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

JUN 17 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of Submitted Mutagenicity Studies on Isoxaben

FROM: Kerry L. Dearfield, Ph.D.
Geneticist
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Margaret Jones
Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Reto Engler, Ph.D.
Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Chemical: Isoxaben CAS# 82558-50-7 Caswell #419F

This reviewer has been requested to examine and summarize mutagenicity studies concerning Isoxaben that have been submitted to OPP. Five studies had been previously submitted and already reviewed (they are the studies with Document numbers). Five additional studies have been submitted and they are the focus of this effort (they are the studies with MRID numbers). The following is a listing of all these studies with their result and classification for acceptance:

Acceptable Studies:

Salmonella assay: negative, Document #005732
Mouse lymphoma gene mutation assay: negative, Document #005750
CHO/aberrations: negative, MRID #404359-01
Mouse micronucleus: presumptive positive, suggested repeat Document #005732
Mouse micronucleus: second study, confirms first study finding, weak positive, MRID #404857-04
SCE/Chinese hamster bone marrow: negative, MRID #404857-03

1

Study Reevaluated:

UDS/primary rat hepatocytes: negative, previously considered acceptable, but no toxicity noted, therefore, could justify higher concentrations and study should be unacceptable, Document #005750

Unacceptable Studies:

Salmonella and E. coli reverse mutation assays: negative, need analytical information for upgrade, MRID #404857-01 (additional study from above Salmonella study)

E. coli reverse mutation assay: negative, additional study from previous report, need analytical information for upgrade, MRID #404857-02

Rat dominant lethal: negative, no concurrent or historical (within 1 year of study) positive control submitted; no toxicity noted, therefore could justify higher dosing, Document #005732

I. Individual assessments

A. Bacterial assays

A Salmonella assay (Document #005732) had been previously reviewed by a separate reviewer. The test article was negative in strains TA98, TA100, TA1535, TA1537 and TA1538 with and without activation from Aroclor-induced rat liver. Concentrations up to 500 ug/plate, the apparent limit of solubility, were tested.

A second Salmonella assay (MRID #404857-01) was submitted with the test article assayed in strains TA98, TA100, TA1535 and TA1537 with and without activation from Aroclor-induced rat liver. Preliminary studies indicated little toxicity to cells up to 5000 ug/plate, but appreciable precipitate was seen at concentrations greater than 500 ug/plate. This concentration was used as the top concentration for the mutation assay. Triplicate plates were used per concentration and the controls appeared adequate. The results were negative. However, this study was considered unacceptable as the purity of the test compound at the time of the assay was not reported. This study can be upgraded to acceptable upon receipt of appropriate information concerning the test article.

Two E. coli reverse mutation assays were performed with the test article (MRID #404857-01 and #404857-02). Both assays were performed with strain WP2 uvrA$^-$. The conditions were the same as the second Salmonella assay reported above. The results were negative, but the study was considered unacceptable as the purity of the test compound at the time of the assay was not reported. These studies can be upgraded to acceptable upon receipt of appropriate information concerning the test article.

B. Mouse lymphoma gene mutation assay (Document #005750)

This study was reviewed earlier by a separate reviewer and was considered negative and acceptable. Mouse lymphoma cells were exposed to test article for four hours with and without activation from Aroclor-induced rat liver at concentrations up to 250 ug/ml without activation and 12 ug/ml with activation. Survivals ranged from 31 - 130% for non-activated conditions and 3 - 104% for activated conditions. The range of concentrations for the without activation portion may need to be extended to obtain higher more acceptable toxicity (10 - 20% range survival). The controls appeared adequate.

C. CHO/aberrations (MRID #404359-01)

The test article was tested for induction of aberrations in cultured Chinese hamster ovary (CHO) cells with and without exogenous activation. A preliminary toxicity test indicated cytotoxicity at 83.5 and 250 ug/ml without activation (percent relative growth was about 30% of controls and the monolayer appeared reduced). No apparent toxicity was seen with activation up to 250 ug/ml, but there was precipitate at this concentration. Therefore, the top concentrations used were 200 ug/ml and 80.1 ug/ml with and without activation, respectively, for the aberration test. For non-activated conditions, cells were exposed for 7.3 or 17.5 hours, washed and then incubated further for a total of 10 or 20 hours from the beginning of compound exposure. Colcemid was added for the last 2 - 2.5 hours of incubation. For activated conditions, cells were exposed for 2 hours with test article and S9, washed and incubated for a total of 10 or 20 hours with Colcemid added for the last 2 - 2.5 hours. All treatments were performed in duplicate and the positive controls appeared adequate. There were no apparent increases in the aberration frequency for any tested condition.

D. Mouse micronucleus assays

The first submitted mouse micronucleus assay (Document #005732) was reviewed earlier by a separate reviewer. It was considered a presumptive positive and it was suggested that an adequate repeat be performed to clarify the initial results. Following toxicity testing, 3 groups of 10 male Swiss mice were intubated twice 24 hours apart at a dose of 5000 mg/kg. They were sacrificed 24, 48 and 72 hours following the second dose. Solvent (peanut oil) and positive (benzene) controls were killed 24 hours after dosing. Two thousand polychromatic erythrocytes (PCEs) per animal were analyzed. No toxicity was noted at this dose, but 2 of 5 animals died in the preliminary test at 10,000 mg/kg. Slight, but statistically significant increases in micronuclei were seen at 24 hour (0.22) and 48 hour (0.17), but not at 72 hour (0.16) versus solvent control (0.10). The

reviewer considered this inconclusive and until subject to a repeat, a presumptive positive. The repeat was recommended to include females and sampling at 12, 24, 36 and 48 hours post dosing.

The second micronucleus test was apparently performed before the repeat suggestion from the previous review was provided as this study also only used male mice. Ten mice per treatment group were given 2 oral doses 24 hours apart at doses of 800, 2000 or 5000 mg/kg. They were killed 24 hours after the second dose and bone marrow harvested. Two thousand cells per animal were examined. Again, statistically significant increases in micronuclei induction were obtained at 800 and 2000 mg/kg (0.25 and 0.22, respectively, versus 0.11 solvent control (peanut oil)). At the 5000 mg/kg dose, there was a less statistically significant increase (0.18; the authors suggest a possible cytotoxic action may mask the genotoxic effect). This study appears to support the previous finding that the test article is a positive, but weak inducer of micronuclei in male mice. Although female mice were still not utilized, these assays together suggest positive activity and are considered acceptable.

