

US EPA ARCHIVE DOCUMENT

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



Office of Pesticide Programs

Friday, September 26, 2008

MEMORANDUM

SUBJECT: Acute Toxicity Review for EPA Reg. No.: 9150 -11
Product Name: Cryocide 20
DP Barcode: D355412

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

Earl Goad 9/26/2008

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

Karen Hicks 52 KPH

September 26, 2008

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

TO: Emily Mitchell PM#32/Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: International Dioxide, Inc.
554 Ten Rod Road
North Kingstown, RI 02852

PRODUCT FORMULATION FROM LABEL:

| <u>PC Codes</u> | <u>Active Ingredient(s):</u> | <u>% by wt.</u> |
|-----------------|---|-----------------|
| 020503 | Chlorine dioxide | 0.72 |
| 069149 | 1-Decanaminium, N-decyl-N,N-dimethyl-, chloride | 0.40 |
| | <u>Other Ingredient(s):</u> | <u>98.88</u> |
| | Total: | 100.00 |

I) BACKGROUND:

The registrant has submitted studies to amend the registration of their product EPA Reg. no.: 9150-11 (Cryocide 20). This product was initially registered as a "me too" registration, apparently with no original studies.

This submission included Acute Oral, Acute Dermal, Acute Inhalation and Primary Skin Irritation studies. A Primary Eye Irritation study was submitted and reviewed May 2007 as a 6(a) (2) action. A Dermal Sensitization study was submitted May of this year as a 6(a) (2) but did not qualify for immediate review and will be reviewed at this time.

This product is a disinfectant as well as an oxidizing agent for use on hard non-porous surfaces to be used in hospitals/medical /dental facilities, nursing homes, industrial and institutional facilities, laboratories, food processing plants and etc. Cryocide 20 is typically diluted with water and additives depending on target use and applied as a liquid wash or coarse spray.

For the purpose of laboratory analysis of this test material, the product has been assigned the code name "H-28516".

A primary review of these original studies was conducted by the Product Science Branch (PSB)/Antimicrobials Division (AD) contractor: Computer Sciences Corporation (CSC), with the exception of the Dermal Sensitization study (which was reviewed entirely by PSB Chemistry and Toxicology Team (CTT)). 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 pH of the product is reported as being pH 9.3 (measured on a 1% solution). However the pH of the concentrate as sold/tested in studies is reported as being pH 10.28, which is definitely alkaline.
- B. The Acute Oral, Dermal and Inhalation Toxicity studies are all acceptable with category IV.
- C. The Primary Skin Irritation study is acceptable, showing severe irritation graded as category II.
- D. The Dermal Sensitization study which was performed using a mouse Local Lymph Node Assay (LLNA) procedure, is acceptable. This product is shown to be a dermal sensitizer.
- E. The previously reviewed Primary Eye Irritation study is cited as category II.

III) The acute toxicity profile for EPA Reg. no.: 9150-11 (Cryocide) is currently:

| Study | MRID Number | Toxicity Category | Status |
|---------------------------|-------------|-------------------|------------|
| Acute Oral Toxicity | 474844-01 | IV | Acceptable |
| Acute Dermal Toxicity | 474844-02 | IV | Acceptable |
| Acute Inhalation Toxicity | 474844-03 | IV | Acceptable |
| Primary Eye Irritation | 471042-01 | II | Acceptable |
| Primary Skin Irritation | 474844-04 | II | Acceptable |
| Dermal Sensitization | 474222-01 | Sensitizer | Acceptable |

IV) LABELING: As required by the current EPA Label Review Manual, August 2007. Other labeling items and terminology, are optional at the discretion of the registrant with the approval of the product regulatory manager.

Keep Out of Reach of Children

- A. The signal word for EPA Reg. 9150-11(Cryocide 20) is **WARNING** based on the category II for Eye and Skin Irritation.
- B. Precautionary labeling:

Hazards to Humans and Domestic Animals:

Causes substantial but temporary eye damage and skin irritation. Do not get in eyes, on skin or on clothing. Wear protective eyewear (goggles or face shield), long-sleeved shirt and long pants, socks and shoes, resistant footwear, and rubber gloves. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. 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.

Note to Physician: Probable mucosal damage may contraindicate the use of gastric lavage.

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.

