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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 29, 2005

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 3008-OR / ORD-X378
DP Barcode: D312490

To: Adam Heyward, PM 34 / Stacey Grigsby
Regulatory Management Branch
Antimicrobials Division (7510C)

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

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

3/29/05

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: Osmose, Inc.

FORMULATION FROM LABEL:

Active Ingredient(s):		% by wt.
Copper 8-quinolinolate		34.18
Other Ingredient(s):		65.82
	Total:	100.00%

I BACKGROUND: Osmose, Inc., has submitted a complete set of six acute toxicity and primary irritation studies to support the registration of their new product, "ORD-X378". The studies were conducted by Product Safety Laboratories, Inc. The MRID Numbers are 464225-03 through 464225-08.

These study reports received an initial review from the PSB/AD/OPP contractor, DynCorp/CSC. CTT/PSB/AD conducted a brief secondary review of the reports and primary reviews.

II RECOMMENDATIONS: PSB findings are:

Each of the six studies is acceptable.

The acute toxicity profile for File Symbol 3008-OR is currently:

Study	MRID Number	Toxicity Category	Acceptability
acute oral toxicity	464225-03	III	Acceptable
acute dermal toxicity	464225-04	III	Acceptable
acute inhalation toxicity	464225-05	IV	Acceptable
primary eye irritation	464225-06	III	Acceptable
primary skin irritation	464225-07	IV	Acceptable
dermal sensitization	464225-08	Nonsensitizer	Acceptable

III LABELING:

1. The signal word is "Caution".

2. The Precautionary Statements should state:

   "Harmful if swallowed, or, absorbed through skin. Causes moderate eye irritation. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling, before eating, drinking, chewing gum or using tobacco. Remove and wash contaminated clothing before reuse."

3. The First Aid Statements should state:

If in eyes:

- Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first five minutes, then continue rinsing.
- Call a Poison Control Center or doctor for treatment advice.

If on skin:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a Poison Control Center or doctor for treatment advice.

If swallowed:

- Call a Poison Control Center or doctor immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by a Poison Control Center or doctor.
- Do not give anything by mouth to an unconscious person.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1, 870.1100)
(UP AND DOWN PROCEDURE)

Product Manager: 34 **Reviewer**: Ian Blackwell
MRID No.: 464225-03 **Study Completion Date**: November 17, 2004
Report No.: 16101

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage: Limit Test: 5,000 mg/kg
Main Test: 175, 550, 1,750 and 5,000 mg/kg (administered as received)
Species: Sprague-Dawley derived, albino rats
(9 female - nulliparous and nonpregnant)
Age: Young adult (9 - 10 weeks)
Weight: 160 - 194 grams at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 19 - 25 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle
Acclimation: 9 - 16 days

Conclusion:

1. LD_{50} (mg/kg): Females = 5,000 mg/kg
(95 % C.I. = 3408 to 20000 mg/kg)

2. Tox. Category: III **Classification**: Acceptable

Procedure (Deviations from §81-1): Due to a technician error, Animal No. 7991 (dosed at 5,000 mg/kg) was inadvertently euthanized on Day 13 of the study. Although the protocol requires that the observations be completed for at least 14 days, the laboratory reports that the gross necropsy revealed no gross abnormalities and that this deviation did not impact the overall outcome of this study. The relative humidity of animal housing was not provided.

Results: For the Main Test, the test substance was administered in sequence as presented below. The decision to proceed with the next animal was based on the survival of the previous animal following dosing.

Limit Test - Reported Mortality

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	7434	5,000	D	D

Main Test - Reported Mortality

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	7470	175	S	S
2	7516	550	S	S
3	7541	1,750	S	S
4	7675	5,000	D	D
5	7918	1,750	S	S
6	7948	5,000	S	S
7	7991*	5,000	S	S
8	8022	5,000	S	S

S - Survival D - Death
* Animal was inadvertently euthanized on Day 13

Observations:

175 mg/kg Dose Level (1 animal), 550 mg/kg Dose Level (1 animal): Both animals survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior.

