonies and increased the mutation frequency in cells exposed to $\geq$ 300 μg/ml in trial 1 and $\geq$ 50 μg/ml in trial 2; however, this occurred in the presence of increasing moderate-to-severe cytotoxicity and small colony development, and may reflect gross chromosomal changes or damage rather than actual mutational changes within the TK gene locus. Although increases in mutant colonies and mutation frequencies occurred in the nonactivated (-S9) assays, both were below the minimum criteria for the test.

Under the conditions of this study, pelargonic acid (technical grade) **is considered weakly positive for inducing mutations at the TK locus of culture mouse L5178Y TK +/- cells in the presence S9-induced metabolic activation.** The study is classified as **Acceptable** and satisfies the requirements for a genotoxicity study under Subdivision M, §152-17.

§152-17 (MRID 436037-02): *In vivo* Mammalian Cytogenetics - Mouse Micronucleus Assay. In an *in vivo* mouse micronucleus assay, groups of ICR mice (15/sex/dose) were administered single oral doses of 1250, 2500, and 5000 mg/kg n-pelargonic acid. The bone marrow cells were harvested 24, 48, and 72 hours post-treatment. No significant increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) were observed in either sex at any dose; thus, **n-pelargonic acid was negative in the micronucleus assay.** This study is **Acceptable**, and satisfies the Subdivision M guideline requirements for an *in vivo* micronucleus assay in mice under §152-17.

§152-17 (MRID 436037-03): In a reverse gene mutation assay (Ames Test) using pelargonic acid, *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to 100, 333, 667, 1000, 3330 or 5000 μg/plate, with and without metabolic activation with rat liver homogenate microsomal fraction S-9.

Cytotoxicity was observed at 5000 μg/plate in the presence of S9, and at 3330 and 5000 μg/plate in the absence of S9 in tester strain TA100 in a dose range-finding study; however, a mutagenic response was not observed in any *Salmonella* strain at any test dose in an initial and confirmatory mutagenicity assays. Therefore, **pelargonic acid was not mutagenic** under the conditions of this study. This study is **Acceptable,** and satisfies the guideline requirement for a gene mutation study under the Subdivision F Guidelines, §152-17.

Although the studies are acceptable, the Registrant must provide information concerning the lot number and purity of the test substances used in the forward (mouse lymphoma) and reverse (Ames Test) mutation assays.

The data evaluation reports are attached.

S. Reilly/7501W/703-308-8265/Pelrgnic.mem/pelargonic acid/BPPD, 5th Fl., Crystal Station; November 6, 1995

Primary Reviewer: Sheryl K. Reilly, Ph.D. Date: 11/6/95
Secondary Reviewer: J. Thomas McClintock, Ph.D. Date: 11/13/95
Biopesticides and Pollution Prevention Division (7501W)

DATA EVALUATION REPORT

STUDY TYPE:	Mammalian cells in culture gene mutation assay in mouse lymphoma cells (§152-17)
TOX. CHEM. NO.:	217500
MRID NUMBER:	436037-01
TEST MATERIAL:	Pelargonic Acid Technical Grade
SYNONYM(S):	Nonanoic acid; Myx 6121 Herbicide; Scythe Herbicide; Thinex Blossom Thinner
STUDY NUMBER:	15656-0-431R
SPONSOR	Mycogen Corporation, San Diego, CA 92121
TESTING FACILITY:	Hazleton Washington, Inc., Vienna, VA 22182
TITLE OF REPORT:	Mutagenicity Test on Pelargonic Acid (Technical Grade) in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay
AUTHOR(S):	Maria A. Cifone, Ph.D.
REPORT ISSUED:	September 14, 1993

EXECUTIVE SUMMARY: In a mouse lymphoma forward mutation assay, target L5178Y TK+/- cells were exposed for 4 hours to nonactivated doses of 150, 200, 300, 600, 800, 1000, 1200,1400 or 1600 μg/ml and S9-activated concentrations of 37.5, 50, 75, 100, 150, 200, 300, 500, or 600 μg/ml of pelargonic acid and evaluated for mutagenic effects. In activated (+S9) cultures, the test substance doubled the number of mutant colonies and increased the mutation frequency in cells exposed to $\geq$ 300 μg/ml in trial 1 and $\geq$ 50 μg/ml in trial 2; however, this occurred in the presence of increasing moderate-to-severe cytotoxicity and small colony development, and may reflect gross chromosomal changes or damage rather than actual mutational changes within the TK gene locus. Although increases in mutant colonies and mutation frequencies occurred in the nonactivated (-S9) assays, both were below the minimum criteria for the test.