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

Product Manager:32
MRID No.: 474844-01

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 13, 2008
Project ID: DuPont-26130

Testing Laboratory: E.I. DuPont de Nemours and Company, Newark, DE
Author: Carol Carpenter, B.A.

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 was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) GLP standards.

Test Material: Cryocide 20
Batch #: 100307123328 / Clear liquid

Dosage: 175 mg/kg, 550 mg/kg, 1,750 mg/kg, and 5,000 mg/kg (administered neat)

Species: 6 Rats; Crt:CD (SD)
Sex: Females.
Age: Young adult (~10 weeks old)
Weight: 194.5-215.6 grams at experimental start
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: Temperature Range: 18-26°C
Humidity Range: 30-70%
Photoperiod: 12-hour light/dark cycle
Acclimation: 3 or 6 days

Conclusion:

1. **Acute Oral LD₅₀ (mg/kg):** Female Rats: >5,000 mg/kg
2. **Toxicity Category:** IV **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 guidelines specify that females used in the study should be nulliparous and non-pregnant. The laboratory did not report on female reproductive status.
- The guidelines state that the temperature of the experimental animal room should be 22±3°C. The lower limit of the animal room temperature range (i.e., 18°C) was below this recommended temperature range. The upper limit of the animal temperature range (i.e., 26°C) was above this recommended temperature range.
- The guidelines state that animals should be kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test animals were quarantined for 3 or 6 days.

Results:

Main Test

| Dosing Sequence | Animal No. | Dose Level (mg/kg) | Short-Term Outcome | Long-Term Outcome |
|------------------------|-------------------|---------------------------|---------------------------|--------------------------|
| 1 | 889 | 175 | S | S |
| 2 | 903 | 550 | S | S |
| 3 | 911 | 1,750 | S | S |
| 4 | 916 | 5,000 | S | S |
| 5 | 926 | 5,000 | S | S |
| 6 | 930 | 5,000 | S | S |

S - Survival

Observations:

No deaths occurred. Diarrhea, stained skin/fur, wet fur, and/or paleness were observed in two rats dosed at 5,000 mg/kg. No other clinical signs of toxicity were observed. No body weight losses occurred after dosing.

Gross Necropsy Findings:

No gross lesions were present in the rats at necropsy.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 32
MRID No.: 474844-02

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 12, 2008
Project ID: DuPont-25848

Testing Laboratory: E.I. DuPont de Nemours and Company, Newark, DE
Author: Carol Carpenter, B.A.

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 was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) GLP standards.

Test Material: Cryocide 20
Batch #: 100307123328 / Clear liquid

Dosage: 5,000 mg/kg (applied neat)

Species: 10 Rats; Crl:CD(SD)

Sex: 5 Males and 5 Females. [The laboratory did not specify whether females were nulliparous and non-pregnant.]

Age: Young adult (~10 weeks old)

Weight: Males: 334.7-370.5 grams; Females: 230.0-245.3 grams; at experimental start

Source: Charles River Laboratories, Inc., Raleigh, NC

Housing: Temperature Range: 18-26°C

Humidity Range: 30-70%

Photoperiod: 12-hour light/dark cycle

Acclimation: At least 6 days

Summary:

1. **Acute Dermal LD₅₀ (mg/kg):** Male and Female Rats: >5,000 mg/kg
2. **The estimated acute dermal LD₅₀ is** greater than 5,000 mg/kg in male and female rats.
3. **Toxicity Category:** IV **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 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.
- The guidelines specify that females used in the study should be nulliparous and non-pregnant. The laboratory did not specify whether females used in the test were nulliparous and non-pregnant.

- The guidelines state that the temperature of the experimental animal room should be 22±3°C for rodents. The lower limit of the animal room temperature range (i.e., 18°C) was below this recommended temperature range. The upper limit of the animal temperature range (i.e., 26°C) was above this recommended temperature range.
- The guidelines state that body weight changes should be calculated and recorded when survival exceeds one day. Individual body weights of test animals were recorded; however, body weight changes were not reported.

Results:

| Dose Level (mg/kg) | Reported Mortality | | |
|-----------------------|-----------------------------|---------|--------|
| | Number Dead / Number Tested | | |
| | Males | Females | Total |
| 5,000 | 0 / 5 | 0 / 5 | 0 / 10 |

Observations:

No deaths occurred. The rats exhibited no clinical signs of systemic toxicity. Body weight loss of approximately 5% of initial weight occurred in one female rat by Day 7. This rat gained weight between Days 7 and 14. No other body weight losses occurred. Epidermal scaling and hyperkeratosis were observed on the test site of 7 rats during the study. No erythema or edema was observed.