E. Rat dominant lethal assay (Document #005732)

This assay was previously reviewed by a separate reviewer. It was considered negative and provisionally acceptable based upon receipt of appropriate positive control data (concurrent or historical (within 1 year of this test)). Male F2 Wistar rats from a 3-generation study were maintained on diets with 0, 0.05, 0.25 and 1.25% test article (0, 500, 2500 and 12,500 ppm or 0, 34, 173 and 932 mg/kg/day, respectively) until they were 19 weeks old. They were then mated to females (1:1) for two 7-day mating periods. Twenty-five males per treatment group were used. Females were sacrificed on day 20 of gestation.

No treatment related toxicity or effect on fertility or mating performance were observed in treated males. No evidence of a dominant lethal effect was suggested by the authors. The data presented in the DER suggest that there is a slight elevation in the percent of postimplantation loss in females mated to treated males. For example, at week 1 at 0.25% intake, there is a 8.36 $\pm$ 2.27 postimplantation loss versus 4.94 $\pm$ 1.00 control, and at week 2 at 0.25% intake, there is a 14.16 $\pm$ 4.69 loss versus 6.84 $\pm$ 1.70 control. Therefore, based on these results and no observed toxicity, higher dosing may have been appropriate.

Also, there were no positive data submitted with this study. Acceptable positive data from a concurrent positive control or positive control data from the same laboratory in the same strain of rat from a study performed within one year of this study would be appropriate.

F. SCE/Chinese hamster bone marrow (MRID #40483?-03)

Chinese hamsters were exposed orally to test article dissolved in peanut oil and then bone marrow analyzed for sister chromatid exchanges (SCE). At time zero, bromodeoxyuridine (BrdU) pellets were implanted and 4 hours later, test article at doses of 800, 2000 and 5000 mg/kg was delivered. At 19 hours, colchicine was given i.p. and at 22 hours, the animals were killed and bone marrow harvested. Bone marrow from 9 male hamsters/treatment group were analyzed with 50 mitoses/animal (25/slide, 2 slides) examined. The results were negative and the positive controls appeared adequate.

G. UDS/primary rat hepatocytes (Document #005750)

Two separate unscheduled DNA synthesis (UDS) assays were performed under similar conditions. These were reviewed earlier by a separate reviewer and considered negative and acceptable. Shortly after rat hepatocytes were prepared, test article in the concentration range of 0.5 - 1000 nmoles/ml was exposed to the cells for 20 hours. Cells were prepared for autoradiography and 20 cells/treatment were counted. Neither cytotoxic or UDS effects were seen in cells in either assay. Positive controls appeared adequate. The original review classified these assays as negative and acceptable. There is a negative response, but it appears that due to the lack of any cytotoxicity, higher concentrations may need to be used.

II. Overall Conclusions

Based upon the acceptable studies submitted for isoxaben, the three areas of mutagenicity testing have been minimally satisfied. The weight-of-evidence suggests that there is not an overt mutagenicity concern for isoxaben at this time. The only positive activity found for isoxaben in any assay was the weak statistically significant activity found in the mouse micronucleus test. The authors suggest that this is due to lower than usual vehicle controls and that the values found in treated animals are within their historical background. This is not an implausible evaluation and the activity itself does not suggest a major effect.

Overall, the data do not suggest a driving need to perform further testing at this time. If further information suggest, additional testing may be necessary in the future, particularly in regards to evaluating the micronucleus response in male and female mice as well as a more carefully dosed dominant lethal assay.

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

MAY 19 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of 5 mutagenicity studies on Isoxaben® (EL-107); Response to registrant letter of 12/4/87 which comments on Toxicology Branch Peer Review of Isoxaben®

TO: Richard Mountfort, PM 23
Registration Division (TS-767)

FROM: Margaret L. Jones Margaret L. Jones 17 May 1988
Review Section III
Toxicology Branch
Hazard Evaluation Division

THROUGH: Marcia van Gemert, Ph.D., Head
Review Section III M. van Gemert 5/19/88
Toxicology Branch

and Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division 5/19/88

Chemical: Isoxaben®; EL-107; Gallery 75 Dry Flowable

Tox. Chem: 419F Record No.: 216479, 216480

Registrant: Elanco

Accession No.: 404857-03, 404857-04, 404359-01
MRID 404857-02, 404857-01

Action Requested: Review 5 mutagenicity studies which were submitted to fulfill the data requirments for mutagenicity testing. In addition, the registrant addresses and raises several issues:

1. Acute oral LD_{50} testing
2. Discussion of genotoxic evidence on Isoxaben®
3. Disagreement with Toxicology Branch Peer Review classification of Isoxaben® as a Category C oncogen (without quantitative risk assessment). Environ Corporation evaluation of the toxicology data on Isoxaben®.

Discussion and Conclusions:

The review of the 5 mutagenicity studies concluded the following:

The in vitro cytogenetic assay using CHO cells was negative for induction of chromosomal aberrations. The study was acceptable.

The in vivo SCE assay using Chinese hamster bone marrow was neative in demonstrating genotoxic potential. The study was acceptable.

Two Ames assays using Salmonella typhimurium and Escherichia coli strains showed Isoxaben® did not induce reverse mutations in the strains tested up to 500 ug/plate (the limit of solubility). The reports did not contain test substance analysis and were therefore unacceptable.

A repeat mouse micronucleus study using males only showed statistically significant increases in micronuclei formation at all doses. The study was acceptable.

The acute toxicity data was properly identified in the recent Registration Standard for Isoxaben. This issue is no longer of concern, however, we appreciate the clarification from the registrant.

In order to address the mutagenicity question for Isoxaben, Toxicology Branch is looking at all the mutagenicity data for this chemical and expects a comprehensive opinion by mid-June. At the same time, we are looking at the issues raised in the independent report (Environ Corp.) which concern the carcinogen classification for this chemical. We will carefully consider each point where the report differs from the EPA assessment and decide whether a reevaluation by the Toxicology Branch Peer Review will be necessary. We will inform the Product Manager, Mr. Mountfort, of the results of our deliberations.

Attachments

Reviewed by: Margaret L. Jones
Review Section III
Toxicology Branch
Hazard Evaluation Divison (TS-769)

Approval: Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-769)

and Kerry Dearfield, Ph.D.
Scientific Mission Support Staff
Toxicology Branch (TS-769)

Chemical: EL-107; Isoxaben; N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (compound 121607) and isomers

Caswell No.: 419F MRID:

Record No.: 216480, 216479 CAS No: 82558-50-7

Accession No.: 404359-01 Guideline: 84-2

Study Type: In vitro cytogenetic assay/chromosome aberration

Citation: Mutagenicity Test on EL-107 (Compound 121607) in an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells

Authors: Murli, H.

