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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

April 21, 1999

MEMORANDUM:

Subject: Acute Toxicity Review for EPA Reg. No.: 10182-UUE / Dolphin Fungicide
DP Barcode: D246439
Case No: 062095

To: Marshall Swindell,
Regulatory Management Branch
Antimicrobials Division (7510C)

From: Ian Blackwell, Biologist
Efficacy Evaluation Team
Efficacy and Science Support Branch
Antimicrobials Division (7510C)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Efficacy and Science Support Branch
Antimicrobials Division (7510C)

Michele Wingfield, Branch Chief
Efficacy and Science Support Branch
Antimicrobials Division (7510C)

Applicant: Zeneca Biocides

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
N-Butyl-1,2-benzisothiazolin-3-one	97.8
Other Ingredient(s):	2.2
Total:	100.0%

BACKGROUND: Zeneca Biocides has submitted four acute toxicity/ irritation studies to support the registration of "Dolphin Fungicide". No acute inhalation toxicity or primary eye irritation study was submitted. The submitted studies were conducted by Zeneca's Central Toxicology Laboratory. The MRID numbers are 443649-15 through 443649-18.

RECOMMENDATIONS: ESSB findings are:

1. The acute oral toxicity, acute dermal toxicity, primary skin irritation and dermal sensitization studies are acceptable.

2. No acute inhalation toxicity study was submitted, nor was a waiver requested for this study. The registrant must submit an acute inhalation toxicity study, or, submit a detailed request for the waiver of the acute inhalation toxicity study.

3. No primary eye irritation study or waiver request was submitted. It is felt that this is because this product was demonstrated to be corrosive in the primary skin (dermal) irritation study. When this occurs, the Agency will waive the primary eye irritation study for that product, if the registrant agrees to accept **toxicity category I** (for primary eye irritation.). Thus, at the registrant's request, ESSB will waive that primary eye irritation study and assign that study a toxicity category I.

The acute toxicity profile for File Symbol 10182-UUE is currently:

acute oral toxicity	III	Acceptable
acute dermal toxicity	III	Acceptable
acute inhalation toxicity		Outstanding (requested)
primary eye irritation		Outstanding (requested)
primary skin irritation	I	Acceptable
dermal sensitization	Sensitizer	Acceptable

LABELING:

No precautionary labeling can be recommended until the outstanding acute inhalation toxicity and primary eye irritation studies are fulfilled.

DATA EVALUATION REPORT

N-BUTYL-1,2-BENZISOTHIAZOLIN-3-ONE

STUDY TYPE: ACUTE ORAL TOXICITY – RATS

Prepared for

Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-171

Primary Reviewer:
H. T. Borges, Ph.D., MT(ASCP), D.A.B.T

Signature: H.T. Borges
Date: AUG 25 1998

Secondary Reviewers:
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Signature: Cheryl B Bast
Date: AUG 25 1998

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: AUG 25 1998

Quality Assurance:
LeeAnn Wilson, M.A.

Signature: L. A. Wilson
Date: AUG 25 1998

Disclaimer

This Data Evaluation Report may have been altered by the Antimicrobials Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Ian Blackwell, M.S. Ian Blackwell, Date 4/15/99
EPA Work Assignment Manager: Peter Thompson, Ph.D._____, Date _____
Antimicrobials Divisions (7510W)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity – Rats
OPPTS 870.1100 [§81-1]

DP BARCODE: D246439 SUBMISSION CODE: S533546
CASE: 062095 TOX. CHEM. NO.: 098951

TEST MATERIAL (PURITY): N-butyl-1,2-benzisothiazolin-3-one (95.5% a.i.)

SYNONYMS: DOLPHIN Fungicide

CITATION: Lees, D. (1996) Substance S123386: Acute oral toxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No: CTL/P/5067, Study No: AR6193, July 25, 1996. MRID 44364915. Unpublished

SPONSOR: Zeneca Biocides, Wilmington, DE

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44364915), groups of fasted young adult Alpk:AP$_f$SD rats (5/sex) were given a single oral dose of 2000, 5000, or 7500 mg/kg DOLPHIN Fungicide (N-butyl-1,2-benzisothiazolin-3-one, 95.5% a.i.) (Lot No. JM 5420/80) and observed for 14 days.