Gross Necropsy Findings:

No gross lesions were present in the rats at necropsy.

**DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(NOSE-ONLY EXPOSURE)**

Product Manager: 32
MRID No.: 474844-03

Reviewer: CSC and Earl Goad (CTT)
Completion Date: July 14, 2008
Project ID: DuPont-25446

Testing Laboratory: Lab Research Ltd., Veszprem, Szabadsagpuszta, Hungary
Author: Krisztina Nagy, M.Sc.

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 was performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001 (III.30) EUM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17).

Test Material: Cryocide 20
Batch #: 100307123328 / Clear liquid

Species: 10 Rats; Wistar CrI:(WI) BR
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (~8-12 weeks old)
Source: Charles River (Europe) Laboratories, Inc., Budapest, Hungary
Weight: Males: 279-302 grams; Females: 220-241 grams; at the time of exposure
Housing: Temperature Range: 22±3°C
Humidity Range: 30-70%
Photoperiod: 12-hour light/dark cycle
Acclimation: 23 days

Concentration:

| Group | Gravimetric Exposure Concentration (mg/L) | Nominal Concentration (mg/L) |
|-------|---|------------------------------|
| I | 5.06 | 9.71 |

Summary:

- LC₅₀ (mg/L) 4-hr exposure:** >5.06 mg/L in male and female rats
- The estimated 4-hr acute inhalation LC₅₀ of Cryocide 20 is greater than 5.06 mg/L in male and female rats.**
- Average MMAD:** 1.87 µm at a 5.06 mg/L exposure level
- 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.

- The following deviation was reported by the laboratory: "Due to equipment failure (probably caused by the high water content of the test material), relative humidity could not be monitored ... because the instrument's sensor was saturated and was assumed to be >95%."
- The following deviation was reported by the laboratory: "Prior to animal exposure the nonvolatile content of the test material was determined to be 3.72%. The normal threshold in our laboratory is for gravimetric analysis to be used when non-volatiles are at least 10%. However, at the request of the Sponsor, the atmosphere quantification in this study was performed by gravimetric analysis. At the dose levels used, the measurements of atmosphere concentrations by gravimetric analysis were considered to be adequate and the study was considered to be valid."
- The guidelines state that the animals should be acclimated and heat stressed minimized. The laboratory does not indicate whether animals were acclimated to exposure conditions and heat stress minimized.

Results:

Reported Mortality

| Exposure Concentration (mg/L) | Number Dead / Number Tested | | |
|-------------------------------|-----------------------------|---------|----------|
| | Males | Females | Combined |
| 5.06 | 1 / 5 | 1 / 5 | 2 / 10 |

Chamber Atmosphere

| Exp. Conc. (mg/L) | Sample | MMAD (µm) | GSD (µm) | Cumulative % of Particles < Effective Cutoff Diameter (µm) ¹ | | | | | | | |
|-------------------|--------|-----------|----------|---|------|------|-------|-------|-------|-------|-------|
| | | | | 0.33 | 0.50 | 0.77 | 1.21 | 1.93 | 3.13 | 5.09 | >5.09 |
| 5.06 | 1 | 1.87 | 1.87 | 0.54 | 1.89 | 7.55 | 18.87 | 40.70 | 73.85 | 97.84 | 100 |

¹Percent of particles smaller than corresponding effective cutoff diameter.

Chamber Environment During Exposure

| | |
|------------------------------------|------------------|
| Exposure Level (mg/L) | 5.06 |
| Chamber Volume (L) | 3.85 |
| Average Total Airflow Volume (Lpm) | 20.2 |
| Air Changes Per Hour | 314.8 |
| Mean Oxygen Content (%) | 20.0-21.0 |
| Temperature Range (°C) | 19.9-20.9 |
| Relative Humidity Range (%) | --- ¹ |

¹Recorded values considered to be spurious due to saturated probe.

Clinical Observations:

Wet fur and fur staining on various occasions were commonly recorded both during and for several hours after exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, are considered not to be biologically significant.