Study Date: August 12, 1987; Study initiated June 9, 1987, completed June 19, 1987

Sponsor: Elanco Products Co.

Laboratory: Hazleton Laboratory America, Inc.
5516 Nicholson Lane, Suite 400
Kensington, Maryland 20895

Study Nos.: HLA Study No.: 9686-0-437, Project No.: 20990

Toxicology Branch Conclusions: EL-107 (compound 121607) was found to be negative for induction of chromosomal aberrations at 80, 40, 20, and 10 ug/ml without S-9 and at 200, 100, 50, and 25 ug/ml with metabolic activation.

Recommendation: Acceptable.

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Detailed Review

Test Compound: EL-107 (compound 121607); Lot No.: 617AS5; Description: white powder (description in cytotoxicity test was "off-white powder"); Date received by Hazleton Laboratories: December 23, 1986; Purity: Analysed 8/1/86, Compound 121607: 91.3% (N-[3-(1-Ethyl1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide) and [redacted]

Dose Selection: The sponsor specified dose levels to be tested in this assay, as reported in the cytotoxicity assay appended to the report. Dose levels to be used in the metabolically activated portion were higher than in the non-activated portion due to variations in observed toxicity.

Doses in non-activated portion: 80, 40, 20, 10, 5 ug/ml
Doses in activated portion: 200, 100, 50, 25, 12.5 ug/ml

Cytotoxicity and precipitation were observed in the non-activated assay in duplicate CHO cultures from 83.5 ug/ml through 250 ug/ml. Cytotoxicity was observed at 250 ug/ml in an initial assay with metabolic activation. However, the results were found to be inconsistent and this portion was repeated. The repeat assay with metabolic activation showed consistent results and no evidence of cytotoxicity was noted although slight precipitation was observed at 250 ug/ml. For this reason, the high dose selected was 200 ug/ml for the activated portion.

Procedure: The purpose of the procedure was to determine the ability of EL-107 to produce chromosomal aberrations in an *in vitro* culture of Chinese Hamster Ovary (CHO) cells with and without metabolic activation.

Protocol no. 437, edition 12 from Hazleton Laboratories America as modified for Lilly Research Laboratories was used to plan the assay. A preliminary assay for cytotoxicity using protocol no. 440, edition 1 from the same source was used to determine doses for the assay.

Solubility of the test substance was first tested by dissolving the substance in dimethyl sulfoxide (DMSO) at 37°C up to 379,000 ug/ml, however, precipitation formed at room temperature. Substance dissolved in DMSO at 296,000, forming a clear yellow solution, but when mixed with culture medium, precipitation was observed. The original desired concentration was 10 mM which was equivalent to 3,320 ug/ml based on a molecular weight of 332, provided by the sponsor. The substance was not soluble at this level, and 250 ug/ml was selected to be the top dose, where some solubility had been observed. Five concentrations were selected from 0.00835 ug/ml through 250 ug/ml (half-log

intervals). The actual doses tested are indicated above. Positive controls with metabolic activation were 2-aminoanthracene for E. coli.

For the chromosomal aberration assay, duplicate cultures were used at each dose level and both assays were conducted usimg 10 and 20 hour fixation times. Single cultures were used for negative control, solvent control, and at two doses of positive control. Chromosome aberrations were evaluated from the four highest doses of activated and non-activated treatment groups and from the highest dose of positive controls.

Mitomycin C (MMC) was used as positive control for the non-metabolically activated groups, and cyclophosphamide (CP) was the positive control used for the metabolically activated groups. The following concentrations were used.

	10 hr. assay	20 hr. assay
MMC	0.5 ug/ml	0.04 ug/ml
	1.0 ug/ml	0.08 ug/ml
CP	25 ug/ml	12.5 ug/ml
	50 ug/ml	17.5 ug/ml

For the non-activated assay, CHO cells were exposed to test article for 7.5 hours. For the activation assay, CHO cells were exposed to test article for 2 hours in the presence of S-9 fraction, (from Aroclor 1254-treated rats). In both assays, test article (and S-9 fraction in the activation assay) was then washed off and cells reincubated with culture medium for the appropriate time interval. Colcemid® was added to the cultures 2.5 hours before termination.

Prior to harvest, visual observations of t xicity were made. Metaphase cells were collected by mitotic shake-off (Terasima and Tolmach, 1961) and then treated with 0.075 M KCl. Fixation and slide preparation followed these steps.

Cells with "good morphology" (as defined by the authors, these cells posessed 19-23 centromeres) were selected for analysis. One hundred cells from each duplicate culture from four dose levels and negative and solvent controls were analysed. Twenty-five cells from positive controls were analysed.

Evaluation included the following five points, as copied from test report 9686-0-437:

1. The overall chromosomal aberration frequencies.
2. The percentage of cells with any aberratons.
3. The percentage of cells with more than one aberraton.
4. Any evidence for increasing amounts of damage with increasing dose, i.e., a positive dose response.
5. The estimated number of breaks involved in the production of the different types of aberrations which were observed, i.e., complex aberrations may have more significance than simple breaks.

Statistical analysis: Fischer's Exact Test with an adjustment for multiple comparisons (Soka? and Rohlf, 1981) to compare percentage of cells with aberrations in each treatment group with the results from the pooled solvent and negative controls. Significance was demonstrated when $p<0.01$.

Reported Results:

Assay without S-9 activation: A precipitate was observed at 80.? ug/ml in both 10 and 20 hour assays. No observations of toxicity were reported. Analyses of results from the 10 and 20 hour assays at 10, 20, 40, and 80 ug/ml concentrations showed no significant increase in chromosomally aberrant cells in either assay.

Assay with S-9 activation: A visible precipitate was noted at dosing and at harvest at 100 and 200 ug/ml in the 10 hour assay and at harvest in the 20 hour assay. Signs of toxicity noted were reductions in monolayer confluence at 200 ug/ml in 10 and 20 hour harvests, at 100 ug/ml in the 10 hour harvest and a moderate reduction in visible mitotic cells at 200 ug/ml in the 10 hour harvest. Concentrations analysed in the 10 and 20 hour harvests were 25, 50, 100, and 200 ug/ml. No significant increases in chromosomally aberrant cells were found in the analysis.

The test report concluded that EL-107(compound 121607) is negative for induction of chromosomal aberrations in CHO cells under conditions of metabolic activation and without metabolic activation.

Toxicology Branch Evaluation:

Results are found in appended pages 1-4 from test report HLA 9686-0-437. Positive controls demonstrated the sensitivity of the cell line used in the assay with and without metabolic activation. True chromosomal aberrations were not produced under the conditions of this assay. Small numbers of chromatid gaps and chromosome gaps were noted. In most cases the percents were small (less than 10%) and the authors did not consider these abnormalities to be true aberrations.

