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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: December 7, 2010

SUBJECT: Revised Hazard Assessment for Tier I Human Health Toxicity in Support of the Registration of **AMV-1018 Technical**, Containing 3-decen-2-one as its Active Ingredient (Amends EPA Memorandum from G. Casciano and C. Walsh through R. Jones to D. Benmhand dated June 16, 2010).

EPA File Symbol Number:	**5481-LAI**
Decision Number:	**425383**
DP Barcode:	**373085**
PC Code:	**068403**
CAS Number:	**10519-33-2**
MRID Numbers:	**479426-09 thru -16, -18**

FROM: Gina M. Casciano, M.S., Biologist /s/ 12/07/2010
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

Colin G. Walsh, M.S., Biologist /s/ 12/07/2010
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

THROUGH: Russell S. Jones, Ph.D., Senior Biologist /s/ 12/07/2010
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Driss Benmhand, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

ACTION REQUESTED

AMVAC Chemical Corporation requests registration of **AMV-1018 Technical**, a new technical grade active ingredient (TGAI) and manufacturing-use product (MP) containing 98.0% of the

new active ingredient (a.i.) 3-decen-2-one, a chemical occurring naturally in some foods and approved as a direct food additive by the U.S. Food and Drug Administration for use as a synthetic flavoring agent and adjuvant (21 CFR 172.515). This TGAI/MP product is for manufacturing-use only; specifically, for formulation into registered end-use products for use in or on agricultural commodities on growing plants. To fulfill Tier I Human Health Toxicity requirements for manufacturing-use products (40 CFR 158.2050), the applicant has submitted acute data, mutagenicity data, and data waiver requests for subchronic and developmental testing.

RECOMMENDATIONS AND CONCLUSIONS

The Tier I Human Health Toxicity submission is **UNACCEPTABLE** but upgradeable. To upgrade the submission to Acceptable, the registrant much resolve the deficiencies listed below:

1. The Acute Toxicity Tier I submission is **ACCEPTABLE**. Studies submitted to fulfill acute toxicity data requirements (Table 1, below) indicate that **AMV-1018 Technical** is Toxicity Category IV for acute oral toxicity and acute dermal toxicity, Toxicity Category III for acute inhalation toxicity, and Toxicity Category II for primary eye irritation and primary dermal irritation. Additional data are not required.

2. The residue study submitted with this application is **UNACCEPTABLE**. Testing for residues of 3-decen-2-one and its degradates did not begin until 16 days post-application. However, the draft product label for end-use product AMV-1018 EP (EPA File Symbol 5481-LTR) indicates that potatoes may be shipped to consumers as early as 24 hours post-treatment (this appears under the Sprout Control Programs section on the label). Therefore, testing for residues beginning 24 hours after application is required. In addition, the applicant did not conduct the residue study using the maximum label application rate. Dietary concerns for 3-decen-2-one and its primary degradates, 2-decanol and 2-decanone, remain.

3. The scientific rationale and cited data submitted to fulfill the 90-day oral toxicity data requirement are **UNACCEPTABLE**. Using the data presented by the applicant in their current residue study, the reviewer calculations indicate that the inclusion of a source of 3-decen-2-one in the diet from pesticidal residues would potentially increase the average daily intake of this chemical 4,000 times based on residues found at 16 days post-application. In addition, the amount of active ingredient that can be applied to the potatoes, based on the maximum label application rates, exceeds the daily dose amounts tested in the 90-day oral studies cited for the three structurally similar compounds. Additional data and/or data waiver rationale must be submitted to determine if the observed residues, including residues at 24 hours post-application, are of toxicological concern.

4. The data waiver request submitted to fulfill the 90-day inhalation toxicity data requirement is

UNACCEPTABLE. The data waiver request is only valid if repeated occupational exposure via the inhalation route is not expected. This is the case when 3 -decen-2-one is used in warehouse air circulation systems (thermal fogging) and the storage facilities remains closed for 24 hours to personnel entry while the thermal fogging in the treated area is re-circulated through the potato pile. No instructions are provided on the AMV-1018EP (EPA File Symbol 5481-LTR) label that indicates when the worker is allowed to reenter the treated area once the ventilation louvers are reopened to the outside after the 24 hour thermal fogging application. The occurrence of inhalation exposure is not clear of the other application method, spray drenching, for AMV-1018 90EC (EPA File Symbol 5481-LTN) and AMV-1018 67.5EC (EPA File Symbol 5481-LAO). Appropriate precautions to mitigate inhalation exposure, including any applicable Restricted Entry Interval (REI) and Personal Protective Equipment (PPE), for all use patterns must be included on all product labels (see NOTES TO RAL 3 and 4, below).

5. The scientific rationale submitted to fulfill the developmental toxicity data requirement is **UNACCEPTABLE**. Significant dietary exposure is expected to occur and it is unclear as it whether or not residues will be of toxicological concern. Additional data and/or data waiver rationale for developmental toxicity must be submitted.

6. The mutagenicity data submitted to fulfill this data requirement are **UNACCEPTABLE**. Study results from the Mouse Lymphoma Assay are equivocal and no other test for mammalian gene mutation or chromosomal aberration was performed. The applicant must resolve this discrepancy.

NOTES TO RAL:

1. The Acute Toxicity data submission (see Conclusion 1, above) places **AMV-1018 Technical** in Toxicity Category II.

2. The label for end-use product AMV-1018 EP (EPA File Symbol 5481-LTR) indicates that potatoes may be shipped to consumers as early as 24 hours post-treatment (this appears under the Sprout Control Programs section on the label). The labels for AMV-1018 90EC (EPA File Symbol 5481-LTN) and AMV-1018 67.5EC (EPA File Symbol 5481-LAO) do not include this information. The applicant must resolve this discrepancy.

3. The labels for AMV-1018 90EC (EPA File Symbol 5481-LTN) and AMV-1018 67.5EC (EPA File Symbol 5481-LAO) mention the use pattern of thermal fogging (under the Use Precautions Sections) but do not include instructions (or worker protection language) for this type of application. The applicant must include complete application instructions and worker protection language for thermal fogging if this is an intended use pattern for these two products. In addition, the REI following thermal fogging should be included on all labels.

4. Since a 90-day inhalation study was not submitted, this use of AMV-1018 90EC (EPA File Symbol 5481-LTN) and AMV-1018 67.5EC (EPA File Symbol 5481-LAO) as spray solutions, as indicated on these product labels, must be accompanied by appropriate worker PPE to mitigate exposure (unless use is restricted to automated spray equipment only) and any applicable REI. Currently, there are no applicator PPE requirements for inhalation on the draft product labels.

5. The amount, frequency, and timing of the proposed application of this biochemical were not included in Tolerance Petition 9F7670. This information appears on the label for AMV-1018 EP (EPA File Symbol 5481-LTR) only. The labels for AMV-1018 90EC (EPA File Symbol 5481-LTN) and AMV-1018 67.5EC (EPA File Symbol 5481-LAO) do not include this information. The applicant must resolve this discrepancy.

SUMMARY OF DATA SUBMITED

Toxicological Profile of 3-Decen-2-one and Hazard Assessment

The data presented in Table 1, below, are a summary of the toxicity data and information submitted to support the registration of the proposed manufacturing-use product AMV-1018 Technical (EPA File Symbol No. 5481-LAI), with contains 98.0% 3-decen-2-one as its active ingredient. At this time, all data requirements have been adequately satisfied with the exception of 90-day oral toxicity, 90-day inhalation toxicity, developmental toxicity, and mutagenicity.

Table 1: Mammalian Toxicology Data Requirements for 3-decen2-one (40 CFR § 158.2050)

Study/OCSPP Guideline No.	Results	Toxicity Category/Description	MRID
Acute oral toxicity (rat) (870.1100)	LD_{50} > 5,000 mg/kg body weight in female rats	Toxicity Category IV	*47942609*
Acute dermal toxicity (rat) (870.1200)	LD_{50} > 5,000 mg/kg body weight in male and female rats	Toxicity Category IV	*47942610*
Acute inhalation toxicity (rat) (870.1300)	LC_{50} 0.52-2.04 mg/L in male rats and > 2.04 mg/L in female rats	Toxicity Category III	*47942611*
Primary eye irritation (rabbit) (870.2400)	Test substance is moderately irritating to the eye.	Toxicity Category II	*47942612*
Primary dermal irritation (rabbit) (870.2500)	Test substance is severely irritating to the skin.	Toxicity Category II	*47942613*
Dermal sensitization (guinea pig) (870.2600)	Test substance is not a dermal sensitizer.	-	*47942614*
Hypersensitivity incidents (885.3400)	None reported.	-	-

Table 1: Mammalian Toxicology Data Requirements for 3-decen2-one (40 CFR § 158.2050)			
Study/OCSPP Guideline No.	**Results**	**Toxicity Category/Description**	**MRID**
90-Day oral toxicity (870.3100)	Waiver rationale submitted.	UNACCEPTABLE; Additional data and/or data waiver rationale is required (see discussion below).	*47942617*
90-Day dermal toxicity (870.3250)	Not required; uses not to involve purposeful application to human skin.	-	*47942617*
90-Day inhalation toxicity (870.3465)	Waiver rationale submitted.	UNACCEPTABLE; Additional data and/or data waiver rationale is required (see discussion below).	*47942617*
Developmental toxicity (870.3700)	Waiver rationale submitted.	UNACCEPTABLE; Additional data and/or data waiver rationale is required (see discussion below).	*47942617*
Mutagenicity (870.5100, 5300 and 5375)	Test substance is negative in bacterial reverse mutation assay. Test substance positive in 24-hr exposure without metabolic activation in an *in vitro* mammalian gene mutation assay (mouse lymphoma cell).	UNACCEPTABLE; Equivocal results are not adequate to fulfill data requirement (see discussion below).	*47942615; 47942616*

Acute Toxicity

All Tier I Human Health Toxicity requirements for Acute Testing were satisfied with studies conducted according to OCSPP Harmonized Test Guidelines. All studies contained signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided. A Data Evaluation Record (DER) was written for each submitted study. DERs appear at the end of this memorandum. The results of the Acute Toxicity testing (summarized in Table 1, above) place **AMV-1018 Technical** in Toxicity Category II. No hypersensitivity incidents or other adverse effects data was reported for the TGAI/MP.

CLASSIFCATION: These data are **ACCEPTABLE**; no additional data are required.

Subchronic Toxicity: 90-day Oral Toxicity

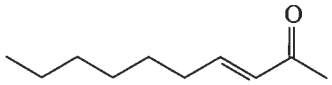
To satisfy the data requirement for 90-day oral toxicity, the applicant has submitted data waiver rationale stating that 3-decen-2-one: 1) occurs naturally in the diet; 2) is approved for use as a direct food additive by the U.S. FDA; 3) is not reported to be associated with subchronic toxicity; 4) is metabolized into innocuous compounds; and 5) is structurally similar to compounds that did not cause adverse effects in rats exposed for 90-days. According to the results of the applicant performed residue study, the inclusion of a source of 3-decen-2-one in the diet from pesticidal use could potentially increase the current average daily intake of this chemical 4,000 times based

on residues found at 16 days post application. In addition, the amount of active ingredient that can be applied to the potatoes, based on the maximum label application rates, exceeds the daily dose amounts tested in the 90-day oral studies cited for the three structurally similar compounds. Therefore, the Agency is requiring additional data and/or data waiver rationale to be submitted to determine if the residues observed, including residues at 24 hours post-application, are of toxicological concern.

The applicant cites the occurrence of 3-decen-2-one in edible porcini mushrooms (Thomas, 1973) and Iberian ham (Timon et al., 1998) (quantitative data not available) without report of incident or safety concern. Its use as a synthetic flavoring agent and adjuvant (21 CFR 172.515) indicates that it may be commonly consumed in other foods, however, specific information regarding this was not provided.

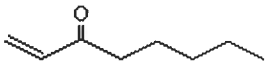
The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has characterized the metabolic pathways of 3-decen-2-one and determined that metabolism of the chemical results in the production of innocuous metabolites. Specifically, 3-decen-2-one is reduced to its corresponding alcohol, which is subsequently conjugated with glucuronic acid and excreted (JEFCA, 2003). In the environment, 3-decen-2-one degrades into the compounds 2-decanol and 2-decanone. The Agency agrees with this characterization.