Under the conditions of this study, pelargonic acid (technical grade) **is considered weakly positive for inducing mutations at the TK locus of culture mouse L5178Y TK +/- cells in the presence S9-induced metabolic activation.** The study is classified as **Acceptable** and satisfies the requirements for a genotoxicity study under Subdivision M, §152-17.

A. MATERIALS:

1. Test Cells: Mouse lymphoma L5178Y TK +/- cells

   Source: Burroughs Wellcome Company, Research Triangle Park, NC
   Periodically checked for mycoplasma contamination
   Periodically checked for karyotype stability
   Periodically "cleansed" against high spontaneous background mutation (to TK-/-) using aminopterin or methotrexate

2. Media

   a) Culture Medium: RPMI 1640 supplemented with Pluronic F68, L-glutamine, sodium pyruvate, antibiotics, and heat-inactivated horse serum (10% by volume).

   b) Treatment Medium: Fischer's medium with the same supplements as the culture medium, except that horse serum was reduced to 5% by volume.

   c) Cloning Medium: The same medium as above, except without Pluronic F68, and up to 20% horse serum. Purified BBL agar was added at a concentration of 0.24 % to achieve a semisolid state.

   d) Selection Medium: Cloning medium with 3 μg/ml TFT.

3. Control Materials:

   Negative Control: Culture medium, with and without S9 activation

   Solvent Control: 1% DMSO in culture medium

   Positive Controls:

   a. Nonactivation: Methyl methane-sulfonate (MMS) at final concentrations of 10 and 15 nl/ml.

   b. Activation: 3-methylcholanthrene (MCA) at final concentrations of 2 and 4 μg/ml

4. Activation Mixture:

a. S9 Fraction: Commercially obtained microsomal fraction S9, derived from homogenates of livers from male Sprague-Dawley rats induced with Aroclor 1254 at a dose of 500 mg/kg, 5 days prior to sacrifice.

b. Activation mixture composition:

Component	Final Concentration in Culture
NADP	3 mM
Isocitrate	15 mM
S9	20 μl/ml

5. Test Material: Nonanoic Acid Technical Grade

Description: Clear liquid
Identification number: HWA Assay No. 15656
Purity: Not provided
Receipt date: May 14, 1993
Stability of compound: Not reported
Solvent used: 1% Dimethyl Sulfoxide (DMSO) in culture medium
Other comments: The test material was stored at room temperature, protected from light. Analytical determinations were performed on 100 mg/ml and 300 mg/ml (in DMSO) stock solutions by gas chromatography to verify actual concentrations.

6. Test Compound Concentrations Used:

a. Cytotoxicity assays: Two preliminary assays were performed, with and without S9 activation. The first trial was not adjusted for pH, but the culture medium was adjusted to 7.0-7.2 for the second trial, and the first one was not used to determine cytotoxicity. The doses tested were 0, 250, 500, 1000, 2000 or 4000 μg/ml. A vehicle control, consisting of 1% DMSO was included. After a 4-hour exposure, the cells were washed twice and resuspended in growth medium. A cell count was made for each culture approximately 24 hours later.

b. Mutation assay:

(1) Nonactivated assay:

a) Trial 1: 150, 300, 600, 1000 or 1200 μg/ml were tested; also tested were 10 or 15 nl/ml MMS and a vehicle control.

b) Trial 2: 200, 300, 600, 800, 1000, 1200, 1400, or 1600 μg/ml were tested along with 0 or 15 nl/ml MMS and a vehicle control.

(2) S9-activated assay:

a) Trial 1: 37.5, 75, 150, 300 or 500 μg/ml were tested; also tested were 2 or 4 μg/ml MCA and a vehicle control.

b) Trial 2: 50, 100, 200, 300, 500, or 600 μg/ml were tested along with 2 or 4 μg/ml MCA and a vehicle control.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells were exposed to the test compound or negative, solvent, or positive controls for 4 hours. The two assays were identical, except the activation assay included 10 ml of the S9 activation mixture during the 4-hour treatment period. The activation mixture was prepared immediately before use.

