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

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

Wednesday, February 25, 2009

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No: 3008-RNR
Product Name: ORD-X170
DP Barcode: D360535

FROM: Earl Goad, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

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

THRU: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

TO: Adam Heyward PM#34/Lisa McKelvin
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: OSMOSE, INC.
980 Ellicott Street
Buffalo, NY 14209

PRODUCT FORMULATION FROM LABEL:

PC Codes	Active Ingredient(s):	% by wt.
022901	Copper Carbonate (Metallic copper equivalent: 33.31%)	57.60
128997	Tebuconazole	1.32
	Other Ingredients:	41.08
	Total:	100.00

I) BACKGROUND:

The registrant has submitted studies (MRID#: 476290-3 thru -8) to satisfy the Acute Toxicity data requirements for registration of EPA file symbol: 3008-RNR (ORD-X170).

This product is a manufacturing use product (MP) antimicrobial wood preservative. It is purposed to control fungal decay of wood products as well as wood rot and wood eating insects. The label use instructs dilution of this product with water to the appropriate concentration for application by pressure treatment or other methods.

A primary review of these original studies was conducted by the Product Science Branch (PSB)/Antimicrobials Division (AD) contractor: Computer Sciences Corporation (CSC). The Chemistry and Toxicology Team (CTT) conducted a brief secondary review to assure that the studies meet EPA/OPP criteria, and is responsible for this memorandum.

II) FINDINGS: PSB findings are:

A. The Acute Oral Toxicity study (Up/Down Procedure) was started with a limit dose of 2,000 where the test animal died. The results of the procedure could only be graded as > 2000 mg/dl or a category III. This is acceptable.

B. The Acute Dermal Toxicity study 2,000 mg/dl is acceptable resulting in a category III.

C. The Acute Inhalation study was acceptable, LC50 > 2.05 mg/L. category IV.

D. Eye Irritation study is acceptable at with a category III, moderately irritating.

E. Dermal Irritation study is acceptable with category IV.

F. ORD-X170 is not a dermal sensitizer.

III) The acute toxicity profile for EPA File Symbol 3008-RNR (ORD-X170) is currently:

Study	MRID Number	Toxicity Category	Status
Acute Oral Toxicity	476290-03	III	Acceptable
Acute Dermal Toxicity	476290-04	III	Acceptable
Acute Inhalation Toxicity	476290-05	IV	Acceptable
Primary Eye Irritation	476290-06	III	Acceptable
Primary Skin Irritation	476290-07	IV	Acceptable
Dermal Sensitization	476290-08	Non-sensitizer	Acceptable

IV) LABELING: **Keep Out of Reach of Children**

A. The signal word for EPA Reg. 3008-RNR (ORD-X170) is **CAUTION** based on the category III for Acute Oral, Dermal Toxicity, and Eye Irritation.

B. Precautionary labeling:

Hazards to Humans and Domestic Animals:

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

C. First Aid Statements:

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 5 minutes, then continue rinsing.
- 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 by a poison control center or doctor.
- Do not give anything to an unconscious person.

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.

Have the product container or label with you when calling a poison control center or doctor or going for treatment.

For emergency information on [product, use, etc.], call the **National Pesticides Information Center** at 1-800-858-7378, 6:30 AM to 4:30 PM Pacific time (PT), seven days a week. During other times, call the poison control center 1-800-222-1222.

D. Corrections to Label:

1) Remove “This Product contains Sodium Nitrite”. It is not found in the formulation (CSF). Communication with Osmose Regulatory Manager (Teri Muchow) confirmed that this ingredient has been replaced.
2) Add precautionary and first aid labeling as above for Eye Irritation.
3) Change “Inert Ingredients” to read “Other Ingredients”.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)
(UP AND DOWN PROCEDURE)

Product Manager: 34
MRID No.: 476290-03

Reviewer: CSC and Earl Goad(CTT)
Study Completion Date: December 11, 2008
Study No.: 26243

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Dosage: Limit Test: 2,000 mg/kg (administered as received)
Main Test: 175, 550, and 2,000 mg/kg (administered as received)