Subchronic oral toxicity studies have been performed on three compounds structurally similar to 3-decen-2-one, and reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). In these studies, the aliphatic secondary ketones, 2-octen-4-one and 1-octen-3-one, and the secondary alcohol, 1-octen-3-ol, were used as test substances; 3-decen-2-one is an unsaturated, aliphatic, secondary ketone. All three 90-day studies revealed no clinical, hematological, or histopathological effects after 90-day exposure in rats to a single daily dose of 6.7 mg/kg test substance (2-octen-4-one and 1-octen3-one), 12 mg/kg (1-octen3-ol, males), or 14 mg/kg (1-octen-3-ol, females) (JECFA, 2003). These results are considered supplemental with regards to the 90-day oral toxicity data requirement for 3-decen-2-one because the amount of a.i. that can be applied to the potatoes, based on the maximum label application rates, exceed the single daily dose amounts given in the studies for these three structurally similar chemicals. In addition, the label indicates that potatoes can be shipped 24 hours post-application and there is no 24 hour residue data from the residue study. The earliest residue data in the residue study is 16 days post-application.

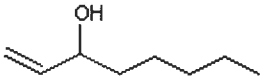


3-Decen-2-one

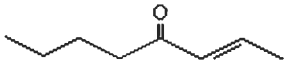
Structures of compounds on which 90-day oral toxicity studies were performed:



1-Octen-3-ol



1-Octen-3-one



2-Octen-4-one

In summary, the information provided to fulfill the requirement for 90-day oral toxicity is inadequate. Residues of 3-decen-2-one and it's degradates were detected up to 60 days post application on the potato internal tissue and potato peel tissue (see discussion below). 3-Decen-2-one was observed in potato internal tissue up to 16 days post treatment (0.85 ng 3-decen-2-one per gram fresh weight potato) and in the peel tissue through the last testing interval of 60 days post treatment (403 ng/g). Residues of the primary degradates, 2-decanol and 2-decanone, were observed in the internal issue (2-decanol only) and the peel tissues (both chemicals) through 60 days post treatment. According to these values, the inclusion of a source of 3-decen-2-one in the diet from pesticidal residues could potentially increase the current average daily intake of this chemical 4,000 times (see development toxicity discussion below) and exceed the daily dose amounts listed in the 90 day oral data cited for the three structurally similar chemicals based on maximum label application rates. Therefore, the Agency is requiring additional data and/or data waiver rationale for the 90-day oral toxicity data requirement be performed on the biochemical to determine if the residues observed, including residues at 24 hours post-application, are of toxicological concern.

No DERs were written for 90-day Oral Toxicity. Please refer to MRID 47942617 for further detail regarding waiver request rationale.

CLASSIFCATION: The waiver request is **UNACCEPTABLE**; additional data and/or data waiver rationale are required. Please refer to #3 under the Recommendations and Conclusions section of this memorandum.

Subchronic Toxicity: 90-day Dermal Toxicity

A 90-day dermal toxicity study was not submitted and is it not required at this time for the use of 3-decen-2-one in the proposed EPs. Prolonged exposure via the dermal route is not anticipated because the product is not purposefully applied to the skin or comparable prolonged human

exposure. The draft product labels for the three end-use products contain instructions for applicator Personal Protective Equipment (PPE) which includes: coveralls worn over long sleeve shirt and long pants, socks, chemical resistant footwear, chemical-resistant gloves, face shield or shielded safety glasses.

No DERs were written for 90-day Dermal Toxicity. Please refer to MRID 47942617 for further detail regarding waiver request rationale.

CLASSIFCATION: These data are **ACCEPTABLE**; no additional data are required.

Subchronic Toxicity: 90-day Inhalation Toxicity

A 90-day inhalation toxicity study was not submitted. This study is not required for the use of 3-decen-2-one in thermal fogging because label instructions limit the likelihood of significant levels of repeated inhalation exposure. Specifically, when 3-decen-2-one is applied to stored product via warehouse air circulation systems and the storage facilities remains closed for 24 hours to personnel entry following application. A re-entry interval following thermal fogging should be included on the labels. However, if the end-use products containing 90% and 67.5% 3-decen-2-one are used as spray solutions, as indicated on the product labels, the possibility of repeated exposure by workers exists, unless PPE or other precautions (such as the use of automated spray equipment only and no workers allowed in the facility during application) is added to these labels. Currently, there are no applicator PPE requirements for inhalation on the draft product labels. The applicant must clarify the discrepancies amongst the product labels.

No DERs were written for 90-day Inhalation Toxicity. Please refer to MRID 47942617 for further detail regarding waiver request rationale.

CLASSIFCATION: The waiver request is **UNACCEPTABLE**; additional data and/or data waiver rationale are required. Please refer to #4 under the Recommendations and Conclusions section of this memorandum.

Developmental Toxicity

To satisfy developmental toxicity testing requirements, the applicant has submitted data waiver rationale stating that 3-decen-2-one: 1) occurs naturally in the diet, 2) is approved by the U.S. FDA as a direct food additive, 3) is metabolized into innocuous compounds, 4) has not been associated in the literature with developmental toxicity, and 5) is within the current intake levels, and well below the threshold levels, identified for flavoring agents in this compound's structural class, as identified by JECFA (2003). Rationale statements 1-4, while true, are not adequate to completely fulfill the developmental toxicity date requirement (see discussion above). The Agency does not agree with statement 5, which is further discussed here.

In the JECFA (2003) report, these agents are divided into structural Classes I and II. 3-Decen-2-one, an unsaturated, aliphatic ketone (see Figure 1, below), is placed in structural class II. The evaluation of these chemicals by structural class implies that they are toxicologically equivalent, although this is not specifically stated in the JECFA (2003) report. The threshold for human intake for chemicals in this class is 540 µg per person per day, as determined by JECFA (2003). The calculated intake values, for all chemicals in this class combined, are 520 µg per person per day in Europe (only 20 µg less than the threshold value for this class of 540 µg) and 120 µg per person per day in the USA. The calculated intake of 3-decen-2-one, in particular, is 0.01 µg/person/day in Europe and not determined for the United States (JECFA, 2003).

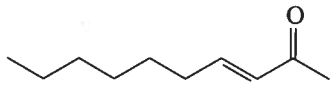


Figure 1: Structure of 3-decen-2-one

The average daily intake (ADI) of 3-decen-2-one, resulting solely from its use in pesticide products as a potato sprout inhibitor, was calculated by the applicant based on the results of an applicant-performed residue study. In the study, potato peel and tissue for 3-decen-2-one residues were analyzed at 16, 23, 30, and 60 days post treatment. It is important to note that this is not agreement with the draft product labels for the associated end-use products containing 3-decen-2-one as their active ingredients, which indicate that the potatoes can be treated and shipped to consumers 24 hours post-application (this appears under the Sprout Control Programs section on the label). Treatment in the residue study was at 0.75 mmol AMV-1018 (containing 98% 3-decen-2-one as its active ingredient) per kg of fresh potato weight. This is equivalent to 0.116 grams of product per kg of potatoes based on reviewer calculations. This is 4 times lower than the max label application rate (see Table 2, below) for thermal fogging. The study revealed residues of 3-decen-2-one in the potato internal tissue up to 16 days post application and in the potato peel up to 60 days post application.

Table 2: Application Rates for End-use Products Containing 3-Decen-2-one as their Active Ingredient.

Product	Percent A.I.	Application Type	Application Rate[1]	Conversion to grams product per kg potatoes
AMV-1018 EP (EPA File Symbol: 5481-LTR)	98.0%	Thermal Fogger (undiluted)	No more than 17.5 fl oz product per ton of potatoes per storage season	0.482 grams product (98.0% pure) per kg of potatoes per storage season
AMV-1018 90EC (EPA File Symbol: 5481-LTN)	90.0%	Spray (diluted)	Mix at a rate of 1.0%-2.0% (v/v) in water and drench or spray at a rate of 0.25-0.5 gallons per ton of potatoes[2]	0.0357 grams product (90% pure) per kg of potatoes[3]

AMV-1018 67.5EC (EPA File Symbol: 5481-LAO)	67.5%	Spray (diluted)	Mix at a rate of 1.3%-2.6% (v/v) in water and drench or spray at a rate of 0.25-0.5 gallons per ton of potatoes[2]	0.0463 grams product (67.5% pure) per kg of potatoes[4]

[1] per product label
[2] Applicant must indicate max number of applications per storage season.
[3] Using 0.5 gallons of a 2.0% (v/v) solution per ton of potatoes.
[4] Using 0.5 gallons of a 2.6% (v/v) solution per ton of potatoes.

Sixteen to 23 days post application, 3-decen-2-one made up 24-25% of measured residues. The other three-quarters of measured residue was almost entirely the degradate 2-decanol. The applicant states that, when evaluated by the criteria of the JECFA committee, this compound would be placed in "structural class I" of aliphatic acyclic secondary alcohol flavoring agents. The reviewers agree with this conclusion. The threshold for human intake for chemicals in this class is 1,800 µg per person per day, as determined by JECFA (2003). The actual intake values, for all chemicals in this class combined, was determined to be 58 µg per person per day in Europe and 16 µg per person per day in the USA. The actual intake of 3-decen-2-one is reported to be 0.01 µg/person/day in Europe and not determined for the United States (JECFA, 2003).

A small amount of a second degradate, 2-decanone, was measured in the potato skin. The applicant states that this degradate is not considered to be of a concern because residues measured are very low (194.46 ng/g max in peel, 0 ng/g in tissue), and would be categorized by the JECFA method as a saturated aliphatic acyclic secondary ketone that is rapidly absorbed and eliminated from the blood (JECFA, 1999).

When average potato intake for women of childbearing age is considered (U.S. EPA, 1997: age group 12-19 years, which is the group with highest potato intake of all adult groups and contains women of child-bearing age), whole potato (skin plus tissue) residue intake levels of 3-decene-2-one, 2-decanol, and 2-decanone are applicant calculated as 41 µg/day, 221 µg/day, and 10 µg/day, respectively. This is well below the structural class threshold levels identified by JECFA (2003); 3-decen-2-one represents structural class II, which has a threshold intake of 540 µg/day, and 2-decanol represent structural class I, which has a threshold intake of 1,800 µg/day. However, a more appropriate comparison is to the current average daily intake of this compound. According to JECFA (2003), the average intake of 3-decen-2-one in Europe is 0.01 µg per person per day, or 0.0002 µg per kg body weight per day. These average daily intake (ADI) values indicate that consumption of residues of 3-decen-2-one at the levels detected in the applicant's residue study, on average, are 4,000 times higher level than the amount currently consumed by the average European. Unfortunately, corresponding ADI values for the U.S. are not listed in the JECFA (2003) report, apparently due to lack of data.

Therefore, the applicant concludes that the estimated exposure to 3-decen-2-one and it's degradates from residues of proposed pesticide application are well within current acceptable

threshold intake levels (and no adverse effects have been reported in the literature) and that, therefore, it is not necessary to perform a guideline study for developmental toxicity. However, based on reviewer calculations, the addition of 3-decen-2-one residues would be a very significant step towards the set threshold value of 540 µg/person/day for JECFA structural class II (whose combined intake total is currently at 120 µg/person/day in the U.S.). Comparatively, a similar move in Europe would exceed the structural class threshold value, since the European daily intake value of structural class II is already 520 µg/person/day.

Consequently, the Agency disagrees with the applicant's conclusion that the estimated exposure to 3-decen-2-one and it's degradates from residues of proposed pesticide application are well within current acceptable threshold intake levels. The Agency estimates that the inclusion of a source of 3-decen-2-one in the diet from pesticidal residues would potentially increase the average daily intake of this chemical 4,000 times (based on current ADIs in Europe). In addition, no residue data was taken at 24 hours post-application (the earliest was 16 days post-application), which would most-likely increase the estimated exposure amount of 3-decen-2-one. Therefore, the information provided to fulfill the requirement for developmental toxicity is inadequate. The Agency is requiring additional data and/or data waiver rationale to be submitted for this biochemical.

No DERs were written for Developmental Toxicity. Please refer to MRID 47942617 for complete waiver request rationale and MRID 47942618 for complete Residue Study.

CLASSIFCATION: The waiver request is **UNACCEPTABLE**; additional data and/or data waiver rationale are required. Please refer to #5 under the Recommendations and Conclusions section of this memorandum.

Mutagenicity

To satisfy mutagenicity testing requirements, the applicant has submitted a bacterial reverse mutation assay (Ames Test) and a *in vitro* mammalian gene mutation (mouse lymphoma) assay. The test substance for each study was AMV-1018 Technical (EPA File Symbol No. 5481-LAI), containing 98.0% 3-decen-2-one. The test substance was found to be negative in the bacterial reverse mutation assay, and appears to be (somewhat equivocally) negative in the mouse lymphoma assay under 4 hour exposure test conditions, with and without metabolic activation, and mutagenic under 24 hour exposure conditions (without metabolic activation). There may be reproducible evidence of mutagenicity in this assay with and without S9 activation. In addition, colony sizing revealed an increase in small colonies in was observed all experiments. An increase in small colonies in addition to high mutant frequencies (as seen in "Experiment II" both with and without metabolic activation) indicates clastogenicity of the test substance. However, there is also evidence of marked cytotoxicity, and the osmolality for all experiments was outside the physiological range. The investigators conclude that, under the conditions of this assay, the test

substance is mutagenic; however, it is not clear that these results, given the increased osmotic pressure in particular and the marked cytotoxicity secondarily, would translate into an *in vivo* system. The Agency is considering the mouse lymphoma assay to be equivocal. The applicant must resolve this discrepancy.

