DATA EVALUATION REPORT (4-9-99)

NOVARON AGZ330

Study Type: Acute Oral (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Kathleen Luck, B.A.	Date 4/9/99
Independent Reviewer	Jennifer Welham, M.S.	Date 4/9/99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

1

EPA Reviewer: T. McMahon, Ph.D. _____, Date 4/20/99
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Novaron AGZ330 (≥99%)

SYNONYMS: none

CITATION: Parcell, B. and L.A. McRea (1998) Acute Oral Toxicity, Rat. Huntingdon Research Centre, Ltd. (Huntingdon, Cambridgshire, England). TSI 78a/940765/AC, September 12, 1994. MRID 44582906. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44582906), groups of fasted, young CD Sprague-Dawley rats (5/sex) were given a single oral dose of Novaron AGZ330 in distilled water at a dose of 5000 mg/kg (50% w/v solution) and observed for 14 days. Piloerection was observed in all rats on the day of dosing and was cleared by day 2[1] of the experiment. Animals gained weight throughout the study. Necropsies of all animals appeared normal.

Based on results of this study, the oral LD_{50} (male, female, combined) exceeds 5000 mg/kg. Therefore, Novaron AGZ330 is classified as TOXICITY CATEGORY IV for acute oral toxicity.

This acute oral study is classified **unacceptable** (upgradable). Individual animal data were not provided for verification of clinical observation and necropsy statements made in the report. This study does not satisfy the guideline requirement for an acute oral study (OPPTS 870.1100) in the rat but can be upgraded following submission of the individual animal data as stated above. It should also be noted that the age of the animals (4-7 weeks) is not in compliance with the guideline requirement of 8-12 weeks of age for testing. While this would normally result in study rejection, the fact that no deaths were observed at a limit dose does not require that the test be repeated. However, rats of 8-12 weeks of age should be used in future acute oral studies.

[1] In this study, days are referred to by total number of days since the experiment began (i.e., dosing occurred on day 1, day 14 post-dosing is referred to day 15 of the experiment). All references to time within this report are consistent with this timing convention.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: D-1-3
   Purity: ≥99%
   CAS #: not given

2. Vehicle
   The test material was administered at a concentration of 50% w/v in distilled water.

3. Test Animals
   Species: rat
   Strain: CD Sprague-Dawley
   Age at dosing: four to seven weeks
   Weight at dosing: 214-235 g (males), 201-208 g (females)
   Source: Harlan Olac Ltd., Bicester, Oxon, England
   Acclimation period: seven days
   Diet: Biosure LAD 1, available *ad libitum*
   Water: source not provided, available *ad libitum*

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: September 29, 1994
   End: October 13, 1994

2. Animal Assignment and Treatment
   Animals were assigned to the test group noted in Table 1. Following an overnight fast, rats were given a single dose of the test material by gavage then observed for mortality and clinical signs of toxicity several times on the day of dosing, twice daily on days 2 through 14 of the experiment, and once on day 15 of the experiment. Animals were weighed prior to dosing (day 1) and on days 8 and 15 of the experiment. On day 15, all animals were sacrificed and a necropsy was performed on each.

TABLE 1. Group Assignment and Mortality

Dose (mg/kg)	Mortality/Animals Treated		
	Males	Females	Combined
5000 mg/kg	0/5	0/5	0/10

4

3. Statistics
None

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality is given in Table 1. No mortalities were reported.

In this study, the oral LD50 was greater than 5000 mg/kg.

B. CLINICAL OBSERVATIONS
According to the authors, piloerection was observed in all rats within five minutes of dosing and at all observations made the day of dosing; it had cleared in all animals by day 2 of the experiment. No other clinical observations were reported. No raw data is included to confirm this.

C. BODY WEIGHT
Animals gained weight throughout the study.

D. NECROPSY
According to the authors, no abnormalities were observed. No raw data is included to confirm this.

E. DEFICIENCIES
Several major deficiencies were noted. The animals were between 4 and 7 weeks old at the beginning of dosing; however, OPPTS guidelines (870.1100) state that young adult rats should be between 8 and 12 weeks old for this test. Furthermore, individual animal data were not provided for clinical observations or necropsy.

In addition, several minor deficiencies were noted. The study report states that homogeneity, stability, and purity of the test substance are the responsibility of the sponsor; however, homogeneity and stability data are not provided. Furthermore, the water source was not adequately characterized. These deficiencies are considered minor because they are unlikely to have influenced the results of the study.

This study is classified as **unacceptable** (upgradable). Individual animal data were not provided for verification of clinical observation and necropsy statements made in the report. This study does not satisfy the guideline requirement for an acute oral study (OPPTS 870.1100) in the rat but can be upgraded following submission of the individual animal data as stated above. Future experiments of acute oral toxicity should utilize rats between 8-12 weeks of age.

DATA EVALUATION REPORT

NOVARON

Study Type: Acute Dermal (Rat)

Prepared for

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4

EPA Reviewer: T. McMahon, Ph.D. _____, Date 4/20/99
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: Peter Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS 870.1200

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Novaron (≥99%)

SYNONYMS: Partially ion-exchange zirconium phosphate; Novaron AG330; Alpha San RC™ 5000

CITATION: Parcell, B. and L. McRae (1994) Acute Dermal Toxicity, Rat. Huntingdon Research Centre, Ltd. (Huntingdon, Cambridgeshire, England). TSI 67-940212/AC, September 12, 1994. MRID 44582907. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44582907), groups of CD Sprague-Dawley rats (5/sex) were dermally exposed to Novaron in distilled water for 24 hours to at a dose of 2000 mg/kg (100% w/v solution). Animals were then observed for 14 days. No mortalities occurred during the study. No clinical signs of systemic toxicity were noted. No irritation or dermal changes were observed. All males and 4/5 females gained weight during the study. One female did not gain weight between day 1[1] and day 8; however, this animal gained weight by study termination. Necropsies revealed no abnormalities.

In this study, the combined dermal LD_{50} is greater than 2000 mg/kg. Therefore, Novaron is TOXICITY CATEGORY IV for acute dermal toxicity.

This acute dermal study is classified as unacceptable (upgradable) because the study report did not contain individual and test group data as required in the guidelines. The study can be upgraded to acceptable upon receipt and validation of individual animal and test group data. The study does not satisfy the guideline requirement for an acute dermal study (OPPTS 870.1200) in the rat.

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

[1] In this study, days are referred to by total number of days since the experiment began (i.e., dosing occurred on day 1, day 14 post-dosing is referred to day 15 of the experiment). All references to time within this report are consistent with this timing convention.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: 7130519
   Purity: ≥99%
   CAS #: not given

2. Vehicle
   The test material was administered in distilled water at a concentration of 100% w/v.

3. Test Animals
   Species: rat
   Strain: CD Sprague-Dawley
   Age at dosing: 7 - 10 weeks
   Weight at dosing: 256 - 310 g (males), 239 - 280 g (females)
   Source: Harlan Olac, Ltd., Bicester, Oxon, England
   Acclimation period: 21 days
   Diet: Biosure LAD 1, available *ad libitum*
   Water: potable water provided by Anglian Water, available *ad libitum*

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: February 17, 1994
   End: March 3, 1994

2. Animal Assignment and Treatment
   Animals were assigned to the test group noted in Table 1. The test material was applied to the clipped back of each animal for 24 hours. The area, which was approximately 10 percent of the total surface area, was then covered in gauze and non-irritative dressing wrapped around the trunk of the animal. Animals were observed for mortality and clinical toxicity (including erythema and edema[2]) several times on day 1, twice daily on days 2 - 14, and once on day 15. Animals were weighed on days 1 (pre-test), 8, and 15. On day 15, all animals were sacrificed and a necropsy was performed on each.

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[2] Erythema and edema were scored using a standard method; however, no citation is provided.

8

TABLE 1. Group Assignment and Mortality

Dose (mg/kg)	Mortality/Animals Treated		
	Males	Females	Combined
2000	0/5	0/5	0/10

3. Statistics
None

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality is given in Table 1. No mortalities were reported.

In this study, the combined dermal LD_{50} is greater than 2000 mg/kg.

B. CLINICAL OBSERVATIONS
According to the authors, no clinical signs of systemic toxicity were noted. No irritation or dermal changes were observed in any of the animals.

C. BODY WEIGHT
All male animals and 4/5 females gained weight during the study. One female animal did not gain weight between day 1 and day 8; however, this animal gained weight from day 8 to day 15.

D. NECROPSY
According to the authors, no abnormalities were reported.

E. DEFICIENCIES
Tabular data of individual and group data for clinical observations and necropsy were not included in the study report; the guidelines state that the data must be included. This deficiency is considered major because the study report cannot be validated against the actual data. However, the study can be upgraded to acceptable upon receipt and validation of the data.

Several minor deficiencies were also noted. The guidelines state that rats must be between 8 and 12 weeks old; the study reports that the animals were between 7 and 10 weeks old. The study report states that homogeneity, stability, and purity of the test substance are the responsibility of the sponsor; however, homogeneity and stability data are not provided. These deficiencies are considered minor because they are unlikely to have influenced the results of the study.

DATA EVALUATION REPORT

EXPERIMENTAL ADDITIVE 9823-37

Study Type: Acute Inhalation (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
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Project Manager (QA/QC Manager)	*Ellen Mantus, Ph.D.*	Date 4/9/99

Contract Number: 68-W6-0022
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EPA Reviewer: T. McMahon, Ph.D. _____, Date 4/20/99
Senior Toxicologist, RASSB/AD (7510W)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat
OPPTS 870.1300

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Experimental Additive 9823-37 (purity not given)

SYNONYMS: None

CITATION: Blagden, S.M. (1998) Acute Inhalation Toxicity, Rat. Safe Pharm Laboratories (Derby, UK). SPL Project No. 656/014, April 15, 1998. MRID 45582908. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 44582908), young adult Sprague-Dawley Crl:CD®BR rats (5/sex) were exposed by inhalation route (nose only) to Experimental Additive 9823-37 for 4 hours at a mean gravimetric concentration of 5.18 mg/L. Animals then were observed for 14 days. One animal died on day 2. All animals exhibited hunched posture, piloerection, and increased respiratory rate following exposure; all but one animal exhibited labored respiration. All effects had cleared by day 6. Five of the nine surviving animals lost weight by day 7, but all nine surviving animals had gained weight by day 14. Necropsy of the animal that died revealed enlarged, abnormally red lungs with dark patches, patchy pallor of the liver, and congestion of the small intestine. Necropsy of the surviving animals revealed a gray mottled appearance of the lungs or dark foci of the lungs in five of the nine animals; four of the nine surviving animals exhibited no abnormalities.

In this study, the combined inhalation LC_{50} was greater than 5.18 mg/L (mean gravimetric concentration). Therefore, Experimental Additive 9823-37 is classified as TOXICITY CATEGORY IV for acute inhalation toxicity.

This acute inhalation study is classified as **unacceptable** (upgradable) and does not satisfy the guideline requirement for an acute inhalation study (OPPTS 870.1300) in the rat.. This study may be upgraded upon receipt of the exact chemical identity and purity of the test material used in this study.

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

11

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: 7170706
   Purity: not given
   CAS #: not given

2. Vehicle
   none

3. Test Animals
   Species: rat
   Strain: Sprague-Dawley Crl:CD®BR
   Age at Dosing: 8 to 10 weeks
   Weight at Dosing: 291-334 g (males), 220-243 g (females)
   Source: Charles River Ltd, Margate, Kent, UK
   Acclimation Period: at least five days
   Diet: Rat and Mouse Expanded Diet No. 1, available *ad libitum* (except during exposure)
   Water: source not provided, available *ad libitum* (except during exposure)
   Housing: in groups of 5 by sex in solid-floor polypropylene cages with stainless steel lids under conditions of controlled temperature and humidity.

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: March 6, 1998
   End: March 24, 1998

2. Exposure Conditions
   During exposure, animals were held in a restraining tube fitted onto a single tier of the exposure chamber. Only the nose of the animal was exposed. The test material was generated as a dust directly into the exposure chamber from a Wright Dust Feed mechanism attached to a compressed air supply. The chamber airflow rate was measured every fifteen minutes during exposure and was maintained at 20 L/minute. Temperature and relative humidity were measured every thirty minutes during exposure; the temperature was 19°F and the relative humidity ranged from 37-42%. Oxygen content during test material delivery was maintained at 19%.

3. Animal Assignment and Treatment
   Animals were assigned to the test group noted in Table 1. The animals were exposed to the test material by nose only exposure for 4 hours. They were observed hourly during exposure, immediately after and one hour after exposure, and daily thereafter for 14 days. The animals were weighed prior to treatment (pre-test) and on days 7 and 14 post-exposure (or at death). On day 14 post exposure, survivors were sacrificed; a necropsy was performed on all animals.

TABLE 1. Group Assignment and Mortality

Nominal Concentration (mg/L)	Gravimetric Concentration (mg/L)	MMAD μm	GSD μm	Mortality/Animals treated		
				Males	Females	Combined
25.8	5.18	1.7	0.54	1/5	0/5	1/10

4. Chamber Concentration and Particle Size

Time to equilibrium was 7 minutes. Nominal test atmosphere concentration was calculated by dividing the total weight of the test material used by the total volume of air passed through the chamber. The gravimetric concentration was determined from samples taken during exposure using Gelman A/E 25 mm glass fiber filters in a filter holder. Results are given in Table 1 above. Mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) were determined using a Marple Cascade impactor. Results are given in Table 1 above.

5. Statistics

Statistical analyses were not performed.

II RESULTS AND DISCUSSION

A. EXPOSURE CHAMBER DATA

From measurement of the test material in the chamber used to expose the rats approximately every 15 minutes during exposure, a mean achieved concentration of 5.18 mg/L was recorded. Particle size analysis showed a MMAD of 1.7 μm, with greater than 91.0% of particles at 4 μm or less.

B. MORTALITY

Mortality is given in Table 1. One male was found dead on day 2 following exposure.

C. CLINICAL OBSERVATIONS

Wet fur, hunched posture, piloerection, and a change in respiration rate (increase 8/10, decrease 2/10) were observed in all animals during exposure. Hunched posture, piloerection, and increased respiratory rate were observed in all animals following exposure; labored respiration was observed in all but one animal following exposure, as well. Other effects exhibited after exposure included a tip-toe gait, lethargy, and noisy respiration. All effects had cleared by day 6.

D. BODY WEIGHT

Five of the nine surviving animals lost weight by day 7. All nine surviving animals gained weight by day 14.

E. NECROPSY

Necropsy of the animal that died following exposure revealed enlarged, abnormally red lungs with dark patches, patchy pallor of the liver, and congestion of the small intestine. Necropsies of the surviving animals revealed gray mottled appearance of the lungs or dark foci of the lungs in five of the nine animals; four of the nine surviving animals exhibited no abnormalities.

F. DEFICIENCIES

There was no information given in the report on the actual chemical identity of the test substance or its purity. This information is essential in order for this study to be considered acceptable.

DATA EVALUATION REPORT

NOVARON

Study Type: Primary Eye Irritation (Rabbit)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

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Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit
OPPTS 870.2400

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Novaron (≥ 99%)

SYNONYMS: Partially ion-exchange zirconium phosphate; Novaron AG-300; AlphaSan™ RC 5000

CITATION: Parcell, B. (1994) Primary Eye Irritation, Rabbit. Huntingdon Research Centre, Ltd. (Huntingdon, Cambridgeshire, England). TSI 69/940296/SE, September 7, 1994. MRID 44582910. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44582910), 40 mg (0.1 ml) of Novaron was instilled into the lower everted lid of six female adult New Zealand White rabbits. Animals were then observed for 72 hours. Irritation was scored by a standard method; however, the citation was not given. No iridal irritation was observed. All animals exhibited chemosis (above normal to obvious swelling) and discharge (slight to considerable). Three animals exhibited corneal opacity (scattered) and five animals exhibited redness (injected vessels to diffuse color). Irritation cleared in 5/6 animals by 48 hours and in all animals by 72 hours.

In this study, Novaron is a slight eye irritant. Irritation cleared in less than 7 days. Therefore, Novaron is classified as TOXICITY CATEGORY III for primary eye irritation.

