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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

October 23, 2007
TXR # 0054508

SUBJECT: **RED-0350-26839: Denatonium benzoate/"Bitrex"**

PC Code: 009106
DP Barcode: D340792, 340795, 340798, 340802, 340815, 340818

FROM: Elissa Reaves, Ph.D. Elissa R 10/23/07
Toxicology Branch
Health Effects Division (7509P)

TO: Andrea Carone, Chemical Review Manager
Special Review and Reregistration Division (7508P)

THROUGH: Jack Housenger, Associate Division Director 10/23/07
Health Effects Division (7509P)

I. CONCLUSIONS

- Five of the acute studies submitted are Acceptable/Guideline
- The acute dermal toxicity study (81-2) is unacceptable due to the test material not properly moistened (MRID 47135803)

II. ACTION REQUESTED

To review six acute studies (MRID 47135802 through 471 35807) for Denatonium benzoate.

III. BACKGROUND

Studies conducted by Safepharm Laboratories, Ltd. Were submitted in support of the denatonium benzoate RED.

IV. RESULTS/DISCUSSION

Therefore the acute toxicity profile for EPA Reg. No. 8682-2 is currently:

Acute Oral	III	Acceptable/Guideline	47135802

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Acute Dermal		Unacceptable	47135803
Acute Inhalation	II	Acceptable/Guideline	47135804
Primary Eye	I	Acceptable/Guideline	47135805
Primary Dermal	III	Acceptable/Guideline	47135806
Skin Sensitization	non-sensitizer	Acceptable/Guideline	47135807

Please refer to the attach DER for additional information on each acute study.

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SCIENTIFIC DATA R
EPA SERIES 36

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

August 21, 2007

MEMORANDUM:

Subject: EPA Reg. No.: 8682-2/Bitrex Technical
DP Barcodes: 340792, 340795, 340798, 340802, 340815, 340818

From: Marianne Lewis, Biologist
Product Reregistration Branch
Special Review and Reregistration Division (7508P)

Marianne Lewis 8/21/07

To: Andrea Carone, CRM
Special Review Branch
Special Review and Reregistration Division (7508P)

Applicant: Johnson Matthey Macfarlan Smith
10 Wheatfield Road
Edinburgh, EH11 2QA
United Kingdom

FORMULATION FROM EPA Reg. No. 8682-2 LABEL:

	% by wt.
Active Ingredient(s):	
Bitrex	99.5%
Inert Ingredient(s):	0.5%
Total	100.0%

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BACKGROUND: In support of the Denatonium Benzoate RED the registrant has submitted acute toxicity studies conducted with their product, EPA Reg. No. 8682-2. The MRID's # are as follows: 471358-02 (81-1), 471358-03 (81-2), 471358-04 (81-3), 471358-05 (81-4), 471358-06 (81-5), 471358-07 (81-6 . The studies were conducted by Safepharm Laboratories, Ltd. The test material used in the studies was the subject product.

RECOMMENDATIONS:

- Five (81-1, 81-3, 81-4, 81-5, 81-6) of the acute toxicity studies submitted are acceptable.
- The acute dermal study (81-2) is unacceptable.

Procedural Deviations:

Acute Dermal Study (81-2): The lab report states: "The appropriate amount of the test material, as received, pre-weighed into a plastic tube, was applied uniformly to an area of shorn skin approximating to 10% of the total body surface area which had previously been moistened with distilled water." This is an unacceptable method of moistening the test material. Dry test materials must be moistened with water before application to ensure good contact and no loss of test material. Dry/solid materials may be moistened in a beaker or other suitable vessel. The laboratory should state the amount of water used to moisten dry test materials. The dry test materials should not be moistened beyond that point which is necessary to assure proper contact with the skin (not runny). This study is unacceptable. A new study should be cited or submitted.