DERs were written for each submitted study and appear at the end of this memorandum.

CLASSIFCATION: The data are **UNACCEPTABLE**; additional data and/or data waiver rationale are required. Please refer to #6 under the Recommendations and Conclusions section of this memorandum.

REFERENCES CITED

Thomas, A.F. (1973) An analysis of the flavor of dried mushroom, Boletus edulis. J. Agric. Food Chem. 21: 955-958.

Timon, M.L, Ventanas, J., Martin, L., Tejada, J.F., Garcia, C. (1998) Volatile compounds in supercritical carbon dioxide extracts of Iberian ham. J. Agric. Food Chem. 46: 5143-5150.

JECFA (1999) Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 42. Saturated aliphatic acyclic secondary alcohols, ketones, and related saturated and unsaturated esters. International Programme on Chemical Safety.

JECFA (2003) Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 50. Secondary alcohols, ketones, and related esters. International Programme on Chemical Safety.

DATA EVALUTATION RECORDS (DERs)

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
Signature: [illegible]
Date: 12/7/2010

EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
Signature: [illegible]
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Acute Oral Toxicity - Rats (OCSPP 870.1100)
MRID NO:	MRID 47942609
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 725 Cranbury Road East Brunswick, NJ 08816
TITLE OF REPORT:	Acute Oral Toxicity Up and Down Procedure in Rats
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27396

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The oral LD_{50} is greater than 5,000 mg/kg body weight in female rats.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

EXECUTIVE SUMMARY:

In an acute oral toxicity study (MRID 47942609), AMV-1018, containing 98.57% of the active ingredient 3-decen-2-one, was administered to four female rats according to the procedures for the performance of an acute oral toxicity limit test, as outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.1100. One animal died within two days of test substance administration. Prior to death, the animal was hypoactive and exhibited ano-genital staining, hunched posture, and soft feces. Gross necropsy revealed red intestines. Surviving animals exhibited similar symptoms, but recovered by Day 6, at which point they appeared active and healthy, gaining body weight over the entire 14-day observation period. No gross abnormalities were noted for these animals when euthanized and necropsied at the conclusion of the 14-day observation period. According to the results of this study, the acute oral LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in female rats. This places AMV-1018 in TOXICITY CATEGORY IV. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Substance:** **AMV-1018**

   Description: Colorless to pale yellow liquid
   Lot No.: KB 147-36-1
   Active Ingredient: 3-decen-2-one
   Purity: 98.57% w/w
   Compound Stability: Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. **Vehicle and/or Positive Control:** Test substance administered via gavage. No positive control (limit test).

3. **Test Animals:**

Number of Animals:	4
Species/Strain:	Rat/Sprague-Dawley derived, albino.
Age/Body Weight:	Young adult (9-10weeks)/170-186 grams at study initiation
Sex:	Female, nulliparous and non-pregnant
Source:	Received from Ace Animals, Inc., Boyertown, PA
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Purina Rodent Chow #5012
Water:	filtered tap water supplied *ad libitum* by dispenser

4. **Environmental Conditions:**

Temperature:	19-23°C
Humidity:	34-69%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	7-15 days

B. STUDY DESIGN

1. **In Life Dates:** Start: April 22, 2009 End: May 15, 2009

2. **Animal Selection:** Healthy, naïve (not previously tested) female rats were selected for testing.

3. **Dose-selection Rationale:** Dosing took place according to the limit test (OCSPP 870.1100). In total, four animals were dosed. Individual doses were calculated based on the initial body weight, taking into account the specific gravity of the test substance.

4. **Treatment Preparation, Administration, and Analysis:** Prior to dosing, test animals were fasted overnight via the removal of feed from the cage. During fasting, rats were examined for health and weight (initial). Test substance was administered to the stomach using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Following administration, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

C. METHODS

1. **Body Weight Measurements:** Individual body weights of animals were collected three times: 1) initial weight before test substance administration, 2) on Day 7 following dosing, and 3) on Day 14 or after death.

2. **Cage-side Observations:** Animals were observed for mortality, signs of gross toxicity, and behavioral changes. Observations took place during the first several hours post dosing and at least once daily thereafter for 14 days or until death occurred. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

3. **Necropsy:** At the end of the 14-day observation period, surviving rats were euthanized via CO_2 inhalation. Gross necropsies were performed on these animals as well as the animal that did not survive the study. Examinations included tissues and organs of the thoracic and abdominal cavities.

II. RESULTS

The first two animals dosed survived, however, the third animal dosed died within two days of test substance administration. Prior to death, the animal was hypoactive and exhibited ano-genital staining, hunched posture, and soft feces. Gross necropsy revealed red intestines. A fourth animal was dosed and survived. Therefore, the limit test outcome was O OX O (where O=survival and X=death). Since three or more animals survived, the LD_{50} for this substance is greater than the test dose (5,000 mg/kg).

All surviving test animals exhibited ano-genital staining and/or were hypoactive, with hunched posture, piloerection, reduced fecal volume, soft feces, and facial stains following test substance administration. However, these animals recovered by Day 6 and appeared active and healthy, gaining body weight over the entire 14-day observation period. No gross abnormalities were noted for these animals when euthanized and necropsied at the conclusion of the 14-day observation period.

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, the acute oral LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in female rats.

B. REVIEWERS COMMENTS

The reviewer agrees with the investigators conclusion that, under the conditions of this study, the acute oral LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in female rats. This places AMV-1018 in TOXICITY CATEGORY IV. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.1100. The study is classified as acceptable/guideline (OCSPP 870.1100) and satisfies the requirements for an acute oral toxicity study in the rat. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010
Signature: Colin Walsh
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Acute Dermal Toxicity - Rats (OCSPP 870.1200)
MRID NO:	MRID 47942610
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 2394 U.S. Highway 130 Dayton, NJ 08810
TITLE OF REPORT:	Acute Dermal Toxicity Study in Rats – Limit Test
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27397
GOOD LABORATORY PRACTICE:	GLP Compliant

CONCLUSION:	The dermal LD_{50} for male and female rats was greater than 5,000 mg/kg body weight.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

EXECUTIVE SUMMARY:

In an acute oral toxicity study (MRID 47942610), AMV-1018, containing 98.57% of the active ingredient 3-decen-2-one, was administered to ten rats (5 male and 5 female) according to the procedures for the performance of a limit test for acute dermal toxicity test, as outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.1200. One animal died within two days of test substance administration. Prior to death, the animal was hypoactive and exhibited prone posture. Gross necropsy revealed extremely red intestines. All surviving test animals exhibited dermal irritation at the dose site between Days 1 and Day 14. Seven of these animals were hypoactive, but all recovered by Day 2. All surviving animals gained body weight over the entire 14-day observation period. No gross abnormalities were noted for the surviving animals when euthanized and necropsied at the conclusion of the 14-day observation period. According to the results of this study, the acute dermal LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in male and female rats. This places AMV-1018 in TOXICITY CATEGORY IV. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Substance: AMV-1018

Description:	Colorless to pale yellow liquid
Lot No.:	KB 147-36-1
Active Ingredient:	3-decen-2-one
Purity:	98.57% w/w
Compound Stability:	Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. Test Animals:

Number of Animals:	10
Species/Strain:	Rat/Sprague-Dawley derived, albino.
Age/Body Weight:	Young adult (8-9 weeks)/ males 217-238 grams and females 182-208 at study initiation

Sex:	5 Male and 5 Female - nulliparous and non-pregnant
Source:	Received from Ace Animals, Inc., Boyertown, PA
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Purina Rodent Chow #5012
Water:	filtered tap water supplied *ad libitum* by dispenser

3. **Environmental Conditions:**

Temperature:	20-23°C
Humidity:	51-66%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	7 days

B. STUDY DESIGN

1. **In Life Dates:** Start: April 28, 2009 End: May 12, 2009

2. **Treatment Preparation and Animal Selection:** On the day prior to test substance application, a group of animals was prepared by clipping hair of the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health including any skin abnormalities. Initial body weights were recorded. From this group ten healthy, naïve (not previously tested) animals were selected for the test – five males and five females.

3. **Dose-selection Rationale:** Individual doses were calculated based on the initial body weight, taking into account the specific gravity of the test substance.

4. **Test Substance Administration and Analysis:** The test substance was administered at 5000 mg/kg of body weight, applied evenly over an area of approximately 2 inches x 3 inches on the dorsal side of the animal where hair had been clipped. Then, the area was covered with a 2 in x 3 in gauze pad. The pad and entire trunk of the animal were wrapped with tape. After 24 hours of exposure to the test substance, the gauze pads were removed and the test sites were gently cleansed of any residual test substance.

C. METHODS

1. **Body Weight Measurements:** Individual body weights of animals were collected three times: 1) initial weight before test substance administration, 2) on Day 7 following dosing, and 3) on Day 14 or after death.

2. **Cage-side Observations:** Animals were observed for mortality, signs of gross toxicity, and behavioral changes. Observations took place during the first several hours post application and at least once daily thereafter for 14 days or until death occurred. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

3. **Necropsy:** At the end of the 14-day observation period, surviving rats were euthanized via CO_2 inhalation. Gross necropsies were performed on these animals as well as the one animal (female) that did not survive the study. Examinations included tissues and organs of the thoracic and abdominal cavities.

II. RESULTS

One female died within two days of test substance application. Prior to death this animal was hypoactive and exhibited prone posture. Gross necropsy of the deceased animal revealed extremely red intestines.

All surviving test animals exhibited dermal irritation at the dose site between Days 1 and Day 14. All surviving animals gained body weight over the entire 14-day observation period. Seven of these animals were hypoactive, but all recovered by Day 2. No gross abnormalities were noted for the surviving animals when euthanized and necropsied at the conclusion of the 14-day observation period.

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, the acute dermal LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in male and female rats.

B. REVIEWERS COMMENTS

The reviewer agrees with the investigators conclusion that, under the conditions of this study, the acute dermal LD_{50} of AMV-1018 is greater than 5,000 milligrams per kg body weight in male and female rats. This places AMV-1018 in TOXICITY CATEGORY IV. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.1200. The study is classified as acceptable/guideline (OCSPP 870.1200) and satisfies the requirements for an acute dermal toxicity study in the rat. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010
Signature: Colin Walsh
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Acute Inhalation Toxicity - Rats (OCSPP 870.1300)
MRID NO:	MRID 47942611
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 2394 U.S. Highway 130 Dayton, NJ 08810
TITLE OF REPORT:	Acute Inhalation Toxicity Study in Rats
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27398

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The acute inhalation LC_{50} is between 0.52 - 2.04 mg/L in male rats and is greater than 2.04 mg/L in female rats.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

EXECUTIVE SUMMARY:

In an acute inhalation toxicity study (MRID 47942611), five male and five female rats were exposed to 2.04mg/L AMV-1018, containing 98.57% of the active ingredient 3-decen-2-one. Testing was in accordance with the procedures for the performance of an acute inhalation toxicity Limit test, as outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.1300. One male died immediately following exposure to the test atmosphere, with no clinical signs observed. Two males died within two days of exposure to the test atmosphere. Surviving animals exhibited clinical signs, however, these animals recovered by Day 10 and appeared active and healthy for the remainder of the study. Due to the treatment related mortality observed, a second test was run with five naïve male rats and an exposure scenario of 0.52mg/L. One animal died within one day of exposure to the test atmosphere. According to these results, the acute inhalation LC_{50} of AMV-1018 is between 0.52 mg/L and 2.04 mg/L in male rats and is greater than 2.04 mg/L in female rats. This places AMV-1018 in TOXICITY CATEGORY III. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Substance:** **AMV-1018**

   Description: Colorless to pale yellow liquid
   Lot No.: KB 147-36-1
   Active Ingredient: 3-decen-2-one
   Purity: 98.57% w/w
   Compound Stability: Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. **Delivery:** Test substance was aerosolized as received from the Sponsor.