One female rat treated with 2000 mg/kg test material was found dead on day 3; 3/5 male rats treated with 5000 mg/kg test material either died or were killed *in extremis* on the day of treatment and 1/5 females was found dead on day 3; and 5/5 male and 5/5 female rats treated with 7500 mg/kg test material died or were killed *in extremis* within 72 hours of treatment. Following treatment, all surviving male and female rats developed slight to moderate piloerection and an upward curvature of the spine. In addition, several rats developed chromodacryorrhea, had urinary incontinence and had stains around the nose or mouth through day 7. In general, male and female rats that died during the study had excess watery fluid in the abdominal cavity, dark livers, and stomachs that were distended with thin walls, red spots, and adhesions. In addition several animals in the 7500 mg/kg group had thoracic cavities filled with a watery fluid. Several rats that survived the study period had abdominal adhesions, thickened stomach walls, nonglandular stomach ulceration, and stomach adhesions. According to the study author, effects noted at necropsy in animals that died during the study are consistent with severe stomach inflammation.

The oral LD_{50} and 95% Confidence Intervals of DOLPHIN Fungicide to Alpk:AP$_f$SD rats are:

Males: 4267 mg/kg (3157-5767 mg/kg)
Females: 4732 mg/kg (2178-12959 mg/kg)

This places the test material in Toxicity Category III.

This acute oral study is classified as acceptable and does satisfy the guideline requirement for an Acute Oral Study (81-1) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: N-butyl-1,2-benzisothiazolin-3-one (95.5% a.i.)

   Description: dark brown liquid
   Lot/Batch #: Blend JM 5420/80

2 Vehicle: corn oil

3. Test animals

   Species: rat
   Strain: Alpk:AP$_f$SD
   Age and/or weight at dosing: young adult; males 231-300 g; females 200-246 g
   Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Acclimation period: ≥ 6 days
   Diet: Rat and Mouse Diet No. 1, Special Diets Services Ltd., ad libitum
   Water: tap water, ad libitum
   Housing: 5/sex
   Environmental conditions:
   Temperature: 21 ± 2°C
   Average Humidity: 40-70%
   Air changes: 25-30/hour
   Photoperiod: 12-hour light/dark

B. STUDY DESIGN and METHODS

1. In life dates

   Start: 02/07/96; end: 04/06/96

2. Animal assignment and treatment

   Animals were assigned to the test groups noted in Table 1. Following an overnight fast, groups of five male and five female rats were given a single gavage dose of 2000 mg/kg, 5000 mg/kg or 7500 mg/kg test material in corn oil at a constant volume of 10 mL/kg. The animals were observed for clinical signs of toxicity within two hours of

dosing, between four and seven hours of dosing, and daily thereafter for the remainder of the study. The animals were weighed before treatment and on study days 7 and 14. Survivors were sacrificed 14 days after treatment and all animals were necropsied.

TABLE 1. Dose, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
2000	0/5	1/5	1/10
5000	3/5	1/5	4/10
7500	5/5	5/5	10/10

3. Statistics

The LD_{50} was calculated by logistic regression and the confidence intervals determined by using a likelihood ratio interval.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality is given in Table 1. One female rat treated with 2000 mg/kg test material was found dead on day 3. Three male rats treated with 5000 mg/kg test material were either found dead or killed *in extremis* on the day of treatment and one female was found dead on day 3. All male and female rats treated with 7500 mg/kg test material died or were killed *in extremis* within 72 hours of treatment.

Based on the study results, the author calculated the following Alpk:AP_fSD rat LD_{50} and 95% Confidence Intervals:

Males: 4267 mg/kg (3157-5767 mg/kg)
Females: 4732 mg/kg (2178-12959 mg/kg)

B. CLINICAL OBSERVATIONS

Following treatment with the test material, all surviving male and female rats developed slight to moderate piloerection and an upward curvature of the spine. In addition, several rats developed chromodacryorrhea, had urinary incontinence and had stains around the nose or mouth. These clinical signs of toxicity persisted through day 7 in some animals.