1,750 mg/kg Dose Level (2 animals): Both animals survived, gained body weight, and appeared active and healthy. Clinical signs observed following administration included ano-genital staining, diarrhea and reduced fecal volume. However, both animals recovered by Day 3 and appeared active and healthy for the remainder of the 14-day observation period.

5,000 mg/kg Dose Level (5 animals): Two animals died within three days of test substance administration. Toxic signs noted prior to death included ano-genital staining, diarrhea, hypoactivity, and hunched posture. Surviving animals exhibited similar clinical signs as well as reduced fecal volume and abdominal distention. However, the survivors recovered from these symptoms by Day 7 and appeared active and healthy for the remainder of the study, gaining body weight over the entire 14-day observation period.

Gross Necropsy Findings: No gross abnormalities were noted for any of the euthanized animals dosed at 175, 550, 1,750 and 5,000 mg/kg dose levels when necropsied at the conclusion of the 14-day observation period. Gross necropsy of the decedents at the 5000 mg/kg dose level revealed discoloration of the intestines.

Statistical Analysis: The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations and/or LD_{50} and confidence limit calculations.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2, 870.1200)
(LIMIT TEST)

Product Manager: 34
MRID No.: 464225-04

Reviewer: Ian Blackwell
Study Completion Date: November 17, 2004
Report No.: 16102

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid

Species: 10 Sprague-Dawley derived, albino rats
(5 / sex; females were nulliparous and nonpregnant)
Age: Young adult (9-10 weeks)
Weight : Males: 298 - 320 grams at experimental start
Females: 188 - 225 grams at experimental start
Source: Ace Animals, Inc. Boyertown, PA
Housing: Temperature Range: 19 - 22 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle
Acclimation: 13 days

Summary:

1. **LD_{50} (mg/kg):**

Males	> 2,000 mg/kg
Females	> 2,000 mg/kg
Combined	> 2,000 mg/kg

2. **The estimated LD_{50} is** > 2,000 mg/kg

3. **Tox. Category:** III **Classification:** Acceptable

Procedure (Deviations From §81-4): No deviations were recorded by the laboratory. The relative humidity of animal housing was not provided.

Results:

Reported Mortality

DOSAGE (mg/kg)	DEATHS / number tested		
	Males	Females	Total
2000	0/5	0/5	0/10

Observations: All animals survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behavior. Light green staining at dose site was noted for three males and two females on Days one and two, and the same was noted for two males and three females on Days one, two and three.

Gross necropsy findings: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3, 870.1300)
LIMIT TEST

Product Manager: 34
MRID No.: 464225-05

Reviewer: Ian Blackwell
Sudy Completion Date: November 17, 2004
Report No.: 16103

Testing Lab-oratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance Statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid

Species: 10 Sprague-Dawley derived, albino rats
(5 / sex; females - nulliparous and nonpregnant)
Age: Young adult (10 - 11 weeks)
Weight: Males: 345 - 363 grams at experimental start
Females: 219 - 250 grams at experimental start
Housing: Temperature Range: 19 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle
Acclimation: 23 days
Source: Ace Animals, Inc., Boyertown, PA

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.02	34.81

Summary:

1. **LC_{50} (mg/L) 4-hr exposure:** Males > 2.02 mg/L
   Females > 2.02 mg/L
   Combined >2.02 mg/L
2. **The estimated LC_{50} is > 2.02 mg/L** (Note: Test animals were exposed for 4 hours and 14 minutes to allow chamber to reach equilibrium.)
3. **MMAD**: 3.5 µm
4. **Tox. Category**: IV **Classification**: Acceptable

Procedure (Deviation From §81-3): No deviations were reported by the laboratory. The relative humidity of animal housing was not provided.

The design, type and dimensions of the exposure chamber were not provided. The oxygen content of chamber was not provided. The relative humidity of the chamber during exposure was 56 - 95 %RH; the guidelines state that the relative humidity should be maintained between 30 - 70 % humidity. The exposure period was extended to 4 hours and 14 minutes to allow the chamber to reach equilibrium (T_{99}).