This study is classified as acceptable. It does satisfy the guideline requirement for a primary eye irritation study (OPPTS 870.2400) in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: 7130519
   Purity: ≥ 99 %
   CAS #: not provided

2. Vehicle
   Test material was administered as provided.

3. Test Animals
   Species: rabbit
   Strain: New Zealand White
   Age at treatment: 13 to 15 weeks
   Weight at treatment: 3.0 - 3.6 kg
   Source: Froxfield (U.K.), Ltd., Petersfield, Hampshire, England
   Acclimation period: not provided
   Diet: SDS Stanrab (P) Rabbit diet, available *ad libitum*
   Water: source not provided, available *ad libitum*
   Housing: individually in plastic cages with perforated floors.

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: April 11, 1994
   End: April 25, 1994
   The animals were not dosed concurrently.

2. Animal Assignment and Treatment
   The eyes of all animals were examined prior to treatment to ensure that there was no pre-existing damage to the cornea or iris, or conjunctival inflammation. For test material administration, a 40 mg (0.1 ml) dose of the test material was instilled into the lower everted lid of one eye of each of six female rabbits. Animals then were observed at 1, 24, 48, and 72 hours after exposure. Irritation of the cornea, iris, and conjunctivae were scored using a standard method; however, a citation was not provided.

II. RESULTS AND DISCUSSION

A. RESULTS

No irritation of the iris was observed in any of the animals. Five animals exhibited a dulling of normal corneal luster at 1 hour and 3/6 animals exhibited scattered areas of opacity at 24 hours. Redness (injected vessels to diffuse color) was observed in all animals at 1 hour, in 3/6 animals at 24 hours, and in 1/6 animals at 48 hours. Chemosis (above normal to obvious swelling) was exhibited in 5/6 animals at 1 hour. Discharge (slight to considerable) was observed in all animals at

1 hour and in 3/6 animals at 24 hours. Irritation cleared in 5/6 animals by 48 hours and in all animals by 72 hours.

B. DEFICIENCIES

Several minor deficiencies were noted. In the study summary (p. 7) and in the results (p. 14), the author states that all animals exhibited chemosis; however, one animal did not exhibit chemosis. This deficiency is considered minor because the toxicity category determination was based on the raw data. In addition, the citation of the method used to score irritation was not provided; however, the method is comparable to the guidelines. Several aspects of the study were not adequately characterized, including the source of the drinking water and the eye in which the test material was instilled. These deficiencies are considered minor because they are unlikely to have influenced results of the study.

DATA EVALUATION REPORT

NOVARON

Study Type: Dermal Sensitization (Guinea Pig)

Prepared for

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EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - (Guinea Pig)
OPPTS 870.2600

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Novaron (≥ 99%)

SYNONYMS: Partially ion-exchange zirconium phosphate, Novaron AG 300; AlphaSan™ RC5000

CITATION: Allan, S. (1994) Skin Sensitization, Guinea Pig. Huntingdon Research Center, Ltd. (Huntingdon, Cambridgeshire, England). TSI 70/940132/SS, September 20, 1994. MRID 44582911. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44582911) with Novaron in distilled water, ten young adult, female Dunkin/Hartley guinea pigs were tested using the GPMT method of Magnusson and Kligman. The test did not produce contact hypersensitivity in any of the previously induced animals. Erythema and edema were not present in any of the previously induced animals.

In this study, Novaron is not a dermal sensitizer.

This study is classified as unacceptable (upgradable) because of deficiencies in study protocol (see Deficiencies section). This study does not satisfy the guideline requirement for a dermal sensitization study (OPPTS 870.2600) in the guinea pig but can be upgraded upon submission of the requested data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: 7130519
   Purity: ≥ 99%
   CAS #: not provided

2. Vehicle and Positive Control
   The test material was administered at a concentration of 0.25% w/v in water during the intradermal induction phase and at a concentration of 60% w/v during the topical induction phase. It was administered in distilled water at concentrations of 60% and 30% w/v during the topical challenge phase. The positive control was cited as formalin. The report presented results of recent studies conducted with formalin at the testing laboratory (Appendix 3, page 25). In these studies, Formalin was administered at a concentration of 10% w/v in water during the topical induction phase, 0.1% w/v in water for the intradermal induction phase, and at concentrations of 5% and 1% w/v in water during the challenge phase.[1] The results of these studies showed evidence of sensitization in the majority of animals tested, supporting the use of these data as positive controls for this assay.

3. Test Animals
   Species: guinea pig
   Strain: Dunkin/Hartley
   Age at start of treatment: six to seven weeks
   Weight at start of treatment: 315 - 410 g
   Source: D. Hall, Newchurch, Staffordshire, England
   Acclimation period: 5 days
   Diet: Vitamin C enriched FD1, available *ad libitum*; hay was given weekly.
   Water: source not specified, available *ad libitum*
   Housing: groups of 5, in suspended metal cages with wire mesh floors.

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: January 26, 1994
   End: February 28, 1994

2. Animal Assignment and Treatment
   The study was conducted according to the GPMT method of Magnusson and Kligman

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[1] The positive control data was a table of results from sixteen previous sensitization tests. All but one of the tests used the doses listed; the remaining test used 3% and 1.5% w/v in water for the challenge phase, 5% and 3% for the second challenge phase, 5% w/v in water for the topical induction phase, and 0.05% for the intradermal induction phase.

(Magnusson, B. and A.M. Kligman. 1970. Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens, Thomas, C.C., Springfield, Illinois.). Prior to induction, a preliminary study was performed to determine the doses for induction and challenge phases. For the preliminary study, two female animals were intradermally exposed to eight concentrations of the test material (10, 7.5, 5.0, 2.5, 1.0, 0.5, 0.25, 0.1% w/v in water) and a vehicle control, and four female animals were dermally exposed to four concentrations of the test material (60, 50, 30, 20% w/v in distilled water). The concentrations selected for the induction phase were 0.25% w/v in water for the intradermal injection and 60% w/v in distilled water for topical application which, according to the study author, was the maximum practical concentration that did not produce irritating effects. The topical challenge concentrations selected were 30% and 60% w/v in distilled water.

For the induction phase, ten female animals were dosed with three pairs of intradermal injections: Freund's complete adjuvant (FCA) diluted with an equal volume of water, the test material at a concentration of 0.25% w/v in water, and the test material at a concentration of 0.25% w/v in equal parts FCA and water. The injections were made from front to back on the right and left side on the clipped back of the animal. Six days after the injection, a 0.2 ml dose of 10% w/w sodium lauryl sulphate in petroleum was rubbed on the injection area. Twenty-four hours later, animals were topically dosed for 48 hours with a 0.4 ml dose of the test material at a concentration of 60% w/v in distilled water. The substance was applied using a patch of Whitman No. 3 paper, secured by tape and an occlusive dressing. In addition, five female control animals were treated using the same method; however, the test material was omitted from the injections and topical application. All animals were left untreated for two weeks.

For the challenge phase, previously induced animals were dermally exposed for twenty-four hours to 0.2 ml doses of the test article at concentrations of 30% and 60% w/v in distilled water. The test material was applied to the clipped and shaved flank of the animal; the 60% w/v dose was applied anterior to the 30% dose. The test material was applied using the same method as described in the topical induction. In addition, the five control animals were treated using the same method. Animals were then observed at 24, 48, and 72 hours for erythema and edema using a standard method (citation not provided).

II. RESULTS AND DISCUSSION

A. INDUCTION REACTIONS AND DURATION

Following the intradermal injection, slight irritation was observed at the test material injection site and necrosis was observed at the FCA and FCA and test material sites. The topical induction produced very slight erythema at all injection sites. Following the intradermal injection in the control animals, necrosis was observed at the two FCA-containing sites; no irritation was observed at the distilled water site. Following the topical induction in the control animals, very slight erythema was observed.

B. CHALLENGE REACTIONS AND DURATION

The test material did not induce contact hypersensitivity in any of the previously induced guinea pigs (incidence = 0/10). Erythema and edema were not observed in any of the previously induced animals. Erythema and edema were not observed in the control animals.

22

C. POSITIVE CONTROL

The study report provides a tabular summary of sixteen recent sensitization tests using formalin. Positive results in all animals (i.e., 10/10 animals) occurred in 13/16 tests; 1/16 tests required a rechallenge, and 2/16 tests had negative or inconclusive animals.

D. DEFICIENCIES

The major deficiency noted in this study was the lack of individual animal data for the induction phase. This is considered to be a major deficiency because the authors observations cannot be validated. These data are required to upgrade this study.

The animals used in the preliminary study appear to have been used in the actual study. According to animal numbers, two control animals received intradermal injections, and three control animals and one test animal received topical treatments of the test material in the preliminary study. Furthermore, the date of the preliminary study was not provided, nor was the amount of time between the preliminary and actual study. However, because no dermal sensitization was observed upon challenge, this deficiency is considered to be minor for this study.

In addition, several aspects of the study were not adequately characterized. The protocol and individual tabular data for the positive control studies were not provided. The justification for using formalin as the positive control was that, according to the author, it is a "known dermal sensitizer." The substance used for the positive control study was not one of the preferred substances listed in the guidelines. The method of scoring erythema and edema was not provided; however, it is comparable to the guidelines. The source of the water was not provided. These are all considered minor because they do not appear to have influenced the outcome of the study.

2-3

DATA EVALUATION REPORT

Partially Ion-Exchange Zirconium Phosphate (NOVARON)

Study Type: Two-Week Palatability Study (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Greg Lawrence, DVM, MS	Date 4-7-99
Independent Reviewer	P. Robinan Gentry, MS	Date 4-7-99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-97
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

24

EPA Reviewer: Tim McMahon, Ph.D. _____, Date 4/27/99

EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Two-week palatability study in the rat

DP BARCODE: D253496 & D253509 SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Partially ion-exchange zirconium phosphate; purity ≥ 99%.

SYNONYMS: NOVARON AG300; AlphaSan® RC5000

CITATION: S. Allen and A. Hawkins, (1994), NOVARON AG300: Two-week palatability study, rat, Huntingdon Research Centre, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire PE 18 6ES, England, Laboratory Report #TSI 75/942358, Study Date: September 13, 1994, MRID# 44582915, Unpublished.

SPONSOR: Milliken Chemical
Division of Milliken & Company
P.O. Box 1927
Spartanburg, S.C. 29304-1927

EXECUTIVE SUMMARY

This study wasdesigned to assess palatibility of NOVARON and to determine suitable doses for a 90-day toxicity study in rats. In this study, NOVARON was administered in the diet to groups of five male and 5 female rats at dose levels of 250, 500, or 1000 mg/kg/day. COntrol groups received basal diet only. Body weight, food consumption, and clinical observations were recorded during the study. All animals were killed and examined macroscopically on day 15. There were no treatment related effect on food consumption, water consumption, mortality, clinical signs of toxicity, body weight or body weight gain, organ weight, or macroscopic observations following the administration of NOVARON AG300 at doses up to and including 1000 mg/kg/day. .

This study is considered to be acceptable (non-guideline).

The LOAEL is > 1000 mg/kg/day. The NOAEL ≥ 1000 mg/kg/day .

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Partially ion-exchange zirconium phosphate (NOVARON AG300)
   Description: White powder
   Lot #: 7130519
   Purity: ≥99% a.i.
   Stability of compound: Reported to be available from the sponsor
   CAS #: Not given
   Structure not available

2. Vehicle and/or positive control: None

3. Test animals
   Species: Rat
   Strain: CD - Sprague-Dawley (Crl:CD BR VAF PLUS™)
   Age: 28 days
   Weight: 69 g upon arrival
   Source: Charles River (UK) Limited, Margate, Kent, UK
   Housing: Animals were housed in groups of 5/cage in suspended stainless steel cages with wire mesh floors.
   Diet: Powdered, Special Diet Services Rat and Mouse No 1 SQC Maintenance Diet ad libitum
   Water: Anglian Water ad libitum
   Environmental conditions: Temperature: 19.5-22°C.
   Humidity: 42-57 %
   Air changes: 19/hour
   Photoperiod: 12 light/12 dark
   Acclimation period: 12 days

B. STUDY DESIGN:

1. In life dates - start: February 21, 1994 end: March 7, 1994

2. Animal assignment

   The authors reported that animals were randomly assigned to four groups/sex (a total of 5 rats/sex/dose-group; Table 1) according to a computer program that insured that the weight distribution within each group was similar. Each animal was identified within each cage with an ear punch and a tail tattoo. Each cage was identified with a color coded card that displayed the study schedule number, cage number, sex, individual animal numbers, and initials of the study director and Home Office Licensees.

Table 1: Study Design and Distribution of Animals into Dose/Sex-Groups

Test Group	Conc. in Diet	Dose to animal mg/kg/day	Main Study 13-weeks	
			male	female
Control	Variable	0	5	5
Low (LDT)	Variable	250	5	5
Mid (MDT)	Variable	500	5	5
High (HDT)	Variable	1000	5	5

3. Dose selection rationale

Dose selection was based on an acute oral toxicity study (HRC Report No. TSI 66/940213). The authors note: " A dosage of 1000 mg/kg/day is the limit level for this study design." However, the authors did not provide any data from this acute oral study.

4. Diet preparation and analysis

Diet admixture was prepared weekly by adding an appropriate amount of a high-dose pre-mixture to plain diet and mixing with a Turbula Mixer. The pre-mixture was prepared by grinding the test article directly into the diet followed by mixing with the Turbula Mixer. The study authors note that the homogeneity, purity, and stability of the test article were the responsibility of the sponsor. Concentration of the test substance in diet, homogeneity of the test substance in diet, and absorption of the test substance were not reported.

There was no analytical data provided in this study that would indicate whether the mixing procedure was adequate or whether the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

For food and water consumption, body weight, organ weight and clinical pathology, if one particular value was predominant (frequency of the mode exceeded 75%), then values different from the mode was analyzed by Fisher's exact test followed by Mantel's trend test. Otherwise, data were evaluated for homogeneity of variance using Bartlett's test. If the variance was homogenous, or if logarithmic transformations could stabilize the variance, then the data were analyzed using a one-way analysis of variance followed by William's test to establish differences between treatment groups. If after data manipulation, the variance remained heterogenous, then data were analyzed using a Kruskal-Wallis analysis of ranks followed by Shirley's test to establish differences between treatment groups.

C. METHODS

1. Observations

Animals were inspected twice daily for mortality and/or clinical signs of toxicity, once in the morning and once in the afternoon.

2. Body weight

Animals were weighed on days 1, 8 and 15.

3. Food and water consumption and compound intake

Food consumption for each cage of 5 animals was determined weekly. Compound intake (mg/kg/day) for each group was calculated on a weekly basis from the food consumption data, body weight, and the dietary inclusion levels of the test substance.

4. Water consumption

Water consumption was measured daily by visual appraisal throughout the treatment period.

7. Sacrifice and Pathology

All animals were sacrificed (cervical dislocation) on schedule (day 14), and were subjected to gross pathological examination. In addition, the liver, kidneys and spleen from each animal were dissected, trimmed and weighed. All macroscopic abnormalities were recorded and the tissues were preserved in 10% buffered formalin.

II. RESULTS

a. Observations

1. Clinical signs of Toxicity : There was no clinical evidence of toxicity that could be attributed to the test substance in any treatment group when compared to the corresponding controls.

2. Mortality - All animals survived to terminal sacrifice.

B. Body weight - There were no statistically significant differences in body weight (Table 2) or body-weight gain (Table 3) in any treatment groups when compared to corresponding control values.

Table 2: Mean Terminal Body Weights Expressed in Grams

Sex	Group/Dose Level (mg/kg/day)			
	0	250	500	1000
Males	300	293	295	291
Females	213	214	211	204

Standard deviations not provided.
Data extracted from Table 1 of the study report, pg 18.

Table 3: Mean Body Weight Gain Expressed in Grams

Sex	Group/Dose Level (mg/kg/day)			
	0	250	500	1000
Males	117	111	114	110
Females	53	54	52	49

Standard deviations not provided.
Data extracted from Table 1 of the study report, pg 18.

C. Food and water consumption and compound intake

1. Food and water consumption - There were no reported differences in food and water consumption that could be attributed to administration of the test article in any treatment group when compared to corresponding control values (Table 4).

Table 4: Mean Weekly Food Intake Expressed as g/rat/week

Sex	Group/Dose Level (mg/kg/day)			
	0	250	500	1000
Week 1				
Males	158	179	159	164
Females	178	157	107	178
Week 2				
Males	163	171	193	179
Females	182	188	107	208

Standard deviations not provided.
Data extracted from Table 2 of the study report, pg 19.