b. After washing, cells were resuspended in 20 ml culture medium and incubated for 2 days (expression period) before cell selection. Cell densities were determined at 24 hours, and cell numbers adjusted to 3 x 10^5 cells/ml to maintain optimal growth rates. If cell density did not reach 4 x 10^5 on the first day after treatment, the cells were returned to the incubator without any adjustment in cell numbers. On day 2, the cell counts were again determined, and appropriate cultures selected for cloning and mutant selection.

c. The cells were seeded at 1x10^6 cells/plate (3 plates with 5 ml each) and cultured for 10–14 days in selection medium to determine the numbers of mutants. To determine cloning efficiency, cells seeded at 200 cells/plate (3 plates) were cultured for 10–12 days in cloning medium. Colony counting and sizing was also performed using an Artek Model 880 colony counter fitted with a 10-turn potentiometer.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

a. Assay validity: The assay was considered valid if (1) the mean cloning efficiency of the negative and solvent control cultures was > 50%, but preferably between 60-130%; (2) the minimum suspension growth of the negative controls for 2 days is 8-fold greater (on average) than the original cell counts; (3) the mutation frequency of the solvent control cultures was between 30 x 10^{-6} and 120 x 10^{-6}, (4) the mutation frequency of the positive control cultures is at least 200 x 10^{-6} ; cell survival must be at least 10-20% of the controls; and (5) the mutant frequencies for at least 3, but preferably 5, treated cultures are determined.

b. Positive result: The test material was considered positive if the mutation frequency was increased at least 2-fold over the solvent control frequency of the test material at a given dose is at least double the background and is dose or toxicity related for at least 3 doses. The result is acceptable only if the relative cloning efficiency is $\geq$ 10% and the number of clones in the selection plating efficiency plates exceeds about 60. It is also considered a positive result if that the mutant frequency for a single dose which is at or near the highest testable toxicity is $\geq$ 4 times the background mutant frequency; this must be confirmed in a second assay, and if not repeatable, this would result in a negative determination at this dose level.

C. REPORTED RESULTS:

1. Cytotoxicity Assay: The test material was less toxic without metabolic activation than with S9 in the cytotoxicity assay. Without activation, total cell killing was observed at 4000 μg/ml , and the 2000 μg/ml was highly toxic, with a cell density of only 0.9% of the vehicle control. With S9 activation, total cell killing was observed at $\geq$ 2000 μg/ml, and the 1000 and 500 μg/ml doses were highly toxic, with cell densities < 5% of vehicle controls.

2. Analytical Determination: Stock solutions of 100 and 300.5 mg/ml of pelargonic acid in DMSO dosing solution were analyzed by gas chromatography to verify actual concentrations, and were found to be approximately 94%-103% of the nominal values.

3. Mutation Assays:

(a) Nonactivated assay: Relative suspension growth generally decreased with increasing dose, from 80% at 150 μg/ml to 9.6% at 1200 μg/ml in trial 1, and 121% at 200 μg/ml to 11% at 1600 μg/ml in trial 2. There

was no cytotoxicity < 600 μg/ml. At 1000 and 1200 μg/ml in trial 1 and 1400 and 1600 μg/ml in trial 2, the number of revertant colonies was increased over the vehicle controls, but not doubled. In trial 1, the mutation frequency at the 1000 μg/ml dose was 85.2 x 10^{-6}, which exceeded the mutation frequency minimum criterion for that trial of 61.6 x 10^{-6}, and thus pelargonic acid appeared to be mutagenic at this dose. However, this was not repeatable in the second trial, and none of the doses (up to 1600 μg/ml) exceeded the mutation frequency minimum criterion of 51 x 10^{-6}, and pelargonic acid was not considered to be mutagenic under the conditions of this study without exogenous metabolic activation.

(b) S9-Activated assay: Relative suspension growth was reduced in a dose-dependent manner at doses, ranging from 94.6% at 37 μg/ml (in trial 1) to 11% at 600 μg/ml (trial 2). Increases in the numbers of mutants per plate were seen at all test material concentrations, and doubled at ≥ 300 μg/ml in trial 1, and at doses ≥ 100 μg/ml in trial 2. Mutation frequencies exceeded the minimum for trial 1 of 136.7 x 10^{-6} at doses ≥ 75 μg/m, and exceeded the minimum of 118.1 x 10^{-6} for trial 2 at all test doses. Colony sizing revealed a bimodal distribution in small and large mutant colonies. An increase in small colonies is consistent with the induction of chromosome deletions.