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number of deaths / number tested		
	Males	Females	Combined
2.02	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exposure conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	% Particles at Effective Cutoff Diameter (Cumulative)						
				0.7 µm	1.1 µm	2.1 µm	3.3 µm	4.7 µm	5.8 µm	9.0 µm
2.02	1	3.6	2.17	1.9	6.8	18.3	42.0	68.8	80.5	92.7
	2	3.4	2.18	2.5	7.9	20.4	44.7	71.3	81.1	91.4

Chamber Environment During Exposure

Exposure Level	2.02 mg/L
Chamber Volume	150 L
Airflow	50.7 lpm
Temperature	21 to 22 ° C
Relative Humidity	56 to 95 %

Clinical Observations: All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. In-chamber animal observations included dyspnea, ocular and nasal discharge, hunched posture, and hypoactivity. Upon removal from the exposure chamber, all animals exhibited abnormal respiration, hunched posture, hypoactivity, nasal discharge (red), and/or reduced fecal volume. However, all of the animals recovered by Day 5 and appeared active and healthy during the remainder of the 14-day observation period.

Gross Necropsy Findings: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§81-4, 870.2400)

Product Manager: 34
MRID No.: 464225-06

Reviewer: Ian Blackwell
Study Completion Date: November 17, 2004
Report No.: 16104

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications except that specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage: 0.1 mL

Species: 3 New Zealand albino rabbits
(2 male and 1 female - nulliparous and nonpregnant)
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NY
Housing: Temperature Range: 20 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Summary:

1. **Toxicity Category**: III
2. **Classification**: Acceptable

Procedure (Deviations From §81-4): No deviations were reported by the laboratory. Young adult rabbits were used for the experiment instead of adult rabbits as recommended in the guidelines. The relative humidity of animal housing was not provided.

Results:

All animals appeared active and healthy. There was no corneal opacity observed in any treated eye during this study. One hour following test substance instillation, all three treated eyes exhibited conjunctivitis, and iritis was present in two animals. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 72 hours. There were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

Incidence of Irritation

Time Post Instillation	Corneal Opacity	Iritis	Conjuctivitis
1 hour	0 / 3	2 / 3	3 / 3
24 hours	0 / 3	0 / 3	3 / 3
48 hours	0 / 3	0 / 3	3 / 3
72 hours	0 / 3	0 / 3	0 / 3

Individual Scores for Ocular Irritation

Observations	Rabbit No.: 12790 (Male)				Rabbit No.: 12791 (Female)				Rabbit No.: 12792 (Male)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Corneal Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
II. Iris	0	0	0	0	1	0	0	0	1	0	0	0
III. Conjunctivae:												
A. Redness	2	1	1	0	2	1	1	0	2	1	1	0
B. Chemosis	1	0	0	0	1	0	0	0	1	0	0	0
C. Discharge	3	1	0	0	3	1	0	0	3	1	0	0

[1] 2 % ophthalmic fluorescein sodium used to verify the absence of corneal opacity.

DATA REVIEW FOR DERMAL IRRITATION TESTING (§81-5, 870.2500)

Product Manager: 34
MRID No.: 464225-07

Reviewer: Ian Blackwell
Study Completion Date: November 17, 2004
Report No.: 16105

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Qualtiy Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included, stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage: 0.5 mL

Species: 3 New Zealand albino rabbits
Sex: Female - nulliparous and non-pregnant
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NY
Housing: Temperature: 19 - 23 °C
Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Summary:

1. **Toxicity Category**: IV
2. **Classification**: Acceptable

Procedure (Deviations From §81-4): No deviations were reported by the laboratory. Relative humidity of animal housing was not reported.