2. Compound consumption (time-weighted average) : The mean test-compound consumption for each dose-group/sex for the duration of the study was calculated using food intake data, body weight data, and nominal test compound concentrations in diet (actual test article concentrations were not determined). The mean results are illustrated in Table 5.

Table 5: Mean Daily Compound Intake Expressed as (mg/kg/day)

Sex	Group/Dose Level (mg/kg/day)			
	0	250	500	1000
Males	0	242	515	1012
Females	0	271	431	1155

Standard deviations not provided.
Data extracted from Table 4 of the study report, pg 21.

G. Sacrifice and Pathology

1. Organ weight

There were no statistically significant differences in organ weight that could be attributed to administration of the test article observed in any of the treatment groups, when compared to the corresponding controls.

Table 6: Absolute Organ Weights Expressed as Mean (g) [1]

Tissue	Group/Dose Level (mg/kg/day)			
	0	250	500	1000
Males				
Liver	12.8	12.3	13.1	12.7
Spleen	0.82	0.98	0.84	0.77
Kidney	2.18	2.20	2.16	2.08
Females				
Liver	8.9	8.4	8.1	8.1
Spleen	0.64	0.58	0.56	0.63
Kidney	1.74	1.74	1.60	1.67

Standard deviations were not provided
Data Extracted from Table 5 of the study report, pg 22.

30

2. Gross pathology

The authors reported that there were no statistically significant macroscopic observations in any treatment group. These results were not provided in the study report.

III. DISCUSSION

This study (MRID#44582915) investigated the palatability of NOVARON AG300 in male and female Sprague-Dawley rats fed a diet containing calculated doses of 250, 500, and 1000 mg/kg/day of the test substance for 14 days. This study also assessed the potential dose levels for use in a 90 day toxicity study.

There were no reported effects on food consumption, water consumption, mortality, clinical signs of toxicity, body weight or body weight gain, organ weight, or macroscopic observations that could be attributed to the administration of NOVARON AG300 in the diet to male and female Sprague-Dawley rats. The objective of this study was to investigate the palatability of NOVARON AG300 and to confirm a suitable high-dosage for use in a subsequent 13-week toxicity study. The authors concluded that based upon the data in this study, NOVARON AG300 is palatable in feed at dose levels up to 10,000 ppm, or 1%. The study authors also concluded from this study that the 1000 mg/kg/day dose was a suitable choice as the high-dose to be used in a subsequent 13-week dietary study.

This study is classified as acceptable (non-guideline) and satisfies the purpose for which it was conducted (palatibility and dose selection for a 90-day toxicity study).

DATA EVALUATION REPORT

Partially Ion-Exchange Zirconium Phosphate (NOVARON AG300)

Study Type: 90-Day Oral Toxicity (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Jeffrey Bounds, Ph.D.	Date 4-7-99
Independent Reviewer	Greg Lawrence, MS, DVM	Date 4-7-99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-97
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

32

EPA Reviewer: Tim McMahon,, Ph.D. _____ , Date 5/27/99
Senior Toxcologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: 90-day Oral Toxicity Study in Rodents OPPTS 870-3100

DP BARCODE: D253496 & D253509 SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Partially ion-exchange zirconium phosphate; purity ≥ 99%.

SYNONYMS: NOVARON AG300; AlphaSan®RC5000

CITATION: S. Allen and A. Hawkins, November 29, 1995, 90-day oral toxicity study, rat, Huntingdon Research Centre, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE 18 6ES, England, Laboratory Report #TSI 76/950471, Study Date: August 24, 1994 - May 26, 1995, MRID# 44582916, Unpublished

SPONSOR: Milliken Chemical
Division of Milliken & Company
P.O. Box 1927
Spartanburg, S.C. 29304-1927

EXECUTIVE SUMMARY

In a 90-day toxicity study (MRID# 44582916), partially ion-exchange zirconium phosphate (NOVARON AG300) was administered to Sprague-Dawley rats (10 rats/sex/dose) as an admixture in diet at dose levels of 0, 30, 300, and 1000 mg/kg/day for 13-weeks.

Administration of NOVARON AG300 in the diet to male and female Sprague-Dawley rats resulted in statistically significant increases in packed cell volume and red blood cell counts, and significant decreases in mean corpuscular volume and Thrombotest time in male treatment groups when compared to the control group. Because the magnitude of these hematological differences were small and only occurred in one sex, they were not considered to be treatment-related. Statistically significant increases alkaline phosphatase were also observed in both mid-dose and high-dose male and female treatment groups when compared to the corresponding control values, however, in the absence of any histological observations, the toxicological significance of these observations is unclear. A statistically significant increase in urinary volume, and a decrease in urinary protein were observed in high-dose females, however, there were no histological observations or changes in indicators of renal function that suggested these were toxic effects. A decrease in spleen weight was observed in high-dose males, and an increase in spleen weight was observed in high-dose females, however, they were not considered treatment-related because the effects were opposite. Additionally, a decrease in testicular weight and epididymides weight were observed in mid-dose and high-dose males, and high-dose males respectively. These changes were considered to be the result of biological variation because the differences were ≤ 10%, and because there were no decreases in

relative testicular or epididymides weights. No differences in macroscopic or microscopic findings that could be attributed to treatment were noted in the high-dose treatment groups when compared to the corresponding controls.

The Systemic LOAEL was > 1000 mg/kg/day. The Systemic NOAEL ≥ 1000 mg/kg/day .

This subchronic toxicity study in the rat is classified as **acceptable** (guideline) and satisfies the OPPTS Guideline [OPPTS 870.3100] for a subchronic toxicity study in rats

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Partially ion-exchange zirconium phosphate (NOVARON AG300)
   Description: White powder
   Lot #: 7130519
   Purity: ≥99% a.i.
   Stability of compound: For the duration of the study.
   CAS #: Not given
   Structure not available

2. Vehicle and/or positive control: None

3. Test animals
   Species: Rat
   Strain: CD - Sprague-Dawley (Crl:CD BR VAF PLUS™)
   Age: 28 days
   Weight: Not specified other than ± 17% of means for males and females
   Source: Charles River (UK) Limited, Margate, Kent, UK
   Housing: Animals were housed in groups of 5/sex/cage in suspended stainless steel wire mesh cages.
   Diet: Powdered, Special Diet Services Rat and Mouse No 1 SQC Maintenance Diet ad libitum
   Water: Source not specified ad libitum

   Environmental conditions: Temperature: 19-24.5°C.
   Humidity: 50-70 %
   Air changes: 19/hour
   Photoperiod: 12 light/12 dark
   Acclimation period: 14 days

B. STUDY DESIGN:

1. In life dates - start: August 31, 1994 end: December 8, 1994

2. Animal assignment

   The authors reported that animals were randomly assigned to four groups/sex (a total of 10/sex/dose-group; Table 1) according to a computer program that insured that the weight distribution within each group was similar. Each animal was identified within each cage with an ear punch and a tail tattoo. Each cage was identified with a color coded card that displayed the study schedule number, cage number, sex, individual animal numbers and initials of the study director.

Table 1: Study Design and Distribution of Animals into Dose/Sex-Groups

Test Group	Conc. in Diet	Dose to animal mg/kg/day	Main Study 13-weeks	
			male	female
Control	Variable	0	10	10
Low (LDT)	Variable	30	10	10
Mid (MDT)	Variable	300	10	10
High (HDT)	Variable	1000	10	10

3. Dose selection rationale

Dose selection was based on the results from an acute oral toxicity study (HRC Report No. TSI 66/940213) and a two-week palatability study (HRC Report No. TSI 75/942358; MRID#44582915) performed at Huntingdon Research Centre. In the 2-week palatability study, there were no adverse toxicological effects or evidence of unpalatability noted in animals receiving 1000 mg/kg/day, and 1000 mg/kg/day was therefore chosen as the highest dose for this 13-week study. The 30 and 300 mg/kg/day doses were thought suitable for producing the dose-response relationship.

4. Diet preparation and analysis

Diet admixture was prepared weekly by adding an appropriate amount of a high-dose pre-mixture to untreated diet. The pre-mixture was prepared by mixing a small quantity of finely sieved (335 *u*) diet with the test article, then adding more finely sieved diet to the mix, then passing this mixture through a coffee grinder, then mixing this mixture with coarser diet (355 *u*) for 5 minutes using a Turbula mixer. Homogeneity was tested prior to the initiation of the experiment on multiple samples taken from the top, middle, and bottom of 25 ppm and 25,000 ppm preparations. Homogeneity (Table 2) was expressed as the mean and a coefficient of variation (CV). The authors noted that stability was tested prior to the initiation of the experiment on 25 and 25,000 ppm samples of admixture stored for 0, 4, 8, and 15 days. Stability was expressed as the mean and as a relative mean error (RME), representing the deviation from the nominal concentration (Table 3). Samples of diets prepared for weeks 1, 6, and 13 of the experiment were analyzed for concentration. Concentrations were reported as the means and as a RME (Table 4).

Table 2: Homogeneity Analysis [1]

Nominal Concentration (ppm)	Mean , CV (%)
25	24.1, 3.29%
25,000	24,700, 4.59%

Table 3: Stability Analysis [1]

Nominal Concentration (ppm)	Days of Storage: Mean, Relative Mean Error (%)			
	0	4	8	15
25	22.8, -8.8%	23.4, -6.4%	23.0, -8.0%	23.0, -8.0%
25,000	25,400, 1.7%	26,000, 3.8%	23,000, -8.0%	24,200, -3.0%

Table 4: Concentration Analysis [1]

Week of Preparation	Group	Nominal Concentration (ppm)	Analyzed Concentration (ppm)	RME (%)
1	2 male	259	270	4.2
	3 male	2622	2693	2.7
	4 male	8953	9565	6.8
	2 female	256	252	-1.6
	3 female	2711	2737	1.0
	4 female	8934	9674	8.3
6	2 male	464	481	3.7
	3 male	4610	4924	6.8
	4 maie	14963	15838	5.8
	2 female	397	408	2.8
	3 female	4046	4223	4.4
	4 female	13471	15158	12.5
13	2 male	607	548	-9.7
	3 male	5935	5450	-8.2
	4 male	19325	18378	-4.9
	2 female	473	431	-8.9
	3 female	4755	4357	-8.4
	4 female	16468	15806	-4.0

[1] Data extracted from Formulation Analysis Report, pg 207.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

For food and water consumption, body weight, organ weight and clinical pathology, if one particular value was predominant (frequency of the mode exceeded 75%), then values different from the mode were analyzed by Fisher's exact test followed by Mantel's trend test. Otherwise, data were evaluated for homogeneity of variance using Bartlett's test. If the variance was homogenous, or if logarithmic transformations could stabilize the variance, then the data were analyzed using a one-way analysis of variance followed by William's test to establish differences between treatment groups. If after data manipulation, the variance remained heterogenous, then data were analyzed using a Kruskal-Wallis analysis of ranks followed by Shirley's test to establish differences between treatment groups.

C. METHODS

1. Observations

Animals were inspected twice daily for mortality and/or clinical signs, once in the morning and once in the afternoon.

2. Body weight

Animals were weighed at randomization, and at weekly intervals starting 1 week prior to study initiation and continuing through week 13.

3. Food and water consumption and compound intake

Food consumption for each cage of 5 animals was determined weekly starting 1 week prior to study initiation and continuing through week 13. Food intake per rat was determined by dividing the amount of food consumed per cage by the number of surviving animals in the cage. Compound intake (mg/kg/day) for each group was calculated on a weekly basis from the food consumption data, body weight, and the dietary inclusion levels of the test substance.

4. Ophthalmoscopic examination

All rats were examined opthalmologically using a Keeler indirect ophthalmoscope before randomization, and all rats in the control and high-dose groups were examined again during week 13.

5. Clinical Pathology

Fasted blood samples were collected via the orbital sinus from all rats prior to terminal sacrifice at week 13 for hematology and clinical chemistry analysis.. Blood was collected in three types of tubes, EDTA anticoagulant tubes for hematology, citrate anticoagulant tubes for

coagulation test, and heparin anticoagulant tubes for biochemical test. The CHECKED (X) parameters were examined (Table 5: Hematology, Table 6: Clinical Chemistry). Additionally, blood film slides were examined for polychromasia, hypochromasia, anisocytosis, and rouleaux formation.

Table 5: Hematological Parameters

X	Hematocrit (HCT)*	X	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements* (Thrombotest)		

* required for 90-day toxicity study in the rat (OPPTS 870.3100)

Table 6: Clinical Chemistry Parameters

X	ELECTROLYTES	X	OTHER
x	Calcium	x	Albumin*
x	Chloride	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus	x	Total Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES@	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase	x	A/G ratio
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)		
x	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for 90-day toxicity study in the rat (OPPTS 870.3100)

@ At least 2 hepatic enzymes should be tested according to OPPTS Guidelines 870.3100

39

6. Urinalysis

Urine was collected from fasted animals housed in metabolic cages prior to sacrifice at week 13. The CHECKED (X) parameters were examined.

Table 7: Urinalysis Parameters

X		X	
	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	pH	x	Blood
x	Sediment (microscopic)		Nitrate
x	Protein	x	Urobilinogen

Not required for 90-day toxicity study in the rat (OPPTS 870.3100)

7. Sacrifice and Pathology

All animals that died and those sacrificed (by carbon dioxide asphyxiation) on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. All tissues were fixed in a 10% buffered formalin solution except for the eyes, which were fixed in Davidson's fixative, and the testes/epididymides, which were fixed in Bouin's solution and then transferred to 70% alcohol. All tissues to be examined histologically were embedded in paraffin wax, sliced into 4 um sections, and stained with hematoxylin and eosin. All tissues from the control and high-dose groups, and all tissues from premature decedent animals were examined. The kidneys, liver and lungs, as well as the all gross lesions from the low and intermediate groups were also examined histologically. The (XX) organs, in addition, were weighed.

40

Table 8: Tissues Collected at Necropsy

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands*	xx	Heart*+	x	Periph.nerve (sciatic)*
x	Esophagus*	x	Bone marrow*	*a*	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*		(mesenteric)	*a*	Eyes*
x	Jejunum*		(cervical)		
x	Ileum*	xx	Spleen*+		GLANDULAR
x	Cecum*	x	Thymus*+	xx	Adrenal gland*+
x	Colon*				Lacrimal gland
x	Rectum*		UROGENITAL	*a*	Mammary gland *
xx	Liver*+	xx	Kidneys*+	x	Parathyroids*
	Gall bladder	x	Urinary bladder*	xx	Thyroids*
x	Pancreas*	xx	Testes*+	x	Harderian gland
		xx	Epididymides*+		
	RESPIRATORY	xx	Prostate*	x	OTHER
x	Trachea*	xx	Seminal vesicle*	x	Bone (sternum and femur)
x	Lung*	xx	Ovaries*+	x	Skeletal muscle (thigh)
x	Nose*	xx	Uterus*+	x	Skin*
x	Pharynx*	*a*	Vagina	x	All gross lesions and masses*
a	Larynx*				

* Histologic examination required for subchronic studies based on OPPTS 870.3100 Guidelines.

+ Organ weight required in subchronic studies.

a The authors indicated that these tissues were collected, but were not examined histologically.

II. RESULTS

A. Observations

1. Clinical signs of Toxicity : There were no apparent differences in clinical signs of toxicity that could be attributed to the test substance in any treatment group. However, the authors did not indicate in the methods (Statistics) whether clinical signs were statistically analyzed.

2. Mortality - All animals survived to terminal sacrifice.

B. Body weight - There were no statistically significant differences in body weight (Table 9) or body-weight gain (Table 10) in any treatment groups when compared to corresponding control values.

C. Food and water consumption and compound intake

1. Food and water consumption - There were no statistically significant differences in food and water consumption noted in any treatment group when compared to corresponding control values (Table 11: Food Consumption).

41

Table 9: Mean Terminal Body Weights; Expressed in Grams

Sex	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
Males	545	539	540	510
Females	319	314	309	318

Standard deviations not provided.
Data extracted from Table 1 of the study report, pg 34.

Table 10: Mean Body Weight Gain for Weeks 0-13; Expressed in Grams and as Percent of Controls

Sex	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
Expressed in Grams (g)				
Males	353	348	349	321
Females	147	149	142	147
Expressed as % Controls				
Males	-	98.5	98.9	91
Females	-	101.4	96.6	100

Standard deviations not provided.
Data extracted from Table 1 of the study report, pg 34.