The acute toxicity profile for EPA Reg. No. 8682-2 is currently:

Acute Oral	III	Acceptable
Acute Dermal		Unacceptable
Acute Inhalation	II	Acceptable
Primary Eye	I	Acceptable
Primary Dermal	III	Acceptable
Skin Sensitization	non sensitizer	Acceptable

DATA REVIEW FOR ACUTE ORAL TOXICITY (§81-1, 870.1100)

Product Manager: John Hebert, 07
MRID No.: 471358-02

Reviewer: Marianne Lewis
Study Completion Date: 7/24/95
Report No.: SPL 799/001

Testing Facility: Safepharm Laboratories, Ltd.
Author: D. Allen

Quality Assurance (40 CFR §160.12): Included

Test Material: Bitrex, white granules

Species: Sprague-Dawley derived rat
Age: young adult
Weight: females = 169 - 175
Source: Charles River (UK) Ltd.

Conclusion:

1. **LD_{50} (mg/kg):** males: 841 mg/kg (707 - 1000 mg/kg)
females: 648 mg/kg (506 - 832 mg/kg)
combined: 749 mg/kg (664 - 845 mg/kg)

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

Procedure (Deviations from §81-1): none

Results:
(401)

Dose mg/kg	(number deaths/number tested)		
	Males	Females	Combined
500	0/5	1/5	1/10
707	0/5	3/5	3/10
1000	5/5	5/5	10/10

Observations:

Dose mg/kg	Time of Death	Clinical Observations
500	1/10 at 30 min.	Hunched posture, lethargy, decreased respiratory rate, labored respiration, ataxia, noisy respiration, signs cleared by day 3 in survivors. Survivors all gained weight.
707	2/10 at 30 min. 1/10 at 2 hrs.	Hunched posture, lethargy, decreased respiratory rate, labored respiration, ataxia, ptosis, signs cleared by day 3 in survivors. Survivors all gained weight.
1000	6/10 at 2 mins. 3/10 at 30 mins. 1/10 at 4 hrs.	6/10 that died within 2 minutes of receiving dose – ataxia and clonic convulsions; 3/10 that died within 30 minutes no observations are noted; 1/10 that died at 4 hrs., hunched posture, lethargy, decreased respiratory rate, labored respiration, ataxia,

Gross Necropsy:

Dose mg/kg	Gross Necropsy Observations
500	Survivors: no observable abnormalities noted; decedent: hemorrhagic lungs, dark liver, dark kidneys, slight hemorrhaging of gastric mucosa
707	Survivors: no observable abnormalities noted; decedents: hemorrhagic lungs, dark liver, dark kidneys, hemorrhaging of gastric mucosa, pale gastric mucosa
1000	10/10 hemorrhagic lungs/dark liver/dark kidneys, 1/10 hemorrhaging of gastric mucosa, 9/10 pale gastric mucosa

DATA REVIEW FOR ACUTE DERMAL TOXICITY (§81-2, 870.1100)

Product Manager: John Hebert, 07
MRID No.: 471358-03

Reviewer: Marianne Lewis
Study Completion Date: 7/24/95
Report No.: SPL 799/2

Testing Facility: Safepharm Laboratories, Ltd.
Author: D. Allen

Quality Assurance (40 CFR §160.12): Included

Test Material: Bitrex, white granules
Species: Sprague-Dawley derived albino rat
Weight: males = 222 - 251 g; females = 202 - 232 g
Age: young adult
Source: Harlan U.K. Ltd.

Summary:

Classification: Unacceptable

Procedure (Deviations From §81-2):

- Test material not properly moistened

Results: **Reported Mortality**

Dosage (mg/kg)	(number deaths/number tested)		
	Males	Females	Combined
2000	0/5	0/5	0/10

Observations: Twenty four hours prior to application of the test material the dorsal area and trunks were clipped free of hair. The neat test material was applied uniformly to the intact test site (approx. 10% of total body surface) which had previously been moistened with distilled water. A piece of surgical gauze (7 x 4 cm) was placed over the test site, covered with a piece of Hypertic and secured with a piece of Blenderm. After 24 hours, the wraps and pads were removed and the test sites were gently cleansed.