D. CONCLUSIONS: Based on the overall results, pelargonic acid, in the presence of S9 metabolic activation, induced a weak mutagenic response in mouse TK +/- lymphoma cells. This occurred in the presence of increasing cytotoxicity, however, and may indicate damage to the chromosome carrying the TK locus, rather than actual mutagenicity at that locus.

E. STUDY DEFICIENCIES: The purity of the test material was not indicated in the study. In addition, it is not clear what lot number was used; the only identification was "HWA Assay No. 15656". The registrant must provide this information.

F. QUALITY ASSURANCE MEASURES: The test was performed under GLP standards. A quality assurance statement was signed and dated March 30, 1995.

Primary Reviewer: Sheryl K. Reilly, Ph.D. SKR Date: 11/6/95
Secondary Reviewer: J. Thomas McClintock, Ph.D. JTM Date: 11/13/95
Biopesticides and Pollution Prevention Division (7501W)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity - *In vivo* Micronucleus Assay in Mice (§152-17)

TOX. CHEM. NUMBER: 217500

MRID NUMBER: 436037-02

DP BARCODE: D218309

CASE NUMBER: 003622

SUBMISSION NUMBER: S489350

TEST MATERIAL: n-Pelargonic Acid

SYNONYMS: nonanoic acid; MYX 6121 Herbicide; Scythe Herbicide; Thinex Blossom Thinner

SPONSOR: Mycogen Corporation, 4980 Carroll Canyon Road, San Diego, CA 92121

STUDY NUMBER: 15656-0-455CO

TESTING FACILITY: Hazleton Washington, Inc., 9200 Leesburg Pike, Vienna, VA 22182

TITLE OF REPORT: Mutagenicity Test on n-Pelargonic Acid *in vivo* Micronucleus Assay

AUTHOR: Hemalatha Murli, Ph. D.

REPORT ISSUED: November 17, 1993 (study completion date)

EXECUTIVE SUMMARY: In an *in vivo* mouse micronucleus assay, groups of ICR mice (15/sex/dose) were administered single oral doses of 1250, 2500, and 5000 mg/kg n-pelargonic acid. The bone marrow cells were harvested 24, 48, and 72 hours post-treatment. No significant increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) were observed in either sex at any dose; thus, **n-pelargonic acid was negative in the micronucleus assay**. This study is **Acceptable**, and satisfied the Subdivision M guideline requirements for an *in vivo* micronucleus assay in mice under §152-17.

A. MATERIALS:

1. Test Material: n-Pelargonic Acid
   Description: Clear, colorless liquid
   Identification: n-Pelargonic Acid New Process Hoop
   Purity: concentration in dosing sample (500 mg/ml) found to be 102% of target (by gas chromatography)
   Receipt date: May 14, 1993
   Structure: CH3 (CH2)7 COOH
   Stability: stable for 24 hours at room temp. (tested in laboratory)
   Contaminants: None listed
   Vehicle used: Corn oil

2. Control Materials:

   Negative: None
   Vehicle: Corn oil (dosing volume of 10 ml/kg) was administered by oral gavage.
   Positive: Cyclophosphamide dissolved in deionized water, dosed at 80 mg/kg.

3. Test Compound:

   Route of administration: oral gavage, dosing volume = 10 ml/kg body weight

   Dose levels used:

   1250, 2500, and 5000 mg/kg, in corn oil

4. Test Animals:

   (a) Species: Mouse Strain: ICR Age: 8 weeks
   Weight range: 21.5-37.6 g (males); 21.4-31.0 g (females)

   (b) Source: Harlan Sprague Dawley, Inc., Frederick, MD

   (c) Number of animals used per test dose: 15/sex/dose vehicle, positive control, and test substance

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

   (a) Test compound: Sampling at 24, 48 and 72 hours post-dosing for all dose levels

   (b) Vehicle control: Sampling at 24 hours, post-dosing

(c) Positive control:
Sampling at 24 hours, post-dosing

2. Scoring of bone marrow PCEs and NCEs:

Frequency of PCEs vs. NCEs was determined by scoring the number per 1000 erythrocytes counted.

Percentage or frequency of Micronucleated cells (not individual micronuclei) was expressed as the % PCEs with micronuclei compared with the total # PCEs (1000) counted.