Species: 6 Rats; Sprague-Dawley derived, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (9-11 weeks old)
Weight: 172-240 grams at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 16-21°C
Relative Humidity: 38-70%
Photoperiod: 12-hour light/dark cycle
Acclimation: 8-21 days

Conclusion:

1. Acute Oral LD_{50} (mg/kg): Female Rats: >2,000 mg/kg

2. Toxicity Category: III Classification: Acceptable

Procedure (Deviations from 870.1100): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendments:
    - Due to the viscosity of the test material the exposure chamber was changed from a 6.7 L to a 28 L nose only chamber.
    - Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines state that the temperature in the experimental animal room should be 22±3°C. The lower limit of the animal room temperature range (i.e., 16°C) was below this recommended range.
- The guidelines recommend an initial dose of 5,000 mg/kg for the limit test. The laboratory started with an initial dose of 2,000 mg/kg.

- The guidelines state that the animals are to be observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours, and daily thereafter. The laboratory stated that the animals were observed during the first several hours post-dosing and at least once thereafter for 14 days after dosing. Data reported (in Table 2 of the report) identify observations made at 1 hour, 3 hours, 4 hours, and 5 hours after dosing on the day of treatment.

Results:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	2,000	D	D

S – Survival; D – Death

Main Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3102	175	S	S
2	3103	550	S	S
3	3104	2,000	S	S
4	3105	2,000	S	S
5	3106	2,000	S	S

S – Survival; D – Death

Observations:
175 mg/kg Dose Level (1 animal): This animal survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

550 mg/kg Dose Level (1 animal): This animal survived, gained body weight, and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

2,000 mg/kg Dose Level (4 animals): One animal died within five days of test substance administration. Toxic signs noted in the decedent prior to death included hypoactivity, hunched posture, and ano-genital staining. Following administration, the surviving animals exhibited clinical signs including ano-genital staining, diarrhea, and/or reduced fecal volume. All survivors recovered from these symptoms by Day 4 and appeared active and healthy for the remainder of the study, gaining body weight over the 14-day observation period.

Gross Necropsy Findings:
175 mg/kg Dose Level (1 animal): No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

550 mg/kg Dose Level (1 animal): No gross abnormalities were noted for the animal when necropsied at the conclusion of the 14-day observation period.

2,000 mg/kg Dose Level (4 animals): Gross necropsy of the decedent revealed discoloration of the lungs and dark intestines distended with fluid and air. No gross abnormalities were noted for any of the euthanized animals necropsied at the conclusion of the 14-day observation period.

Statistical Analysis:
The Acute Oral Toxicity (Guideline 425) Statistical Program (Westat, 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
(OPPTS 870.1200)

Product Manager: 34
MRID No.: 476290-04

Reviewer: CSC and Earl Goad(CTT)
Completion Date: December 11, 2008
Study No.: 26244

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Dosage: 2,000 mg/kg (applied as received)

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-9 weeks old)
Weight: Males: 221-238 grams; Females: 176-194 grams; at experimental start
Source: Ace Animals, Inc., Boyertown, PA
Housing: Temperature Range: 19-23°C
Humidity Range: 30-66%
Photoperiod: 12-hour light/dark cycle
Acclimation: 7 days

Summary:

1. Acute Dermal LD_{50} (mg/kg): Male and Female Rats: >2,000 mg/kg

2. The estimated acute dermal LD_{50} is greater than 2,000 mg/kg in male and female rats.

3. Toxicity Category: III Classification: Acceptable

Procedure (Deviations from 870.1200): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendment: Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female animals at the same time.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
2,000	0 / 5	0 / 5	0 / 10

Observations:
All animals survived exposure to the test substance, appeared normal and healthy, and gained body weight during the study. Apart from green staining noted for the dose site of all males between Days 1 and 5 (or Days 1 and 3), there were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior.