Table 11: Mean Food Intake for Weeks 0-13; Expressed in Grams and as Percent of Controls

Sex	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
Expressed as Grams (g)				
Males	2542	2475	2490	2414
Females	1884	1874	1803	1831
Expressed as % Controls				
Males	-	97%	98%	95%
Females	-	99%	96%	97%

Standard deviations not provided.
Data extracted from Table 2 of the study report, pg 35.

2. Compound consumption (time-weighted average) : The mean test-compound consumption for each dose-group/sex for the duration of the study was calculated using food intake data, body weight data, and test compound concentrations in diet. The mean results are illustrated in Table 12.

Table 12: Mean Compound Intake (mg/kg/day)

Sex	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
Males	0	30	300	998
Females	0	30	299	994

Standard deviations not provided.
Data extracted from Table 5 of the study report, pg 38.

D. Ophthalmoscopic examination - There were no ophthalmological observations that could be attributed to administration of the test-article.

E. Clinical Pathology

1. Hematology - A statistically significant increase in packed cell volume was reported in all treated males, when compared to the controls (Table 13). A statistically significant increase in red blood cell count, and a statistically significant decrease in mean corpuscular volume was reported in the mid-dose and high-dose males, while a decrease in Thrombotest time was reported in high-dose males. However, these differences were small (< 10%), and did not occur in female treatment groups, and were therefore considered to be biological variation. Additionally, a decrease in clotting time is not considered toxicologically significant.

2. Clinical chemistry - Statistically significant increases in total protein and alkaline phosphatase were observed in high-dose males, while a statistically significant increase in cholesterol was reported in mid-dose and high-dose males (Table 14). Also, a statistically significant decrease in phosphorous was reported in high-dose males when compared to the control group. Statistically significant increases in cholesterol and alkaline phosphatase, and decreases in total protein, albumin, and creatinine were observed in high-dose females, when compared to controls. A statistically significant increase in alkaline phosphatase, and a statistically significant decrease in albumin was also reported for mid-dose females. The changes in total protein were opposite in males and females, and the changes in albumin and creatinine (female only) were small in magnitude, and therefore were not considered to be biologically significant. The majority of individual cholesterol and alkaline phosphatase values for males and females were reported to be within the normal range for rats of this strain and age, and were also considered not to be treatment-related.

Table 13: Statistically Significant Hematological Observations Expressed as Mean[1]

Observation	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
	Males			
Packed cell volume (%)	56	58*	58*	57*
Red blood cell count (10^6/ml)	7.3	7.6	7.8**	7.9**
Mean corpuscular volume (%)	77	77	74**	73**
Thrombotest time	24 (sec)	24	23	22**

* denotes significantly different from controls ($p \leq 0.05$)
** denotes significantly different from controls ($p \leq 0.01$)
[1] standard deviations were not provided
Data extracted from Table 7 of the study report, pg 40.

Table 14 Statistically Significant Clinical Chemistry Observations Expressed as Mean[1]

Observation	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
	Males			
Total Protein (g/dl)	6.8	6.7	7.0	7.1*
Alkaline phosphatase (mU/ml)	183	154	188	226*
Phosphorus	4.2	4.0	4.0	3.9*
Cholesterol (mg/dl)	85	75	115**	129**

44

Observation	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
	Females			
Total Protein (g/dl)	7.3	7.2	7.2	6.9**
Albumin (g/dl)	3.4	3.4	3.1*	3.1**
Creatinine (mg/dl)	0.6	0.5	0.6	0.5*
Alkaline phosphatase (mU/ml)	96	82	141*	129*
Cholesterol (mg/dl)	78	76	88	115**

* denotes significantly different from controls ($p \leq 0.05$)
** denotes significantly different from controls ($p \leq 0.01$)
[1] standard deviations were not provided
Data extracted from Table 8 of the study report, pg 41.

F. Urinalysis - No differences in urinalysis parameters that could be attributed to administration of the test article were noted in any treatment group after 13 weeks of treatment when compared to corresponding control values. A statistically significant increase in urinary volume, and a significant decrease in urinary protein was observed in high-dose females, however, because there were no histological observations in the kidney or changes in indicators of renal function, these observations were not considered to be toxicologically significant.

G. Sacrifice and Pathology

1. Organ weight

Statistically significant decreases in absolute spleen weight and absolute testes weight were noted in mid-dose and high-dose males (Table 15). Additionally, absolute epididymides weight were statistically significantly decreased, and relative heart weight was statistically significantly increased in high-dose males when compared to the control values. A statistically significant increase in absolute spleen weight was observed in mid-dose and high-dose female treatment groups. The decreases in testicular weights and epididymides weights, and the increase in relative heart weight noted in high-dose males were most likely due to a slight decrease in body weight reported for high-dose males. Additionally, there were no histological findings in the testes, epididymides, or heart. Therefore these changes were considered to be biological variation. The significant changes in spleen weights were opposite in males and females, and were therefore not considered biologically significant.

Table 15: Statistical Differences in Organ Weights Expressed as Mean (g) [1]

Tissue	Group/Dose Level (mg/kg/day)			
	0	30	300	1000
Males				
Spleen	1.06	0.92	0.84*	0.84*
Testes				
Right	1.87	1.79	1.69*	1.71*
Left	1.90	1.82	1.71*	1.70*
Epididymides				
Right	0.64	0.67	0.63	0.63
Left	0.69	0.68	0.66	0.62*
Heart (relative)	1.61	1.58	1.65	1.80**
Females				
Spleen	0.58	0.58	0.70*	0.66*

* denotes significantly different from controls ($p \leq 0.05$)
** denotes significantly different from controls ($p \leq 0.01$)
[1] standard deviations were not provided
Data extracted from Table 10 of the study report, pg 43-44.

2. Gross pathology

Our reviewers noted that there appeared to be an increase in the incidence of green discoloration of the pancreas (0, 0, 10, 10 for control, low-, mid-, and high-dose), and an increase in the incidence of dark red coloration of the harderian gland (0, 0, 8, 10) in mid-dose and high-dose females. Our reviewers also noted that the incidence of enlarged cervical lymph nodes appeared to be increased in high-dose males (2, 5, 3, 7). However, there did not appear to be a dose-response relationship and enlarged lymph nodes accompanied by plasmacytosis is common in this strain of rat.

3. Microscopic pathology

a) Non-neoplastic - There were no changes in the incidence of microscopic observations in any treatment group that could be attributed to administration of the test article. However, our reviewers did note an increase in the incidence of plasmacytosis in the cervical lymph nodes of treated males (1, 5, 2, 6), and an increase in the incidence of fibrosis and inflammatory cells in the harderian gland in mid-dose and high-dose females (0, 0, 5, 6).

4/6

III. DISCUSSION

The study (MRID#44582916) investigated the toxicity of NOVARON AG300 in male and female Sprague-Dawley rats fed a diet containing calculated doses of 30, 300, and 1000 mg/kg/day of the test substance for 90 days.

Following administration of NOVARON AG300 in the diet to male and female Sprague-Dawley rats, a statistically significant increase in packed cell volume was reported in all treated males, when compared to the controls. A statistically significant increase in red blood cell count, and a statistically significant decrease in mean corpuscular volume was reported in the mid-dose and high-dose males, while a decrease in Thrombotest time was reported in high-dose males. However, these differences were small (< 10%), and did not occur in female treatment groups, and were therefore considered to be biological variation. Statistically significant increases in total protein and alkaline phosphatase were observed in high-dose males, while a statistically significant increase in cholesterol was reported in mid-dose and high-dose males. Also, a statistically significant decrease in phosphorous was reported in high-dose males when compared to the control group. Statistically significant increases in cholesterol and alkaline phosphatase, and decreases in total protein, albumin, and creatinine were observed in high-dose females, when compared to controls. A statistically significant increase in alkaline phosphatase, and a statistically significant decrease in albumin was also reported for mid-dose females. The changes in total protein were opposite in males and females, and the changes in albumin and creatinine (female only) were small in magnitude, and therefore were not considered to be biologically significant. The majority of individual cholesterol and alkaline phosphatase values for males and females were reported to be within the normal range for rats of this strain and age by the authors, and were also considered not to be treatment-related. Statistically significant decreases in absolute spleen weight and absolute testes weight were noted in mid-dose and high-dose males. Additionally, absolute epididymides weight were statistically significantly decreased, and relative heart weight was statistically significantly increased in high-dose males when compared to the control values. A statistically significant increase in absolute spleen weight was observed in mid-dose and high-dose female treatment groups. The decrease in testicular weights and epididymides weights were < 10%, and were therefore considered to be biological variation. The significant changes in spleen weights were opposite in males and females, and were therefore not considered biologically significant. The increase in relative heart weights is equivocal, however, there were no histological findings at the high-dose and the authors reported that the majority of the individual values were within the normal weight range for rats of this strain and age. Therefore these changes were not considered to be treatment-related. Our reviewers noted that there appeared to be an increase in the incidence of green discoloration of the pancreas (0, 0, 10, 10 for control, low-, mid-, and high-dose), and an increase in the incidence of dark red coloration of the harderian gland (0, 0, 8, 10) in mid-dose and high-dose females. Our reviewers also noted that the incidence of enlarged cervical lymph nodes appeared to be increased in high-dose males (2, 5, 3, 7). However, there did not appear to be a dose-response relationship and enlarged lymph nodes accompanied by plasmacytosis is common in this strain of rat. There were no changes in the incidence of microscopic observations in any treatment group that could be attributed to administration of the test article. However, our reviewers did note an increase in the incidence of plasmacytosis in the cervical lymph nodes of treated males (1, 5, 2, 6), and an increase in the incidence of fibrosis and inflammatory cells in the harderian gland in mid-dose and high-dose females (0, 0, 5, 6).

The doses administered in this study were chosen based upon the results of an acute toxicity study (results not provided in the study report), and a 2-week palatability study (MRID#44582915) wherein

it was reported that NOVARON AG300 administered in the diet at doses up to 1000 mg/kg/day resulted in no observable toxic effects. Likewise, this 90-day study also reported no observable toxic effects that could be attributed to the administration of the test article at doses up to 1000 mg/kg/day.

B. Study deficiencies

* The authors did not histologically examine the larynx, vagina, spinal chord, eyes, or mammary gland as required by OPPTS 870.3100 guidelines. This deficiency had moderate impact on the classification of this study because the effects on these tissues resulting from administration of the test article could not be evaluated.

* It does not appear that the study authors statistically analyzed the macroscopic observations or the microscopic observations in this study. This deficiency had minor impact on the classification of this study because it did not appear to impact the interpretation of the data.

* The study authors do not indicate that each animal received a detailed clinical examination prior to the beginning of the experiment as required by OPPTS 870.3100. This deficiency had minimal impact on the classification of this study because it did not appear to impact the interpretation of the data.

DATA EVALUATION REPORT

EXPERIMENTAL ADDITIVE NUMBER 9823-37

Study Type: Developmental (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Jennifer Welham *Jennifer Welham, M.S.*	Date 4/5/99
Independent Reviewer	Greg Lawrence *Greg Lawrence, D.V.M., M.S.*	Date 4-5-99
Project Manager (QA/QC Manager)	Ellen Mantus *Ellen Mantus, Ph.D.*	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

EPA Reviewer: T. McMahon, Ph.D. _____, Date _____.
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: Peter Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat
OPPTS 870.3700

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Experimental Additive Number 9823-37 (unknown)

SYNONYMS: Silver Sodium Hydrogen Zirconium Phosphate (10% silver) ; AlphaSan™ RC5000

CITATION: Wood, E. and N. Doleman. (1999). Supplementary submission of data requested for a pending application. Safepharm Laboratories Limited, Derby, UK. SPL Project Number 656/017, January 7, 1999. MRID 44740101. Unpublished.

SPONSOR: Milliken Chemical, Spartanburg, SC

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44740101) Experimental Additive Number 9823-37 in carboxymethyl cellulose (1%) was administered to mated female Sprague-Dawley CD rats (25/dose) by gavage at dose levels of 0, 100, 300, or 1000 mg/kg/day from days 6 through 15 of gestation.

There were no treatment-related deaths or clinical signs of toxicity. Body weight gain, food consumption, and reproductive parameters were not affected by treatment. **The maternal NOAEL is 1000 mg/kg/day. The maternal LOAEL was not established.**

External, visceral, and skeletal fetal examinations revealed no treatment-related effects. **The developmental NOAEL is 1000 mg/kg/day. The developmental LOAEL was not established.**

This developmental toxicity study in the rat is classified as acceptable and it satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700) in rats. Although the updated guidelines specify the dosing period to last until GD19, the lack of maternal or developmental effects in this study mitigates concern for any effects missed after GD15.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Experimental Additive Number 9823-37
   Description: white powder
   Lot #: 7170706
   Purity: not provided
   CAS #: not provided

2. Vehicle: Carboxymethyl cellulose (1%)
   Description: not provided
   Lot/Batch #: not provided
   Purity: 1%

3. Test Animals
   Species: rat
   Strain: Sprague-Dawley CD
   Age at mating: not provided
   Weight at mating: not provided; 200-276 g at allocation
   Source: Charles River (UK) Limited, Margate, Kent, UK
   Housing: individually in polypropylene cages with stainless steel grid floors and tops; softwood chips used as bedding
   Diet: SQC Rat and Mouse Expanded Diet No. 3, *ad libitum*
   Water: tap, *ad libitum*
   Environmental conditions:
   Temperature: 19-23 °C
   Humidity: 40-70%
   Air changes: 15/hour
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): 0 days (allocation to study was performed on the day of animal arrival)

B. PROCEDURES AND STUDY DESIGN

1. In Life Dates
   Start: April 17, 1998
   End: May 13, 1998

2. Mating:
   Time-mated female rats were obtained from Charles River (UK) Limited. The day of observation of positive evidence of mating was gestation day 0. The females were delivered to the laboratory between gestation days 1 and 3.

3. Animal Assignment
   Animals were assigned to dose groups as indicated in Table 1. Assignment was random using a procedure based on stratified body weight to ensure comparable group mean body weights for each treatment group.

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Volume (ml/kg)	Concentration (mg/ml)	Number of Females
Vehicle Control	0	10	0	25
Low (LDT)	100	10	10	25
Mid (MDT)	300	10	30	25
High (HDT)	1000	10	100	25

4. Dose Selection Rationale

The selection of doses was based on the results of a range-finding study (MRID 44698001) in which groups of eight mated female Sprague-Dawley CD rats were given silver sodium hydrogen zirconium phosphate (10% silver) in carboxymethyl cellulose (1%) by gavage at dose levels of 0, 100, 300, or 1000 mg/kg/day from days 6 through 15 of gestation. Clinical observations, body weight and food consumption records, and analysis of reproduction data indicated no significant treatment-related maternal toxic effects. External examination revealed no significant treatment-related fetal toxic effects. Taking into account the limit dose of 1000 mg/kg/day for substances of low toxicity specified in the guidelines (OPPTS 870.3700), higher doses were not selected for the definitive study.

5. Dosage Preparation and Analysis

During the study, test substance formulations were prepared daily by combining appropriate amounts of the test substance with carboxymethyl cellulose (1%) and mixing with a Silverson mixer/homogenizer. The formulations were used immediately.

The homogeneity of the test substance in carboxymethyl cellulose (1%) was evaluated using top, middle, and bottom samples. The stability of the test substance in carboxymethyl cellulose (1%) was evaluated for a period of two hours at ambient temperature in light. The concentrations of the test substance formulations were evaluated three times during the study (start, middle, and end). Results of these determinations were presented in the report on pages 171-172 and are summarized below:

Results

Homogeneity Analysis: 98-104% of nominal

Stability Analysis: 97-101% of nominal

Concentration Analysis: 89-104% of nominal (day 1); 94-105% od nominal (day 9); 96-102% of nominal (day 17).

The analytical data indicate that the mixing procedure was adequate, the compound was stable in the vehicle, and the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage Administration
All doses were administered once daily by gavage, on gestation days 6 through 15, in a volume of 10 ml/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations
The animals were checked for mortality twice daily during the work week and once daily on weekends. Clinical observations were recorded once daily; an additional observation was made one hour after dosing during the treatment period. Body weight data were recorded on gestation days 3, 6, 7, 8, 9, 12, 15, 18, and 20. Food consumption data were recorded for discrete periods, gestation days 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20. Surviving dams were sacrificed on day 20 of gestation by carbon dioxide asphyxiation. Each animal was examined externally and internally for macroscopic abnormalities. The ovaries and uteri of pregnant females were removed and the following data were recorded: gravid uterine weight, number of corpora lutea, and number, position, and type of intrauterine implantation. Implantation types were classified as early death, late death, or dead fetus.