C. BODY WEIGHT

Following a single oral dose of 2000 mg/kg, male and female rats exceeded their initial weight by day 8 and continued to gain weight through the remainder of the study. Surviving male and female rats from the 5000 mg/kg group exceeded their initial bodyweight by study end.

D. NECROPSY

Of five male and four female rats in the 2000 mg/kg group that survived the study period, two males and one female had abdominal adhesions, two males and two females had thickened stomach walls, two males and three females had nonglandular stomach ulceration, and two males and one female had stomach adhesions. The one female of the 2000 mg/kg group that died during the study had excess watery fluid in the abdominal cavity, a dark liver, and a distended stomach with a thin wall, red spots, and adhesions.

No treatment-related effects were found in male and female rats of the 5000 mg/kg group that survived the study. Three male rats of the 5000 mg/kg group that died during the study period had dark livers, stomachs filled with fluid, and empty rectums. The female of the 5000 mg/kg group that died during the study had a discolored diaphragm, a distended stomach filled with fluid, and the stomach, ileum, and jejunum were filled with discolored contents.

Of male and female rats in the 7500 mg/kg group, three males and two females had dark or mottled lungs, four males and one female had distended stomachs, and one male and one female had stomachs filled with fluid, discolored stomach contents, and/or red spots on the stomach wall. In addition, the thoracic cavities of three males and one female were filled with a watery fluid.

According to the study author, effects noted at necropsy in animals that died during the study are consistent with severe stomach inflammation.

E. DEFICIENCIES

None identified.

DATA EVALUATION REPORT

N-BUTYL-1,2-BENZISOTHIAZOLIN-3-ONE

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-171

Primary Reviewer: H. T. Borges, Ph.D., MT(ASCP), D.A.B.T	Signature: H.T Borges Date: AUG 2 5 1998
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T	Signature: Cheryl B Bast Date: AUG 2 5 1998
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: AUG 2 5 1998
Quality Assurance: LeeAnn Wilson, M.A.	Signature: L. A. Wilson Date: AUG 2 5 1998

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

EPA Reviewer: Ian Blackwell, M.S. Ian Blackwell, Date 4/15/99
EPA Work Assignment Manager: Peter Thompson, Ph.D._____, Date _____
Antimicrobials Division (7510W)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS 870.1200 [§81-2]

DP BARCODE: D246439
CASE: 062095

SUBMISSION CODE: S533546
TOX. CHEM. NO.: 098951

TEST MATERIAL (PURITY): N-butyl-1,2-benzisothiazolin-3-one (93.4% a.i.)

SYNONYMS: DOLPHIN Fungicide

CITATION: Lees, D. (1996) Substance S123386: Acute dermal toxicity study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No: CTL/P/4992, Study No: CR3269, July 30, 1996. MRID 44364916. Unpublished

SPONSOR: Zeneca Biocides, Wilmington, DE

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44364916), groups of young adult Alpk:AP$_f$SD rats (5/sex) were given a single dermal dose of 2000 mg/kg DOLPHIN Fungicide (N-butyl-1,2-benzisothiazolin-3-one, 93.4% a.i.) (Lot No. NBY5708/28) and observed for 14 days.

None of the animals died during the study and no clinical signs of toxicity were observed. However, slight to moderate erythema at the application site was observed through day 7 on all rats. In addition, desquamation, scabbing, skin thickening and wrinkling at the application site were noted on all animals through the study period. Most male and female rats lost weight initially, but all male rats regained and continued to gain weight through the remainder of the study. By the end of the study, 2/5 female rats had not recovered their pretreatment weight.

Based on the study results, the dermal LD_{50} of DOLPHIN Fungicide to male, female, and male and female Sprague Dawley rats is > 2000 mg/kg. This places the test material in Toxicity Category III.

This acute dermal toxicity study is classified as acceptable and satisfies the guideline requirements for an acute dermal toxicity study (81-2) in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-butyl-1,2-benzisothiazolin-3-one (93.4% a.i.)