According to the study results, EL-107 (compound 121607) appears to be negative for induction of chromosome aberrations in Chinese hamster ovary cells.

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Pages 12 through 15 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

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X FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Margaret L. Jones
Review Section III
Toxicology Branch
Hazard Evaluation Division (TS-769)

Approval: Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-769)

and Kerry Dearfield, Ph.D.
Scientific Mission Support Staff
Toxicology Branch (TS-769)

Chemical: EL-107; Isoxaben MRID:

Caswell No.: 419 CAS No: 82558-50-7

Record No.: 216479, 216480 Guideline: 84-2

Study Type: Sister chromatid exchange in Chinese hamsters (in vivo)

Citation: Mutagenicity Test on EL-107 (Compound 121607) Research for Possible Mutagenic Potentiality of EL-107 Using the Technique of Sister Chromatid Exchanges in the Chinese Hamster

Authors: Siou, G., Lerond-Conan, L., El Haitem, M., LaCrampe, M.

Study Date: October 31, 1984; Report submitted to EPA December 1987

Sponsor: Eli Lilly, 203 Bureaux de la Colline, 92213 Saint Cloud, France

Laboratory: C.E.R.T.I., Histopathology Laboratory
59 Ave. de Paris, 78000 Versailles

Study No: C.E.R.T.I. 886

Toxicology Branch Conclusions: EL-107 did not demonstrate a genotoxic potential as measured in the counting of sister chromatid exchanges in bone marrow cells from male Chinese hamsters administered 800, 2000, or 5000 mg/kg test substance. Sensitivity of the test species was demonstrated using methylmethanesulfonate (MMS). No conclusions can be drawn about the effect of EL-107 on female Chinese hamsters.

Recommendation: Acceptable

Detailed Review

Test Compound: EL-107, Batch No. HC2-2G6-118; Description: white, slightly water-soluble, powder; Purity: analysed 12/8/82 and amended 8/11/83, 82.2% compound 121607, [redacted] The compounds are identified as follows: 121607: N-[3-(1-Ethyl-1-methylpropyl)-5-isoxazolyl]2,6-dimethoxybenzamide; [redacted]

Test animals: Adult male Chinese hamsters (Cricetulus griseus) were used, weighing about 30 g and from the Lessieux Company (95 Bray-Lu). Animals were acclimated for one week, and housed 5/cage in polypropylene cages. Animals were fed UAR complete food (Ref. 105) and "drinking water" (source unspecified). Composition of food was approximately 70% cereal, sugar and fat, 11% vegetable proteins, 14% animal proteins and 4% vitaminized mineral compound. Each animal was given 15-20 g/day depending on age and weight and water ad libitum.

Animals were assigned to one of 5 groups with 10-12 animals per group. Negative (vehicle) controls received peanut oil, positive controls received methylmethanesulfonate (MMS) and treated animals received either 800, 2000, or 5000 mg/kg EL-107. Table I adapted (from Tables I and II from test report 886) shows the number of animals treated, the number examined, and the fate of other animals which did not reach examination. The study authors indicated the "quality of certain preparations was insufficient" as the reason fewer animals were examined than treated. As stated in the test report, the samples from some animals had low mitotic indices or sister chromatids were poorly differentiated. For this reason, such preparations were not examined.

Preliminary Toxicity Test: Doses of 10,000 and 5000 mg/kg were used in five animals each and no mortality was observed at either dose. The study authors stated that anesthetic shock and/or administration of 5-bromodeoxyuridine (5-BrdU) can weaken animals, as found in previous experiments, therefore 5000 mg/kg was chosen to be the high dose for the experiment.

Dose Selection: Rationale for selection of 800 and 2000 mg/kg was not stated in the test report. The rationale for selection of 5000 as the high dose appears in preliminary toxicity section. Ten to 12 animals were treated as follows:

Batch I:	negative control- peanut oil	
Batch II:	positive control- MMS	32.5 mg/kg
Batch III:	EL-107	800 mg/kg
Batch IV:	EL-107	2000 mg/kg
Batch V:	EL-107	5000 mg/kg

Doses were administered in a volume of 0.25 ml per 10 g of bodyweight.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Principle of the study: Mutagenic agents increase the already spontaneous rate of sister chromatid exchanges (SCE). Bone marrow cells are examined after in vivo incorporation of 5-bromodeoxyuridine, a structural analogue of thymidine. Any differences in sister chromatids, which correspond to differences in incorporation of 5-BrdU, can be counted following staining techniques. Fifty mitoses per animal were examined by two observers on two different slides, each reading 25 diploid mitoses (22 chromosomes).

Statistical analysis of results: Results were analysed using a statistical program on an Apple IIe micro-computer. Two averages were compared using Student's t test or the u test of Mann and Whitney where variances were not similar.

Study results and author's conclusions:

Mitoses in the metaphase stage were selected for examination. Several animals showed low mitotic index or insufficient number of marked mitoses and were not examined. Animals with these deficiencies were observed in all groups (4 in vehicle control, 2 in positive control, 3 in the high and low dose and 1 in the mid dose group of animals treated with EL-107) and the authors concluded these deficiencies were not compound related. The positive control batch demonstrated the sensitivity of the test species, with increased numbers of SCE (18.0 $\pm$ 2.1) compared to vehicle controls (6.1 $\pm$ 0.3). The authors concluded the animals responded normally to a genotoxic reference substance. Results are summarized in Table I. Average numbers of SCE per group were as follows:

Batch III	800 mg/kg	6.0 $\pm$ 0.3
Batch IV	2000 mg/kg	6.2 $\pm$ 0.4
Batch V	5000 mg/kg	5.7 $\pm$ 0.4

Upon statistical analysis, none of the animals treated differed significantly from the negative (vehicle) control group and in these analyses, $p>0.05$, according to the Student t test.

The study authors concluded no genotoxic potentiality was demonstrated in the SCE study using Chinese hamsters administered EL-107.

Toxicology Branch conclusions:

Toxicology Branch agrees with the study authors. However, the conclusions must be confined to statements about male Chinese hamsters since no females were tested. Toxicology Branch also agrees that 5000 mg/kg is a sufficiently high dose to have used in this study. MMS demonstrated the sensitivity of the test species.