2. Fetal Evaluations
At necropsy, each fetus was weighed, sexed, and examined macroscopically for any external findings. Placental weights were also recorded. Approximately one half of the fetuses per dam were fixed in Bouin's solution, transferred to 90% industrial methylated spirits (IMS), and examined for visceral abnormalities under a low power binocular microscope according to the method of Wilson. The remaining fetuses were placed in 70% IMS and then eviscerated; the skeletons were stained with alizarin red according to the method of Dawson and examined microscopically for skeletal abnormalities.

D. DATA ANALYSIS

1. Statistical Analyses
Maternal body weight change and food consumption were evaluated using Levene's test for homogeneity of variance and one-way analysis of variance. Because all values showed no significant intergroup differences in homogeneity of variance or in the subsequent analysis of variance, further intergroup comparisons were not necessary.

All caesarian necropsy parameters and fetal parameters, including group incidence of skeletal or visceral anomalies were evaluated using Kruskall-Wallis non-parametric analysis of variance. A subsequent pair-wise analysis of control values against treated values using the Mann-Whitney U test was conducted where significance occurred.

2. Indices
The following indices were calculated from cesarean section records of animals in the study:

Preimplantation loss (in %):

$$\frac{\text{number of corpora lutea - number of implantations}}{\text{number of corpora lutea}} \times 100$$

Postimplantation loss (in %):

$$\frac{\text{number of implantations - number of live fetuses}}{\text{number of implantations}} \times 100$$

3. Historical Control Data
Historical control data were provided to allow comparison with concurrent controls. The data indicate the occurrence of fetal external, visceral, and skeletal anomalies in controls from recently conducted studies. Information about the study designs such as the dates of study conduct, the strain and source of the animals, and the vehicle and route of administration were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations
One mid-dose (300 mg/kg/day) female exhibited clinical signs of respiratory distress and was subsequently killed *in extremis* on day 15. According to the study authors, necropsy findings in this animal were consistent with dosing trauma. There were no other deaths or clinical signs of toxicity.

2. Body Weight
Body weight gain data are summarized in Table 2. Body weight gain in all dose groups was comparable to controls throughout the study.

3. Food Consumption
There were no significant differences in food consumption of treated groups relative to the control group throughout the study.

4. Gross Pathology
Gross necropsy of the female sacrificed *in extremis* on day 15 revealed clear fluid and fibrinous adhesions within the thorax and congested lungs. According to the study authors, these findings are consistent with dosing trauma. Necropsy observations in surviving animals (increased renal pelvic cavication, fluid and pus in the kidney, and ovarian follicular cyst) were not related to dose and are commonly observed in this strain of rat and, therefore, are considered incidental.

54

TABLE 2 Mean Maternal Body Weight Gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)							
	0 (22)		100 (23)		300 (23)		1000 (21)	
	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)
Pretreatment: Days 3-6	21.1	(4.7)	22.2	(6.5)	21.0	(6.3)	23.1	(6.2)
Treatment: Days 6-15	56.2	(8.3)	58.7	(8.5)	56.1	(9.8)	56.8	(11.1)
Entire Study: Days 3-20	107.4	(14.5)	103.3	(21.8)	102.9	(16.8)	108.2	(19.0)

[a]Data extracted from SPL Project Number 656/017, Table 2

5. Cesarean Section Data

The reproduction data are summarized in Table 3. A statistically significant ($p<0.01$) higher mean percentage of males per litter was noted in the high-dose group (1000 mg/kg/day) compared to controls. There were no significant differences in treated groups compared to the control group in pregnancy rate, number of corpora lutea, number of implantation sites, number of live fetuses, early or late resorptions, fetal weight, and preimplantation or postimplantation loss.

TABLE 3 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)							
	0		100		300		1000	
	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)	mean	(s.d.)
# Animals Assigned (Mated)	25		25		25		25	
# Animals Pregnant	22		23		23		21	
Pregnancy Rate (%)	88		92		92		84	
# Nonpregnant	3		2		2		4	
Maternal Wastage								
# Died	0		0		1		0	
# Died Pregnant	0		0		0		0	
# Died Nonpregnant	0		0		1		0	
# Aborted	0		0		0		0	
# Premature Delivery	0		0		0		0	
Total # Corpora Lutea	345		332		350		327	
Corpora Lutea/Dam	15.7	(2.5)	15.1	(2.7)	15.2	(1.7)	15.6	(2.1)

55

Observation	Dose (mg/kg/day) 0 mean	(s.d.)	100 mean	(s.d.)	300 mean	(s.d.)	1000 mean	(s.d.)
Total # Implantations	315		307		306		298	
Implantations/Dam	14.3	(1.7)	13.3	(2.5)	13.3	(1.5)	14.2	(2.6)
Total # Litters	22		23		23		21	
Total # Live Fetuses	289		294		289		281	
Live Fetuses/Dam	13.1	(1.9)	12.7	(2.7)	12.6	(1.8)	13.4	(2.7)
Total # Dead Fetuses[b]	–	–	–	–	–	–	–	–
Dead Fetuses/Dam[b]	–	–	–	–	–	–	–	–
Total # Resorptions	26		12		17		17	
Early	20		9		17		16	
Late[b]	6		3		0		1	
Resorptions/Dam	1.18	(1.76)	0.52	(0.59)	0.74	(1.14)	0.81	(1.29)
Early	0.91	(1.57)	0.39	(0.50)	0.74	(1.14)	0.76	(1.30)
Late[b]	0.27	(0.70)	0.13	(0.34)	0.00	(0.00)	0.05	(0.22)
Litters with Total Resorptions	0		0		0		0	
Mean Fetal Weight (g)	3.71	(0.33)	3.80	(0.19)	3.74	(0.29)	3.77	(0.27)
Males	–	–	–	–	–	–	–	–
Females	–	–	–	–	–	–	–	–
Sex Ratio (% Male)	43.0	(11.2)	46.9	(15.9)	50.4	(15.9)	56.8**	(12.7)
Preimplantation Loss (%)	7.9	(7.6)	12.1	(12.1)	12.1	(9.5)	9.2	(11.9)
Postimplantation Loss (%)	7.9	(11.2)	4.2	(5.5)	5.5	(8.6)	5.6	(8.8)

[a]Data extracted from SPL Project Number 656/017, Tables 4 and 5 and Appendix V
[b]Fetal deaths were combined with late resorptions in the study report
**Significantly different from control group ($p<0.01$)

B. DEVELOPMENTAL TOXICITY

1. External Examination
The results of the external examination are summarized in Table 4a. External fetal examinations revealed no treatment-related effects. A greater proportion of external findings were noted in the control group.

2. Visceral Examination
The results of the visceral examination are summarized in Table 4b. Visceral fetal examinations revealed no treatment-related effects. The proportion of affected fetuses was comparable for all treated groups and the control group.

TABLE 4a External Examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1000
#Fetuses (litters) examined	289 (22)[b]	293 (23)	289 (23)	281 (21)
Total affected	9 (6)	1 (1)	5 (3)	1 (1)
Fetus small	7 (5)	1 (1)	3 (2)	1 (1)
Fetus pale	1 (1)	0 (0)	0 (0)	0 (0)
Fetal edema	1 (1)	0 (0)	0 (0)	0 (0)
Agnathia/astomia	1 (1)	0 (0)	0 (0)	0 (0)
Placenta small	0 (0)	0 (0)	1 (1)	0 (0)
Placenta large	0 (0)	0 (0)	1 (1)	0 (0)
Placentae fused	2 (1)	0 (0)	2 (1)	0 (0)
Excess amniotic fluid	1 (1)	0 (0)	0 (0)	0 (0)

[a]Data extracted from SPL Project Number 656/017, Table 6
[b]Fetal (litter) incidence

TABLE 4b Visceral Examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1000
#Fetuses (litters) examined	148 (22)[b]	151 (23)	151 (23)	144 (21)
Total affected	54 (22)	48 (23)	42 (23)	35 (21)
Head				
Ovoid lens(es) of eye(s)	7 (6)	5 (5)	6 (4)	3 (3)
Enlarged/dilated brain ventricle(s)	7 (4)	12 (8)	7 (6)	5 (5)
Submenigeal hemorrhage	0 (0)	0 (0)	0 (0)	1 (1)
Neck/Thorax				
Small lobe(s) of thyroid	2 (2)	1 (1)	0 (0)	0 (0)
Undescended lobe(s) of thymus	13 (9)	7 (7)	12 (8)	12 (6)

57

Observations	Dose (mg/kg/day)			
	0	100	300	1000
Abdomen				
Unilateral/bilateral small/no development of renal papilla(e)	26 (12)	21 (9)	20 (9)	11 (6)
Unilateral/bilateral increased renal pelvic cavitation	3 (2)	1 (1)	3 (3)	0 (0)
Unilateral/bilateral kinked and/or dilated ureter(s)	18 (10)	20 (9)	17 (10)	8 (5)
Immature liver	1 (1)	0 (0)	4 (4)	0 (0)
Pale liver	1 (1)	0 (0)	0 (0)	0 (0)
Extra lobulation of liver median lobe	4 (3)	2 (2)	0 (0)	2 (2)
Large intrahepatic hemorrhage in liver	0 (0)	1 (1)	0 (0)	0 (0)
Blood in abdominal cavity	1 (1)	0 (0)	0 (0)	1 (1)
Other				
Marked subcutaneous edema	1 (1)	0 (0)	0 (0)	0 (0)

[a]Data extracted from SPL Project Number 656/017, Table 7
[b]Fetal (litter) incidence

3. Skeletal Examination

There were no treatment-related significant differences in the level of fetal skeletal development as measured by the number of ribs, number of fully ossified sternebrae, number of post lumbar vertebral centra, number of post lumbar vertebral arches, number of metacarpals, number of metatarsals, and fontenelle size. The results of the skeletal examination are summarized in Table 4c. Skeletal fetal examinations revealed no treatment-related effects. The proportion of affected fetuses was comparable for all treated groups and the control group.

TABLE 4c Skeletal Examinations[a]

Observations	Dose (mg/kg/day)			
	0	100	300	1000
#Fetuses (litters) examined	141 (22)[b]	142 (23)	138 (23)	137 (21)
Total affected	100 (22)	103 (23)	120 (23)	102 (21)

Observations	Dose (mg/kg/day)			
	0	100	300	1000
Head/Neck				
Incomplete ossification of one cranial bone	41 (20)	30 (15)	40 (19)	33 (17)
Incomplete ossification of more than one cranial bone	19 (11)	34 (16)	36 (16)	32 (10)
Incomplete ossification of one facial bone	3 (2)	3 (1)	6 (5)	6 (4)
Incomplete ossification of more than one facial bone	6 (4)	3 (3)	8 (7)	9 (3)
Irregular ossification of one cranial bone	15 (9)	6 (4)	16 (9)	12 (8)
Irregular ossification of more than one cranial bone	9 (4)	11 (4)	16 (9)	13 (7)
No ossification of hyoid	27 (14)	36 (14)	37 (16)	30 (12)
Incomplete ossification of hyoid	1 (1)	11 (7)	12 (8)	12 (9)
Irregular ossification of hyoid	2 (2)	2 (2)	3 (2)	4 (3)
Ribs				
Unilateral/bilateral rudimentary 14th rib(s)	6 (5)	2 (1)	6 (4)	6 (6)
Vertebrae				
One thoracic vertebral centrum semi-bipartite	31 (17)	22 (13)	25 (15)	24 (16)
More than one thoracic vertebral centrum semi-bipartite	11 (7)	14 (10)	24 (12)	19 (10)
One thoracic vertebral centrum bipartite	4 (4)	2 (2)	2 (2)	3 (2)
Pelvic Girdle				
Incomplete ossification of pubis(es)	2 (1)	2 (1)	5 (5)	5 (4)
No ossification of pubis(es)	5 (2)	1 (1)	4 (3)	6 (2)
Irregular ossification of pubis(es)	0 (0)	0 (0)	1 (1)	3 (2)
Sternebrae				
No ossification of one sternebra	7 (5)	2 (1)	5 (3)	1 (1)
Incomplete ossification of one sternebra	8 (5)	2 (2)	4 (3)	4 (3)
No ossification of more than one sternebra	2 (1)	1 (1)	3 (3)	4 (1)
Incomplete ossification of more than one sternebra	4 (3)	1 (1)	3 (3)	1 (1)
Irregular ossification of one sternebra	4 (3)	3 (2)	1 (1)	2 (2)
Irregular ossification of more than one sternebra	4 (3)	0 (0)	3 (3)	1 (1)

[a]Data extracted from SPL Project Number 656/017, Table 9
[b]Fetal (litter) incidence

59

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

1. Maternal Toxicity
The authors concluded that, under the conditions of this study, the administration of Experimental Additive Number 9823-37 in carboxymethyl cellulose (1%) at doses up to 1000 mg/kg/day resulted in no maternal toxicity. There were no treatment-related deaths or clinical signs of toxicity. The observation of respiratory distress in one mid-dose (300 mg/kg/day) female (subsequently killed *in extremis*) was attributed to dosing trauma. Body weight gain, food consumption, and reproductive parameters were not affected by treatment. A higher mean percentage of males per litter was noted in the high-dose group (1000 mg/kg/day) compared to controls, which according to the authors, was incidental.

2. Developmental Toxicity
The authors concluded that, under the conditions of this study, the administration of Experimental Additive Number 9823-37 in carboxymethyl cellulose (1%) at doses up to 1000 mg/kg/day resulted in no significant effects on the growth and development of offspring. There were no intergroup variances in the distribution or proportion of external. visceral, or skeletal anomalies.

B. REVIEWER'S DISCUSSION

1. Maternal Toxicity
The reviewer agrees with the authors' conclusion that no treatment-related maternal effects occurred in this study. Based on the necropsy findings, it is likely that the mid-dose female sacrificed *in extremis* suffered injury from dosing. The increased percentage of male pups in the high-dose group has no apparent biological or toxicological significance. Therefore, the NOAEL for maternal toxicity is 1000 mg/kg/day. The LOAEL was not established.

2. Developmental Toxicity
The reviewer agrees with the authors' conclusion that no treatment-related developmental effects occurred in this study. The greater proportion of external findings noted in the control group is most likely incidental. There were no changes in the proportion or distribution of visceral or skeletal anomalies observed. Therefore, the NOAEL for developmental toxicity is 1000 mg/kg/day. The LOAEL was not established.

C. STUDY DEFICIENCIES

The current OPPTS guidelines (OPPTS 870.3700) recommend that the test substance be administered at a minimum from the day of implantation to the day prior to cesarean section. However, in this study, the test article was administered on gestation days 6-15, with cesarean sections conducted on day 20. Therefore, potential effects that may have been associated with exposures late in gestation could not be assessed, i.e., effects that may have been identified had the test article been administered until gestation day 19. While this deficiency is noted and should not be repeated, the lack of any maternal or developmental effects in this study lessens concern for any effects which could have been missed during GD15-GD19.

Other study deficiencies include:

- The purity of the test substance was not provided.
- The lot/batch number of the vehicle was not provided.
- Because the animals had already been mated upon receipt from the breeding facility, the age and weight of the test animals at mating was not provided. However, the weight of the animals at the time of allocation to study groups was provided.
- The number of fetal deaths was combined with the number of late resorptions and reported as "late deaths" in the study report.
- Group fetal body weight data were not presented by sex. Only the combined values were listed in the summary table.
- Fetal external, soft tissue, and skeletal anomalies were not categorized as malformations or variations. The results indicate only the total number of fetuses affected.
- The historical control data do not include information such as the dates of study conduct, strain and source of the animals, vehicle, and route of administration.

These deficiencies are considered minor, however, because they are unlikely to have influenced the study results.

GI

DATA EVALUATION REPORT

NOVARON

Study Type: Bacterial Reverse Mutation Test

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Kristin Jacobson, MSPH	Date 4/7/99
Independent Reviewer	Greg Lawrence, DVM	Date 4-7-99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

62

EPA Reviewer: T. McMahon, Ph.D. _____ Date 4/27/99
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Bacterial reverse mutation test (OPPTS 870.5100)

DP BARCODE: D253496, D253509 SUBMISSION CODE: S557116, S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): NOVARON (≥99% active ingredient, a.i.)