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 (OPPTS 870.1300)
(NOSE-ONLY EXPOSURE)

Product Manager: 34
MRID No.: 476290-05

Reviewer: CSC and Earl Goad(CTT)
Completion Date: December 8, 2008
Study No.: 26245

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (10-11 weeks old)
Source: Ace Animals, Inc., Boyertown, PA
Weight: Males: 325-397 grams; Females: 222-280 grams; at experimental start
Housing: Temperature Range: 20-23°C
Humidity Range: 30-63%
Photoperiod: 12-hour light/dark cycle
Acclimation: 24 days

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.05	23.16

Summary:

1. LC_{50} (mg/L) 4-hr exposure: >2.05 mg/L in male and female rats

2. The estimated 4-hr acute inhalation LC_{50} of ORD-X170 is greater than 2.05 mg/L in male and female rats.

3. Average MMAD: 3.55 µm at the 2.05 mg/L exposure level

4. Toxicity Category: IV Classification: Acceptable

Procedure (Deviations from 870.1300): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendments: (1) Due to the viscosity of the test substance, a 28 liter nose-only inhalation chamber was utilized instead of a 6.7 liter nose-only inhalation chamber; and (2) Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is exposed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups at the same time.
- The guidelines state that the animals should be acclimated to the chamber and heat stressed minimized. The laboratory does not indicate whether animals were acclimated to exposure conditions and heat stress minimized.
- The guidelines state that three to four measurements should be taken during exposure if chamber concentrations and MMAD values taken during the trial run measurements are not within 10 percent of each other. Chamber concentrations ranged from 1.61 and 3.48 mg/L during the pre-test trials. MMAD values were reported for two of the five trial runs. The laboratory conducted only two sample measurements during the test, instead of the three to four measurements recommended in the guidelines.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds 1 day. Individual body weights of test animals were recorded; however, body weight changes were not reported.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Combined
2.05	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	Cumulative % of Particles < Effective Cutoff Diameter (µm)[1]								
				0.0	0.4	0.7	1.1	2.1	3.3	4.7	5.8	9.0
2.05	1	3.7	1.97	0.0	0.2	1.0	4.8	16.0	36.3	63.3	75.8	90.6
	2	3.4	1.92	0.0	0.4	1.5	5.4	17.0	38.6	68.0	78.8	91.0

[1]Percent of particles smaller than corresponding effective cutoff diameter

Chamber Environment During Exposure

Exposure Level (mg/L)	2.05
Chamber Volume (L)	28
Average Total Airflow Volume (Lpm)[1]	33.7
Air Changes Per Hour	72
Mean Oxygen Content (%)	not reported
Temperature Range (°C)	20-22
Relative Humidity Range (%)	42-46

[1]Total air = compressed filtered air + compressed mixing air

Clinical Observations:
All animals survived exposure to the test atmosphere. Immediately following exposure and over the 14-day observation period, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. Although two female animals lost body weight through Day 7, all animals gained weight over the entire 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 ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 34
MRID No.: 476290-06

Reviewer: CSC and Earl Goad(CTT)
Completion Date: December 11, 2008
Study No.: 26246

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Dosage: 0.1 mL (instilled as received)

Species: 3 Rabbits; New Zealand, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 16-21°C
Humidity Range: 38-45%
Photoperiod: 12-hour light/dark cycle
Acclimation: 5 days

Summary:

1. Toxicity Category: III (mildly irritating)

2. Classification: Acceptable

Procedure (Deviations from 870.2400): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendment: Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines recommend that testing be performed using healthy adult albino rabbits. Testing was performed using young adult albino rabbits (specific age not provided).

Results:
All animals appeared active and healthy. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

There was no corneal opacity or iritis observed in any treated eye during this study. One hour after test substance instillation, all three treated eyes exhibited conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by Day 4 (study termination). The Maximum Mean Total Score of ORD-X170 is 6.0. Under the conditions of this study, ORD-X170 is classified as mildly irritating to the eye.