3. Slide Preparation: At 24, 48, and 72 hours after administration of the test material or the vehicle, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the vehicle and positive control groups was 24 hours. Bone marrow cells were flushed from both femurs of each animal with fetal calf serum and centrifuged. Supernatants were discarded; pellets were resuspended in residual supernatant, spread onto slides and air dried. The slides were fixed in methanol, stained with May-Grunwald and Giemsa solutions, coverslipped, coded and scored.

4. Statistical Methods: The results were evaluated for statistical significance using an analysis of variance on the square root arcsine transformation, performed on the proportion of cells with micronuclei/animal. Tukey's Studentized range test with adjustment for multiple comparisons was used at each harvest time to determine which dose groups, if any, were significantly different ($p < 0.05$) from the vehicle control. Analyses were performed separately for each harvest time and sex, and also at each harvest time for the sexes combined.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant increase ($p \leq 0.05$) in micronucleated polychromatic erythrocytes (MPEs) at any test dose compared to the vehicle control was seen.

C. REPORTED RESULTS:

Analytical determinations: Results from the gas chromatographic determinations of dosing solutions indicated that actual concentrations were between 101% and 102% of the nominal concentrations.

Animal observations: One female mouse died during the study (5000 mg/kg dose group, 24 hour observation period); the cause of death was unknown. All other animals remained healthy during the study.

Micronucleus assay: Representative findings from the micronucleus assay are shown in Table 1, below (photocopy of page 18 of the study). The test compound did not increase the % micronucleated PCEs at any dose or interval. By contrast, the

positive control (30 mg/kg CP) induced a significant ($p \leq 0.05$) increase in the % micronucleated PCEs in both sexes.

D. CONCLUSIONS: In this study, n-Pelargonic Acid was adequately tested and found to be negative in this *in vivo* micronucleus assay.

E. QUALITY ASSURANCE MEASURES: The test was performed under, and a quality assurance statement was signed and dated November 17, 1993.

TABLE 1

MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: Mycogen Corporation TEST ARTICLE: n Pelargonic Acid

ASSAY: 15656

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
VEHICLE CONTROL Corn oil	10 ml/kg	24	0.06 ± 0.04	0.06 ± 0.04	0.06 ± 0.03	0.38 ± 0.13	0.73 + 0.10
		48	0.06 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.49 ± 0.07	0.74 ± 0.07
		72	0.08 ± 0.05	0.08 ± 0.02	0.08 ± 0.02	0.45 ± 0.06	0.66 + 0.10
POSITIVE CONTROL Cyclophosphamide	80 mg/kg	24	2.00 ± 0.13*	2.22 ± 0.31*	2.11 ± 0.16*	0.28 ± 0.06	0.63 ± 0.07
TEST ARTICLE	1250 mg/kg	24	0.12 ± 0.04	0.14 ± 0.04	0.13 ± 0.03	0.51 ± 0.13	0.67 ± 0.11
		48	0.02 ± 0.02	0.12 ± 0.06	0.07 ± 0.03	0.49 ± 0.04	0.58 ± 0.16
		72	0.06 ± 0.04	0.06 ± 0.04	0.06 ± 0.03	0.76 ± 0.07	0.32 ± 0.06
	2500 mg/kg	24	0.02 ± 0.02	0.06 ± 0.04	0.04 ± 0.02	0.41 ± 0.11	0.75 ± 0.12
		48	0.10 ± 0.03	0.08 ± 0.04	0.09 ± 0.02	0.31 ± 0.06	0.47 ± 0.16
		72	0.04 ± 0.04	0.04 ± 0.02	0.04 ± 0.02	0.69 ± 0.09	0.48 ± 0.15
	5000 mg/kg	24	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.42 ± 0.09	0.81 ± 0.06
		48	0.04 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.65 ± 0.08	0.76 ± 0.13
		72	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.50 ± 0.14	0.41 ± 0.08

* Significantly greater than the corresponding vehicle control, $p<0.05$.