3. **Test Animals:**

Number of Animals:	15
Species/Strain:	Rat/Sprague-Dawley derived, albino.
Age/Body Weight:	Young adult (9-11 weeks)/males 267-346 grams and females 210-248 grams at study initiation
Sex:	10 males and 5 females (nulliparous and non-pregnant)
Source:	Received from Ace Animals, Inc., Boyertown, PA
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Purina Rodent Chow #5012
Water:	filtered tap water supplied *ad libitum* by dispenser

4. Apparatus and Equipment:

Exposure Apparatus:	Nose-only inhalation chamber, internal volume 6.7 liters
Air Supply:	Filtered air supplied by compressor and air tank (mixing)
Ambient Conditions:	Humidity-Temperature Indicator/Monitor
Particle Size:	Eight-stage Anderson Cascade Impactor
Chamber Concentration:	Gravimetric samples collected using glass fiber filters attached to vacuum pump via filter holder and tubing
Treatment of Exhaust Air:	Air is discharged from the chamber through a 0.5-inch tube attached to the base unit of the chamber. Treatment of exhausted air was not reported.

5. Environmental Conditions:

Temperature:	19-23°C
Humidity:	50-67%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	13-20 days

B. STUDY DESIGN

1. **In Life Dates:** Start: April 20 and May 11, 2009 End: May 25, 2009

2. **Pre-test Trials:** Prior to study initiation, pre-test trials were conducted to establish procedures to achieve target chamber concentration (2.0 mg/L and 0.5 mg/L) and desired particle size distribution (less than or equal to 4 µm). Results revealed conditions favorable to achieve 2.04 mg/L chamber concentration at particle size distribution of 2.8 µm, and 0.52 mg/L chamber concentration at particle size distribution 2.5 µm.

3. **Test Conditions:** Temperature and humidity within the chamber as well as the room were recorded every 15 minutes during the first hour of exposure and every 30 minutes thereafter. Chamber concentration measurements were taken via gravimetric measurements from the breathing zone of the animals, six times during each exposure. For each exposure level, the animals were exposed to the targeted chamber concentration for at least 4 hours. Nominal concentration of test substance was 13.72 mg/L for the 2.0 mg/L study, and 6.73 mg/L for the 0.5 mg/L study. Actual (gravimetric) concentrations of test substance in the breathing zone were 2.04 mg/L and 0.52 mg/L, respectively.

4. **Animal Selection, Assignment, and Dose-selection Rationale:** On the day of exposure and the day prior to exposure, the rats were examined for health and were weighed. Five male and five female rats were selected for the 2.04 mg/L exposure. Based on the results of this exposure, five male rats only were selected for the 0.52 mg/L exposure.

5. **Treatment Preparation, Administration, and Analysis:** For each exposure level, animals were exposed to the target chamber concentration for at least 4 hours (extended beyond 4 hours to allow the chamber to reach equilibrium). Chamber was then operated for an additional 15 minutes with clean air. Animals were removed, fur cleaned of excess test material, and returned to their cages.

C. METHODS

1. **Body Weight Measurements:** Individual body weights of animals were collected three times: 1) initial weight before test substance administration, 2) on Day 7 following dosing, and 3) on Day 14 or after death.

2. **Cage-side Observations:** Animals were observed for mortality, signs of gross toxicity, and behavioral changes. Observations took place during the first several hours post dosing and at least once daily thereafter for 14 days or until death occurred. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

3. **Necropsy:** At the end of the 14-day observation period, surviving rats were euthanized via CO_2 inhalation. Gross necropsies were performed on these animals as well as the animal that did not survive the study. Examinations included tissues and organs of the thoracic and abdominal cavities.

II. RESULTS

At the 2.04 mg/L exposure level, one male died immediately following exposure to the test atmosphere, with no clinical signs observed prior to death. Two males died within two days of exposure to the test atmosphere, displaying signs of hypoactivity, abnormal respiration, nasal discharge, facial staining, hunched posture, and reduced fecal volume, and were cold to the touch. The surviving animals exhibited hypoactivity, hunched posture, abnormal respiration, reduced fecal volume, nasal and oral discharge and/or facial staining. However, these animals recovered by Day 10 and appeared active and healthy for the remainder of the study. Gross necropsy of the dead animals revealed edema, discoloration of the lungs and liver, gas distended yellow intestines, and/or rigor mortis. No gross abnormalities were noted for the surviving animals when euthanized and necropsied at the conclusion of the 14-day observation period.

At the 0.52 mg/L exposure level, one animal died within one day exposure to the test atmosphere. Prior to death, this animal was hypoactive and exhibited irregular respiration and moist rales. The surviving animals also exhibited hypoactivity, irregular respiration, and rales, as well as hunched posture, reduced fecal volume, and facial and/or ano-genital staining. However, these animals recovered by Day 7 and appeared active and healthy, gaining body weight over the entire 14-day observation period. Gross necropsy of the dead animal revealed extremely red lungs. No gross abnormalities were noted for the surviving animals when euthanized and necropsied at the conclusion of the 14-day observation period.

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, the acute inhalation LC_{50} of AMV-1018 is between 0.52 mg/L and 2.04 mg/L in male rats and is greater than 2.04 mg/L in female rats.

B. REVIEWERS COMMENTS

This study first used the Limit test approach for acute inhalation (five males and five females dosed at 2 mg/L). Due to the test substance related mortality seen as a result of this test (3 male deaths), further testing was required. The investigators' then dosed five male rats only at 0.52mg/L. One animal death was observed. Due to the results of these tests, the reviewer agrees with the investigators conclusion that, under the conditions of this study, the acute inhalation LC_{50} of AMV-1018 is between 0.52 mg/L and 2.04 mg/L in male rats and is greater than 2.04 mg/L in female rats. This places AMV-1018 in TOXICITY CATEGORY III. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.1300. The study is classified as acceptable/guideline (OCSPP 870.1300) and satisfies the requirements for

an acute inhalation toxicity study in the rat. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010
Signature: Colin Walsh
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Acute Eye Irritation (OCSPP 870.2400)
MRID NO:	MRID 47942612
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 725 Cranbury Road East Brunswick, NJ 08816
TITLE OF REPORT:	Primary Eye Irritation Study in Rabbits
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27399

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Test substance is moderately irritating to the eye.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY II

EXECUTIVE SUMMARY:

In an acute eye irritation study (MRID 47942612), three female rabbits were exposed to 0.1 mL AMV-1018, which contains the active ingredient 3-decen-2-one at 98.57%. Testing was performed according to the procedures for the performance of a step-wise acute eye irritation test, as outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2400. One hour after test substance instillation, all three treated eyes exhibited corneal opacity and conjunctivitis, and two treated eyes exhibited iritis. Severity decreased over time, with all animals free of ocular irritation by Day 10. All animals appeared active and healthy during the study, with no signs of toxicity aside from the eye irritation. The Maximum Mean Total Score (MMTS) for ocular irritation in this study is 24.3. According to these results, AMV-1018 is classified as moderately irritating to the eye. This places AMV-1018 in TOXICITY CATEGORY II. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Substance:** **AMV-1018**

   Description: Colorless to pale yellow liquid
   Lot No.: KB 147-36-1
   Active Ingredient: 3-decen-2-one
   Purity: 98.57% w/w
   Compound Stability: Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. **Delivery and Control:** The test substance was instilled into the right eye of the test animal. Substance was instilled as received by the Sponsor. The left eye remained untreated and served as a control.

3. **Test Animals:**

Number of Animals:	3
Species/Strain:	Rabbit/New Zealand albino
Age/Body Weight:	Young adult
Sex:	Female, nulliparous and non-pregnant
Source:	Received from Robinson Services, Inc. Clemmons, NC
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Pelleted Purina Rabbit Chow #5326
Water:	filtered tap water supplied *ad libitum* by dispenser

4. **Environmental Conditions:**

Temperature:	20-22°C
Humidity:	36-70%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	12 or 19 days

B. STUDY DESIGN

1. **In Life Dates:** Start: May 18, 2009 End: June 8, 2009

2. **Animal Selection:** Prior to test irritation, a group of female rabbits under went ocular examination involving a white light source and fluorescein dye. Three healthy, naïve animals (not previously tested) without pre-existing ocular irritation were selected for the test.

3. **Treatment Preparation, Administration, and Analysis:** Study was conducted according to a step-wise procedure, observing the effects of installation on one animal before enrolling two more. Prior to all instillations, 2-3 drops of ocular anesthetic were placed into both eyes. Then, one-tenth of a millimeter of the test substance was instilled into the conjunctival sac of the right eye. The left eye remained untreated and served as a control. Ocular irritation was evaluated using a high-intensity white light and scored according to Draize, *et al.* (1944).

C. METHODS

1. **Body Weight Measurements:** Not taken.

2. **Cage-side Observations:** Animals were observed for signs of gross toxicity, and behavioral changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

3. **Necropsy:** Not performed.

II. RESULTS

One hour after test substance instillation, all three treated eyes exhibited corneal opacity and conjunctivitis, and two treated eyes exhibited iritis. Severity decreased over time, with all animals free of ocular irritation by Day 10. All animals appeared active and healthy during the study, with no signs of toxicity aside from the eye irritation. The Maximum Mean Total Score (MMTS) for ocular irritation in this study is 24.3.

III. DISCUSSION AND CONCLUSION

D. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, AMV-1018 is moderately irritating to the eye.

E. REVIEWERS COMMENTS

This study followed the step-wise procedure for an acute eye irritation study, as set forth in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2400. Three animals were dosed, with all three exhibiting eye irritation and no other signs of gross toxicity or abnormal behavior. Irritation cleared by Day 10. Therefore, the reviewer recommends that AMV-1018 be placed in TOXICITY CATEGORY II. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.2400. The study is classified as acceptable/guideline (OCSPP 870.2400) and satisfies the requirements for acute eye irritation in the rabbit. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

F. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

IV. REFERENCES

Draize, J.H., Woodward, G. and Calvery, H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther*. 82:377-390.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)
EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010
Signature: Colin Walsh
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Acute Dermal Irritation (OCSPP 870.2500)
MRID NO:	MRID 47942613
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 2725 Cranbury Road East Brunswick, NJ 08816
TITLE OF REPORT:	Primary Skin Irritation Study in Rabbits
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27400

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Test substance is severely irritating to the skin.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY II

EXECUTIVE SUMMARY:

In an acute dermal irritation study (MRID 47942613), three male rabbits were exposed for four hours to 0.5 mL AMV-1018, which contains the active ingredient 3-decen-2-one at 98.57%. Testing was performed according to the procedures outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2500. Animals exhibited severe irritation (severe erythema and moderate edema) through 72 hours post patch removal. Irritation was no longer observed by Day 14. The Primary Dermal Irritation Index (PDII) calculated for this test substance was 5.5. According to the results of this study, AMV-1018 is classified as severely irritating to the skin and is placed in TOXICITY CATEGORY II. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Substance: AMV-1018

Description:	Colorless to pale yellow liquid
Lot No.:	KB 147-36-1
Active Ingredient:	3-decen-2-one
Purity:	98.57% w/w
Compound Stability:	Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. Test Animals:

Number of Animals:	3
Species/Strain:	Rabbit/New Zealand albino
Age/Body Weight:	Young adult
Sex:	Male
Source:	Received from Robinson Services, Inc. Clemmon, NC
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Pelleted Purina Rabbit Chow #5326

Water: filtered tap water supplied *ad libitum* by dispenser

3. **Environmental Conditions:**

Temperature:	19-22°C
Humidity:	40-66%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	6 days

B. STUDY DESIGN

1. **In Life Dates:** Start: April 28, 2009 End: May 12, 2009

2. **Treatment Preparation and Animal Selection:** On the day prior to test substance application, a group of animals was prepared by clipping hair of the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health including any skin abnormalities. From this group three healthy male animals, with no pre-existing skin irritation, were selected for the test.

3. **Test Substance Administration and Analysis:** Five-tenths of a milliliter of test substance was administered to one 6 cm^2 dose site (hair previously clipped). Then, the area was covered with a 1 inch x 1 inch gauze pad. The pad and entire trunk of the animal were wrapped with tape. Elizabethan collars were placed on animals to avoid ingestion/inhalation of the test substance. After 4 hours of exposure to the test substance, the gauze pads and collars were removed and the test sites were gently cleansed of any residual test substance. Skin irritation was scored according to Draize, *et al.* (1944).

C. METHODS

1. **Body Weight Measurements:** Not taken.

2. **Cage-side Observations:** Animals were observed for signs of gross toxicity, and behavioral changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern.

3. **Necropsy:** Not performed.

II. RESULTS

One hour after patch removal, all three treated sites exhibited well-defined to severe erythema

and moderate edema. Severe irritation was observed through 72 hours post patch removal. Irritation (erythema and edema) was gone by Day 14. The Primary Dermal Irritation Index (PDII) calculated for this test substance was 5.5.

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, AMV-1018 is classified as severely irritating to the skin.