SYNONYMS: NOVARON AG 300; AlphaSan™ RC5000; silver sodium hydrogen zirconium phosphate

CITATION: Jones, E. and R.A. Grant. (1994) Application for pesticide registration: AlphaSan™ RC 5000, EPA File Symbol 11631, Volume 14, Bacterial mutation assay. Huntingdon Research Centre, Ltd. (Cambridgeshire, England). HRC Study No. TSI 72/941424, May 18, 1994. MRID 44582912. Unpublished.

SPONSOR: Milliken Chemical, Spartanburg, SC

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 44582912), strains TA1535, TA1537, TA1538, TA98, and TA100 of *S. typhimurium* were exposed to suspensions of NOVARON (≥99% a.i.) at concentrations of 0, 1.5, 5, 15, 50, 150, and 500 µg/plate in the presence and absence of mammalian metabolic activation (S9 from Aroclor-induced rat liver).

NOVARON was tested up to cytotoxic concentrations (≥50 µg/plate -S9 and ≥500 µg/plate +S9), as demonstrated by reduced revertant counts and/or effects on the background lawn of growth. In two independent assays, there were no appreciable increases in revertants in any strain above the corresponding solvent controls. However, under nonactivated conditions an inadequate number of dose levels was evaluated, and therefore firm conclusions cannot be reached. Positive control compounds elicited the appropriate response in all strains. **There was no evidence of an induced increase in mutant colonies over background in the presence or absence of S9 activation.**

Although less than five analyzable concentrations of the test material were evaluated under nonactivated conditions (as required by OPPTS Test Guideline 870.5100), there was at least one concentration available under these test conditions to show that the compound is non-mutagenic. Therefore, this study can be considered **acceptable** and it satisfies the guideline requirement for a reverse mutation assay.

COMPLIANCE: Signed and dated GLP, QA, and Data Confidentiality statements were provided. A Flagging Statement was not provided. [An additional GLP statement provided by the Sponsor incorrectly stated that the study does not meet the requirements of 40 CFR Part 160 (because "this document does not contain the report of a study and therefore does not fall under the requirements of 40 C.F.R. Part 160.")]

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: NOVARON
   Description: Odorless, fine white powder
   Lot/Batch #: 7130519
   Purity: ≥99% a.i.
   Stability: Stable "indefinitely" (but expires 1 year after manufacture)
   CAS # : Not provided
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: The test material was stored at room temperature in the dark. Dosing solutions were prepared on the day of use. Analytical determinations of achieved test concentrations or of homogeneity of stock solutions were not performed. The average particle diameter of the test material was 0.9 µm.

2. Control Materials:
   Negative: Untreated
   Solvent/final concentration: DMSO/ 0.1 mL/plate
   Positive: Nonactivation:

Sodium azide	____ µg/plate TA1535, TA100
2-Nitrofluorene	2 µg/plate TA1538
2-Nitrofluorene	1 µg/plate TA98
9-Aminoacridine	80 µg/plate TA1537
Other (list):	
N-Ethyl-N'-nitro-N-nitrosoguanidine	5 µg/plate TA1535
N-Ethyl-N'-nitro-N-nitrosoguanidine	3 µg/plate TA100

   Activation:

2-Aminoanthracene	2 µg/plate TA1535, TA1537
2-Aminoanthracene	0.5 µg/plate TA1538, TA98
2-Aminoanthracene	1 µg/plate TA100
Other (list):	

3. Activation: S9 derived from

x Aroclor 1254 (as Arachis oil)	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The rat liver S9 homogenate was prepared by the testing laboratory and stored frozen until use. Prior to use, the metabolic capability of the S9 mix was evaluated with 7,12-dimethylbenzanthracene and 2-aminoanthracene. The S9 mix was prepared as follows:

69

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9 homogenate	10% (v/v)

4. Test organisms: *S. typhimurium* strains
__ TA97 _x_ TA98 _x_ TA100 _ TA102 _ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538 ; list any others:

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test compound concentrations used:

a) Preliminary cytotoxicity tests: Four concentrations of the test material (5, 50, 500, and 5000 µg/plate +/- S9) were evaluated in the five tester strains, using single plates per dose, per strain, per condition.

b) Main assay: Two independent trials were conducted in the five tester strains, with 1.5, 5, 15, 50, 150 and 500 µg/plate +/- S9. (The lowest dose, 1.5 µg/plate, was not evaluated under S9-activated conditions in the confirmatory trial. Triplicate plates were prepared per dose, per strain, per condition.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
_x_ standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification
__ spot test
__ other

2. Protocol: Aliquots of the selected test material concentration, bacterial suspension, and S9 mix (or phosphate buffer for nonactivated conditions) were mixed with histidine-deficient overlay agar, and plated in triplicate on minimal agar. Sterility controls (for the test material, S9 mix, and phosphate buffer) were also prepared. The plates were incubated at 37°C for 3 days and scored for revertant colonies using a Seescan automatic colony counter. The background lawn of growth was also assessed. For each treatment level, the mean (and standard deviation) revertant colony count was determined.

3. Evaluation Criteria: The assay was considered positive if there was a reproducible, dose-related, ≥2-fold increase in revertants above the concurrent solvent control in any strain. The findings were considered negative if revertant counts at all dose levels, in all strains, were <1.5 times the solvent control count. If equivocal results were obtained (*i.e.*, increases ≥1.5-fold that were not dose-related and not reproducible), additional testing may have been conducted using

protocol modifications such as a narrower dose range or different S9 concentrations, or the data would have been analyzed statistically using the methods of the UKEMS Guidelines as described by Mahon et al. (1989).[1]

II. REPORTED RESULTS

A. Solubility: Information provided by the Sponsor indicated that the test material was soluble in water only at concentrations less than 10 µg/L (and even less soluble in organic solvents). The study authors reported that NOVARON formed a suspension in water or DMSO at 50 mg/mL (a concentration corresponding to a final concentration of 5000 µg/plate). The preliminary cytotoxicity assay was conducted with concentrations up to 5000 µg/plate, using DMSO as the solvent.

B. Preliminary Cytotoxicity Assay: Under nonactivated conditions, an incomplete lawn of growth (with no revertant colonies) was noted on plates for all five tester strains exposed to 50 or 500 µg/plate -S9, and no lawn was reported at the highest dose level, 5000 µg/plate -S9. A lesser degree of cytotoxicity was noted in the presence of S9, with revertant counts comparable to controls at 5 or 50 µg/plate +S9 (in all five strains). At 500 or 5000 µg/plate +S9, the bacterial lawn was incomplete or absent and no revertant colonies were reported.

Based on these findings, the initial mutagenicity assay was conducted with concentrations up to 5000 µg/plate +/-S9.

C. Mutagenicity Assay:

The results of the initial and confirmatory mutagenicity assays are summarized in Tables 1 and 2, respectively.

Under nonactivated conditions in the initial trial, extreme cytotoxicity was noted at 50 µg/plate in strains TA1537, TA1538, and TA98, and at the 150 µg/plate dose level no revertants were reported in any strain. Thus, there were only three or four analyzable concentrations evaluated under nonactivated conditions. No increases in revertants (relative to controls) were reported in any strain.

The same concentrations were evaluated in the confirmatory trial under nonactivated conditions, and in this second trial the 50-µg/plate dose level was toxic to all strains, resulting in three analyzable concentrations. In agreement with the initial nonactivated trial, revertant counts in treated groups were comparable to controls. By contrast, the positive controls elicited the expected positive responses in each of the tester strains in both the initial and confirmatory trial.

The test material was less cytotoxic under S9-activated conditions. In both the initial and confirmatory trial under S9-activated conditions, revertant counts were comparable to the corresponding controls for all strains at 500 µg/plate +S9, the highest dose tested. (Our reviewers

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[1]Mahon, G.A.T., M.H.L. Green, B. Middleton, et al. (1989) Analysis of data from the microbial colony assay. In Kirkland, D.J. (Ed.). *UKEMS Subcommittee on Guidelines for Mutagenicity Testing. Report. Part III. Statistical Evaluation of Mutagenicity Test Data.* Cambridge, England: Cambridge University Press.

noted that in the preliminary toxicity assessment, the 500-µg/plate dose level caused an incomplete lawn of growth in all five strains, and no revertant colonies were reported. As designed, the study did not provide information on whether any revertant colonies occurred on these plates with incomplete background lawns of growth.) There were no increases in revertant colonies above the corresponding control counts at any dose level. The positive controls yielded the expected positive response in each tester strain. Results for the sterility control plates were not reported and were assumed by our reviewers to be unexceptional (*i.e.*, the dosing solutions, phosphate buffer, and S9 mix were shown to be sterile).

The study authors concluded the test material was not mutagenic in this bacterial test system.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. We assess that the study authors' interpretation of the data was correct. NOVARON was evaluated over a range of concentrations that caused cytotoxicity (as evidenced by reduced revertant counts or incomplete background lawn) at concentrations ≥50 µg/plate - S9 and ≥500 µg/plate +S9. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls for each strain. The study's design allowed assessment of only three or four analyzable concentrations under nonactivated conditions, however. We conclude, therefore, that NOVARON was not mutagenic in this bacterial mutation assay in the presence of metabolic activation. Interpretation of the results from testing in the absence of S9 activation is limited due to the low number of analyzable concentrations included in the study's design.

This study is classified as acceptable. Because of cytotoxicity at higher dose levels, only three or four dose levels were evaluated for mutagenic potential in both the initial and the confirmatory trial under nonactivated conditions. Although the guidelines state that at least five concentrations be available for analysis, there is at least one concentration showing no evidence of mutagenicity under non -activated conditions. Therefore, the study satisfies the requirements for OPPTS Guideline 870.5100 for bacterial reverse mutation assay.

B. Study deficiencies: Because the study's design did not allow for at least five analyzable concentrations of test material in at least one of the trials under nonactivated conditions (*i.e.*, did not accommodate the extent of cytotoxicity that had been observed in the preliminary toxicity and initial mutagenicity trials), the resulting data are considered minimal for interpretation of mutagenicity. However, as there was at least one analyzable concentration, the study can be considered acceptable. for a bacterial reverse mutation assay.

Table 1. Results of the Initial Bacterial Reverse Mutation Assay with NOVARON

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
Untreated	--	—	14±0.6	9±2.0	10±2.5	25±4.4	101±5.5
	--	+	16±3.5	8±1.0	16±3.0	27±4.6	124±5.0
Solvent Control							
Dimethyl sulfoxide	0.1 mL	—	14±1.5	9±1.7	9±1.2	25±4.9	96±5.1
	0.1 mL	+	13±5.2	8±0.0	16±3.0	27±3.5	119±16.1
Positive Controls							
ENNG	various[b]	—	292±19.3	--	--	--	322±33.5
9-AC	80 µg	—	--	>500[e]	--	--	--
2-NF	various[c]	—	--	--	250±25.0	159±6.4	--
2-AA	various[d]	+	163±7.6	65±10.8	198±14.2	218±18.3	533±19.6
Test Material							
NOVARON	1.5 µg	—	10±1.2	8±2.6	11±2.6	21±4.2	97±10.0
	5 µg	—	7±2.1	11±0.6	8±2.5	22±2.6	89±12.1
	15 µg	—	12±4.0	10±1.2	8±2.9	20±3.8	88±8.7
	50 µg[f]	—	9±2.0	toxic	toxic	toxic	98±4.6
NOVARON	50 µg[g]	+	12±5.0	9±1.5	15±2.3	23±3.6	114±7.2
	150 µg	+	17±2.1	8±0.6	14±3.1	28±1.2	102±13.9
	500 µg[h]	+	17±2.3	9±0.6	14±2.1	24±3.1	92±4.5

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100

Abbreviations used: ENNG = N-Ethyl-N'-nitro-N-nitrosoguanidine 9-AC = 9-Aminoacridine 2-NF = 2-Nitrofluorene 2-AA = 2-Aminoanthracene

a Means and standard deviations of revertant counts from triplicate plates.
b ENNG was used at 5 µg/plate -S9 for strain TA1535 and at 3 µg/plate -S9 for strain TA100.
c 2-NF was used at 2 µg/plate -S9 for strain TA1538 and at 1 µg/plate -S9 for strain TA98.
d 2-AA was used at various S9-activated concentrations (2 µg/plate for TA1535 and TA1537; 0.5 µg/plate for TA1538 and TA98; and 1 µg/plate for TA100).
e Reported as too many colonies to count accurately. Based on other revertant counts, our reviewers assume these plates had >500 colonies.
f Higher doses (150 and 500 µg/plate -S9) were toxic in all tester strains (*i.e.*, incomplete background lawn, with no revertants reported).
g Results for lower levels (1.5, 5, or 15 µg/plate +S9) did not suggest a mutagenic effect.
h Highest dose tested.

NOTE: Data were extracted from the study report, pp. 19-22.

Table 2. Results of the Confirmatory Bacterial Reverse Mutation Assay with NOVARON

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
Untreated	--	—	15±2.5	12±1.0	16±0.6	23±4.2	107±17.8
	--	+	15±1.5	7±0.6	10±1.2	25±5.5	124±12.5
Solvent Control							
Dimethyl sulfoxide	0.1 mL	—	12±2.3	8±1.5	13±4.0	23±2.9	104±7.5
	0.1 mL	+	16±4.0	12±5.1	15±2.5	27±2.5	117±11.5
Positive Controls							
ENNG	various[b]	—	318±46.1	--	--	--	371±28.4
9-AC	80 µg	—	--	>500[e]	--	--	--
2-NF	various[c]	—	--	--	479±21.9	187±28.9	--
2-AA	various[d]	+	139±12.2	114±9.3	209±52.8	238±7.8	578±27.1
Test Material							
NOVARON	1.5 µg	—	10±2.5	12±0.6	12±2.1	27±4.6	93±8.7
	5 µg	—	11±3.6	10±3.5	10±2.6	22±1.2	95±10.0
	15 µg[f]	—	11±0.6	9±0.6	10±4.2	17±3.0	93±6.8
NOVARON	50 µg[g]	+	16±2.6	6±1.5	16±1.0	30±4.6	99±5.6
	150 µg	+	16±1.7	8±2.3	13±2.1	25±5.0	100±6.2
	500 µg[h]	+	9±3.2	8±1.5	13±4.5	25±2.9	103±2.5

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100

Abbreviations used: ENNG = N-Ethyl-N'-nitro-N-nitrosoguanidine 9-AC = 9-Aminoacridine 2-NF = 2-Nitrofluorene 2-AA = 2-Aminoanthracene

a Means and standard deviations of revertant counts from triplicate plates.
b ENNG was used at 5 µg/plate -S9 for strain TA1535 and at 3 µg/plate -S9 for strain TA100.
c 2-NF was used at 2 µg/plate -S9 for strain TA1538 and at 1 µg/plate -S9 for strain TA98.
d 2-AA was used at various S9-activated concentrations (2 µg/plate for TA1535 and TA1537; 0.5 µg/plate for TA1538 and TA98; and 1 µg/plate for TA100).
e Reported as too many colonies to count accurately. Based on other revertant counts, our reviewers assume these plates had >500 colonies.
f Higher doses (50, 150 and 500 µg/plate -S9) were toxic in all tester strains (*i.e.*, incomplete background lawn, with no revertants reported).
g Results for lower levels (5 or 15 µg/plate +S9) did not suggest a mutagenic effect.
h Highest dose tested.

NOTE: Data were extracted from the study report, pp. 23-26.

DATA EVALUATION REPORT

NOVARON

Study Type: Mammalian Erythrocyte Micronucleus Test

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Kristin Jacobson, MSPH	Date 4/7/99
Independent Reviewer	Greg Lawrence, DVM	Date 4-7-99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

EPA Reviewer: T. McMahon, Ph.D. Date 7/4/95
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian erythrocyte micronucleus test; OPPTS 870.5395

DP BARCODE: D253496, D253509 SUBMISSION CODE: S557116, S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): NOVARON (≥99% active ingredient, a.i.)

SYNONYMS: AlphaSan™ RC5000; partially ion-exchanged zirconium phosphate; silver sodium hydrogen zirconium phosphate; NOVARON AG-300

CITATION: Proudlock, R.J. and K. Taylor. (1994) Application for pesticide registration: AlphaSan RC5000. Volume 16: Mouse micronucleus test. Huntingdon Research Centre, Ltd. (Cambridgeshire, England). HRC Study No. TSI 74/941459, September 15, 1994. MRID 44582914. Unpublished.