Primary Reviewer: Sheryl K. Reilly, Ph.D. Date: 11/6/95
Secondary Reviewer: J. Thomas McClintock, Ph.D. Date: 11/13/95
Biopesticides and Pollution Prevention Division (7501W)

DATA EVALUATION REPORT

STUDY TYPE:	Mutagenicity - *Salmonella typhimurium* Reverse Mutation Assay, Ames Test (§152-17)
TOX. CHEM. NO.:	217500
MRID NUMBER:	436037-03
TEST MATERIAL:	Pelargonic Acid Technical Grade
SYNONYM(S):	Nonanoic acid; Myx 6121 Herbicide; Scythe Herbicide; Thinex Blossom Thinner
STUDY NUMBER:	15656-0-401R
SPONSOR:	Mycogen Corporation, San Diego, CA 92121
TESTING FACILITY:	Hazleton Washington, Inc., Vienna, VA 22182
TITLE OF REPORT:	Mutagenicity Test on Pelargonic Acid (Technical Grade) in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)
AUTHOR(S):	Timothy E. Lawlor, M.A.
REPORT ISSUED:	November 29, 1993 (study completion date)

EXECUTIVE SUMMARY: In a reverse gene mutation assay (Ames Test) using pelargonic acid, *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to 100, 333, 667, 1000, 3330 or 5000 μg/plate, with and without metabolic activation with rat liver homogenate microsomal fraction S-9.

Cytotoxicity was observed at 5000 μg/plate in the presence of S9, and at 3330 and 5000 μg/plate in the absence of S9 in tester strain TA100 in a dose range-finding study; however, a mutagenic response was not observed in any *Salmonella* strain at any test dose in an initial and confirmatory mutagenicity assays. Therefore, **pelargonic acid was not mutagenic** under the conditions of this study. This study is **Acceptable,** and satisfies the guideline requirement for a gene mutation study under the Subdivision F Guidelines, §152-17.

A. MATERIALS:

1. Test Material: Pelargonic (nonanoic) Acid, Technical Grade

Description: Clear liquid
Lot Number: Not provided
Purity: Not provided
Receipt date: May 14, 1993
Stability of compound: Not reported
Vehicle: Dimethyl Sulfoxide (DMSO)
Concentration (in DMSO): 100 mg/mL

2. Control Materials:

Vehicle Control: 50 μL DMSO (99%) per plate, with and without S9 activation

Positive Control Materials:

a. Nonactivation:

Sodium azide:	2 μg/plate (TA1535, TA100)
ICR-191;	2 μg/plate (TA1537)
2-Nitrofluorene:	1 μg/plate (TA98, TA1538)

b. Activation: 2-Aminoanthracene: 2.5 μg/plate (all strains)

3. Activation Mixture:

a. S9 Fraction: Commercially obtained microsomal fraction S9, derived from homogenates of livers from male Sprague-Dawley rats induced with Aroclor 1254 at a dose of 500 mg/kg, 5 days prior to sacrifice.

b. Activation mixture composition:

Component:	Volume:
Water	0.70 mL
1M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 mL
0.25 M Glucose-6-phosphate	0.02 mL
0.10 M NADP	0.04 mL
0.2 M $MgCl_2$/0.825 M KCl	0.04 mL
S-9 homogenate	0.10 mL
	1.00 mL

4. Test Strains: The following *S. typhimurium* strains were used in this study to detect mutagens which revert histidine dependence to histidine independence by frameshift mutation and base substitution (obtained from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley):

TA98, TA1537, and TA1538 (detect frameshift mutagens)
TA100 (detects frameshift and base substitution mutagens)
TA1535 (detects base substitution mutagens)

6. Media:

a. Culture Broth: Vogel-Bonner salt solution, supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2 (dry powder).

b. Bottom Agar (25 mL per 15 x 100 mm petri dish): Vogel-Bonner minimal medium E, supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose.

c. Overlay Agar: 0.7% agar (w/v) and 0.5% NaCl (w/v), supplemented with 10 mL of 0.5 mM histidine/biotin solution per 100 mL agar. When S9 mix is required, 2.0 mL of the supplemented top agar is used in the overlay; in nonactivated test cultures, water is added to the supplemented top agar (0.5 mL/2 mL top agar) to assure that the final top agar and amino acid supplement concentrations are equal in activated and non-activated test cultures.

B. TEST PERFORMANCE:

1 Test Compound Concentrations Used:

a. Dose Range-finding Study: Doses of 6.7, 10, 33, 66.7, 100, 333, 667, 1000, 3330, and 5000 μg/plate were evaluated with and without S9 activation with an overnight culture of strain TA100. One plate was used per dose, per condition.

b. Mutation assay: Doses of 100, 333, 667, 1000, 3330, and 5000 μg/plate were evaluated, with and without S9 activation. Each dose was tested in triplicate.

c. Analytical Determinations: Analytical determinations of the highest concentration stock dosing solution (100 mg/mL) as well as a sample of the vehicle used in each trial of the mutagenicity assay indicated that test material concentrations were within 84.1 to 81.3% of theoretical concentrations. It was not thought to impact the results of the study,

however, since the biological endpoint of cytotoxicity was clearly demonstrated at the maximum dose in the initial and confirmatory assays (see results section, below).