B. REVIEWERS COMMENTS

This study followed the step-wise procedure for an acute dermal irritation study, as set forth in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2500. Animals exhibited severe irritation through 72 hours post patch removal. Therefore, the reviewer agrees with the investigators conclusion that, under the conditions of this study, AMV-1018 is classified as severely irritating to the skin. This places AMV-1018 in TOXICITY CATEGORY II. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.2500. The study is classified as acceptable/guideline (OCSPP 870.2500) and satisfies the requirements for acute dermal irritation in the rabbit. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

IV. REFERENCES

Draize, J.H., Woodward, G. and Calvery, H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther*. 82:377-390.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010

EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: Colin Walsh
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Skin Sensitization (OCSPP 870.2600)
MRID NO:	MRID 47942614
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98.57%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	Eurofins/Product Safety Laboratories 2394 U.S. Highway 130 Dayton, NJ 08810
TITLE OF REPORT:	Dermal Sensitization in Guinea Pigs (Buehler Method)
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	July 24, 2009
STUDY NO:	27401

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Test substance is not a dermal sensitizer.
CLASSIFICATION:	ACCEPTABLE

EXECUTIVE SUMMARY:

In a dermal sensitization study (MRID 47942614), twenty healthy guinea pigs were exposed to topical applications of AMV-1018, which contains the active ingredient 3-decen-2-one at 98.57%. Testing was performed according to the Buehler Method, as outlined in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2600. Twenty test animals were dosed three times each week for three weeks with either undiluted test substance or 75% w/w test substance in mineral oil. Twenty-seven days after the first dose was given, test animals and ten additional control animals were given a challenge dose of 1% w/w test substance in mineral oil to determine sensitization to the test material. No positive reactions were observed following challenge in either the test or the control animals. According to these results, AMV-1018 is classified as not a dermal sensitizer. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Substance:** **AMV-1018**

   Description: Colorless to pale yellow liquid
   Lot No.: KB 147-36-1
   Active Ingredient: 3-decen-2-one
   Purity: 98.57% w/w
   Compound Stability: Expected to be stable for the duration of testing, outcome of stability test not available at time of study.

2. **Delivery and Control:** The test substance was topically applied (0.4 mL) either undiluted or at 75% w/w in mineral oil for an initial induction period. Test substance at 1% w/w in mineral oil was applied for the challenge application. A naïve control group of ten animals was treated at challenge only.

3. **Test Animals:**

Number of Animals:	38
Species/Strain:	Guinea pigs/Hartley albino
Age/Body Weight:	Young adult, 318-412 grams at experimental start
Sex:	Female, nulliparous and non-pregnant
Source:	Received from Elm Hill Breeding Labs, Chelmsford, MA
Housing:	Singly, in suspended stainless steel cages with mesh floor
Diet:	Pelleted Purina Guinea Pig Chow #5025
Water:	filtered tap water supplied *ad libitum* by dispenser

4. **Environmental Conditions:**

Temperature:	19-22°C
Humidity:	42-74%
Photoperiod:	12-hour light/dark cycle
Acclimation Period:	5-7 days

B. STUDY DESIGN

1. **In Life Dates:** Start: April 17, 2009 End: May 28, 2009

2. **Animal Selection:** On the day prior to test substance application, a group of animals was prepared by clipping hair of the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health including any skin abnormalities. Initial body weights were recorded. From this group, eight healthy animals were selected for the preliminary irritation group, twenty for the test group, and ten for the naïve control group. Females only were chosen for the test and naïve control groups.

3. **Dose Selection:** Dose was based on the results of a preliminary irritation test. Dose concentrations of 100%, 75%, 50%, 25%, 12%, 6%, 3%, and 1% (dilutions in mineral oil) were applied at 0.4 mL to an area of clipped fur using an occlusive chamber. The test area was then wrapped for 6 hours. The occlusive chamber was removed after 6 hours and the test area was cleaned to remove any residual test substance. Test sites were examined and scored approximately 24 hours after application. The highest non-irritating concentration that produced a response in 4 guinea pigs was the 1% dilution, therefore, this concentration was chosen for the challenge phase.

4. **Treatment Preparation, Administration, and Analysis:** Twenty test animals were dosed three times each week for three weeks with 0.4mL of either undiluted test

substance or 75% w/w test substance in mineral oil. Twenty-seven days after the first dose was given, test animals and ten additional control animals were given a challenge dose (1% w/w) to determine sensitization to the test material. Scores were given for incidence and for severity, based on the methods of Ritz and Buehler (1980). Sensitizer classification was based on the criterion of Robinson, *et al.* (1990).

C. METHODS

1. **Body Weight Measurements:** Individual body weights (test group and control group) were recorded prior to experimental start and again on the day after challenge.

2. **Cage-side Observations:** Not performed.

3. **Necropsy:** Not performed.

II. RESULTS

No positive reactions were observed following challenge in either the test or the control animals. (Very faint erythema was seen in some animals, but this is not considered a positive reaction).

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, AMV-1018 is not a dermal sensitizer.

B. REVIEWERS COMMENTS

This study followed the Buehler method for skin sensitization determination, as set forth in the U.S. EPA Health Effects Test Guidelines, OCSPP 870.2600. No positive reactions were observed following challenge in either the test or the control animals. Therefore, the reviewer agrees with the investigators conclusion that AMV-1018 is not a contact sensitizer. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.2600. The study is classified as acceptable/guideline (OCSPP 870.2600) and satisfies the requirements for skin sensitization in the guinea pig. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

IV. REFERENCES

Ritz, H. and Buehler, E. (1980) Planning, conduct, and interpretation of guinea pig sensitization patch tests. *Current Concepts in Cutaneous Toxicity*, V.A. Drill and P. Lazar (Eds.), Academic Press, New York, 1980, pg 25.

Robinson, M., Nusair, T., Fletcher, E., and Ritz, H. (1990) A review of the Buehler Guinea Pig Sensitization Test and its use in risk assessment process for human skin sensitization. *Toxicology*, 61: 91-107.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010

EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	Bacterial Reverse Mutation Test (*Salmonella typhimurium)* OCSPP 870.5100
MRID NO:	MRID 47942616
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstrabe 6/8 82152 Planegg, Germany
TITLE OF REPORT:	Reverse Mutation Assay Using Bacteria
AUTHOR:	Dr. Barbara Wallner
STUDY COMPLETED:	July 7, 2009
STUDY NO:	091460

GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Test substance is non-mutagenic (negative) in this assay.
CLASSIFICATION:	ACCEPTABLE

EXECUTIVE SUMMARY:

In a reverse mutation assay (MRID 47942616), AMV-1018, containing 98% of the active ingredient 3-decen-2-one, was investigated for its potential to induce gene mutations via a plate incorporation test (Experiment I), and a pre-incubation test (Experiment II). Each experiment was conducted with five tester strains of *Salmonella typhimurium* (TA 98, TA 100, TA 1535, TA 1537, and TA 102), six different test substance concentrations (0.0316, 0.100, 0.316, 1.0, 2.5 and 5.0 µL/plate, and control scenario), and with and without metabolic activation. According to the results of this study, no biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with AMV-1018 at any concentration level, neither in the presence or absence of metabolic activation, in either experiment. In the pre-incubation experiments, cytotoxicity was noted in all five tester strains at a dose concentration of 5.0 µL/plate without metabolic activation and in tester strain TA 102 at a dose concentration of 5.0 µL/plate with metabolic activation. The reference mutagens employed in the control scenarios induced a distinct increase in revertant colonies, indicating the validity of the experiments. Therefore, the test substance is considered to be non-mutagenic in this bacterial reverse mutation assay. The study classification is **ACCEPTABLE.**

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Substance: AMV-1018

Description:	Pale yellow liquid
Lot No.:	3D2-2009/01
Active Ingredient:	3-decen-2-one
Purity:	98% w/w
Compound Stability:	Expected to be stable for the duration of testing, outcome of stability test not available at time of study.
CAS No of TGAI:	10519-33-2

2. **Positive Controls:** The reference mutagens sodium azide, 4-nitro-o-phenylene-diamine, and methyl methane sulfonate were tested in one or two of the test strains without metabolic activation. The reference mutagen 2-aminoanthracene was tested in all five test strains with metabolic activation. The OCSPP Test Guideline for this assay (OCSPP 870.5100) recommends that 2-Aminoanthracene not be used as the sole indicator of the efficacy of the S9 mix, and that a pro-mutagen requiring metabolic activation by microsomal enzymes also be used. The study authors did not follow this recommendation, however, the study is still considered to be acceptable.

3. **Test System:** Five different strains of the bacterium *Salmonella typhimurium.*

Strain:	Characteristic:
TA 98	frame shift mutations
TA 100	base-pair substitutions
TA 1535	base-pair substitutions
TA 1537	frame shift mutations
TA 102	base-pair substitutions

All strains contain mutations in the histidine operon, thereby imposing a requirement for histidine in the growth medium. They also all contain the deep rough (rfa) mutation, which deletes the polysaccharide side chain of the lipopolysaccharides of the bacterial cell surface, thus increases the permeability of larger substances, and the uvrB mutation, which is a deletion of the uvrB gene coding for a protein of the DNA nucleotide excision repair system resulting in an increased sensitivity in detecting many mutagens. Tester strains TA 98, TA 100 and TA 102 contain the R-factor plasmid pkM101. These strains are reverted by a number of mutagens that are detected weakly or not at all in the parent strains.

B. METHODS

1. **Preparation of Bacteria:** Samples of each tester strain were grown by culturing for 12h at 38.5 °C in nutrient broth (8 g nutrient broth + 5 g NaCl) to the late exponential or early stationary phase of growth (appox. 10^9 cells/mL). For strains TA 98, TA 100, and TA 102, a solution of 125 µL ampicillin (10 mg/mL) was added in order to retain the phenotypic characteristics of the strains.

2. **Preparation of Medium:** Vogel-Donner Medium E agar plates with 2% glucose were either prepared by BSL BIOSERVICE GmbH or provided by a supplier. The investigators indicate that quality controls were performed and sterilization was at 121°C in an autoclave. Overlay agar containing L-histidine and biotin was prepared and sterilized at 121°C in an autoclave.

3. **Preparation of Metabolic Activation (S9):** The S9 homogenate was prepared at the facility by inducing male Wistar rats with Phenobarbital (80 mg/kg bw) and β-Naphthoflavone (100 mg/kg bw) for three consecutive days by oral route. The protein concentration in the S9 preparation was 33 mg/mL. The S9 mix was prepared according to Ames et al. (1973).

C. STUDY DESIGN

1. **Exposure Concentrations:** A pre-experiment was conducted for toxicity of the test substance at eight concentration levels. The following six were chosen for the main experiments: 0.0316, 0.100, 0.316, 1.0, 2.5 and 5.0 µL/plate.

2. **Controls:** Three plates per experiment were prepared for each of the following scenarios: solvent control, negative control, and reference mutagen solution (positive control).

3. **Procedures:** For each strain and dose level, three plates were used. For each experiment, after solidification, the plates were inverted and incubated in the dark at 37°C for at least 24 hours.

    a. **Plate Incorporation Experiment:** The following materials were mixed in a test tube and poured over the surface of a minimal agar plate: 100µL test solution at one of six concentration levels for each of three controls, 500 µL of either S9 mix or S9 substitution buffer (for testing without metabolic activation), 100 µL of bacteria suspension, and 2000 µL of overlay agar.

    b. **Pre-Incubation Experiment:** 100 µL of the test substance preparation was preincubated with the tester strains (100 µL) and sterile buffer or the S9 (500 µL) for 60 minutes at 37°C prior to adding the overlay agar (2000 µL) and pouring onto the surface of a minimal agar plate.

4. **Data and Reporting:** Data was collected using a ProtoCOL colony counter if conditions were favorable. If precipitation of the test substance precluded automatic counting, and for tester strains with low spontaneous mutation frequency, counting was done by hand. Evaluation of results for validity/mutagenicity was done in accordance with OCSPP Test Guideline 870.5100. Historical laboratory control data of the negative and the positive controls were included.

II. RESULTS

The investigators reported that no dose-response related or biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with

AMV-1018 at any concentration level, neither in the presence or absence of metabolic activation, in either experiment. In the pre-incubation experiments, cytotoxicity was noted in all five tester strains at a dose concentration of 5.0 µL/plate without metabolic activation and in tester strain TA 102 at a dose concentration of 5.0 µL/plate with metabolic activation. The reference mutagens employed in the control scenarios induced a distinct increase in revertant colonies, indicating the validity of the experiments. Therefore, the test substance is considered to be non-mutagenic in this bacterial reverse mutation assay.

III. DISCUSSION AND CONCLUSION

A. INVESTIGATORS CONCLUSIONS

The investigators conclude that, under the conditions of this study, AMV-1018 is non-mutagenic.