Significant clinical signs commonly noted on the day of exposure included labored noisy respiration and increased respiratory rate. One day after exposure, one male animal was found dead. One female animal was found dead on Day 2. Lack of grooming was commonly observed among all surviving animals. Noisy respiration, labored respiration, and sneezing were common among surviving animals. During the last week of the observation period, these signs continued but less often and milder in intensity. On Day 14, one of the four surviving males and three of the four surviving females still demonstrated slight sneezing.

All surviving males and one female showed slight body weight loss during Week 1 of the study. Normal development was noted for all animals from Day 11 of the observation period.

Gross Necropsy Findings:

No treatment-related macroscopic abnormalities were detected in animals necropsied at study termination. Occasional instances of pin-prick sized hemorrhages of the lungs were noted; however, this finding is considered to be related to the termination procedure. Two incidences of pyelectasis of the kidneys in male animals were noted; this is a common background finding. Occasional mild hydrometra was noted in females; this finding occurs sporadically in laboratory-maintained rats.

In animals found dead during the study, the following findings were recorded at necropsy: reddish mottled lungs, gas-filled stomachs and intestines, and nutmeg-like patterned livers.

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 32
MRID No.: 474844-04

Reviewer: CSC and Earl Goad (CTT)
Completion Date: June 12, 2008
Project ID: DuPont-25929

Testing Laboratory: E.I. DuPont de Nemours and Company, Newark, DE
Author: Carol Carpenter, B.A.

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 was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) GLP standards.

Test Material: Cryocide 20
Batch #: 100307123328 / Clear liquid

Dosage: 0.5 mL (applied as received)

Species: 3 Rabbits; New Zealand, white

Sex: 3 Males.

Age: Young adult (Ages not reported)

Weight: 2,845-3,275 grams

Source: Covance Research Products, Denver, PA

Housing: Temperature Range: t6-22°C

Humidity Range: 30-70%

Photoperiod: t2-hour light/dark cycle

Acclimation: At least 7 days

Summary:

1. **Toxicity Category:** II
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 guidelines recommend that testing be performed using healthy adult animals. Testing was performed using young adult animals (specific age not provided).

Results:

No clinical signs of toxicity were observed in any of the rabbits during the study. Erythema, edema, thickening, hyperkeratosis, epidermal scaling, and sloughing were observed on the test site of all 3 rabbits. Hyperkeratosis is considered to be a severe dermal effect. Dermal irritation was still evident on the test site of the 3 rabbits on Day t4 (normal study termination). The rabbits were further evaluated to assess reversibility of the dermal effects. All dermal irritation cleared in 2 rabbits by Day 17 and in the remaining rabbit by Day 24.

Incidence of Irritation

| Time after Patch Removal | Erythema | Edema |
|--------------------------|----------|-------|
| 1 hour | 3 / 3 | 0 / 3 |
| 24 hours | 3 / 3 | 0 / 3 |
| 48 hours | 3 / 3 | 0 / 3 |
| 72 hours | 3 / 3 | 3 / 3 |
| Day 7 | 3 / 3 | 2 / 3 |
| Day 10 | 3 / 3 | 0 / 3 |
| Day 14 | 3 / 3 | 0 / 3 |
| Day 17 | 1 / 3 | 1 / 3 |
| Day 24 | 0 / 1 | 0 / 1 |

Individual Skin Irritation Scores

| Animal No. | Sex | Erythema / Edema | | | | | | | | |
|--------------|-----|--------------------------|-----|-----|-----|------------------|------------------|------------------|---------|-----|
| | | Time After Patch Removal | | | | | | | | |
| | | Hours | | | | Day | | | | |
| | | 1 | 24 | 48 | 72 | 7 | 10 | 14 | 17 | 24 |
| 53 | M | 2/0 | 2/0 | 2/0 | 3/2 | 2/2 ¹ | 1/0 ² | 1/0 | 0/0 | --- |
| 46 | M | 2/0 | 2/0 | 2/0 | 3/2 | 3/1 ¹ | 2/0 ² | 2/0 ³ | 0/0 | --- |
| 47 | M | 2/0 | 2/0 | 2/0 | 3/2 | 2/0 ¹ | 2/0 ² | 1/0 | 1/1 | 0/0 |
| Total | | 6/0 | 6/0 | 6/0 | 9/6 | 7/3 | 5/0 | 4/0 | 1/1 | 0/0 |
| Mean | | 2/0 | 2/0 | 2/0 | 3/2 | 2.3/1 | 1.7/0 | 1.3/0 | 0.3/0.3 | 0/0 |