Results: All animals appeared active and healthy. One hour after patch removal, all three treated sites exhibited very slight erythema and very slight edema. The overall incidence and severity of irritation decreased thereafter. All animals were free of dermal irritation by 72 hours. There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
1 hour	3 / 3	3 / 3
24 hours	3 / 3	0 / 3
48 hours	2 / 3	0 / 3
72 hours	0 / 3	0 / 3

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (§81-6, 870.2600)
(BUEHLER METHOD)

Product Manager: 34 **Reviewer**: Ian Blackwell
MRID No.: 464225-08 **Study Completion Date**: November 17, 2004
Report No.: 16106

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, with the following exceptions:

- Specific information related to the stability, charcaterization identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.
- The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during the historical positive control study were not determined.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)

Species: 35 Hartley albino guinea pigs
Sex: Male and Female; Females were nulliparous and nonpregnant
Age: Young adult
Weight: Males: 342 to 424 grams at experimental start
Source: Elm Hill Breeding Labs, Chelmsford, MA
Housing: Temperature Range: 18 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Method: Buehler method

Summary:

1. **Based on the results of this study, the test substance is not considered to be a contact sensitizer.** (Study evaluated erythema only.)

2. **Classification**: Acceptable

Procedure (Deviation From §81-6): No deviations were reported by the laboratory. Relative humidity information of animal housing was not provided. Study evaluated only erythema. No observations were reported for edema.

Procedure:
Preliminary Irritation: Five animals were used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping (Oster model #A5-small) the dorsal area and flanks of

each guinea pig. This area was divided into four test sites on each animal and the test substance was applied as neat (100%) and also as diluted with distilled water to yield w/w concentrations of 75%, 50%, 25%, 12% and 6%. Each concentration was applied as 0.4 mL to a test site using an occlusive Hill Top Chamber. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed. Approximately 24 hours after application, each site was evaluated for local reactions (erythema). From these results, the HNIC selected for the challenge phase was a 12% w/w mixture in diluted water.

Induction Phase: Once each week for three weeks, 0.4 mL of the undiluted test substance was applied to the left side of each test animal using an occluive 25-mm Hill Top Chamber. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual substance. Approximately 24 hours and 48 hours after each induction application, readings were made of local reactions (erythema).

Challenge Phase: 27 days after the first induction dose, 0.4 mL of a 12% w/w mixture of the test substance in distilled water (HNIC) was applied to a naive site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 hours and 48 hours after the challenge application.

Results:
Based on the results of this study (erythema only), the laboratory states that the test substance is not considered to be a contact sensitizer.

	Sensitization Response Indices (Erythema)			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0 / 20	0 / 20	0.25	0.05
Naive Control Animals	0 / 10	0 / 10	0.30	0.05

[1] Animals with scores greater than 0.5
[2] Sum of erythema scores divided by the number of animals evaluated.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1, 870.1100)
(UP AND DOWN PROCEDURE)

Product Manager: Adam Heyward
MRID No.: 464225-03

Reviewer: Karen Hicks
Study Completion Date: November 17, 2004
Report No.: 16101

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material:	ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage:	Limit Test: 5,000 mg/kg
	Main Test: 175, 550, 1,750 and 5,000 mg/kg (administered as received)
Species:	Sprague-Dawley derived, albino rats
	(9 female - nulliparous and nonpregnant)
Age:	Young adult (9 - 10 weeks)
Weight:	160 - 194 grams at experimental start
Source:	Ace Animals, Inc., Boyertown, PA
Housing:	Temperature Range: 19 - 25 °C
	Relative Humidity: information not provided
	Photoperiod: 12-hour light/dark cycle
Acclimation:	9 - 16 days

Conclusion:

1. **LD_{50} (mg/kg)**: Females = 5,000 mg/kg
(95 % C.I. = 3408 to 20000 mg/kg)

2. **Tox. Category**: III **Classification**:

Procedure (Deviations from §81-1): Due to a technician error, Animal No. 7991 (dosed at 5,000 mg/kg) was inadvertently euthanized on Day 13 of the study. Although the protocol requires that the observations be completed for at least 14 days, the laboratory reports that the gross necropsy revealed no gross abnormalities and that this deviation did not impact the overall outcome of this study. The relative humidity of animal housing was not provided.