B. REVIEWERS COMMENTS

The reviewer agrees with the investigators conclusion that, under the conditions of this study, AMV-1018 is non-mutagenic. There were no significant deviations from U.S. EPA Health Effects Test Guidelines, OCSPP 870.5100. The study is classified as acceptable/guideline (OCSPP 870.1500) and satisfies the requirements for a reverse mutation assay using bacteria. Signed and dated Data Confidentiality, Good Laboratory Practice (GLP) Compliance, and Quality Assurance statements were provided.

C. STUDY DEFICIENCIES

No significant study deficiencies that would invalidate the study outcome were found.

IV. REFERENCES

Ames, B.N., Durston, E.W., Yamasaki, E. and Lee, F.D. (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. (USA) 70: 2281-2285.

EPA Primary Reviewer:
Gina M. Casciano, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/2010

EPA Secondary Reviewer:
Colin G. Walsh, **Biochemical Pesticides Branch**
Biopesticides and Pollution Prevention Division (7511P)

Signature: [illegible]
Date: 12/7/10

DATA EVALUATION RECORD

STUDY TYPE:	In vitro Mammalian Cell Gene Mutation Test (OCSPP 870.5300)
MRID NO:	MRID 47942615
DP BARCODE NO:	373086
DECISION NO:	425385
SUBMISSION NO:	864619
TEST SUBSTANCE:	AMV-1018 active ingredient: 3-decen-2-one purity: 98%
PC CODE:	068403
SPONSOR:	AMVAC CHEMICAL CORPORATION 4100 East Washington Blvd. Los Angeles, CA 90023-4406
TESTING FACILITY:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstrabe 6/8 82152 Planegg, Germany
TITLE OF REPORT:	In vitro Mammalian Cell Gene Mutation Assay (Thymidine Kinase Locus/$TK^{+/-}$) in Mouse Lymphoma L5178Y Cells with AMV-1018
AUTHOR:	Michael Kraft
STUDY COMPLETED:	September 1, 2009

STUDY NO:	091462
GOOD LAB PRACTICE:	GLP (Good Laboratory Practice) Compliant
CONCLUSION:	Test substance is considered to be mutagenic under the conditions of the 24 assay without metabolic activation, presented here. Other results remain equivocal.
CLASSIFICATION:	ACCEPTABLE

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the TK $^{+/-}$ locus (MRID 47942615), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to AMV-1018 (3-decen-2-one; 98.57% a.i.) in dimethyl sulfoxide (DMSO) at the following concentrations: 0.05, 0.15, 0.22, 0.28, 0.31, 0.34, 0.37 mM (+ S9) for 4 hours; 0.005, 0.01, 0.02, 0.05, 0.10, 0.12, 0.14, 0.16 mM (- S9) for 4 hours; 0.10, 0.15, 0.19, 0.23, 0.27, 0.31, 0.35, 0.38 mM (+ S9) for 4 hours; and 0.0001, 0.0007, 0.004, 0.007, 0.014, 0.028, 0.034, 0.04 mM (- S9) for 24 hours. The S9 fraction (for metabolic activation) was derived from the livers of male Wistar rats induced with Phenobarbital (80 mg/kg bw) and β-Naphthoflavone (100 mg/kg bw). The solvent DMSO served as a negative control in the presence and absence of S9. Benzo(α)pyrene (BP) served as a positive control in the presence of S9. Ethylmethanesulfonate (EMS) and methylmethanesulfonate (MMS) served as positive controls in the absence of S9.

Selection of test substance concentrations were based on a pre-experiment for cytotoxicity. No precipitation of the test substance was noted in the experiments. Growth inhibition was noted in all experiments (+/- S9), with marked cytotoxicity seen in several cases (one incident less than 10%, several less than 20%). The pH values for the highest concentrations tested were within the physiological range. The osmolality for the solvent controls as well as for the highest test concentrations was found to be 465 mosmol/kg. Thus, the osmolality was above the physiological range.

The reviewer agrees with the investigators conclusions that, under the conditions of this assay, the test substance AMV-1018 (3-decen-2-one) is mutagenic. However, it is not clear that the results, given the increase osmotic pressure in particular and the marked cytotoxicity secondarily, would translate into an *in vivo* system. In addition, equivocal results in the experiments with metabolic activation should be clarified with a follow-up experiment.

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP 870.5300) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AMV-1018

Description: Pale yellow liquid
Batch #: 3D2-2009/01
Purity: 98% a.i.
CAS # of TGAI: 10519-33-2

2. Control Materials

Negative control: The solvent dimethylsulfoxide (DMSO, 1%) served as the negative control.
Positive control: Non-activated: Ethylmethanesulfonate (EMS, 200 µg/mL and 500 µg/mL)
Methylmethanesulfonate (MMS, 10 µg/mL)
Activated: Benzo[a]pyrene (B[a]P, 2.5-3.5 µg/mL)

3. Activation: The S9 fraction was derived from male Wistar rats (age, weight, and supplier not reported).

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbital		Mouse		Lung
			None		Hamster		Other
		X	Other (β-Naphthoflavone)		Other		

The S9 fraction was prepared at BSL BIOSERVICE GmbH (the testing facility). Quality control measures included a *Salmonella typhimurium* assay for biological activity and a sterility test. A stock of the supernatant containing microsomes was frozen in ampoules of 2 and 4.5 mL and stored at ≤ -75°C. For preparation of the S9 mix, supernatant was thawed and mixed with cofactors to reach the following concentrations: 8 mM $MgCl_2$, 33 mM KCl, 5 mM Glucose-6-phosphate, and 5 mM NADP, in a solution of 100 mM sodium-phosphate buffer, pH 7.4.

4. Test Cells: Mammalian cells in culture

X	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		list any others

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

Media: Growth medium - RPMI 1640 medium, supplemented with 15% horse serum, 100 µg/mL penicillin/streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine, 25 mM HEPES, and 250 µg/mL amphotericin B; Treatment medium - growth medium with 3% horse serum for short-term exposure and 7.5% horse serum for long-term exposure; and Selection medium - growth medium supplemented with 5 µg/mL trifluorothymidine.

5. Locus Examined

	[X]	**Thymidine kinase (TK)**	[ ]	**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**	[ ] **Na^+/K^+ ATPase**
Selection agent:	[ ]	Bromodeoxyuridine (BrdU)	[ ]	8-azaguanine (8-AG)	[ ] Ouabain
	[ ]	Fluorodeoxyuridine (FdU)	[ ]	6-thioguanine (6-TG)	
	[X]	Trifluorothymidine (TFT; 5 µg/mL)			

6. Test Compound Concentrations

Pre-test for Cytotoxicity, Activated:	0.005, 0.01, 0.05, 0.1, 0.2, 0.4 mM (0.77, 1.54. 7.71, 15.43, 30.85, 61.70 µg/mL)*
Pre-test for Cytotoxicity, Non-Activated:	0.005, 0.01, 0.05, 0.1, 0.2 mM (0.77, 1.54. 7.71, 15.43, 30.85 µg/mL)*
Mutagenicity Assay, Activated (4 hr):	0.05, 0.15, 0.22, 0.25, 0.28, 0.31, 0.34, 0.37 mM (7.71, 23.14, 33.94, 38.56, 43.19, 47.82, 52.45, 57.07 µg/mL)*
Mutagenicity Assay, Non-Activated (4 hr)	0.005, 0.01, 0.02, 0.05, 0.10, 0.12, 0.14, 0.16 mM (0.77, 1.54, 3.09, 7.71, 15.43, 18.51, 21.60, 24.68 µg/mL)*
Mutagenicity Assay, Activated (4 hr):	0.10, 0.15, 0.19, 0.23, 0.27, 0.31, 0.35, 0.38 mM (15.43, 23.14, 29.31, 35.48, 41.65, 47.82, 53.99, 58.62 µg/mL)*
Mutagenicity Assay, Non-Activated (24 hr):	0.0001, 0.0007, 0.004, 0.007, 0.014, 0.028, 0.034, 0.04 mM (0.015, 0.108, 0.617, 1.080, 2.160, 4.319, 5.245, 6.170 µg/mL)*

*molecular weight of the test substance is 154.25 g/mol

B. TEST PERFORMANCE

1. Cell Treatment

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (activated and non-activated) in Experiment I, and for 4 hours (activated) and 24 hours (non-activated) in Experiment II.

b. After washing, cells were cultured during the expression period for 72 hours after the 4 hour experiments and 48 hours after the 24 hour experiment at 37°C in 5% CO_2/95% humidified air before cell selection.

c. After expression, a statistical number of 1.6 cells/well (192 wells/group) were cultured for 6 days without selective agent to determine cloning efficiency (viability), and 2000

cells/well (384 wells/group) were cultured for 11-14 days in selection medium to determine numbers of mutants.

2. Statistical Methods:

Mutant frequencies were calculated from the natural log (ln) comparison of the dividend of negative cultures to total cultures seeded, of the selective medium over the non-selective medium (see below). No further statistical methods (i.e. comparison or regression) were performed.

$$\text{Mutation frequency} = \frac{-\ln [NC / TC \text{ (selective medium)}]}{-\ln [NC / TC \text{ (non-selective medium)}]} \times 800$$

NC: number of negative cultures
TC: total number of cultures seeded

3. Evaluation criteria

a. Assay validity

According to the study authors, the assay was considered valid if the following criteria were met:

- The mutant frequencies in the negative or solvent control cultures were in the range of BSL BIOSERVICE historical control data (about 82 to 253 mutants per 10^6 cells).
- The positive controls (EMS, MMS, and B[a]P) induce significant increases (at least 2-fold increase of mutant frequencies related to the comparable negative control values and higher than the historical range of negative controls) in the mutant frequencies.
- At least three out of four 96-well-plates from the TFT resistance-testing portion of the experiment are scorable.
- The absolute cloning efficiency ([number of positive cultures x 100]/ total number of seeded cultures) of the negative and/or solvent controls is >50%.

b. Positive result

According to the study authors, the test article was considered mutagenic if:

- There was a clear and dose-related increase in the mutant frequency.
- There was a biologically relevant response (at least a 2-fold increase of mutant frequencies related to the respective negative control values and higher than the historical range of negative controls) for at least one of the dose groups.
- If combined with a positive effect in the mutant frequency, an increased occurrence of

small colonies (slow growth colonies) indicated by a low large/small colonies ratio (1.5 times the ratio of clastogenic controls MMS and/or B[a]P) is an indication for potential clastogenic effects and/or chromosomal aberrations.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: The pre-test for cytotoxicity was performed on solvent controls, negative controls, and the test material concentrations reported above (section I.6.). Observation of test material precipitation was not reported. Marked cytotoxicity (<10-20% relative survival) was seen at the highest test material concentration with and without metabolic activation (6.60% RSG at 0.4 mM with metabolic activation; 4.74% RSG at 0.2 mM without metabolic activation). When comparing the results of these experiments across test material concentration levels, higher cytotoxicity is observed in the experiments without metabolic activation than with metabolic activation. This data is reported in Tables 1-2 of the study report (page 24 of the study report) and are included as an Attachment to this DER.

B. MUTAGENICITY ASSAY

The main experiment consisted of four parts:

Experiment I, with metabolic activation (4 hr assay)
Experiment I, without metabolic activation (4 hr assay)
Experiment II, with metabolic activation (4 hr assay)
Experiment II, without metabolic activation (24 hr assay)

Test compound concentrations for each experiment are listed above (section I.6.). The pH values of the highest concentrations tested were within the physiological range. The osmolality for the solvent controls as well as for the highest concentrations were found to be 465 mosmol/kg. Thus, the osmolality was above the physiological range. No precipitation of the test substance was noted in the experiments.

Dose-response related growth inhibition was observed in all four experimental trials. Mutant frequency (i.e. mutation factor, MF) was determined for all test substance concentrations, and colony sizing was performed for the highest concentrations of the test item and for the negative and positive controls. EMS (500 and 200 µg/mL), MMS (10 µg/mL) and B[a]P (3.5 µg/mL) were used as positive controls and showed distinct and biologically relevant effects in mutation frequency in all experiments.

In Experiment I with metabolic activation, all mutant values were within the range of historical control data of the test facility (93-253 mutants per 10^6 cells, Table 16, page 40 of the study report). The study authors report that no dose-response relationship could be

observed, however, the reviewer notes a dose-response related increase at the three highest test substance concentrations. The study authors report that the mutation frequencies found in the groups treated with the test item did not show a biologically relevant increase as compared to the solvent controls. This is based upon a mutation factor upper limit of 2.0 (when compared to the solvent controls) for a negative outcome. The highest mutation factor observed was 1.91 and was seen in the highest dose group for this experiment (0.37mM, RTG of 12.22%). In addition, colony sizing (performed on the three highest dose groups) revealed an increased number of small colonies as compared to the negative and solvent controls. However, because no corresponding mutagenicity was found in this experiment (i.e. MF > 2.0), the investigators concluded that no clear statement could be made regarding these results, and therefore, to clarify these findings an independent repetition experiment was performed (see Experiment II with metabolic activation). The data for this experiment appears in Tables 3-5 (pages 25-26) of the study report, and are attached to this DER.