SPONSOR: Milliken Chemical, Spartanburg, SC

EXECUTIVE SUMMARY:

In a CD-1 mouse bone marrow micronucleus assay (MRID 44582914), 5 animals/sex/dose/sacrifice interval were treated via oral gavage with NOVARON (≥99% a.i.) in a 1% (w/v) aqueous methylcellulose vehicle at doses of 0, 1250, 2500, and 5000 mg/kg. Bone marrow cells were harvested at 24, 48, and 72 hours post-treatment.

There were no overt signs of toxicity during the study, with the exception of transient, slight piloerection immediately after dosing, and NOVARON was tested up to 5000 mg/kg (*i.e.*, well above the limit dose of 2000 mg/kg). There was some indication of cytotoxicity to bone marrow cells at the 48-hour sampling interval, with a significant ($p<0.001$), dose-related decrease in the proportion of polychromatic (immature) erythrocytes (*i.e.*, the PCE:NCE ratio). The frequency of micronucleated polychromatic erythrocytes was comparable in treated and control animals. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as unacceptable, because too few erythrocytes were evaluated per animal. It does not satisfy the requirements for OPPTS Guideline 870.5395 for mammalian erythrocyte micronucleus test data. The study may be upgradable, if the prepared slides are still available for re-analysis.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not included in the study report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: NOVARON
   Description: White powder
   Lot/Batch #: 7130519
   Purity: ≥99% a.i.
   Stability of compound: Not reported
   CAS #: Not reported
   Solvent used: Methyl cellulose vehicle, 1% w/v (aqueous)
   Other comments: The test material was stored at room temperature in the dark. Dosing solutions were prepared on the day of use. The stability and homogeneity of the test material (and of the test material in the vehicle) were not determined. Analyses of achieved concentrations were not conducted.

2. Control Materials:
   Negative: Not used
   Vehicle/Final volume/Route of administration: Methyl cellulose (aqueous solution, 1% w/v)/ 20 mL/kg / Oral gavage
   Positive/Final dose(s)/Route of administration: Mitomycin C/ 12 mg/kg / Oral gavage

3. Test compound administration:
   Volume of test substance administered: 20 mL/kg
   Route of administration: Oral gavage
   Dose levels used:
   Preliminary toxicity test: 5000 mg/kg
   Micronucleus assay: 1250, 2500, and 5000 mg/kg

4. Test animals:
   a. Species: Mouse Strain CD-1 Age (on arrival): ≈35 days
      Weight (on arrival): 22-24 g
      Source: Charles River U.K. Ltd., Margate, Kent, England
   b. No. animals used per dose, per sacrifice interval: 5 males; 5 females
   c. Properly maintained? Yes

B. TEST PERFORMANCE

1. Preliminary Toxicity Assay: Two animals per sex were administered 5000 mg/kg of the test material and monitored for mortality and clinical signs of toxicity for 72 hours.

1. Treatment and Sampling Times:
   a. Test compound
      Dosing: X once ___ twice (24 hr apart)
      ___ other (describe):
      Sampling (after last dose): ___ 6 hr ___ 12 hr
      X 24 hr X 48 hr X 72 hr ___ other (describe):

79

b. Negative and/or vehicle control
Dosing: _X_ once ___ twice (24 hr apart)
___ other (describe):
Sampling (after last dose): ___ 6 hr ___ 12 hr
_X_ 24 hr _X_ 48 hr _X_ 72 hr ___ other (describe):

c. Positive control
Dosing: _X_ once ___ twice (24 hr apart)
___ other (describe):
Sampling (after last dose): ___ 6 hr ___ 12 hr
_X_ 24 hr ___ 48 hr ___ 72 hr ___ other (describe):

2. Tissues and Cells Examined:

_X_ bone marrow ___ other (list):

No. of polychromatic erythrocytes (PCEs) examined per animal: 1000
No. of normochromatic erythrocytes (NCEs) examined per animal: Number observed in 1000 total erythrocytes (NCEs and PCEs); the frequency of micronucleated NCEs was also determined.
Other (if other cell types examined, describe):

3. Details of Slide Preparation: At 24, 48, or 72 hours post-administration, animals were sacrificed by cervical dislocation and bone marrow from each femur was mixed with one drop of fetal calf serum and smeared directly onto a slide (*i.e.*, one slide per femur, two slides per animal). Slides were air-dried, fixed in methanol, and stained with 10% Giemsa solution. Slides were coded prior to scoring.

4. Statistical Methods: The incidence of micronucleated PCEs in treatment groups was compared to the corresponding control group using the Kruskal Wallis test; a Wilcoxon rank-sum test was used to evaluate the results for the positive control. Dose-response trends were evaluated using the Jonckheere's test. Data for males and females were combined for analysis.

5. Evaluation Criteria: The test material was considered positive in this assay if there was a substantial (*i.e.*, values outside the historical control range), dose-related, statistically significant ($p<0.01$) increase in the frequency of micronucleated PCEs above the vehicle control group. (Historical control values for the frequency of micronucleated PCEs were provided in Appendix 3 of the study report, p. 26.) Similarly, the test material was considered cytotoxic to bone marrow cells if it caused a substantial, dose-related, statistically significant ($p<0.01$) decrease in the PCE:NCE ratio at both the 48 and 72 hour sacrifice intervals.

II. REPORTED RESULTS

A. Preliminary Toxicity Assay: With the exception of slight piloerection, noted in all four animals during the first 2 hours post-dosing, there were no other clinical signs of toxicity observed. Based on these findings, the micronucleus assay was conducted with dose levels of 1250, 2500, and 5000 mg/kg.

B. Micronucleus Assay

As noted in the preliminary toxicity trial, no clinical signs of toxicity were evident in treated animals, with the exception of slight piloerection during the first few hours post-dosing. Among animals sacrificed 48 hours post-treatment, there was a slight but significant ($p<0.001$) decrease in the PCE:NCE ratio and this decrease was also significantly dose-related ($p<0.001$) (Table 1). At both the 24-hour and 72-hour sacrifice intervals, however, there was no apparent effect related to treatment, with PCE:NCE ratios comparable in treated and control animals.

The results of the micronucleus assay are summarized in Table 1. There were no appreciable increases in the frequency of micronucleated PCEs (or micronucleated NCEs) in any treatment group. By contrast, the positive control (mitomycin C, 12 mg/kg) caused a clearly significant ($p<0.001$) increase in micronucleated PCEs, as well as a significant ($p<0.01$) reduction in the PCE:NCE ratio.

The study authors concluded that NOVARON did not show any evidence of chromosome-damaging activity in this *in vivo* micronucleus test.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We assess that the study was well conducted and that the study authors' interpretation of the data was correct. NOVARON was evaluated at dose levels above the limit dose (*i.e.*, >2000 mg/kg), but failed to induce an increase in the incidence of micronucleated PCEs. Reduced PCE:NCE ratios were noted in treated animals sacrificed 48 hours post-dosing, with a significant ($p<0.001$) reduction in the highest dose group, and a significant ($p<0.001$) dose-response trend across the three dose groups. Although the performing laboratory's criteria for a cytotoxic response require a significant depression in the PCE:NCE ratio at 48 *and* 72 hours, the ratios obtained in this study at the one sacrifice interval are suggestive of a treatment-related cytotoxic effect in bone marrow cells. Furthermore, current OPPTS Guidelines do not require sampling as long as 72 hours post-treatment. The positive control elicited the expected response. We conclude, therefore, that NOVARON was negative in this *in vivo* micronucleus assay.

The study is classified as unacceptable, however, because too few PCEs were scored per animal: current OPPTS Guidelines (870.5395) require the analysis of 2000 PCEs per animal (to determine the frequency of micronucleated cells), rather than the 1000 PCEs per animal evaluated in this study. The study may be upgradable if the prepared slides can be re-analyzed and sufficient cells are available for scoring.

B. Study Deficiencies: As discussed above, OPPTS Guideline 870.5395 requires that 2000 PCEs per animal be scored for micronucleated PCEs. Because only 1000 PCEs per animal were evaluated in this study, the study is classified unacceptable, but may be upgradable if the prepared slides are still available for re-analysis.

One other deficiency was noted in study reporting: Guidelines (870.5395) require both mean and standard deviation values for the frequency of micronucleated cells. Because data for individual animals were provided in the study report, our reviewers were able to calculate the standard

deviations for each treatment group, and so this deficiency did not affect our interpretation of the study.

Table 1. Results of the Micronucleus Assay in Mice with NOVARON

Substance	Dose/kg	Exposure Time (hours)	Number of PCEs Analyzed[a]	PCE:NCE Ratio	Incidence of mPCEs per 1000 PCEs (mean ± S.D.)[b]	Incidence of mNCEs per 1000 NCEs (mean ± S.D.)[b]
Vehicle Control						
1% Methylcellulose	20 mL	24	10,000	1.074	1.8±1.8	0.8±1.1
		48	10,000	1.170	1.0±1.6	1.2±1.8
		72	10,000	0.897	0.4±0.7	1.1±1.3
Positive Control						
Mitomycin C	12 mg	24	10,000	0.811*	53.3±18.3**	1.4±1.5
Test Material						
NOVARON	1250 mg	24	10,000	1.037	1.2±1.1	0±0
		48	10,000	1.070	1.2±1.0	0.2±0.6
		72	10,000	1.089	0.7±0.8	0.3±0.7
NOVARON	2500 mg	24	10,000	1.039	0.7±0.5	0.4±0.9
		48	10,000	0.820	1.4±1.4	0.9±1.2
		72	10,000	1.085	1.2±1.3	1.2±2.1
NOVARON	5000 mg	24	10,000	1.096	0.9±0.9	1.3±1.1
		48	10,000	0.637**[c]	0.3±0.5	0.5±1.0
		72	10,000	1.186	1.0±0.7	0.2±0.6

Substance	Dose/kg	Exposure Time (hours)	Number of PCEs Analyzed[a]	PCE:NCE Ratio	Incidence of mPCEs per 1000 PCEs (mean ± S.D.)[b]	Incidence of mNCEs per 1000 NCEs (mean ± S.D.)[b]

Abbreviations used: PCE = polychromatic erythrocyte NCE = normochromatic erythrocyte
mPCE = micronucleated PCE mNCE=micronucleated NCE
* Significantly ($p<0.01$) different from control.
**Significantly ($p<0.001$) different from control
a 1000 PCEs were analyzed per animal, and there were 10 animals (5/sex) per treatment group, per sacrifice interval.
b Means and standard deviations were calculated by our reviewers; tabulated means may differ slightly from values in study report due to rounding.
c A significant ($p<0.001$) dose-related trend was observed at the 48-hour interval.
NOTE: Data were extracted from the study report, p. 19.

DATA EVALUATION REPORT

NOVARON

Study Type: *In vitro* mammalian cell gene mutation test in mouse lymphoma (L5178Y) cells

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Kristin Jacobson, MSPH	Date 4/7/99
Independent Reviewer	Greg Lawrence, DVM	Date 4-7-99
Project Manager (QA/QC Manager)	Ellen Mantus, Ph.D.	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

80

EPA Reviewer: T. McMahon, Ph.D. Date 4/21/99
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cell gene mutation test in mouse lymphoma L5178Y cells; OPPTS 870.5300

DP BARCODE: D253496, D253509 SUBMISSION CODE: S557116, S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): NOVARON (≥99% active ingredient, a.i.)

SYNONYMS: AlphaSan™ RC5000; Partially ion-exchange zirconium phosphate; silver sodium hydrogen zirconium phosphate

CITATION: Adams, K. (1994) Application for pesticide registration: AlphaSan™ RC 5000, EPA File Symbol 11631. Volume 15: Mammalian cell mutation assay. Huntingdon Research Centre, Ltd. (Cambridgeshire, England). HRC Study No. TSI 73/941431,[1] June 16, 1994. MRID 44582913. Unpublished.

SPONSOR: Milliken Chemical Co., Spartanburg, SC

EXECUTIVE SUMMARY:

In a mammalian cell gene mutation assay at the thymidine kinase (TK) locus (MRID 44582913), mouse lymphoma L5178Y cells were exposed *in vitro* to NOVARON (≥99% a.i.) at concentrations between 1 and 50 µg/mL in the absence of mammalian metabolic activation and at concentrations between 5 and 100 µg/mL in the presence of metabolic activation (Aroclor-induced rat liver S9). Cultures exposed to dose levels of 1, 5, 15, 20, and 25 µg/mL -S9 and 10, 25, 50, 75, and 100 µg/mL +S9 were cloned for mutant selection in one or both trials.

NOVARON was tested up to either cytotoxic concentrations (≥50 µg/mL - S9) or the limit of solubility (100 µg/mL +S9) in two independent assays. Mutation frequencies (MFs) in treated cultures were significantly ($p<0.05$) increased relative to the corresponding solvent controls under nonactivated conditions. These increases were not considered biologically significant, however, because they represented less than two-fold increases above the control MFs and were within (or just slightly higher than) the performing laboratory's historical control MF range. The positive controls induced the appropriate response. **There was no evidence of a biologically relevant increase in induced mutant colonies over background.**

[1]The HRC Study No. was listed as 74/941431 on the report cover, but the study report was consistently labeled as 73/941431 throughout the remainder of the report.

This study is classified as acceptable. It does satisfy the requirements for OPPTS Test Guideline 870.5300 for *in vitro* mammalian cell gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging Statement was not provided in the study report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: NOVARON
   Description: Odorless white powder
   Lot/Batch #: 7130519
   Purity: ≥99% a.i.
   Stability of compound: Not reported
   CAS #: Not reported
   Solvent used: Deionized water
   Other comments: The test material was stored at room temperature in the dark. Dosing solutions were prepared immediately prior to use. Stability (of the test material or the test material in the solvent) was not determined. Achieved concentrations were not verified analytically. The average particle diameter of the test material was 0.9 µm.

2. Control Materials:
   Negative: Not used
   Solvent/final concentration: Deionized water/ 1% (v/v)
   Positive:
   Nonactivation: Ethyl methanesulphonate was dissolved in deionized water to attain a final concentration of 500 µg/mL.
   Activation: 20-Methylcholanthrene was dissolved in dimethyl sulfoxide and used at a final concentration of 2.5 µg/mL.

3. Activation: S9 derived from adult male Sprague Dawley-derived

X Aroclor 1254 (as Arachis oil)	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none	___ hamster	___ other	
___ other	___ other		

The S9 homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to metabolize 20-methylcholanthrene. The S9 mix was prepared as follows:

Component	Concentration
NADP	1.6 mM
Isocitric acid	8.7 mM
S9 homogenate	5% (v/v)

f 2

4. Test Cells: mammalian cells in culture
   _X_ mouse lymphoma L5178Y cells
   ___ Chinese hamster ovary (CHO) cells
   ___ V79 cells (Chinese hamster lung fibroblasts)
   ___ other (list):

   Properly maintained? Yes
   Periodically checked for Mycoplasma contamination? Not reported
   Periodically checked for karyotype stability? Not reported
   Periodically "cleansed" against high spontaneous background? Yes

   Media: For general cell culture, RPMI 1640 supplemented with 10% heat-inactivated donor horse serum (HiDHS), 0.1% Syncperonic F68, sodium pyruvate (0.011%), L-glutamine (200 mM), gentamicin and sodium bicarbonate buffer was used. During the treatment period, the RPMI 1640 medium was supplemented only with 5% HiDHS, L-glutamine and gentamicin, and buffered with HEPES and bicarbonate. Cloning and selection media (*i.e.*, media during the expression period) contained 0.02% Syncperonic F68 and 30% HiDHS; additionally, 4% Noble agar was added to the (semi-solid) cloning medium.

5. Locus Examined:
   _X_ thymidine kinase (TK)
   Selection agent:_____ bromodeoxyuridine (BrdU)
   _____ fluorodeoxyuridine (FdU)
   4 µg/mL trifluorothymidine (TFT)

   __ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
   Selection agent:_____ 8-azaguanine (8-AG)
   _____ 6-thioguanine (6-TG)
   __ Na^+/K^+ ATPase
   Selection agent:_____ ouabain
   __ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

   a) Preliminary cytotoxicity assay: Nine concentrations (0.5, 1, 3, 6, 12, 25, 50, 75, and 100 µg/mL +/- S9) were evaluated using single cultures per dose, per condition.

   b) Main assay: Duplicate cultures were established per dose, per condition, at the following concentrations:

      Nonactivated conditions:
      Initial assay: 0.5, 1, 2.5, 5, 10, 15, 20, and 25 µg/mL. Cultures exposed to 5, 15, 20, or 25 µg/mL were cloned for mutant selection.