Table I. Treatment groups for Chinese hamsters administered EL-107 and results [Adapted from Tables I and II from study report C.E.R.T.I. 886]

Dose	Number of animals				
	Treated	Dead	Killed	Examined	Average Number of SCE m + 2 Sm
Negative Control peanut oil	11	0	11	7	6.1 + 0.3
Positive Control MMS 32.5 mg/kg	9	0	9	7	18.0 ± 2.1*
EL-107					
800 mg/kg	12	0	12	9	6.0 ± 0.3
2000 mg/kg	10	0	10	9	6.2 ± 0.4
5000 mg/kg	12	0	12	9	5.7 ± 0.4

*Statistically significant difference at $p<0.01$ (Mann and Whitney)

Reviewed by: Margaret L. Jones Margaret L. Jones 17 May 1988
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Hazard Evaluation Divison (TS-769)

Approval: Marcia van Gemert, Ph.D., Head M. van Gemert 5/19/88
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and Kerry Dearfield, Ph.D. Kerry Dearfield 5.17.88
Scientific Mission Support Staff
Toxicology Branch (TS-769)

Chemical: EL-107; Isoxaben;

Caswell No.: 419F MRID: N/A

Record No.: 216480, 216479 CAS No: 82558-50-7

Accession No.: 404857-04 Guideline: 84-2

Study Type: Mouse micronucleus

Citation: Mutagenicity Test on EL-107 (Compound 121607) Research for Possible Genotoxic Potentiality of EL-107 Using the Micronucleus Technique in the Mouse

Authors: Siou, G., Lerond-Conan, L., et. al.

Study Date: November 20, 1984; Report submitted December 9, 1987

Sponsor: Eli Lilly, 203 Bureau de la Colline, 92213 Saint Cloud

Laboratory: Laboratoire d'Histopathologie - CERTI
59 Avenue de Paris
78000 Versailles France 953.49.76

Study Nos.: C.E.R.T.I 894

Toxicology Branch Conclusions: This repeat study in which EL-107 was administered to male mice at 0, 800, 2000, and 5000 mg/kg confirmed an earlier presumptive positive result in males. The results showed statistically significant increases in micronuclei formation at all doses (See discussion of discrepancies in study report). The study confirms the preliminary conclusion that EL-107 induces a cytogenetic effect in male mice.

The repeat study provides no information on females. However, this deficiency does not compromise the positive result. If further mutagenicity testing is done the Agency would recommend using both males and females.

Recommendation: Acceptable.

Detailed Review

Background and Objective: A previous study with EL-107 (study 871, 9/6/84) using the micronucleus test by the same laboratory had found a statistically significant increase in the rate of formation of polychromatophilic erythrocytes in animals killed at 24 and 48 hours but not at 72 hours. A repeat study was performed in November, 1984 to confirm or invalidate the results of the earlier study.

In the Toxicology Branch Evaluation of the earlier study (No. 871), several recommendations were made for the repeat study (see Mauer review of 1/8/87, appended pages 1-3) which included testing an equal number of females as males, demonstrate cytotoxicity at an an MTD (higher than 5000 but less than 10000 mg/kg), and start sampling at 12 hours post dosing.

Since the repeat assay was performed before the original assay was reviewed, none of these recommendations could be incorporated into the repeat assay.

Test Compound: EL-107 (compound 121607); Batch No. H-02-2G6-118; Description: white slightly water-soluble powder; Purity: analysed 12/8/82 and amended 8/11/83, 82.2% compound 121607, [redacted] The compounds are identified as follows: 121607: N-[3-(1-Ethyl-1-methylpropyl)5-isoxazolyl]-2,6-dimethoxybenzamide; [redacted]

Dose Selection: Doses used in the previous study were selected to be used in the repeat assay. Fifty male mice were divided into 5 groups of 10 each and distributed as follows:

Group 1: vehicle control using peanut oil

Group 2: EL-107; 2 doses of 800 mg/kg

Group 3: EL-107; 2 doses of 2000 mg/kg

Group 4: EL-107; 2 doses of 5000 mg/kg

Group 5: Positive control, benzene; 2 doses of 1.25 ml/kg

Doses were administered in a volume of 0.25 ml/mg bw. Animals were dosed twice, 24 hours apart and were killed 24 hours after the second dose. The apparent justification for using males only was a statement by the authors that age and sex can condition sensitivity of the animals to a clastogenic agent (p 12, test report 894).

Cytotoxicity: Results of an earlier study were used to select doses for the repeat assay. A separate test for cytotoxicity was apparently not performed in this assay.

Assessment: Bone marrow polychromatophilic erythrocytes (PCE, 2000 from each animal) were scored for the presence of micronuclei (1000 each by two different examiners). Statistical analysis of results was performed using an Apple IIe microcomputer program which compared two averages with the Student's t test or with the Mann-Whitney U test, where variances were not similar.

Study results and authors' conclusions:

Results are shown in appended page 4 from test report 894. The positive control, benzene, demonstrated the sensitivity of the test species to a clastogenic substance.

EL-107 results were similar to the original assay. Statistically significant increases in PCE carriers of micronuclei were noted at 300 and 2000 mg/kg but the increase at 5000 mg/kg was not found statistically significant. The authors concluded the results could not enable elimination of the hypothesis of genotoxic potential of EL-107, which was noted in the previous study.

Discrepancies in test report and Toxicology Branch Evaluation:

A discrepancy in the test report was found in the comparison of high dose and negative (peanut oil) controls. The translation from French to English erroneously transcribed numbers and the direction of symbols. Appended pages 5 and 6 show pages 29 and 76 of the test report. The discrepancies appear below each table, as follows:

1. "p>0.05 difference non significative" is translated as follows, "non-significant difference p<0.01" (Appended p.5)
2. "p>0.05 difference non significative" is translated as follows, "non-significant difference p<0.05" (Appended p.6)

The authors apparently consider results statistically significant only if $p<0.01$. The Toxicology Branch policy is to interpret as significant, differences where $p<0.05$. In this example, the average of values at the high dose was 0.185 ± 0.06. Comparing this value to the negative control value of 0.11 ± 0.04 gives a "t" value of 1.979 with 18 degrees of freedom and a corresponding p value of 0.032. Toxicology Branch therefore interprets the result at 5000 mg/kg as statistically significantly greater than negative (vehicle) controls ($p<0.05$).

The repeat study used only males and apparently did not choose sufficiently high dose levels (to demonstrate the MTD), however, these deficiencies do not compromise the positive result. The study results demonstrate EL-107 is clastogenic in male mice, and the results were compound but not dose-related.