   Description: brown liquid
   Lot/Batch #: NBY5708/28
   Specific gravity: 1.090

2 Vehicle: none

3. Test animals

   Species: rat
   Strain: Alpk:AP_fSD
   Age and/or weight at dosing: young adult; males 216-263 g; females 208-219 g
   Source: Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire
   Acclimation period: ≥ 6 days
   Diet: Rat and Mouse Diet No. 1, Special Diets Services Ltd., ad libitum
   Water: tap water, ad libitum
   Housing: individually
   Environmental conditions:
   Temperature: 21 ± 2°C
   Average Humidity: 40-70%
   Air changes: 25-30/hour
   Photoperiod: 12-hour light/dark

B. STUDY DESIGN and METHODS

1 In life dates

   Start: 08/17/95; end: 08/31/95

2. Animal assignment and treatment

   Animals were assigned to the test groups noted in Table 1. An area of 5 × 10 cm over the dorso-lumbar region of each rat was shaved 16-32 hours before treatment. The undiluted test material was spread evenly over the shaved area at a volume of 2 mL/kg. A piece of 4-ply gauze was placed over the treatment area and the site occluded with a piece of plastic film. The site was further secured by wrapping the trunk with an adhesive bandage. Twenty-four hours later, the dressings and gauze were removed and the skin wiped with moistened cotton wool to remove excess test material. The rats were observed for clinical signs of toxicity and mortality once on the day of dosing and daily thereafter for the duration of the 14-day study. At the end

of the study, the survivors were sacrificed and necropsied. Body weights were recorded immediately before treatment and on study days 3, 6, 8, and 15.

TABLE 1. Doses, mortality/animals treated			
Dose (mg/kg)	Males	Females	Combined
2000	0/5	0/5	0/10

3. Statistics - Calculation of a dermal LD_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

As shown in Table 1, none of the animals died during the study. Therefore, the dermal LD_{50} of DOLPHIN Fungicide for male, female, and male and female Alpk:AP_fSD rats is >2000 mg/kg. This places the test material in Toxicity Category III.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed. However, slight to moderate erythema at the application site was observed through day 7 on all rats. In addition, desquamation, scabbing, skin thickening and wrinkling at the application site were noted on all animals through the study period.

C. BODY WEIGHT

Most male and female rats lost weight during the first three days of treatment. After day three all male rats had regained their pretreatment weight and continued to gain weight through the remainder of the study. Two of five female rats did not recover their pretreatment weight by the end of the study.

D. NECROPSY

Other than desquamation and scabbing at the application site, no treatment-related effects were noted.

E. DEFICIENCIES

None identified.

DATA EVALUATION REPORT

N-BUTYL-1,2-BENZISOTHIAZOLIN-3-ONE

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT

Prepared for

Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Life Sciences Division
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Oak Ridge, TN 37831
Task Order No. 98-171

Primary Reviewer: H. T. Borges, Ph.D., MT(ASCP), D.A.B.T	Signature: H.T Borges Date: AUG 2 5 1998
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T	Signature: Cheryl B Bast Date: AUG 2 5 1998
Robert H. Ross, M.S., Group Leader	Signature: Robert H Ross Date: AUG 2 5 1998
Quality Assurance: LeeAnn Wilson, M.A.	Signature: L. A. Wilson Date: AUG 2 5 1998

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

EPA Reviewer: Ian Blackwell, M.S. Ian Blackwell, Date 4/16/99
EPA Work Assignment Manager: Peter Thompson, Ph.D. _____, Date _____
Antimicrobials Division (7510W)

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS 870.2500 [§81-5]

DP BARCODE: D246439 SUBMISSION CODE: S533546
CASE: 062095 TOX. CHEM. NO.: 098951

TEST MATERIAL (PURITY): N-butyl-1,2-benzisothiazolin-3-one (93.4% a.i.)