      Confirmatory assay: 1, 5, 10, 15, 20, 25, 37.5, and 50 µg/mL. Cultures exposed to 1, 15, 20, or 25 µg/mL were cloned for mutant selection.

F3

S9-Activated conditions:

Initial assay: 5, 10, 15, 20, 25, 50, 75, and 100 µg/mL. Cultures exposed to 25-100 µg/mL were cloned for mutant selection.

Confirmatory assay: 10, 15, 20, 25, 37.5, 50, 75, and 100 µg/mL. Cultures exposed to 10, 25, 50, or 100 µg/mL were cloned for mutant selection.

B. TEST PERFORMANCE

1. Cell treatment:
   a) Cells exposed to test compound, solvent or positive controls for: 3 hours (nonactivated and activated conditions).

   b) After washing, cells were cultured for 2 days (expression period) before cell selection.

   c) After expression, $1x10^6$ cells/dish (3 dishes/culture, 2 cultures/treatment group) were cultured for 12 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/culture, 2 cultures per treatment group) were cultured for 12 days without selective agent to determine cloning efficiency. For the solvent controls, four (rather than two) replicate cultures were used.

2. Statistical Methods: MF data were analyzed with a weighted analysis of variance using the method described by Arlett et al. (1989)[2] in the UKEMS Guidelines.

3. Evaluation Criteria: The test material was considered positive in this assay if it induced a reproducible, dose-related, statistically significant increase in the MF over background that was at least two-fold higher than the corresponding control MF and the induced MF was at least 150 mutants per 10^6 surviving cells (*i.e.*, exceeded the upper limit of the performing laboratory's historical control range).

II. REPORTED RESULTS

A. Solubility: The study author reported that the maximum concentration of test material that could be suspended in the solvent (deionized water) was 10 mg/mL. The final concentration attained using this 10-mg/mL stock solution was 100 µg/mL, and this was the highest concentration selected for the preliminary cytotoxicity assay.

B. Preliminary Cytotoxicity Assay: "Very very slight precipitation" was noted in cultures containing ≥12 µg/mL +/-S9. Under nonactivated conditions, dose levels ≥25 µg/mL were severely cytotoxic, with ≤4% relative suspension growth compared to the solvent control. Relative growth at lower concentrations (0.5-12 µg/mL -S9) was ≥86%.

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[2]Arlett, C.F., D.M. Smith, G.M. Clarke, et al. (1989) Mammalian cell gene mutation assays based upon colony formation. In Kirkland, D.J. (Ed.). *UKEMS Sub-Committee on Guidelines for Mutagenicity Testing. Report. Part III. Statistical Evaluation of Mutagenicity Test Data.* Cambridge, England: Cambridge University Press.

The test material was less cytotoxic under S9-activated conditions, with ≥78% relative growth noted at concentrations between 0.5 and 50 µg/mL, and 51-63% relative growth at the two highest concentrations, 75 and 100 µg/mL +S9.

Accordingly, the initial mutagenicity assay was conducted with concentrations ranging up to 25 µg/mL - S9 and up to 100 µg/mL +S9.

C. Mutagenicity Assay:

Under nonactivated conditions, relative suspension growth was decreased in a dose-related manner in both the initial and confirmatory trials (Table 1). In the initial trial, the highest dose tested (25 µg/mL -S9) resulted in a relative suspension growth of 54%. Relative viability in the individual cultures in the initial trial ranged from 62% to 90%, and relative total survival (*i.e.*, the product of relative suspension growth and relative viability) ranged from 37% to 73%. Statistically significant ($p<0.05$ and $p<0.01$) increases in the MF were noted at the two highest doses (Table 1). These increases were small, however, representing just 1.4- and 1.8-fold increases above the control. Furthermore, the induced MFs were within the performing laboratory's historical control range (*i.e.*, <150 mutants per 10^6 surviving cells). These increases were therefore not considered biologically significant.

In the confirmatory trial under nonactivated conditions, cells were treated with a higher range of concentrations (37.5 and 50 µg/mL -S9), but relative suspension growth for both of these higher concentrations was ≤10% and these cultures were therefore not cloned for mutant selection. Relative total survival ranged from 19% to 74% in cultures exposed to 1-25 µg/mL -S9 in this trial. Statistically significant ($p<0.05$) increases were again noted in the MF at two dose levels (15 and 20 µg/mL -S9, but not in cultures treated with 25 µg/mL -S9) (Table 1). As noted for the initial trial, these increases were small (1.5- and 2.1-fold above the solvent control), and were approximately within the historical control range. In addition, the largest increase, 154 mutants per 10^6 survivors, occurred in the presence of appreciable cytotoxicity (relative total survival of 19%). The increased MFs observed in the confirmatory nonactivated trial were therefore not considered biologically significant. In both the initial and confirmatory trials, the positive controls elicited the expected responses, with MFs increased 7.8- to 11.4-fold above the corresponding solvent controls.

In the presence of S9 activation, relative suspension growth and relative total survival were greater than 50% at all dose levels, with the exception of cultures exposed to 37.5, 75 and 100 µg/mL in the confirmatory trial (Table 2). MFs in treated cultures were comparable to the corresponding solvent control, with the exception of the 100-µg/mL cultures in the confirmatory trial for which the MF was significantly ($p<0.05$) increased above the solvent control. This increase was very small (a 1.2-fold increase above the solvent control), however, and was well within the historical control range of 150 mutants per 10^6 survivors. In addition, this apparent increase in the MF occurred in the presence of a low relative total survival at this dose level, 33%. As noted for the increased MFs that occurred under nonactivated conditions, this statistically significant increase in the MF was not considered to be biologically meaningful. The positive control induced highly significant ($p<0.001$) increases in the MF in both trials, although relative total survival was reduced to 17-18% in each trial.

AS-

Results from the four assays (*i.e.*, the initial and confirmatory trials, with and without S9 activation) were also presented graphically in the study report (pp. 27-28). These figures clearly illustrated the occurrence of increased MFs only when survival was decreased, and also that the induced MFs never exceeded 160 mutants per 10^6 surviving cells.

The study author concluded that NOVARON did not demonstrate mutagenic potential in this *in vitro* mammalian cell mutagenicity assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. We agree with the study author's interpretation of the data. In two independent assays, NOVARON was tested either up to cytotoxic concentrations (≥50 µg/mL - S9) or the limit of solubility (100 µg/mL +S9), but failed to induce biologically significant increases in the MF, in either the presence or absence of S9 activation. Statistically significant ($p<0.05$) increases in the MF that occurred in both nonactivated trials and also in the confirmatory S9-activated trial were not considered biologically relevant because of the small magnitude of the increases (≤2-fold higher than controls and within the historical control range) and/or the presence of appreciable cytotoxicity. The positive controls induced highly significant ($p<0.001$) increases in MFs in each trial that were 7.6- to 11.4-fold higher than the solvent controls. We conclude, therefore, that NOVARON was tested over an appropriate range of concentrations and was not genotoxic in this mammalian cell gene mutation assay.

This study is classified as acceptable. It does satisfy the requirements for OPPTS Test Guideline 870.5300 for *in vitro* mammalian cell gene mutation data.

B. Study deficiencies: One reporting deficiency was identified:

- It was not reported whether the cell line was periodically inspected for *Mycoplasma* contamination or for karyotype stability.

This minor deficiency is not considered to have affected the overall outcome or integrity of the study, in light of the appropriate responses reported in solvent control and positive control cultures in the two independent trials.

8 6

Table 1. Results of the Mouse Lymphoma Mutation Assays (Initial and Confirmatory) with NOVARON under Nonactivated Conditions

Substance	Dose/mL	Relative Suspension Growth (%)	Relative Viability (%)[a]	Relative Total Survival (%)[b]	Average Total Mutant Colonies per Culture	Mutation Frequency per 10^6 Survivors[c]
Solvent Control: Deionized Water						
Initial Assay	10 uL	100	100	100	218	82
Confirm. Assay	10 uL	100	100	100	220	74
Positive Control: Ethyl methanesulfonate						
Initial Assay	500 µg	52	48	25	1142	936***
Confirm. Assay	500 µg	70	59	41	1024	583***
Test Material: NOVARON						
Initial Assay[d]	5 µg	95	72	70	189	100
	15 µg	86	86	73	193	85
	20 µg	58	81	47	248	115*
	25 µg	54	68	37	269	149**
Confirm. Assay[e]	1 µg	85	88	74	214	82
	15 µg	58	91	52	291	108*
	20 µg	45	52	23	234	154*
	25 µg	31	60	19	188	109

Substance	Dose/mL	Relative Suspension Growth (%)	Relative Viability (%)[a]	Relative Total Survival (%)[b]	Average Total Mutant Colonies per Culture	Mutation Frequency per 10^6 Survivors[c]

*Significantly higher than control, $p<0.05$ **Significantly higher than control, $p<0.01$ ***Significantly higher than control, $p<0.001$

a Average of duplicate cultures; calculated by our reviewers.
b Relative total survival = (Relative suspension growth) x (Relative viability) x 100%
c Average of duplicate cultures.

$$\text{Mutation Frequency} = \frac{(200 \text{ cells})(3 \text{ plates/culture})}{(\text{total number viable cells})} \div \frac{(\text{total number of mutant colonies})}{(1\times10^6 \text{ cells/plate})(3 \text{ plates/culture})}$$

d Four concentrations were selected for cloning; other levels (0.5, 1, 2.5, and 10 µg/mL) did not suggest a cytotoxic effect.
e Four concentrations were selected for cloning; other lower levels (5 or 10 µg/mL) did not suggest a cytotoxic effect, whereas higher levels (37.5 and 50 µg/mL) resulted in ≤10% relative survival.

Data were extracted from the study report, pp. 21-24.

Table 2. Results of the Mouse Lymphoma Mutation Assays (Initial and Confirmatory) with NOVARON under S9-Activated Conditions

Substance	Dose/mL	Relative Suspension Growth (%)	Relative Viability (%)[a]	Relative Total Survival (%)[b]	Average Total Mutant Colonies per Culture	Mutation Frequency per 10^6 Survivors[c]
Solvent Control: Deionized Water						
Initial Assay	10 uL	100	100	100	260	98
Confirm. Assay	10 uL	100	100	100	220	89
Positive Control: Ethyl methanesulfonate						
Initial Assay	500 µg	42	40	17	780	744***
Confirm. Assay	500 µg	56	32	18	668	858***
Test Material: NOVARON						
Initial Assay[d]	25 µg	97	84	81	256	116
	50 µg	76	86	65	234	103
	75 µg	79	78	61	222	107
	100 µg	63	88	55	260	112
Confirm. Assay[e]	10 µg	94	87	81	184	85
	25 µg	90	90	80	162	73
	50 µg	78	74	56	156[f]	85
	100 µg	43	76	33	204	109*

Substance	Dose/mL	Relative Suspension Growth (%)	Relative Viability (%)[a]	Relative Total Survival (%)[b]	Average Total Mutant Colonies per Culture	Mutation Frequency per 10^6 Survivors[c]

*Significantly higher than control, $p<0.05$ **Significantly higher than control, $p<0.01$ ***Significantly higher than control, $p<0.001$

a Average of duplicate cultures; calculated by our reviewers.
b Relative total survival = (Relative suspension growth) x (Relative viability) x 100%
c Average of duplicate cultures.

$$\text{Mutation Frequency} = \frac{(200 \text{ cells})(3 \text{ plates/culture})}{(\text{total number viable cells})} \div \frac{(\text{total number of mutant colonies})}{(1\text{x}10^6 \text{ cells/plate})(3 \text{ plates/culture})}$$

d Four concentrations were selected for cloning; lower levels (5, 10, 15, and 20 µg/mL) did not suggest a cytotoxic effect.
e Four concentrations were selected for cloning; other intermediate levels (15, 20, 37.5 or 75 µg/mL) were exposed to the test material but were not cloned.
f One cloning plate was lost to contamination; data shown were adjusted by performing laboratory to accommodate lost plate.

Data were extracted from the study report, pp. 25-28.

DATA EVALUATION REPORT

NOVARON

Study Type: Primary Dermal Irritation (Rabbit)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	*Kathleen Luck, B.A.*	Date 4/9/99
Independent Reviewer	*Jennifer Welham, M.S.*	Date 4/9/99
Project Manager (QA/QC Manager)	*Eilen Mantus, Ph.D.*	Date 4/9/99

Contract Number: 68-W6-0022
Work Assignment No.: 3-17
EPA Project Manager: Peter Thompson

Disclaimer

This review may have been changed following contractor's submission to the Antimicrobial Division of the Office of Pesticide Programs.

91

EPA Reviewer: T. McMahon, Ph.D. _____, Date _____
Senior Toxicologist, RASSB/AD (7510C)
EPA Work Assignment Manager: P. Thompson, Ph.D.
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS 870.2500

DP BARCODE: D253496 & D253509
SUBMISSION CODE: S557116 & S557132
P.C. CODE: 072560

TEST MATERIAL (PURITY): Novaron (≥ 99%)

SYNONYMS: Partially ion-exchange zirconium phosphate, Novaron AG-300; AlphaSan™ RC5000

CITATION: Parcell, B. (1994) Primary Dermal Irritation, Rabbit. Huntingdon Research Centre, Ltd. (Huntingdon, Cambridgshire, England). TSI 68/940295/SE, September 7, 1994. MRID 44582909. Unpublished.

SPONSOR: Milliken Chemical

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44582909), six female adult New Zealand White rabbits were dermally exposed on the shaved dorso-lumbar region to 0.5 g of Novaron (≥ 99%) for 4 hours. Examination of the skin site was then made approximately 30 minutes after removal of the test substance, and again at 24, 48, and 72 hours. Erythema and edema were scored by a standard method (citation not provided). There was no evidence of erythema or edema in any of the rabbits used in this study.

In this study, Novaron is not a dermal irritant. Therefore, Novaron is classified as TOXICITY CATEGORY IV for primary dermal irritation.

This study is classified as acceptable. It does satisfy the guideline requirement for a primary dermal irritation study (OPPTS 870.2500) in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material
   Description: white powder
   Lot/Batch #: 7130519
   Purity: ≥ 99%
   CAS #: not given

2. Vehicle
   Novaron was administered as supplied by the sponsor.

3. Test Animals
   Species: rabbit
   Strain: New Zealand White
   Age at treatment: 10 to 14 weeks
   Weight at treatment: 2.4 to 3.3 kg
   Source: Froxfield U.K., Ltd., Petersfield, Hampshire, England
   Acclimation period: not given
   Diet: SDS Stanrab (P) Rabbit Diet, available *ad libitum*
   Water: source not given, available *ad libitum*
   Housing: individually in plastic cageswith perforated floors.

B. STUDY DESIGN AND METHODS

1. In Life Dates
   Start: April 5, 1994
   End: April 8, 1994

2. Animal Assignment and Treatment
   Approximately 24 hours prior to test material application, the hair was removed with electric clippers from the dorso-lumbar region of each rabbit, exposing an area 10cm x 10cm. Then, 0.5ml of the undiluted test substance was applied under a 25mm x 25mm gauze pad which had been moistened with 0.5ml distilled water to one intact skin site per rabbit. The gauze was secured with Elastoplast elastic adhesive and the animals returned to their respective cages. Exposure was for four hours. Following the exposure period, the semi-occlusive dressing and gauze pad were removed and the test site was washed with warm water. Erythema and edema were scored using a standard method; however, a citation was not provided. Evaluations were made at 30 minutes after exposure, and again at 24, 48, and 72 hours after exposure.

93

II. RESULTS AND DISCUSSION

A. RESULTS

There was no evidence of erythema and edema in any of the animals throughout the observation period.

B. DEFICIENCIES

Several minor deficiencies were noted. A citation was not provided for the method used to score dermal irritation; however, the method is comparable to the guidelines. In addition, the study report does not adequately characterize several aspects of the study, including acclimation period of the animals and source of the drinking water. These deficiencies are all considered minor, because it is unlikely that they influenced the results of the study.