¹Thickening, hyperkeratosis, and/or epidermal scaling

²Epidermal scaling and/or sloughing

³Sloughing

Summary of Skin Irritation Scores¹

| | Time After Patch Removal | | | | | | | | |
|--------------------------------|--------------------------|----|----|----|-----|-----|-----|-----|----|
| | Hours | | | | Day | | | | |
| | 1 | 24 | 48 | 72 | 7 | 10 | 14 | 17 | 24 |
| Erythema | 2 | 2 | 2 | 3 | 2.3 | 1.7 | 1.3 | 0.3 | 0 |
| Edema | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0.3 | 0 |
| TOTAL (PDI)² | 2 | 2 | 2 | 5 | 3.3 | 1.7 | 1.3 | 0.6 | 0 |

¹Average values for three rabbits.

²PDI = Average Erythema + Average Edema

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(LOCAL LYMPH NODE ASSAY)

Product Manager: 32
MRID No.: 474222-01

Reviewer: Earl Goad
Completion Date: May 2, 2008
Report No.: DuPont-25439

Testing Laboratory: E.I. du Pont de Nemours and Company, Newark, Delaware

Author: Denise Hoban, B.A, MLT (ASCP)

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 was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) GLP standards. Control preparations used in this study were not analyzed by the laboratory for concentration, uniformity, or stability.

Test Material: Cryocide 20 (Clear Liquid)
Lot No.: t00307123328
(Analysis of the test substance was performed by Case Consulting Laboratories, Inc. Whippany, New Jersey.
CCL Study number 3280-48)

Positive Control Material: 25% (v/v) Alpha-Hexylcinnamaldehyde (HCA), blended in vehicle (propylene glycol)

Vehicle: Propylene glycol

Species: 30 Mice; CBA/JHsd

| Group: | Dosage (% Test Material) | Number/Group |
|---------------|---|---------------------|
| 1 | Vehicle Control (100% Propylene glycol) | 5 Females |
| 2 | 5% Test Material | 5 Females |
| 3 | 25% Test Material | 5 Females |
| 4 | 50% Test Material | 5 Females |
| 5 | 100% Test Material | 5 Females |
| 6 | Positive Control Group (25% HCA) | 5 Females |

Age: Approximately 11 weeks old at start of study

Weight: 21.7 - 24.1 grams on initial dose day

Source: Harlan Sprague-Dawley, Frederick, Maryland

Housing: Temperature: 18-26°C

Humidity: 30-70%

Photoperiod: 12-hour light/dark cycle

Acclimation: At least 6 days

Method: Local Lymph Node Assay in Mice

Summary:

1. **Based on these findings and on the evaluation system used, Cryocide 20 is considered to be a positive 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 guidelines state that, in all instances, the tester must document that appropriate techniques were used to facilitate test substance adherence to the mouse ear for adequate exposure duration. The laboratory did not document the techniques used to facilitate test substance adherence.
- The procedure for preparation of the lymph node single cell suspension was not reported
- Sample preparation for counting the resulting single cell suspensions was not elaborated.

Procedure:

Test Substance Preparation and Administration: Healthy mice were released from quarantine prior to testing. Five females were selected for each of four treatment groups (Groups I-IV). On Days 1, 2, and 3, each test animal in its group received an open application of 25 μ L of an appropriate dilution (5%, 25%, or 50%) of the test substance, or the undiluted test substance, to the dorsum of both ears. The vehicle control group (5 females) was treated in the same way as the test animals, but with vehicle alone (propylene glycol) instead of test substance. The positive control group (5 females) was treated with 25% alpha-hexylcinnamaldehyde(HCA) blended in test vehicle (propylene glycol).

All test and control animals were given a two-day rest period on Days 4 and 5.

Injection of Tritiated Methyl-Thymidine

On Day 6 of the study, all test and control animals were injected in the tail vein with 250 μ L of 0.01 M phosphate-buffered saline (PBS), containing 20 μ Ci of 3 H Thymidine. Five hours after the injection, the animals were sacrificed, the draining auricular lymph nodes were excised and pairs from each individual animal were processed.