SYNONYMS: DOLPHIN Fungicide

CITATION: Lees, D. (1996) Substance S123386: Skin irritation to the rabbit. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No: CTL/P/4969, Study No: EB4412, July 30, 1996. MRID 44364917. Unpublished

SPONSOR: Zeneca Biocides, Wilmington, DE

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44364917), 0.5 mL of DOLPHIN Fungicide (N-butyl-1,2-benzisothiazolin-3-one, 93.4% a.i.) (Lot No. NBY5708/28) was applied to a shaved area on the left flank of one female New Zealand white rabbit. The treatment area was covered with a gauze pad held in place with tape that in turn was overwrapped with rubber sheeting. Four hours later, the sheeting, tape, and gauze pad were removed. The sites were scored for erythema and edema 0.5-1 hours and 1, 2, 3, 8, 14, 17, and 21 days after treatment according to the Draize method.

The test material induced very slight to well-defined erythema at the application site within 30-60 minutes after removal of the bandage that persisted through study day 17. Severe edema was also observed at the site within 30-60 minutes before resolving by day 8. Additional signs of irritation were observed and included desquamation, sparse hair growth, new skin formation, scabbing, and skin thickening and wrinkling. Histopathological examination of skin from the application site showed acanthosis and marked sub-epithelial fibrosis indicating that the test material caused severe and permanent damage. Because of this response, no additional animals were treated.

In this study, DOLPHIN Fungicide was corrosive to the skin of female New Zealand white rabbits and is placed in TOXICITY CATEGORY I.

This study is classified as acceptable and satisfies the guideline requirement for a primary dermal irritation study (81-5) 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: N-butyl-1,2-benzisothiazolin-3-one (93.4% a.i.)

   Description: brown liquid
   Lot/Batch #: NBY5708/28
   Specific gravity: 1.090

2 Vehicle: none

3. Test animals

   Species: rabbit
   Strain: New Zealand White
   Sex: female
   Age and/or weight at dosing: young adult; female 2.53 kg
   Source: Charles River UK Limited, Margate, Kent, UK
   Acclimation period: ≥ 6 days
   Diet: STANRAB SQC Rabbit Diet, Special Diets Services Ltd., ad libitum
   Water: tap water, ad libitum
   Housing: individually
   Environmental conditions:
   Temperature: 17 ± 2°C
   Average Humidity: 40-70%
   Air changes: 25/hour
   Photoperiod: 12-hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start 09/05/95; end 09/26/95

2. Animal assignment and treatment

   Approximately 24 hours before treatment, a 7 ×13 cm area over the left flank of one rabbit was shaved. The undiluted test material (0.5 mL) was applied to a 6 cm^2 area within the shaved site and covered with 8-ply gauze secured with surgical tape. The site was further secured by wrapping impermeable rubber sheeting around the trunk of the animal. Four hours later, the wrapping and gauze were removed and the application site cleansed with moistened cotton wool. The application site was scored for irritation according to the Draize method 0.5-1.0 hours and 1, 2, 3, 8, 14, 17, and 21 days after treatment.

II. RESULTS AND DISCUSSION

A. Very slight to well-defined erythema was observed at the application site within 30-60 minutes after removal of the bandage and persisted through study day 17. Severe edema was also observed at the site within 30-60 minutes. The edema resolved by day 8. Additional signs of irritation were observed and included desquamation, sparse hair growth, new skin formation, scabbing, and skin thickening and wrinkling. Because of the persistent skin irritation, a sample from the site of test material application and a sample taken from the opposite flank were excised and submitted for histopathological examination. Skin at the application site showed acanthosis and marked sub-epithelial fibrosis indicating that the test material caused severe and permanent damage to the rabbit skin during the four hour application. Because of this response, no additional animals were treated.

Based on the study results, the test material is classified as corrosive to the skin of female New Zealand white rabbits and is placed in Toxicity Category I.

B. DEFICIENCIES

None identified.

DATA EVALUATION REPORT

N-BUTYL-1,2-BENZISOTHIAZOLIN-3-ONE

STUDY TYPE: DERMAL SENSITIZATION – GUINEA PIG

Prepared for

Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-171

Primary Reviewer:
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Signature: H.T Borges
Date: AUG 2 5 1998

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Date: AUG 2 5 1998

Robert H. Ross, M.S., Group Leader

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Date: AUG 2 5 1998

Quality Assurance:
LeeAnn Wilson, M.A.