In Experiment I without metabolic activation, all mutant values found were within or slightly above the range of historical control data of the test facility (82-238 mutants per 10^6 cells, Table 16, page 40 of the study report). A slight dose-response relationship could be observed. The highest mutant value of 265.61 mutants/10^6 cells at a concentration of 0.16 mM was regarded as not biologically relevant due to a mutagenicity factor (when compared to the solvent controls) of only 1.85. RTG for this dose level was 13.17%. Colony sizing was performed and all dose groups were considered as not clastogenic. The data for this experiment appears in Tables 6-8 (pages 27-28) of the study report, and are attached to this DER.

In Experiment II with metabolic activation, the mutant values at doses of 0.27, 0.31, and 0.38 mM clearly exceeded the range of the historical control data (93-253 mutants per 10^6 cells, Table 16, page 40 of the study report), and a slight dose-response relationship was observed. In two dose groups (0.31 and 0.38 mM) the threshold value of 2.0 for the mutation factor was slightly exceeded. The mutation factor (compared to the solvent control values) at a concentration of 0.31 mM (with a RTG of 36.5 1%) was 2.22, and at a concentration of 0.38 (with RTG of 9.2%) was 2.15. The investigators conclude that, since in no mutagenicity was evaluated up to a RTG of 12.22% in Experiment I with metabolic activation, the results for the experiments with metabolic activation are equivocal (i.e., inconclusive). For colony sizing, increases in the number of small colonies noted at doses of 0.31 mM and 0.38 mM lead the investigators to conclude a clastogenic effect of the test item because the threshold value for mutagenicity was exceeded in these same dose groups. The data for this experiment appears in Tables 9-11 (pages 29-30) of the study report, and are attached to this DER.

In Experiment II without metabolic activation, all mutant values found up to a dose of 0.014 mM (2.160 µg/mL) were within the range of the historical control data (82-238 mutants per 10^6 cells, Table 16, page 40 of the study report), however, doses of 0.028, 0.034, and 0.04

mM (4.319, 5.245, and 6.170 µg/mL) clearly exceeded the historical control range. In addition, in these dose groups the threshold MF value of 2.0 (when compared to the solvent control value) was exceeded. Mutation factors for the doses with mutants above historical control data were 2.84 at 0.028 mM with a RTG of 29.93%, 3.63 at 0.034 mM with a RTG of 17.51%, and 4.13 at 0.04 mM with a RTG of 13.16%. These are considered by the study authors to be significant, indicating mutagenicity of the test compound in this 24-hour assay. Colony sizing revealed an increase of small colonies at the 0.034 mM dose, leading the investigators to conclude that this "is a hint at clastogenicity of the test item" because corresponding mutagenicity was found in this dose group. The data for this experiment appears in Tables 13-15 (pages 32-33) of the study report, and are attached to this DER.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The investigators conclude that the test item AMV-1018 is mutagenic in the mouse lymphoma thymidine kinase locus (cell line L5178Y) without metabolic activation. Results for the experiments with metabolic activation are considered to be equivocal (i.e., inconclusive).

B. REVIEWER COMMENTS:

OCSPP Test Guideline 870.5300 states that the criterion for determining a positive result in the *in vitro* Mammalian Cell Gene Mutation Test includes biological relevance and a concentration-related, reproducible increase in mutant frequency. Furthermore, the Guideline indicates that although most studies will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgment about the activity of the test substance, with results remaining equivocal or questionable regardless of the number of times the experiment is repeated. In the case of AMV-1018 (3-decen-2-one) described above, the numbers of mutants per 10^6 cells exceeded the laboratory's historical control data at particular dose levels in experiments with and without metabolic activation. In Experiment II without metabolic activation, these results exhibit a clear dose-response relationship. In addition, colony sizing revealed an increase in small colonies in all experiments performed. An increase in small colonies in addition to high mutant frequencies, as seen in Experiment II both with and without metabolic activation, indicates clastogenicity of the test substance.

However, the Guideline also states that care should be taken to avoid conditions which would lead to results not reflecting intrinsic mutagenicity, which therefore would not be biologically relevant when translated to a living system. Such positive results which do not reflect intrinsic mutagenicity may arise from changes in pH, osmolality, or high levels of

cytotoxicity (OCSPP 870.5300; Scott, 1991; Moore, 2003). In the assay in question, the investigators report that pH values of the highest concentrations tested were within the physiological range, however, osmolality for the solvent controls as well as for the highest concentrations of test substance were both found to be 465 mosmol/kg. Thus, the osmolality was above the physiological range.

With regards to cytotoxicity, the Guideline states that if the maximum concentration of test substance investigated is based on cytotoxicity (rather than solubility) then it should result in approximately 10–20 percent, but not less than 10 percent, relative survival (i.e., relative total growth, RTG). This principle was generally adhered to in the assay, with only one dose level investigated (0.38 mM, Experiment II with metabolic activation) producing a RTG of less than 10%. However, of MF values exceeding 2.0 (five in total), three occurred at RTG levels less than 20%. This may be of significance when determining the validity of the assay. The Mouse Lymphoma Assay (MLA) MLA Workgroup of the International Workshop on Genotoxicity Tests (IWGT), assembled in 2002, considered the general issue of biological relevance in the assay and concluded that very small increases in MF can often be seen at high cytotoxicity levels, and that mutations induced under these circumstances would not normally occur in vivo and thus are not considered by the Workgroup to be biologically relevant (Moore, 2003). Cytotoxicity levels less than 20% and osmolality outside the physiological range are of concern to the reviewer.

The OCSPP Guideline states that a positive result for the *in vitro* mammalian cell gene mutation test which indicates mutagenicity and is reproducible is most meaningful. There appears to be reproducible evidence of mutagenicity in this assay with and without S9 activation. However, there is also evidence of marked cytotoxicity, and the osmolality for all experiments was outside the physiological range. The reviewer agrees with the investigators conclusions that, under the conditions of this assay, the test substance AMV-1018 (3-decen-2-one) is mutagenic. However, it is not clear that these results, given the increase osmotic pressure in particular and the marked cytotoxicity secondarily, would translate into an *in vivo* system. In addition, equivocal results from the experiments with metabolic activation should be clarified with a follow-up experiment.

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. STUDY DEFICIENCIES: None

D. REFERENCES CITED

Moore, M.M. et al. (2003) Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: International Workshop on Genotoxicity Tests Workgroup Report – Plymouth UK, 2002. *Mutation Research* 540: 127-140.

Scott, D. et al. (1991) Genotoxicity Under Extreme Culture Conditions. A report from ICPEMC Task Group 9. *Mutation Research* 257: 147–204.

ATTACHMENT

Pre-test for Toxicity:
Tables 1-2 from page 24 of MRID 47620516

Main Experiment I, with metabolic activation:
Tables 3-5 from pages 25-26 of MRID 47620516

Main Experiment I, without metabolic activation:
Tables 6-8 from pages 27-28 of MRID 47620516

Main Experiment II, with metabolic activation:
Tables 9-11 from pages 29-30 of MRID 47620516

Main Experiment II, without metabolic activation:
Tables 13-15 from pages 32-33 of MRID 47620516

Table 1: Pre-test for toxicity, with metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 4 h after Treatment	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Relative Suspension Growth (RSG) [%]
NC1	0	300000	352000	1060000	1570000	99.87
NC2		300000	357000	1030000	1650000	101.52
S1	0	300000	342000	1040000	1600000	100.00
S2		300000	357000	1060000	1520000	
1	0.005	300000	349000	1040000	1610000	102.31
2	0.01	300000	344000	1030000	1580000	100.88
3	0.05	300000	332000	1020000	1450000	95.00
4	0.1	300000	326000	941000	1600000	98.49
5	0.2	300000	321000	721000	1520000	72.81
6	0.4	300000	190000	165000	198000	6.60

NC: Negative control
S: Solvent control
RSG: [(value TSG / value TSG of corresponding controls) x 100]
TSG: [(value 24 h x value 48 h) / (value 4 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$]

Table 2: Pre-test for toxicity, without metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 4 h after Treatment	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Relative Suspension Growth (RSG) [%]
NC1	0	300000	327000	1020000	1610000	99.57
NC2		300000	358000	1070000	1750000	103.70
S1	0	300000	355000	1030000	1650000	100.00
S2		300000	330000	1060000	1650000	
1	0.005	300000	317000	972000	1550000	94.23
2	0.01	300000	336000	886000	1580000	82.60
3	0.05	300000	258000	619000	1410000	67.07
4	0.1	300000	223000	321000	1330000	37.96
5	0.2	300000	133000	95200	106000	4.74

NC: Negative control
S: Solvent control
RSG: [(value TSG / value TSG of corresponding controls) x 100]
TSG: [(value 24 h x value 48 h) / (value 4 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$]

Table 3: Toxicity Data, with metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 4 h after Treatment	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Total Suspension Growth (TSG)[a]	Relative Suspension Growth (RSG)[b] [%]	Relative Cloning Efficiency (RCE)[c] [%]	Relative Total Growth (RTG)[d] [%]
NC1	0	300000	330000	869000	1740000	15.10	94.32	103.05	97.19
NC2		300000	336000	857000	1720000	14.62	91.36	108.10	96.93
S1	0	300000	293000	880000	1620000	16.22	100.00	100.00	100.00
S2		300000	307000	898000	1620000	15.80			
5	0.05	300000	302000	872000	1620000	15.59	97.41	103.05	100.38
7	0.15	300000	289000	756000	1670000	14.56	90.97	98.17	89.31
8	0.22	300000	268000	623000	1620000	12.55	78.42	107.93	84.64
9	0.25	300000	258000	550000	1590000	11.30	70.58	106.71	75.32
10	0.28	300000	260000	447000	1430000	8.20	51.20	103.66	53.07
11	0.31	300000	186000	273000	1090000	5.86	36.61	101.83	37.28
12	0.34	300000	189000	213000	641000	3.39	21.19	100.61	21.32
13	0.37	300000	146000	143000	306000	2.10	13.09	93.29	12.22
B[a]P	3.5 µg/mL	300000	314000	466000	1360000	6.73	42.03	103.66	43.57

NC: Negative control
S: Solvent control
a: TSG = [(value 24 h x value 48 h) / (value 4 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$
b: RSG = [(value TSG / value TSG of corresponding controls) x 100]
c: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
d: RTG = (RSG x RCE)/100
B[a]P: Benz(a)pyrene

Table 4: Mutagenicity Data, with metabolic activation

		Cloning Efficiency (CE)			Mutagenicity Data						
					Number of cultures / 96 wells						
Test Group	Concentration [mM]	Plate 1[a]	Plate 2[a]	RCE [%][f]	Plate 1[a]	Plate 2[a]	Plate 3[a]	Plate 4[a]	Mean	Mutants/ 10^6 cells[g]	Mutation factor
NC1	0	86	83	103.05	32	30	19	25	26.50	121.78	
NC2		87	87	108.10	40	35	34	27	34.00	147.76	
S1	0	85	81	100.00	35	35	29	29	32.00	162.23	
S2		80	82		32	30	36	26	31.00	168.06	
5	0.05	84	85	103.05	32	32	29	28	30.25	142.69	0.86
7	0.15	82	79	98.17	34	34	32	36	34.00	191.81	1.16
8	0.22	86	91	107.93	37	38	34	34	35.75	146.18	0.89
9	0.25	90	85	106.71	41	35	35	39	37.50	163.45	0.99
10	0.28	88	82	103.66	40	39	36	41	39.00	192.50	1.17
11	0.31	86	81	101.83	37	46	47	38	42.00	225.79	1.37
12	0.34	85	80	100.61	47	47	44	35	43.25	244.20	1.48
13	0.37	78	75	93.29	48	47	40	44	44.75	315.01	1.91
B[a]P	3.5 µg/mL	85	85	103.66	64	63	74	64	66.25	432.60	2.62

NC: Negative control
S: Solvent control
e: Number of cultures with cell growth.
f: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
g: Mutation frequency = (-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)])x800
B[a]P: Benz(a)pyrene

Table 5: Colony Sizing, with metabolic activation

Test Group	Concentration [mM]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
NC1	0	106	84	22	3.82
NC2		136	111	25	4.44
S1	0	128	98	30	3.27
S2		124	93	31	3.00
11	0.31	168	95	73	1.30
12	0.34	173	100	73	1.37
13	0.37	179	101	78	1.29
B[a]P	3.5 µg/mL	265	133	132	1.01