2. Preparation and Storage of Test Strains: The test organisms were properly maintained and checked for appropriate genetic markers (histidine dependence, rfa mutation, and ampicillin resistance). The mean number of spontaneous revertants characteristic of the respective strains were determined by plating 100 μL aliquots of the culture with the vehicle on selective media. The test organisms were prepared for storage and testing as follows:

   a. Frozen permanent stocks: DMSO (0.09 mL/mL of culture) was added to fresh overnight cultures of each tester strain, and small aliquots (0.5-1.5 mL) were stored at $\leq$-70°C.

   b. Master plates: Each tester strain from a frozen permanent stock was streaked onto minimal agar supplemented with 260 μM histidine, 3 μM biotin, and 25 μg/mL ampicillin (strains TA100 and TA98 only). Master plates were stored at 5 $\pm$ 3°C.

   c. Preparation of cultures used in test procedures: Colonies from appropriate master plates were inoculated into a flask containing culture broth. The flasks were then placed in a shaker/incubator at 37 $\pm$ 2°C overnight. Cultures were harvested in late log phase, which was determined spectrophotometrically by monitoring culture turbidity; the target density was approximately 0.5 x 10^9 cells/mL.

3. Sterility controls: 50 μL of the most concentrated test article dilution was plated onto selective agar to test for sterility; the S9 mix was tested by plating 0.5 mL on selective agar.

4. Range-Finding and Mutation Assays: The method used was a plate incorporation assay (Ames Test). Similar procedures were used for the range-finding and mutation assays. For the non-activated tests, 100 μL of tester strain, 50 μL vehicle, positive control, or test substance was added to 13 x 100 mm tubes containing 2.5 mL molten selective top agar. When S9 was used, 500 μL of S9 mix, 100 μL tester strain, and 50 μL of the vehicle, positive control, or test substance was added to 2.0 mL of the molten selective top agar. The mixture in the tubes were vortexed, then poured onto a layer of minimal bottom agar (25 mL) in a 15 x 100 mm petri dish. After the overlay had solidified, the plates were inverted and incubated at 37 $\pm$ 2°C for 48 $\pm$ 8 hours. The plates were either immediately counted after incubation, or stored at 5 $\pm$ 3°C until counted. Revertant counts and the condition of the background lawn of growth (relative to the vehicle controls) was determined

for each plate.

5. Evaluation Criteria:

a. Validity: The assay was considered valid if: (1) the genetic markers for the appropriate tester strains (rfa wall mutation, ampicillin resistance) were verified; (2) the mean number of revertant colonies in the vehicle control fell within the provided expected spontaneous range for each tester strain (8-60, TA98; 60-240, TA100;4-45, TA1535; 2-25, TA1537; and 3-35, TA1538); (3) the density of tester strain cultures were $\geq$ 0.5 x 10^9 bacteria/mL; (4) the positive controls induced a $\geq$ 3-fold increase in the number of revertants (with or without S9 activation); and (5) a minimum of three nontoxic dose levels was available for analysis.

b. Positive response: The test material was considered positive if it induced a reproducible, dose-related increase in mutant colonies of strains TA98 or TA100 that was at least $\geq$ 2-fold higher than the solvent control, or $\geq$ 3-fold higher than the solvent control for strains TA1535, TA1537, or TA1538.

C. REPORTED RESULTS:

1. Dose Range-finding Assay Results: Cytotoxicity was observed at 5000 μg per plate in the presence of S9 activation, and at 3330 and 5000 μg per plate in the non-activated cultures. This was evidenced by a reduction in the numbers of revertants per plate and/or a thinning of the bacterial background lawn. The 6 doses used in the mutagenicity assay were based on the range-finding assay.

2. Mutation Assay Results: The summaries of the two mutation assays are presented in Tables 3 and 5, attached (photocopied from pages 30 and 32 of the study submission). All criteria for a valid study were met, and no positive increases in the number of histidine revertants per plate were observed with any of the tester strains, with or without metabolic activation.