Signature: L. A. Wilson
Date: AUG 2 5 1998

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EPA Reviewer: Ian Blackwell, M.S. Ian Blackwell, Date 4/21/99
EPA Work Assignment Manager: Peter Thompson, Ph.D._____, Date _____
Antimicrobials Division (7510W)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea Pig
OPPTS 870.2600 [§81-6]

DP BARCODE: D246439 SUBMISSION CODE: S533546
CASE: 062095 TOX. CHEM. NO.: 098951

TEST MATERIAL (PURITY): N-butyl-1,2-benzisothiazolin-3-one (95.5% a.i.)

SYNONYMS: DOLPHIN Fungicide

CITATION: Lees, D. (1996) Substance S123386: Skin sensitisation to the guinea pig. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No: CTL/P/5046, Study No: GG6736, July 30, 1996. MRID 44364918. Unpublished

SPONSOR: Zeneca Biocides, Wilmington, DE

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44364918) with DOLPHIN Fungicide (N-butyl-1,2-benzisothiazolin-3-one, 95.5% a.i.) (Batch No. Blend JM 5420/80), 20 young adult female Dunking Hartley guinea pigs were induced and challenged according to the Magnusson & Kligman Maximization Method.

Dermal irritation scores following intradermal and topical induction were not provided. Twenty-four hours after challenge with undiluted and 50% test material, none of the test or naive control animals developed a positive dermal response. Forty-eight hours after challenge with the undiluted test material, 14/20 test guinea pigs had developed slight to moderate erythema while no erythema was present on the naive control animals. By 72 hours after challenge with the undiluted test material, slight to moderate erythema was present on 16/20 test animals and 5/10 naive control animals. Forty-eight hours after challenge with 30% test material, 8/20 test animals had developed slight to moderate erythema and by 72 hours after challenge, slight erythema was present on 1/20 animals. None of the naive control animals developed erythema following challenge with 30% test material.

Based on the study results, **DOLPHIN Fungicide is a sensitizer to female Albino Crl (HA) BR guinea pigs.**

This study is classified as acceptable and does satisfy the guideline requirement for a dermal sensitization study (81-6) in the guinea pig. Although the date of the positive control study was not provided and dermal responses following intradermal and topical induction were not provided, these omissions would not invalidate the study results.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-butyl-1,2-benzisothiazolin-3-one (95.5% a.i.)

Description: dark brown liquid
Lot/Batch #: Blend JM 5420/80

2. Vehicle and positive control

Corn oil; Freund's Complete Adjuvant; hexylcinnamaldehyde (positive control)

3. Test animals

Species guinea pig
Strain: Albino Crl (HA) BR
Age and weight at start of treatment: young adult, females 301-419 g
Source: Charles River UK Limited, Margate, Kent, UK
Acclimation period: ≥ 6 days
Diet: Guinea Pig FD1 Diet, Special Diets Services, 1 Stepfield, Witham, Essex, ad libitum
Water: tap water, ad libitum
Housing: 2/suspended cage
Environmental conditions:
Temperature: 17 ± 2°C
Humidity: 40-70%
Photoperiod: 12 hour light/dark
Air Changes: 25/hour

B. STUDY DESIGN AND METHODS

1. In life dates

Start: 03/21/96; end: 04/17/96

2. Animal assignment and treatment

The definitive study was conducted according to the Magnusson & Kligman Maximization method with two groups of guinea pigs. One group of twenty female guinea pigs served as the test animals while another group of 10 female guinea pigs remained untreated until challenge and served as the naive controls. Shortly before the first induction, an area of approximately 5 × 5 cm was shaved on the scapular region of the test and control animals. A row of three paired intradermal injections (0.1 mL) of 1:1 Freund's Complete Adjuvant in corn oil; 0.1% w/v test material in corn oil; and 0.1% w/v test material in 1:1 Freund's Complete Adjuvant in corn oil were made on each side of the midline of the test

animals. The naive control animals were treated similarly with the exception corn oil was substituted for the test material. The injection sites were evaluated for erythema 24 and 48 hours later.