Suspension Preparation and DPM Determination

Single cell suspensions were prepared and incubated overnight at 2-8 $^{\circ}$ C. Suspensions were counted on a beta counter and reported as disintegrations per minute (DPM).

Data Evaluation

Stimulation Indices (SI) for each exposure group were calculated by dividing the mean DPM of individuals in each group by the mean of the vehicle control group. A positive response measured as $SI \geq 3.0$, the validity of the result was evaluated considering the dose response, and as appropriate the statistical significance. Positive control data was not included in the statistical validation of test group response. Various statistical tests were employed to confirm the significance of the results.

Body Weights and Observations

Individual body weights were recorded on Day 1 prior to dosing and Day 6 prior to injection. All test and control animals were observed daily for clinical signs of toxicity and any signs of excessive irritation at the test site.

Results:

There was no effect on body weight gain in test group animals during the study. All animals appeared normal for the duration of the study.

The Stimulation Index (SI) or Test/Vehicle Control Ratio derived for each test group based on the group mean DPM are provided below. The test substance produced a SI of ≥ 3 in one group of test animals and, therefore, is considered a sensitizer (defined as producing a positive response). The estimated Test Substance Concentration with an $SI=3$ is 55% Test Material.

| Animal Group | Test Substance Concentration | n | Mean DPM/Group | S.D. (DPM) | Test/Vehicle Control Ratio |
|--------------------|------------------------------|---|----------------|------------|----------------------------|
| 1.Vehicle Control | NA | 5 | 396.2 | 145.7 | NA |
| 2.Test Group I | 5% | 5 | 567.2 | 401.1 | 1.43 |
| 3.Test Group II | 25% | 5 | 388.2 | 248.9 | 0.98 |
| 4. Test Group II | 50% | 5 | 775.2 | 247.6 | 1.96 |
| 5.Test Group III | 100% | 5 | 4985.6 | 3147.9 | 12.58 |
| 6.Positive Control | NA (25% HCA) | 5 | 5368.8 | 2591.9 | 13.55 |

NA - Not applicable

DPM – disintegrations/minute.

Group 6 data (Positive control) was not included in statistical analysis of test substance groups, rather data is used to confirm animal sensitization potential and validate procedures.

Body Weights and DPM Counts

| Animal Number | Day of Study | | DPM Count |
|--|---------------|---------------|-----------|
| | Day 1 Weights | Day 6 Weights | |
| Vehicle Control (100% Propylene glycol) | | | |
| 106 | 21.9 | 21.2 | 157 |
| 107 | 23.1 | 23.5 | 545 |
| 108 | 22.5 | 21.7 | 467 |
| 109 | 23.7 | 22.5 | 393 |
| 110 | 24.0 | 23.6 | 419 |
| Test Group I – 5% conc. | | | |
| 206 | 23.6 | 24.7 | 1167 |
| 207 | 21.8 | 21.2 | 514 |
| 208 | 24.1 | 23.6 | 137 |
| 209 | 22.9 | 22.1 | 720 |
| 210 | 23.1 | 22.7 | 298 |
| Test Group II – 25% conc. | | | |
| 306 | 22.6 | 22.0 | 500 |
| 307 | 23.1 | 22.7 | 73 |
| 308 | 23.5 | 23.7 | 505 |
| 309 | 21.8 | 22.7 | 188 |
| 310 | 24.0 | 23.4 | 675 |
| Test Group III – 50% conc. | | | |
| 406 | 23.8 | 24.0 | 436 |
| 407 | 23.3 | 22.2 | 645 |
| 408 | 22.6 | 23.1 | 1006 |
| 409 | 23.5 | 22.7 | 769 |
| 410 | 21.7 | 22.2 | 1020 |
| Test Group IV – 100% conc. | | | |
| 506 | 24.0 | 23.2 | 2420 |
| 507 | 23.6 | 22.7 | 4119 |
| 508 | 23.2 | 23.6 | 7641 |
| 509 | 22.5 | 21.3 | 1842 |
| 510 | 22.5 | 23.0 | 8906 |
| Positive Control- 25% HCA | | | |
| 606 | 23.0 | 23.1 | 5090 |
| 607 | 23.7 | 23.7 | 5671 |
| 608 | 22.2 | 22.3 | 4089 |
| 609 | 23.4 | 23.8 | 2513 |
| 610 | 24.1 | 23.1 | 9481 |