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			Severity – Mean Score
	Corneal Opacity	Iritis	Conjunctivae	
1 hour	0 / 3	0 / 3	2 / 3	6.0
24 hours	0 / 3	0 / 3	0 / 3	2.7
48 hours	0 / 3	0 / 3	0 / 3	0.7
72 hours	0 / 3	0 / 3	0 / 3	0.7
Day 4	0 / 3	0 / 3	0 / 3	0

Individual Scores for Ocular Irritation

Observations	Rabbit No. 3401 (Female) Time After Treatment				
	1 hour	24 hours	48 hours	72 hours	Day 4
I. Corneal Opacity	0	0[1]	0	0	0
II. Iris	0	0	0	0	0
III. Conjunctivae					
A. Redness	1[2]	0	1	1	0
B. Chemosis	0	0	0	0	0
C. Discharge	1	0	0	0	0
Observations	Rabbit No. 3402 (Female) Time After Treatment				
	1 hour	24 hours	48 hours	72 hours	Day 4
I. Corneal Opacity	0	0[1]	0	0	0
II. Iris	0	0	0	0	0
III. Conjunctivae					
A. Redness	2[2]	1	0	0	0
B. Chemosis	0	0	0	0	0
C. Discharge	1	1	0	0	0
Observations	Rabbit No. 3403 (Female) Time After Treatment				
	1 hour	24 hours	48 hours	72 hours	Day 4
I. Corneal Opacity	0	0[1]	0	0	0
II. Iris	0	0	0	0	0
III. Conjunctivae					
A. Redness	3[2]	1	0	0	0
B. Chemosis	0	0	0	0	0
C. Discharge	1	1	0	0	0

[1]2% ophthalmic fluorescein sodium used to verify the absence of corneal opacity
[2]Green staining around eye

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 34
MRID No.: 476290-07

Reviewer: CSC and Earl Goad(CTT)
Completion Date: December 11, 2008
Study No.: 26247

Testing Laboratory: Eurofins | Product Safety Laboratories, East Brunswick, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA).

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Dosage: 0.5 mL (applied as received)

Species: 3 Rabbits; New Zealand, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (specific age not provided)
Weight: Information not provided (and not required)
Source: Robinson Services, Inc., Clemmons, NC
Housing: Temperature Range: 19-20°C
Humidity Range: 53-70%
Photoperiod: 12-hour light/dark cycle
Acclimation: 6 days

Summary:

1. Toxicity Category: IV (non-irritating)

2. Classification: Acceptable

Procedure (Deviations from 870.2500): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendment: Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines recommend that testing be performed using healthy adult animals. Testing was performed using young adult animals (specific age not provided).

Results:
All animals appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

There was no dermal irritation observed at any treated site during this study.

The Primary Dermal Irritation Index for ORD-X170 was calculated to be 0.0. [Scores for observations made during the first 30-60 minutes, 24, 48, and 72 hours were used in this calculation.] Under the conditions of this study, ORD-X170 is classified as non-irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
30-60 minutes	0 / 3	0 / 3
24 hours	0 / 3	0 / 3
48 hours	0 / 3	0 / 3
72 hours	0 / 3	0 / 3

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema			
		Time After Patch Removal			
		30-60 minutes	24 hours	48 hours	72 hours
3501	F	0 / 0	0 / 0	0 / 0	0 / 0
3502	F	0 / 0	0 / 0	0 / 0	0 / 0
3503	F	0 / 0	0 / 0	0 / 0	0 / 0
Total		0 / 0	0 / 0	0 / 0	0 / 0
Mean		0.0 / 0.0	0.0 / 0.0	0.0 / 0.0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	30-60 minutes	24 hours	48 hours	72 hours
Erythema	0.0	0.0	0.0	0.0
Edema	0.0	0.0	0.0	0.0
TOTAL (PDI)[2]	0.0	0.0	0.0	0.0

[1]Average values for three rabbits
[2]PDI = Average Erythema + Average Edema

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 34
MRID No.: 476290-08

Reviewer: CSC and Earl Goad(CTT)
Completion Date: December 11, 2008
Study No.: 26248

Testing Laboratory: Eurofins | Product Safety Laboratories, Dayton, NJ
Author: Jennifer Durando, B.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), with the following exception: "The stability, uniformity of mixture, and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Eurofins | Product Safety Laboratories historical positive control study were not determined."