Appended page 1 MRID 84-2 007948 genotoxicity 84-2

TB 7-0037
(7-0139)

TOXICOLOGY BRANCH: DATA REVIEW

Caswell: 419F
EPA Chem: 125851

Chemical: Isoxaben (EL-107)

Study Type: Mutagenicity - Chromosome aberrations in vivo (mouse micronucleus)

Citation: Test for Genotoxicity of EL-107 Using a Micronucleus Technique in the Mouse

Accesion No: 265739

MRID: N/A

Sponsor: Eli Lilly France SA

Testing Lab: C.E.R.T.I. (G. Siou, L. Lerond-Conan, M. el Haitem, and M. Lacrampe)

Study No: 871

Date: September 6, 1984

Test Material:

EL-107 (Lot No. HO2-266-118), wettable powder (% ai not stated), suspended in peanut oil for oral administration.

TB Conclusion/Evaluation:

Inconclusive. Presumptive positive results should be confirmed in a repeat assay, employing additional procedures as outlined below (TB Evaluation).

Procedures:

Following toxicity testing, three groups of ten adult male Swiss mice (25-30 g) were intubated twice 24 hours apart with test material at a dose of 5000 mg/kg/day and sacrificed 24, 48, and 72 hours following the second dose. Negative controls (a fourth group given peanut oil vehicle only), and a fifth group of ten males administered benzene as reference clastogen (positive control) were killed 24 hours after dose. Bone marrow polychromatic erythrocytes (PCE, 2000 from each animal) were examined for the presence of micronuclei, and group means of micronucleated PCE compared statistically by Student's t-test and the Mann-Whitney (U) test.

Study Results:

In a preliminary dose-selection test, two of five animals died following two doses of 10,000 mg/kg 24 hours apart, and all treated mice showed signs of prostration. Therefore, a schedule of 2X 5000 mg/kg was chosen as the MTD.

No animals died in the main test (no evidence of clinical toxicity was reported). Group mean percent of PCE with micronuclei of benzene-treated mice was significantly elevated (5.54, $p < 0.01$) over peanut oil controls (0.10) by both statistical analyses. In EL-107 treated groups, slight but significant ($p < 0.01$) increases were found in both the 24-hour (0.22) and 48-hour (0.17) groups, but not at 72 hours (0.16).

Study Conclusions:

The author concluded there may be a "slight clastogenic effect of EL-107" in the mouse, and suggest further study (at 24 hours) to assess the validity of this initial assay.

TB Assessment:

The inclusion of individual animal data support the summary results presented, indicating a consistently slight but statistically sigificant increase in micronucleated PCE over controls at the 24-hour sacrifice following a dose of 5000 mg/kg. We note that no justification for not testing females was offered. Further, although the single level used was not shown to be a MTD (no clinical toxicity was discussed, nor evidence that a sufficient concentration of test material was absorbed to cause cytotoxicity at the target), we concur with the study author's conclusion that EL-107 induces a cytogenetic effect. Thus, the study can be considered inconclusive evidence for a presumptively positive result in males, deserving of a repeat study to confirm this effect.

We recommend the following procedural details for the repeat study:

1. An equal number of females be tested with EL-107.

2. Evidence of cytotoxicity (e.g., altered ratios of PCE to NCE) be demonstrated at an MTD (higher than 5000 mg/kg, but less than 10,000 mg/kg).

Appended page 3

007948

3. Sampling bone marrow cells be started as early as 12 hours after dosing, and other groups at 24, 36 and 48 hours postdose.

Reviewed by: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

01-07-87

Judith W. Hauswirth
1/8/87

ISOXABEN 125851

Page_____ is not included in this copy.

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Reviewed by: Margaret L. Jones Margaret L Jones 11 May 88
Review Section III
Toxicology Branch
Hazard Evaluation Divison (TS-769)

Approval: Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-769) M. van Gemert 5/19/88

and Kerry Dearfield, Ph.D.
Scientific Mission Support Staff
Toxicology Branch (TS-769) Kerry Dearfield 5.17.88

Chemical: EL-107; Isoxaben; N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (compound 121607) and isomers

Caswell No.: 419F MRID:

Record No.: 216480, 216479 CAS No: 82558-50-7

Accession No.: 404857-01 Guideline: 84-2

Study Type: Ames Assay

Citation: The Effect of EL-107 (Compound 121607) on the Induction of Reverse Mutations in Salmonella Typhimurium and Escherichia Coli Using the Ames Test

Authors: Gries, C.L., Rexroat, M.A., Probst, G.S.

Study Date: September 9, 1987

Sponsor: Elanco Products Co.

Laboratory: Toxicology Division, Lilly Research Labs,
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Study Nos.: 840924AMS 1378
870615AMS 1378A

Toxicology Branch Conclusions: Isoxaben did not induce increased mutations in Salmonella or E. coli strains at concentrations up to 500 ug/plate (solubility limit) with or without activation. Test article characterization (purity) was not reported. Referenced characterization was dated 1983 and may no longer be valid. Updated analysis will be necessary to upgrade the classification. No replicate assay was done, however, the positive controls demonstrated the sensitivity of the tester strains, therefore a replicate will not be required.

Recommendation: Unacceptable. Purity of the test compound at the time of the assay should be reported in order to upgrade to acceptable.

Detailed Review

Test Compound: EL-107, a mixture of isomers including primarily compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; [redacted]

Lot No. Z10025; Purity: Not reported (see discussion); Description: Not reported

Discussion: Referenced analysis from 1983 (Rutherford) should be updated to demonstrate the current purity of the test article used in the present assay. Characterization from 1983 may no longer be valid.

Dose Selection: Strain TA 100 was tested in 8 concentrations of EL-107 from 50 ug/plate up to 5000 ug/plate with and without metabolic activation using two plates per concentration. EL-107 was found not to be toxic to TA 100 at all levels tested, however, precipitation was observed at doses from 1000 to 5000 ug/plate. Therefore, 500 ug/plate was selected as the high dose for the assay. A preliminary test with E. coli strain was apparently not performed as was recommended in several protocols for this assay.

Procedure: The procedure was an in vitro assessment of the mutagenic potential of technical EL-107 in which strains of Salmonella typhimurium (TA 1535, TA 1537, TA 98, TA 100, histidine-dependent auxotrophs) and Escherichia coli (WP2uvrA$^-$, tryptophan-dependent auxotrophs) were exposed to 0, 31, 62.5, 125, and 500 ug/plate of EL-107 in DMSO. DMSO was used as solvent control at 0.05 ml/plate at the beginning of plating and at termination of plating.

The assays were done in the presence and absence of metabolic activation using liver extracts of Fischer 344 rats treated with Aroclor 1254.

In the preliminary range-finding study (840924AMS 1378) no toxicity was observed up to 5000 ug/plate. Precipitation of test substance was observed at 1000 ug/plate and above. Therefore, the next lowest dose, 500 ug/plate, was selected to be the high dose for the full assay. In a separate test for precipitation of EL-107, precipitation was noted in one plate of two tested without activation and none with metabolic activation at 500 ug/plate.