5,000 mg/kg Dose Level (5 animals): Two animals died within three days of test substance administration. Toxic signs noted prior to death included ano-genital staining, diarrhea, hypoactivity, and hunched posture. Surviving animals exhibited similar clinical signs as well as reduced fecal volume and abdominal distention. However, the survivors recovered from these symptoms by Day 7 and appeared active and healthy for the remainder of the study, gaining body weight over the entire 14-day observation period.

Gross Necropsy Findings: No gross abnormalities were noted for any of the euthanized animals dosed at 175, 550, 1,750 and 5,000 mg/kg dose levels when necropsied at the conclusion of the 14-day observation period. Gross necropsy of the decedents at the 5000 mg/kg dose level revealed discoloration of the intestines.

Statistical Analysis: The *Acute Oral Toxicity (Guideline 425) Statistical Program* (Weststat, version 1.0, May 2001) was used for all data analyses including: dose progression selections, stopping criteria determinations and/or LD_{50} and confidence limit calculations.

Results: For the Main Test, the test substance was administered in sequence as presented below. The decision to proceed with the next animal was based on the survival of the previous animal following dosing.

Limit Test - Reported Mortality

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	7434	5,000	D	D

Main Test - Reported Mortality

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	7470	175	S	S
2	7516	550	S	S
3	7541	1,750	S	S
4	7675	5,000	D	D
5	7918	1,750	S	S
6	7948	5,000	S	S
7	7991*	5,000	S	S
8	8022	5,000	S	S

S - Survival D - Death
* Animal was inadvertently euthanized on Day 13

Observations:

175 mg/kg Dose Level (1 animal), 550 mg/kg Dose Level (1 animal): Both animals survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior.

1,750 mg/kg Dose Level (2 animals): Both animals survived, gained body weight, and appeared active and healthy. Clinical signs observed following administration included ano-genital staining, diarrhea and reduced fecal volume. However, both animals recovered by Day 3 and appeared active and healthy for the remainder of the 14-day observation period.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2, 870.1200)
(LIMIT TEST)

Product Manager: Adam Heyward
MRID No.: 464225-04
Reviewer: Karen Hicks
Study Completion Date: November 17, 2004
Report No.: 16102

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid

Species:	10 Sprague-Dawley derived, albino rats (5 / sex; females were nulliparous and nonpregnant)
Age:	Young adult (9-10 weeks)
Weight :	Males: 298 - 320 grams at experimental start Females: 188 - 225 grams at experimental start
Source:	Ace Animals, Inc. Boyertown, PA
Housing:	Temperature Range: 19 - 22 °C Relative Humidity: information not provided Photoperiod: 12-hour light/dark cycle
Acclimation:	13 days

Summary:

1. **LD_{50} (mg/kg):** Males > 2,000 mg/kg
   Females > 2,000 mg/kg
   Combined > 2,000 mg/kg

2. **The estimated LD_{50} is** > 2,000 mg/kg

3. **Tox. Category:** III **Classification:**

Procedure (Deviations From §81-4): No deviations were recorded by the laboratory. The relative humidity of animal housing was not provided.

Results:

Reported Mortality

DOSAGE (mg/kg)	DEATHS / number tested		
	Males	Females	Total
2000	0/5	0/5	0/10

Observations: All animals survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behavior. Light green staining at dose site was noted for three males and two females on Days one and two, and the same was noted for two males and three females on Days one, two and three.

Gross necropsy findings: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3, 870.1300)
LIMIT TEST

Product Manager: Adam Heyward
MRID No.: 464225-05

Reviewer: Karen Hicks
Sudy Completion Date: November 17, 2004
Report No.: 16103

Testing Lab-oratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance Statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications except that the specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid

Species: 10 Sprague-Dawley derived, albino rats
(5 / sex; females - nulliparous and nonpregnant)
Age: Young adult (10 - 11 weeks)
Weight: Males: 345 - 363 grams at experimental start
Females: 219 - 250 grams at experimental start
Housing: Temperature Range: 19 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle
Acclimation: 23 days
Source: Ace Animals, Inc., Boyertown, PA

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.02	34.81

Summary:

1. **LC_{50} (mg/L) 4-hr exposure:** Males > 2.02 mg/L
   Females > 2.02 mg/L
   Combined >2.02 mg/L
2. **The estimated LC_{50} is > 2.02 mg/L** (Note: Test animals were exposed for 4 hours and 14 minutes to allow chamber to reach equilibrium.)
3. **MMAD**: 3.5 µm
4. **Tox. Category**: IV **Classification**:

Procedure (Deviation From §81-3): No deviations were reported by the laboratory. The relative humidity of animal housing was not provided.