D CONCLUSIONS: Under the conditions of this study, pelargonic acid (technical) was negative for mutagenicity in all *Salmonella typhimurium* strains tested, with and without metabolic activation.

E. STUDY DEFICIENCIES: The lot number and purity of the test substance used in this study must be provided by the Registrant.

F QUALITY ASSURANCE MEASURES: The test was performed under GLP, and a quality assurance statement was signed and dated November 29, 1993.

HAZLETON
WASHINGTON

TABLE 3
MUTAGENICITY ASSAY RESULTS
SUMMARY

TEST ARTICLE ID: Pelargonic Acid (Technical Grade)

EXPERIMENT ID: 15656-B1

DATE PLATED: 16-Jun-93 VEHICLE: DMSO

DATE COUNTED: 25-Jun-93 PLATING ALIQUOT: 50 µl

		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
	DOSE/PLATE	TA98		TA100		TA1535		TA1537		TA1538		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL		20	1	109	16	11	3	8	4	14	4	1
TEST ARTICLE	100 µg	23	1	90	10	14	5	7	4	11	1	1
	333 µg	19	8	108	6	11	3	9	3	9	5	1
	667 µg	18	6	100	12	9	4	6	3	12	5	1
	1000 µg	20	4	96	6	12	3	6	3	9	2	1
	3330 µg	14	3	50	7	7	3	0	1	2	1	5
	5000 µg	5	2	10	5	3	1	0	0	0	0	5
POSITIVE CONTROL **		768	213	1065	116	144	9	211	40	1346	20	1
MICROSOMES: None												
VEHICLE CONTROL		10	6	94	9	11	2	7	2	10	3	1
TEST ARTICLE	100 µg	17	5	91	6	13	5	7	2	11	5	1
	333 µg	11	6	87	3	8	1	6	3	8	1	1
	667 µg	10	4	81	4	8	3	6	2	9	2	1
	1000 µg	9	4	68	13	7	3	4	2	6	2	1
	3000 µg	7	3	58	4	4	2	1	1	0	0	2
	5000 µg	5	1	21	14	3	2	0	0	0	0	
POSITIVE CONTROL ***		192	22	545	64	416	28	332	15	309	56	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
TA1538 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

HAZLETON
WASHINGTON

TABLE 5
MUTAGENICITY ASSAY RESULTS
SUMMARY

TEST ARTICLE ID: Pelargonic Acid (Technical Grade)

EXPERIMENT ID: 15656-C1

DATE PLATED: 30-Jun-93 VEHICLE: DMSO

DATE COUNTED: 06-Jul-93 PLATING ALIQUOT: 50 µl

		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
	DOSE/PLATE	TA98		TA100		TA1535		TA1537		TA1538		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL		21	5	96	19	9	3	7	1	15	5	1
TEST ARTICLE	100 µg	24	3	82	15	13	4	5	3	14	2	1
	333 µg	17	5	112	9	11	4	6	2	17	4	1
	667 µg	18	5	97	9	7	3	5	0	9	2	1
	1000 µg	12	1	90	13	9	5	4	1	14	4	1
	3330 µg	8	1	65	21	5	2	0	0	5	2	1
	5000 µg	4	3	32	4	1	1	0	0	0	0	1
POSITIVE CONTROL **		837	43	1028	153	134	18	210	14	1126	30	1
MICROSOMES: None												
VEHICLE CONTROL		14	2	94	21	10	2	7	4	8	5	1
TEST ARTICLE	100 µg	14	4	80	19	9	2	4	2	8	3	1
	333 µg	9	2	81	5	9	2	4	2	8	3	1
	667 µg	15	4	78	6	6	3	5	1	5	1	1
	1000 µg	9	1	62	14	6	1	2	3	4	3	1
	3330 µg	5	2	47	6	2	2	0	0	0	0	3
	5000 µg	5	4	11	6	2	3	0	0	0	0	3
POSITIVE CONTROL ***		171	36	425	28	357	13	296	17	255	18	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
TA1538 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced
4 = extremely reduced 5 = absent 6 = obscured by precipitate
sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

13544

R140145

Chemical: Nonanoic acid

PC Code:
217500

HED File Code: 41500 BPPD Tox/Chem
Memo Date: 11/13/1995
File ID: DPD218309
Accession #: 000-00-9002

HED Records Reference Center
3/23/2007