Solvent controls consisted of plates with 0.05 ml/plate of DMSO, one each per strain with and without metabolic activation at initiation and at termination of the study.

Positive controls without metabolic activation were N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) for S. typhimurium strains TA

1535 and TA 100 and for E. Coli strain WP2uvrA⁻, 2-nitrofluorene (2NF) for S. typhimurium strain TA 98, 9-aminoacridine (9AmAc) for S. typhimurium strain TA 1537. Positive controls with metabolic activation were 2-aminoanthracene for all strains of S. typhimurium and E. coli.

Tester strains of S. typhimurium were provided by Professor Bruce N. Ames and E. coli by Dr. M.H.L. Green and were maintained in the testing labs for several years.

Cultures of S. typhimurium (4 tester strains) and E. coli were incubated for 48 hours at 37°C with 5 concentrations of EL-107 ranging from 31 ug/plate to 500 ug/plate and with solvent control (DMSO, 0.05 ml/plate). Three plates per strain per dose (with and without metabolic activation) were used in one assay. A replicate assay was apparently not performed.

The authors assess as positive results when a dose-related increase in revertants is observed where the number of revertants exceeds control value by two-fold (for strains TA 98, TA 100, and WP2uvrA⁻) or at least three-fold (for strains TA 1535 and TA 1537) for two successive concentrations of test substance. No statistical analysis was done due to the author's observation that investigators disagree as to the relevance and appropriateness of statistical treatment of results.

Results: Results are summarized in Appended page 1 from the test report (Study 870615AMS 1378A) with and without metabolic activation. There was no apparent dose-related increase in revertant colony counts in strains dosed with EL-107. Positive controls demonstrated the sensitivity of the tester strains. Precipitation was observed at 500 ug/plate and was the reason noted for slight increases in colony counts at this dose. Increases in counts for strains TA 1535 and TA 98 with S-9 were larger than for other doses, however, there was no clear evidence of a compound-related effect, as defined by the authors.

Toxicology Branch Evaluation: There was no clear evidence of a dose-related effect of EL-107 on the induction of revertant colonies of tester strains of S. typhimurium and E. coli with and without metabolic activation. A replicate study was apparently not performed and would have confirmed the negative finding. A toxicity trial should have been performed using the strain of E. coli as well as the strain of S. typhimurium in order to investigate the level of toxicity of EL-107. However, since the compound precipitates at 500 ug/plate and above, this was apparently the highest level which could be tested under the Ames method. Since positive controls demonstrated the sensitivity of the tester strains, and since three plates per strain per dose were tested, a replicate study will not be required. An updated analysis of the test substance used in this assay will be required to upgrade the study.

Appended page 007946

TABLE 5. AN EVALUATION OF EL-107 (COMPOUND 121607) FOR THE INDUCTION OF BACTERIAL MUTATION USING THE AMES TEST, STUDY 870615AMS1378A.

Treatment	ug/plate	Revertant Colony Counts (Mean ± S.D.)[a] TA1535	TA1537	TA98	TA100	WP2uvrA⁻
TEST WITHOUT METABOLIC ACTIVATION						
EL-107	500	16 ± 2[e]	14 ± 2[e]	32 ± 3[e]	102 ± 12[e]	22 ± 2[e]
	250	17 ± 1	9 ± 0	34 ± 7	95 ± 4	21 ± 2
	125	17 ± 2	10 ± 2	27 ± 6	89 ± 3	19 ± 2
	62.5	16 ± 4	9 ± 2	27 ± 7	89 ± 1	23 ± 4
	31	16 ± 1	9 ± 3	29 ± 2	87 ± 2	22 ± 7
DMSO[b]	0.05 ml	15 ± 1	9 ± 1	24 ± 3	98 ± 13	28 ± 6
DMSO[c]	0.05 ml	13 ± 2	9 ± 1	27 ± 3	91 ± 3	24 ± 3
ENNG[d]	10	630 ± 21				
ENNG[d]	5	203 ± 18			1173 ± 102	2163 ± 25
9AmAc[d]	100		2032 ± 52		585 ± 50	1276 ± 91
9AmAc[d]	50		1004 ± 15			
2NF[d]	5			893 ± 43		
2NF[d]	0.5			140 ± 20		
TEST WITH METABOLIC ACTIVATION						
EL-107	500	21 ± 3[e]	18 ± 3[e]	69 ± 14[e]	124 ± 9[e]	32 ± 4[e]
	250	10 ± 2	10 ± 2	46 ± 4	101 ± 12	29 ± 7
	125	12 ± 4	12 ± 3	51 ± 12	110 ± 7	26 ± 3
	62.5	16 ± 1	9 ± 3	45 ± 8	100 ± 5	21 ± 3
	31	13 ± 2	10 ± 2	55 ± 3	110 ± 4	25 ± 2
DMSO[b]	0.05 ml	16 ± 1	7 ± 3	42 ± 10	91 ± 2	23 ± 3
DMSO[c]	0.05 ml	12 ± 2	9 ± 3	39 ± 11	91 ± 3	21 ± 3
2AA[d]	2.5	126 ± 10	124 ± 11	1425 ± 100	1159 ± 110	
2AA[d]	1.25	66 ± 10	56 ± 5	762 ± 33	561 ± 30	
2AA[d]	10					414 ± 16
2AA[d]	5					90 ± 29

[a] Mean ± standard deviation of counts from triplicate plates.
[b] DMSO control for the tester strain plated at the initiation of plating.
[c] DMSO control for the tester strain plated at the termination of plating.
[d] In the non-activated test ENNG served as the positive control for strains TA1535, TA100, and WP2uvrA⁻; 9AmAc was the positive control for strain TA1537; and 2NF served as the positive control for strain TA98. In the activated test, 2AA served as the positive control for all tester strains.
[e] Chemical precipitate present on plates and contributed to the count recorded.

Reviewed by: Margaret L. Jones Margaret L Jones 17 May 1988
Review Section III
Toxicology Branch
Hazard Evaluation Divison (TS-769)

Approval: Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-769) M. van Gemert 5/19/88

and Kerry Dearfield, Ph.D.
Scientific Mission Support Staff Kerry Dearfield 5-17-88
Toxicology Branch (TS-769)

Chemical: EL-107; Isoxaben; N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (compound 121607) and isomers

Caswell No.: 419F MRID:

Record No.: 216480, 216479 CAS No: 82558-50-7

Accession No.: 404857-02 Guideline: 84-2

Study Type: Ames Assay

Citation: The Effect of EL-107 (Compound 121607) on the Induction of Reverse Mutations in Escherichia Coli Strain WP2uvrA Using the Ames Test

Authors: Gries, C.L., Rexroat, M.A., Probst, G.S.