Test Material: ORD-X170
Lot # FN1-55 / Light green, opaque aqueous dispersion

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)
Historical data – Completed on July 10, 2008

Species:	44 Guinea pigs; Hartley, albino	
Sex:	Range-Finding:	1 Female, 3 Males
	Test Group:	20 Females
	Naïve Control Group – Challenge:	10 Females
	Naïve Control Group – Rechallenge:	10 Females
	Females were nulliparous and non-pregnant.	
Age:	Young adult (specific age not reported)	
Weight:	Test and Naïve Control Groups: 334-408 grams at experimental start	
Source:	Elm Hill Breeding Labs, Chelmsford, MA	
Housing:	Temperature Range:	19-22°C
	Humidity Range:	41-70%
	Photoperiod:	12-hour light/dark cycle
Acclimation:	5-12 days	

Method: Buehler Method

Summary:

1. Based on these findings and on the evaluation system used, ORD-X170 is not considered to be a contact sensitizer.

2. Classification: Acceptable

Procedure (Deviations from 870.2600): The deviations recorded here are minor deficiencies in reporting or slight departures from study guidelines, and may not necessarily affect study outcome, with exceptions noted in the above memo.

- No procedure deviations were reported.
- The laboratory reported the following protocol amendment: Due to his unavailability, the Study Director was replaced so that this report could be finalized in a timely manner.
- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.

Procedure:
Preliminary Irritation Testing: A group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was applied neat (100%) and also diluted with distilled water to yield w/w concentrations of 75%, 50%, and 25%. Each concentration was applied (0.4 mL) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. The HNIC selected for the challenge phase was 100%.

Preparation and Selection of Animals: On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy, naïve animals (not previously tested) without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty-seven days after the first induction dose, four tenths of a milliliter of the test substance (100%, HNIC) was applied to a naïve 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 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical

environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the “naïve control” group.

Rechallenge Phase: Due to ambiguous results for the test animals following challenge, it was necessary to conduct a rechallenge using the same concentration of the test substance (100%, HNIC). Seven days after the primary challenge, a rechallenge was conducted. The undiluted test substance was applied to a naïve site on the right side of each animal as a rechallenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the rechallenge application according to the scoring system. An additional group of ten guinea pigs was placed on test at rechallenge to serve as a naïve control group and was treated with the test substance.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, EPSL Study #25331, was performed by Eurofins | Product Safety Laboratories. Testing was completed on July 10, 2008. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described above.

Results:
Induction Phase:
Test Animals (undiluted test substance): Very faint erythema (0.5) was noted for some test sites during the induction phase.

Historical Positive Control Animals (HCA applied undiluted): Very faint to faint erythema (0.5-1) was noted for all positive control sites during the induction phase.

Challenge Phase:
Test Animals (undiluted test substance): Six of twenty test sites exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge application. These indications did not persist through 48 hours. Very faint erythema (0.5) was observed at fourteen of twenty test sites 24 hours after challenge. Similar irritation persisted at six test sites through 48 hours.

Naïve Control Animals (undiluted test substance): Very faint erythema (0.5) was noted for three of ten naïve control sites 24 hours following the challenge application. Similar irritation persisted at one site through 48 hours.

Historical Positive Control Animals (HCA applied undiluted): Four of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar indications persisted at two sites through 48 hours.

Historical Naïve Control Animals (HCA applied undiluted): Very faint erythema (0.5) was noted for one naïve control site 24 hours after challenge. Irritation cleared from the affected site by 48 hours.

Rechallenge Phase:
Test Animals (undiluted test substance): Nine of twenty test sites exhibited very faint erythema (0.5) 24 hours after rechallenge. Similar irritation persisted at four sites through 48 hours.

Naïve Control Animals (undiluted test substance): Very faint erythema (0.5) was noted for four of ten naïve control sites 24 hours following the challenge application. Similar irritation persisted at one site through 48 hours.