One week after the intradermal injections, the same site on each animal was again shaved. A filter paper patch (2 × 4 cm) containing 0.2-0.3 mL 30% w/v test material in corn oil was applied to the shaved site of each test animal. The filter paper was secured in place with surgical tape which in turn was overlain with an adhesive bandage secured with PVC tape. The test material was allowed to remain in contact with the skin for 48 hours. Naive control animals were treated similarly with the exception the filter paper patch contained corn oil. Erythema at the application site was evaluated 24 hours after removal of the bandage.

Two weeks after the second induction, an area of approximately 15 × 15 cm was shaved on both flanks of each test and naive control animal. Occlusive dressings were prepared for each animal consisting of two pieces of filter paper (1 × 2 cm) stitched to a piece of rubber sheeting. The undiluted test material (0.05-0.1 mL) was applied to one of the dressings and placed on the shaved left flank and 30% w/v test material in corn oil (0.05-0.1 mL) was applied to a second dressing and applied to the shaved right flank. The dressings were covered with an adhesive bandage secured with PVC tape for 24 hours. Both challenge sites on the test and naive control animals were examined 1, 2, and 3 days after removal of the bandage.

A positive control was not run concurrently with the present study. The study report states that positive control studies are run at six month intervals and the results of the most recent was included with the report. However, the date of the study was not reported. In the positive control study, the animals were intradermally induced with 0.3% w/v positive control in corn oil, topically induced with undiluted positive control, and challenged with 50% w/v positive control in corn oil.

II. RESULTS AND DISCUSSION

A. INDUCTION REACTIONS AND DURATION

Although the study report indicates that the test sites were evaluated following intradermal and topical induction, the results were not presented in the report.

B. CHALLENGE REACTIONS AND DURATION

Twenty-four hours after challenge with undiluted and 50% test material, none of the test or naive control animals developed a positive dermal response. Forty-eight hours after challenge with the undiluted test material, 14/20 test guinea pigs had developed slight to moderate erythema while no erythema was present on the naive control animals. By 72 hours after challenge with the undiluted test material, slight to moderate erythema was present on 16/20 test animals and 5/10 naive control animals. Forty-eight hours after

challenge with 30% test material, 8/20 test animals had developed slight to moderate erythema at the test site. By 72 hours after challenge with 30% test material, slight erythema was present on 1/20 animals. None of the control animals developed erythema following challenge with 30% test material.

Based on the study results, the test material is a moderate sensitizer to Albino Crl (HA) BR guinea pigs.

C. POSITIVE CONTROL

According to the study report, 15/20 animals developed a positive response following induction and challenge with hexylcinnamaldehyde.

D. DEFICIENCIES

The date of the positive control study was not reported and the dermal responses recorded following induction with the test material were not provided. These would not invalidate the study results.

ACUTE TOX ONE-LINER

1. PC CODE: 098951

2. CURRENT DATE: April 21, 1999

3. TEST MATERIAL: EPA File Symbol 10182-UUE; N-Butyl-1,2-benzisothiazolin-3-one ... 97.8%

Study/Species/Lab/ Study#/Date	MRID No.	Results	Tox. Cat.	Core Grade
acute oral toxicity / rat / Central Toxicology Laboratory / AR6193 / 7-25-96	443649-15	LD_{50} (mg/kg) Males = 4267 (3157-5767) Females= 4732 (2178-12959)	III	A
acute dermal toxicity / rat / Central Toxicology Laboratory / CR3269 / 7-30-96	443649-16	LD_{50} > 2,000 mg/kg	III	A
primary skin irritation / rabbit / Central Toxicology Laboratory / EB4412 / 7-30-96	443649-17	Corrosive. Subepthelial fibrosis, severe edema, scabbing, desquamation, sparse hair growth.	I	A
dermal sensitization / guinea pig / Central Toxicology Laboratory/ GG6736 / 7-30-96	443649-18	Moderate dermal sensitizer	---	A

A = Acceptable
U = Unacceptable
S = Supplementary
V = self-Validated