Study Date: September 9, 1987

Sponsor: Elanco Products Co.

Laboratory: Toxicology Division, Lilly Research Labs,
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Study Nos.: 840924AMS 1378
870615AMS 1378B

Toxicology Branch Conclusions: Isoxaben did not induce increased mutations in E. Coli strains at concentrations up to 500 ug/plate (solubility limit) with or without metabolic activation. Test article characterization (purity) was not reported. Referenced characterization was dated 1983 and may no longer be valid. Updated analysis will be necessary to upgrade the classification. No replicate assay was done, however, the positive controls demonstrated the sensitivity of the method, therefore a replicate assay will not be required. Toxicity trial should have been conducted using the E. coli tester strain rather than S. typhimurium.

Recommendation: Unacceptable. Purity of the test compound at the time of the assay should be reported in order to upgrade to acceptable.

Detailed Review

Test Compound: EL-107, a mixture of isomers including primarily compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; [redacted]

Lot No. Z10025; Purity: Not reported (see discussion); Description: Not reported

Discussion: Referenced analysis from 1983 (Rutherford) should be updated to demonstrate the current purity of the test article used in the present assay. Characterization from 1983 may no longer be valid.

Dose Selection: Salmonella typhimurium strain TA 100 was tested in 8 concentrations of EL-107 from 50 ug/plate up to 5000 ug/plate with and without metabolic activation using two plates per concentration. EL-107 was found not to be toxic to S. typhimurium strain TA 100 at all levels tested, however, precipitation was observed at doses from 1000 to 5000 ug/plate. Therefore, 500 ug/plate was selected as the high dose for the assay. A preliminary test with E. coli to determine the toxicity of the test compound, as recommended in various protocols for this assay, to the tester strain was apparently not performed. Since the E. coli strain was tested in this assay, it is unclear why the trial toxicity test was done using S. typhimurium.

Procedure: The procedure was an in vitro assessment of the mutagenic potential of technical EL-107 in which a strain of Escherichia coli (WP2uvrA$^-$, tryptophan-dependent auxotrophs) was exposed to 0, 31, 62.5, 125, and 500 ug/plate of EL-107 in DMSO. DMSO was used as solvent control at 0.05 ml/plate at the beginning of plating and at termination of plating.

The assays were done in the presence and absence of metabolic activation using liver extracts of Fischer 344 rats treated with Aroclor 1254.

In the preliminary range-finding study (840924AMS 1378) [see dose selection] precipitation was observed at 1000 ug/plate and above. The next lowest dose, 500 ug/plate was selected to be the high dose for the full assay. In a separate test for precipitation of EL-107, precipitation was noted in one plate of two tested without activation and none with metabolic activation at 500 ug/plate.

Solvent controls consisted of plates with 0.05 ml/plate of DMSO, one each per strain with and without metabolic activation at initiation and at termination of the study.

Positive controls without metabolic activation were N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) for E. Coli strain WP2uvrA$^-$.

Positive controls with metabolic activation were 2-aminoanthracene for E. coli.

Tester strain of E. coli was provided by Dr. M.H.L. Green and were maintained in the testing labs for several years.

Cultures of E. coli were incubated for 48 hours at 37°C with 5 concentrations of EL-107 ranging from 31 ug/plate to 500 ug/plate and with solvent control (DMSO, 0.05 ml/plate). Three plates per strain per dose (with and without metabolic activation) were used in one assay. A replicate assay was apparently not performed.

The authors assess as positive results when a dose-related increase in revertants is observed where the number of revertants exceeds control value by two-fold for E. coli strain WP2uvrA⁻ for two successive concentrations of test substance. No statistical analysis was done due to the author's observation that investigators disagree as to the relevance and appropriateness of statistical treatment of results.

Results: Results are summarized in Appended page 1 from the test report (Study 870615AMS 1378B) with and without metabolic activation. There was no apparent dose-related increase in revertant colony counts in strains dosed with EL-107. Positive controls demonstrated the sensitivity of the tester strains. Precipitation was observed at 500 ug/plate and was the reason noted for slight increases in colony counts at this dose. There was no clear evidence of a compound-related effect.

Toxicology Branch Evaluation: There was no clear evidence of a dose-related effect of EL-107 on the induction of revertant colonies of the tester strain of E. coli with and without metabolic activation. A replicate study was apparently not performed and would have confirmed the negative result. A toxicity trial should have been performed using the E. coli tester strain, not S. typhimurium, as reported. However, since the compound was observed to precipitate at 500 ug/plate and above, this was apparently the highest level which could be tested by the assay. Since three plates per strain per dose were tested, and since positive controls demonstrated the sensitivity of the tester strain, a replicate study will not be required. An updated analysis of the test substance used in this assay will be required to upgrade the study.

Appendix 1

007943

TABLE 5. AN EVALUATION OF EL-107 (COMPOUND 121607) FOR THE INDUCTION OF BACTERIAL MUTATION IN E. COLI STRAIN WP2uvrA⁻ USING THE AMES TEST, STUDY 870615AMS1378B.

Treatment	µg/plate	Revertant Colony Counts (Mean ± S.D.)[a] WP2uvrA⁻
TEST WITHOUT METABOLIC ACTIVATION		
EL-107	500	20 ± 3[e]
	250	19 ± 4[e]
	125	22 ± 1[e]
	62.5	22 ± 1[e]
	31	28 ± 6[e]
DMSO[b]	0.05 ml	20 ± 0
DMSO[c]	0.05 ml	20 ± 1
ENNG[d]	10	1961 ± 103
ENNG[d]	5	1145 ± 87
TEST WITH METABOLIC ACTIVATION		
EL-107	500	33 ± 12[e]
	250	23 ± 3[e]
	125	27 ± 4[e]
	62.5	26 ± 3[e]
	31	26 ± 5[e]
DMSO[b]	0.05 ml	23 ± 1
DMSO[c]	0.05 ml	20 ± 1
2AA[d]	10	380 ± 20
2AA[d]	5	91 ± 6

[a] Mean ± standard deviation of counts from triplicate plates.
[b] DMSO control for the tester strain plated at the initiation of plating.
[c] DMSO control for the tester strain plated at the termination of plating.
[d] In the non-activated test ENNG served as the positive control for tester strain WP2uvrA⁻, while 2AA served as the positive control for the activated test.
[e] Chemical precipitate present on plates and contributed to the count recorded.