The design, type and dimensions of the exposure chamber were not provided. The oxygen content of chamber was not provided. The relative humidity of the chamber during exposure was 56 - 95 %RH; the guidelines state that the relative humidity should be maintained between 30 - 70 % humidity. The exposure period was extended to 4 hours and 14 minutes to allow the chamber to reach equilibrium (T_{99}).

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number of deaths / number tested		
	Males	Females	Combined
2.02	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exposure concentration (mg/L)	Sample	MMAD (µm)	GSD (µm)	% Particles at Effective Cutoff Diameter (Cumulative)						
				0.7 µm	1.1 µm	2.1 µm	3.3 µm	4.7 µm	5.8 µm	9.0 µm
2.02	1	3.6	2.17	1.9	6.8	18.3	42.0	68.8	80.5	92.7
	2	3.4	2.18	2.5	7.9	20.4	44.7	71.3	81.1	91.4

Chamber Environment During Exposure

Exposure Level	2.02 mg/L
Chamber Volume	150 L
Airflow	50.7 lpm
Temperature	21 to 22 ° C
Relative Humidity	56 to 95 %

Clinical Observations: All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. In-chamber animal observations included dyspnea, ocular and nasal discharge, hunched posture, and hypoactivity. Upon removal from the exposure chamber, all animals exhibited abnormal respiration, hunched posture, hypoactivity, nasal discharge (red), and/or reduced fecal volume. However, all of the animals recovered by Day 5 and appeared active and healthy during the remainder of the 14-day observation period.

Gross Necropsy Findings: No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§81-4, 870.2400)

Product Manager: Adam Heyward
MRID No.: 464225-06

Reviewer: Karen Hicks
Study Completion Date: November 17, 2004
Report No.: 16104

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications except that specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage: 0.1 mL

Species: 3 New Zealand albino rabbits
(2 male and 1 female - nulliparous and nonpregnant)
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NY
Housing: Temperature Range: 20 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Summary:

1. **Toxicity Category**: III

2. **Classification**:

Procedure (Deviations From §81-4): No deviations were reported by the laboratory. Young adult rabbits were used for the experiment instead of adult rabbits as recommended in the guidelines. The relative humidity of animal housing was not provided.

Results:

All animals appeared active and healthy. There was no corneal opacity observed in any treated eye during this study. One hour following test substance instillation, all three treated eyes exhibited conjunctivitis, and iritis was present in two animals. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 72 hours. There were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

Incidence of Irritation

Time Post Instillation	Corneal Opacity	Iritis	Conjuctivitis
1 hour	0 / 3	2 / 3	3 / 3
24 hours	0 / 3	0 / 3	3 / 3
48 hours	0 / 3	0 / 3	3 / 3
72 hours	0 / 3	0 / 3	0 / 3

Individual Scores for Ocular Irritation

Observations	Rabbit No.: 12790 (Male)				Rabbit No.: 12791 (Female)				Rabbit No.: 12792 (Male)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Corneal Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
II. Iris	0	0	0	0	1	0	0	0	1	0	0	0
III. Conjunctivae:												
A. Redness	2	1	1	0	2	1	1	0	2	1	1	0
B. Chemosis	1	0	0	0	1	0	0	0	1	0	0	0
C. Discharge	3	1	0	0	3	1	0	0	3	1	0	0

[1] 2 % ophthalmic fluorescein sodium used to verify the absence of corneal opacity.