NC: Negative control
S: Solvent control
B[a]P: Benz(a)pyrene

Table 6: Toxicity Data, without metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 4 h after Treatment	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Total Suspension Growth (TSG)[a]	Relative Suspension Growth (RSG)[b] [%]	Relative Cloning Efficiency (RCE)[c] [%]	Relative Total Growth (RTG)[d] [%]
NC1	0	300000	330000	1030000	1690000	17.58	115.95	105.04	121.79
NC2		300000	336000	1090000	1570000	16.98	111.95	105.64	118.26
S1	0	300000	301000	856000	1590000	15.07	100.00	100.00	100.00
S2		300000	312000	887000	1610000	15.26			
4	0.005	300000	329000	859000	1630000	14.19	93.55	90.80	84.94
5	0.01	300000	293000	789000	1620000	14.54	95.69	99.70	95.60
6	0.02	300000	288000	677000	1720000	13.48	88.87	104.45	92.83
7	0.05	300000	236000	497000	1550000	10.88	71.75	93.77	67.28
8	0.10	300000	204000	271000	1180000	5.78	38.14	96.14	36.67
9	0.12	300000	166000	199000	681000	4.10	27.05	97.92	26.49
10	0.14	300000	151000	138000	426000	2.82	18.60	93.18	17.33
11	0.16	300000	145000	122000	307000	2.12	13.96	94.36	13.17
EMS	500 µg/mL	300000	304000	750000	1430000	11.76	77.55	100.89	78.24
MMS	10 µg/mL	300000	314000	783000	1580000	13.13	86.60	100.30	86.86

NC: Negative control
S: Solvent control
a: TSG = [(value 24 h x value 48 h) / (value 4 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$
b: RSG = [(value TSG / value TSG of corresponding controls) x 100]
c: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
d: RTG = (RSG x RCE)/100
EMS: Ethylmethanesulfonate
MMS: Methylmethanesulfonate

Table 7: Mutagenicity Data, without metabolic activation

		Cloning Efficiency (CE)			Mutagenicity Data						
Test Group	Concentration [mM]	Plate 1[e]	Plate 2[e]	RCE [%][f]	Number of cultures / 96 wells[e] Plate 1[e]	Plate 2[e]	Plate 3[e]	Plate 4[e]	Mean	Mutants / 10^6 cells[g]	Mutation factor
NC1	0	86	91	105.04	23	29	20	30	25.50	96.88	
NC2		89	89	105.84	29	21	23	24	24.25	88.96	
S1	0	89	85	100.00	26	35	30	30	30.25	127.92	
S2		83	80		28	29	31	32	30.00	158.58	
4	0.005	84	69	90.80	30	33	29	23	28.75	178.64	1.25
5	0.01	86	82	99.70	22	24	29	31	26.50	124.27	0.87
6	0.02	86	90	104.45	26	26	20	28	25.00	97.12	0.68
7	0.05	75	83	93.77	19	24	36	22	25.25	141.04	0.98
8	0.10	79	83	96.14	36	41	31	35	35.75	200.76	1.40
9	0.12	81	84	97.92	42	37	38	28	36.25	193.38	1.35
10	0.14	78	79	93.18	45	39	36	31	37.75	234.81	1.64
11	0.16	82	77	94.36	46	37	46	41	42.50	265.61	1.85
EMS	500 µg/mL	84	86	100.89	86	84	84	78	83.00	738.31	5.15
MMS	10 µg/mL	85	84	100.30	61	66	57	63	61.75	388.56	2.71

NC: Negative control
S: *Solvent control*
e: Number of cultures with cell growth.
f: RCE = {(mean value positive cultures / mean value positive cultures of corresponding controls) x 100}
g: Mutation frequency = {-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)]}x800
EMS: Ethylmethanesulfonate
MMS: Methylmethanesulfonate

Table 8: Colony Sizing, without metabolic activation

Test Group	Concentration [mM]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
NC1	0	102	74	28	2.64
NC2		97	78	19	4.11
S1	0	121	108	13	8.31
S2		120	105	15	7.00
9	0.12	145	91	54	1.69
10	0.14	151	100	51	1.96
11	0.16	170	90	80	1.13
MMS	10 µg/mL	247	103	144	0.72

NC: Negative control
S: *Solvent control*
MMS: Methylmethanesulfonate

Table 9: Toxicity Data, with metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 4 h after Treatment	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Total Suspension Growth (TSG)[a]	Relative Suspension Growth (RSG)[b] [%]	Relative Cloning Efficiency (RCE)[c] [%]	Relative Total Growth (RTG)[d] [%]
NC1	0	300000	327000	1040000	1880000	19.93	111.88	95.91	107.11
NC2		300000	346000	1050000	1830000	18.51	103.73	100.58	104.33
S1	0	300000	348000	1010000	1920000	18.57	100.00	100.00	100.00
S2		300000	353000	1030000	1760000	17.12			
4	0.1	300000	283000	808000	1820000	17.32	97.06	100.00	97.06
5	0.15	300000	272000	783000	1830000	17.56	98.39	94.74	93.22
6	0.19	300000	276000	680000	1850000	15.19	85.13	95.32	81.15
7	0.23	300000	270000	599000	1800000	13.31	74.59	99.42	74.15
8	0.27	300000	251000	429000	1690000	9.63	53.95	95.91	51.74
9	0.31	300000	241000	349000	1570000	7.58	42.47	85.96	36.51
10	0.35	300000	235000	226000	899000	3.83	21.44	97.66	20.93
11	0.38	300000	235000	181000	437000	1.86	10.42	88.30	9.20
B[a]P	3.5 µg/mL	300000	310000	513000	1690000	9.32	52.24	63.74	33.30

NC: Negative control
S: Solvent control
a: TSG = [(value 24 h x value 48 h) / (value 4 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$
b: RSG = [(value TSG / value TSG of corresponding controls) x 100]
c: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
d: RTG = (RSG x RCE)/100
B[a]P: Benz(a)pyrene

Table 10: Mutagenicity Data, with metabolic activation

		Cloning Efficiency (CE)			Mutagenicity Data						
					Number of cultures / 96 wells						
Test Group	Concentration [mM]	Plate 1	Plate 2	RCE [%]	Plate 1	Plate 2	Plate 3	Plate 4	Mean	Mutants/ 10^6 cells[g]	Mutation factor
NC1	0	77	87	95.91	36	33	25	34	32.00	168.48	
NC2		83	89	100.58	30	41	29	34	33.50	151.80	
S1	0	89	90	100.00	31	42	40	30	35.75	138.41	
S2		77	86		33	33	36	37	34.75	190.20	
4	0.1	89	82	100.00	31	41	41	43	39.00	188.45	1.15
5	0.15	87	75	94.74	38	41	45	31	38.75	222.77	1.36
6	0.19	76	87	95.32	33	34	35	32	33.50	181.65	1.11
7	0.23	88	82	99.42	49	35	40	48	43.00	219.37	1.34
8	0.27	84	80	95.91	45	54	59	43	50.25	307.97	1.87
9	0.31	78	69	85.96	52	39	54	41	46.50	365.24	2.22
10	0.35	83	84	97.66	44	40	45	50	44.75	246.30	1.50
11	0.38	77	74	88.30	51	41	46	52	47.50	353.79	2.15
B[a]P	3.5 µg/mL	50	59	63.74	67	75	71	75	72.00	1322.40	8.05

NC: Negative control
S: Solvent control
e: Number of cultures with cell growth.
f: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
g: Mutation frequency = {-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)]}x800
B[a]P: Benz(a)pyrene

Table 11: Colony Sizing, with metabolic activation

Test Group	Concentration [mM]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
NC1	0	128	107	21	5.10
NC2		134	115	19	6.05
S1	0	143	111	32	3.47
S2		139	116	23	5.04
9	0.31	186	125	61	2.05
10	0.35	179	110	69	1.59
11	0.38	190	135	55	2.45
B[a]P	3.5 µg/mL	288	183	105	1.74

NC: Negative control
S: Solvent control
B[a]P: Benz(a)pyrene

Table 13: Toxicity Data, without metabolic activation

Test Group	Concentration [mM]	Number of Cells Seeded	Number of Cells 24 h after Treatment	Number of Cells 48 h after Treatment	Number of Cells 72 h after Treatment	Total Suspension Growth (TSG)[a]	Relative Suspension Growth (RSG)[b] [%]	Relative Cloning Efficiency (RCE)[c] [%]	Relative Total Growth (RTG)[d] [%]
NC1	0	300000	1100000	1630000	1200000	29.88	141.19	95.43	134.73
NC2		300000	980000	1560000	1120000	25.48	120.38	92.57	111.44
S1	0	300000	847000	1520000	1130000	21.46	100.00	100.00	100.00
S2		300000	852000	1470000	1160000	20.87			
2	0.0001	300000	906000	1410000	1160000	21.29	100.59	98.86	99.44
4	0.0007	300000	835000	1490000	1120000	20.74	97.97	99.43	97.41
6	0.004	300000	763000	1640000	1160000	20.86	98.53	95.43	94.03
7	0.007	300000	692000	1470000	1220000	16.95	80.10	95.43	76.44
8	0.014	300000	535000	1460000	1170000	13.02	61.51	95.43	58.70
10	0.028	300000	359000	1180000	1090000	7.06	33.36	89.71	29.93
11	0.034	300000	285000	885000	1030000	4.33	20.43	85.71	17.51
12	0.04	300000	200000	637000	897000	3.19	15.05	87.43	13.16
EMS	200 µg/mL	300000	708000	1480000	1130000	17.46	82.51	80.57	66.48
MMS	10 µg/mL	300000	672000	1030000	862000	11.54	54.50	52.57	28.65

NC: Negative control
S: Solvent control
a: TSG = [(value 24 h x value 48 h) / (value 0 h x value 24 h*)]; * : for value 24 h > $3x10^5$ then value 24 h = $3x10^5$
b: RSG = [(value TSG / value TSG of corresponding controls) x 100]
c: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
d: RTG = (RSG x RCE)/100
EMS: Ethylmethanesulfonate
MMS: Methylmethanesulfonate

Table 14: Mutagenicity Data, **without** metabolic activation

		Cloning Efficiency (CE)			Mutagenicity Data						
					Number of cultures / 96 wells						
Test Group	Concentration [mM]	Plate 1[e]	Plate 2[e]	RCE [%][f]	Plate 1[e]	Plate 2[e]	Plate 3[e]	Plate 4[e]	Mean	Mutants/ 10^6 cells[g]	Mutation factor
NC1	0	86	81	95.43	36	42	31	32	35.25	179.57	
NC2		82	80	92.57	31	34	33	31	32.25	176.43	
S1	0	90	85	100.00	33	23	35	28	29.75	122.40	
S2		86	89		26	29	32	28	28.75	117.46	
2	0.0001	89	84	98.86	33	35	34	42	36.00	162.56	1.36
4	0.0007	86	88	99.43	27	29	28	27	27.75	115.30	0.96
6	0.004	84	83	95.43	37	35	22	37	32.75	163.74	1.37
7	0.007	84	83	95.43	29	30	33	32	31.00	153.03	1.28
8	0.014	86	81	95.43	41	41	27	31	35.00	177.95	1.48
10	0.028	82	75	89.71	45	47	56	50	49.50	340.70	2.84
11	0.034	77	73	85.71	53	62	58	43	54.00	435.14	3.63
12	0.04	76	77	87.43	57	61	63	60	60.25	495.78	4.13
EMS	200 µg/mL	71	70	80.57	89	81	88	71	82.25	1172.73	9.78
MMS	10 µg/mL	42	50	52.57	74	77	78	79	77.00	1986.63	16.57

NC: Negative control
S: Solvent control
e: Number of cultures with cell growth.
f: RCE = [(mean value positive cultures / mean value positive cultures of corresponding controls) x 100]
g: Mutation frequency = {-ln [negative cultures/total wells (selective medium)] / -ln [negative cultures/total wells (non selective medium)]}x800
EMS: Ethylmethanesulfonate
MMS: Methylmethanesulfonate

Table 15: Colony Sizing, **without** metabolic activation

Test Group	Concentration [mM]	Wells with at least 1 colony	Large colonies	Small colonies	Quotient Large/Small
NC1	0	141	119	22	5.41
NC2		129	115	14	8.21
S1	0	119	96	23	4.17
S2		115	92	23	4.00
10	0.028	198	141	57	2.47
11	0.034	216	104	112	0.93
12	0.04	241	132	109	1.21
MMS	10 µg/mL	308	123	185	0.66

NC: Negative control
S: Solvent control
MMS: Methylmethanesulfonate

cc: G. Casciano, C. Walsh, R. S. Jones, D. Benmhend, BPPD Science Review File, IHAD/ARS
G. Casciano, Biologist, FT, PY-S: 12/07/2010