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals – Challenge	6 / 20	0 / 20	0.65	0.15
Naïve Control Animals – Challenge	0 / 10	0 / 10	0.15	0.05
Test Animals – Rechallenge	0 / 20	0 / 20	0.23	0.10
Naïve Control Animals – Rechallenge	0 / 10	0 / 10	0.20	0.05

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

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge		Rechallenge	
	1		2		3					
Concentration	100%		100%		100%		100%		100%	
Hours[1]	24[2]	48[2]	24[2]	48[2]	24[2]	48[2]	24	48	24	48
Animal No. / Sex										
Test Group										
3601 / F	0	0	0	0	0	0	0.5	0	0.5	0.5
3602 / F	0	0	0	0	0	0	1	0.5	0.5	0.5
3603 / F	0	0	0	0	0	0	0.5	0	0	0
3604 / F	0	0	0	0	0	0	1	0.5	0	0
3605 / F	0	0	0	0	0	0	1	0.5	0	0
3606 / F	0	0	0	0	0	0	0.5	0	0.5	0
3607 / F	0	0	0	0	0	0	0.5	0	0	0
3608 / F	0.5	0	0	0	0	0	0.5	0	0.5	0
3609 / F	0.5	0.5	0	0	0	0	0.5	0	0	0
3610 / F	0.5	0	0	0	0	0	0.5	0	0.5	0
3611 / F	0.5	0.5	0.5	0	0.5	0	0.5	0	0	0
3612 / F	0	0	0	0	0	0	0.5	0	0	0
3613 / F	0	0	0	0	0	0	1	0.5	0.5	0
3614 / F	0.5	0	0.5	0	0	0	0.5	0	0	0
3615 / F	0	0	0	0	0.5	0	1	0.5	0.5	0.5
3616 / F	0.5	0.5	0.5	0	0	0	0.5	0	0.5	0.5
3617 / F	0	0	0	0	0	0	0.5	0.5	0	0
3618 / F	0.5	0	0	0	0	0	0.5	0	0.5	0
3619 / F	0	0	0	0	0	0	1	0	0	0
3620 / F	0	0	0	0	0	0	0.5	0	0	0

[1]Hours after induction, challenge, or rechallenge dose
[2]Light green staining at all dose sites

Challenge / Rechallenge Control Group Skin Reaction Scores

Treatment	Induction						Challenge		Rechallenge	
Phase	1		2		3					
Concentration	100%		100%		100%		100%		100%	
Hours[1]	24	48	24	48	24	48	24	48	24	48
Animal No. / Sex										
Naïve Group – Challenge										
3621 / F	--	--	--	--	--	--	0	0	--	--
3622 / F	--	--	--	--	--	--	0	0	--	--
3623 / F	--	--	--	--	--	--	0	0	--	--
3624 / F	--	--	--	--	--	--	0	0	--	--
3625 / F	--	--	--	--	--	--	0.5	0	--	--
3626 / F	--	--	--	--	--	--	0	0	--	--
3627 / F	--	--	--	--	--	--	0.5	0	--	--
3628 / F	--	--	--	--	--	--	0	0	--	--
3629 / F	--	--	--	--	--	--	0.5	0.5	--	--
3630 / F	--	--	--	--	--	--	0	0	--	--
Naïve Group – Rechallenge										
3631 / F	--	--	--	--	--	--	--	--	0.5	0
3632 / F	--	--	--	--	--	--	--	--	0.5	0
3633 / F	--	--	--	--	--	--	--	--	0	0
3634 / F	--	--	--	--	--	--	--	--	0.5	0.5
3635 / F	--	--	--	--	--	--	--	--	0.5	0
3636 / F	--	--	--	--	--	--	--	--	0	0
3637 / F	--	--	--	--	--	--	--	--	0	0
3638 / F	--	--	--	--	--	--	--	--	0	0
3639 / F	--	--	--	--	--	--	--	--	0	0
3640 / F	--	--	--	--	--	--	--	--	0	0

[1]Hours after challenge or rechallenge dose