DATA REVIEW FOR DERMAL IRRITATION TESTING (§81-5, 870.2500)

Product Manager: Adam Heyward
MRID No.: 464225-07

Reviewer: Karen Hicks
Study Completion Date: November 17, 2004
Report No.: 16105

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Qualtiy Assurance statement was provided. A statement of Good Laboratory Practice (GLP) compliance also was included, stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, except that specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Dosage: 0.5 mL

Species: 3 New Zealand albino rabbits
Sex: Female - nulliparous and non-pregnant
Age: Young adult
Source: Robinson Services, Inc., Clemmons, NY
Housing: Temperature: 19 - 23 °C
Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Summary:

1. **Toxicity Category**:

2. **Classification**:

Procedure (Deviations From §81-4): No deviations were reported by the laboratory. Relative humidity of animal housing was not reported.

Results: All animals appeared active and healthy. One hour after patch removal, all three treated sites exhibited very slight erythema and very slight edema. The overall incidence and severity of irritation decreased thereafter. All animals were free of dermal irritation by 72 hours. There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
1 hour	3 / 3	3 / 3
24 hours	3 / 3	0 / 3
48 hours	2 / 3	0 / 3
72 hours	0 / 3	0 / 3

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (§81-6, 870.2600)
(BUEHLER METHOD)

Product Manager: Adam Heyward
MRID No.: 464225-08

Reviewer: Karen Hicks
Study Completion Date: November 17, 2004
Report No.: 16106

Testing Laboratory: Product Safety Laboratories
Author: Daniel J. Merkel, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance also was included stating that the study meets the requirements of 40 CFR Part 160 and OECD specifications, with the following exceptions:

- Specific information related to the stability, charcaterization identity and verification of the test substance concentration as received and tested is the responsibility of the study sponsor.
- The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during the historical positive control study were not determined.

Test Material: ORD-X378 / Lot #FN1-19 / greenish-brown, opaque liquid
Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)

Species: 35 Hartley albino guinea pigs
Sex: Male and Female; Females were nulliparous and nonpregnant
Age: Young adult
Weight: Males: 342 to 424 grams at experimental start
Source: Elm Hill Breeding Labs, Chelmsford, MA
Housing: Temperature Range: 18 - 23 °C
Relative Humidity: information not provided
Photoperiod: 12-hour light/dark cycle

Method: Buehler method

Summary:

1. **Based on the results of this study, the test substance is not considered to be a contact sensitizer.** (Study evaluated erythema only.)

2. **Classification**:

Procedure (Deviation From §81-6): No deviations were reported by the laboratory. Relative humidity information of animal housing was not provided. Study evaluated only erythema. No observations were reported for edema.

Procedure:
Preliminary Irritation: Five animals were used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping (Oster model #A5-small) the dorsal area and flanks of each guinea pig. This area was divided into four test sites on each animal and the test substance was applied as neat (100%) and also as diluted with distilled water to yield w/w concentrations of 75%, 50%, 25%, 12% and 6%. Each concentration was applied as 0.4 mL to a test site using an occlusive Hill Top Chamber. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed. Approximately 24 hours after application, each site was evaluated for local reactions (erythema). From these results, the HNIC selected for the challenge phase was a 12% w/w mixture in diluted water.

Induction Phase: Once each week for three weeks, 0.4 mL of the undiluted test substance was applied to the left side of each test animal using an occluive 25-mm Hill Top Chamber. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual substance. Approximately 24 hours and 48 hours after each induction application, readings were made of local reactions (erythema).

Challenge Phase: 27 days after the first induction dose, 0.4 mL of a 12% w/w mixture of the test substance in distilled water (HNIC) was applied to a naive site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 hours and 48 hours after the challenge application.

Results:
Based on the results of this study (erythema only), the laboratory states that the test substance is not considered to be a contact sensitizer.

	Sensitization Response Indices (Erythema)			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0 / 20	0 / 20	0.25	0.05
Naive Control Animals	0 / 10	0 / 10	0.30	0.05

[1] Animals with scores greater than 0.5

[2] Sum of erythema scores divided by the number of animals evaluated.