In 9/10 no signs of skin irritation were noted during the study. One female had necrosis, severe erythema, very slight edema, and formation of eschar lasting throughout the study.

Gross Necropsy Findings: No observable abnormalities were noted.

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

Product Manager: John Hebert, 07
MRID No.: 471358-04

Reviewer: Marianne Lewis
Study Completion Date: 8/23/95
Report No.: SPL 799/006

Testing Facility: Safepharm Laboratories, Ltd.
Author: S. Blagden

Quality Assurance (40 CFR §160.12): Included

Test Material: Bitrex, white granules (ground prior to dose in ultra-centrifugal mill)

Species: Sprague-Dawley derived rat
Weight: males = 200 - 237 g; females = 200 - 229 g
Age: young adult
Source: Charles River (UK) Ltd.

Summary:

1. **LC_{50} (mg/L):** 0.20 mg/L (0.13 – 0.36 mg/L)

2. **MMAD:** 3.0 µm **GSD:** 0.41

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

Procedure (Deviation From §81-3): none

Results: **Reported Mortality**

Exposure Concentration	(number deaths/number tested)		
	Males	Females	combined
0.13 mg/L	0/5	0/5	0/10
0.36 mg/L	5/5	5/5	10/10
0.77 mg/L	5/5	5/5	10/10

Chamber Atmosphere		
Dose Level mg/L	MMAD	GSD
0.13	2.7 µm	0.39
0.36	2.8 µm	0.44
0.77	3.0 µm	0.41

Chamber Environment	Dose Level mg/L	Dose Level mg/L	Dose Level mg/L
	0.13	0.36	0.77
Chamber Volume	30 L	30 L	30 L
Airflow	16 Lpm	16 Lpm	16 Lpm
Temperature (°C)	21 - 22	22 - 23	20 - 21
Relative Humidity %	29 - 34	24 - 34	31 - 45

Clinical Observations:

Dose mg/L	Time of Death	Clinical Observations
0.13	N/A	Labored respiration, increased respiratory rate, wet fur, hunched posture, piloerection, pallor of extremities, lethargy, ptosis, decreased respiratory rate, clinical signs cleared by day 4. All gained weight during study
0.36	5/10 at 3 hrs 5/10 killed *in extremis* 1 hr. after exposure	Labored respiration, increased respiratory rate, wet fur, hunched posture, piloerection, pallor of extremities, lethargy, ptosis, ataxia, decreased respiratory rate, gasping respiration, noisy respiration
0.77	10/10 between 2 and 3 hrs. of exposure	Labored respiration, decreased respiration, wet fur, increased respiratory rate, pallor of extremities

Gross Necropsy Findings:

Dose mg/L	Gross Necropsy Observations
0.13	No observable abnormalities noted
0.36	10/10 abnormally red lungs, 3/10 dark patches on lungs
0.77	10/10 abnormally red lungs, 7/10 dark patches on lungs

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

Product Manager: John Hebert, 07
MRID No.: 471358-04

Reviewer: Marianne Lewis
Study Completion Date: 8/23/95
Report No.: SPL 799/006

Testing Facility: Safepharm Laboratories, Ltd.
Author: S. Blagden

Quality Assurance (40 CFR §160.12): Included
Test Material: Bitrex, white granules
Dosage: 0.1 g (ground to fine powder prior to application)
Species: New Zealand albino rabbit
Sex: 1 male
Weight: 2.84 kg
Age: young adult
Source: David Percival Ltd.

Summary:

Toxicity Category: I

Classification: Acceptable

Procedure (Deviations From §81-4): none

Results:

Observations	(number "positive"/number tested)						
	Hours				Days		
	1	24	48	72	7	14	21
Corneal Opacity	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Iris	1/1	1/1	1/1	1/1	0/1	0/1	0/1
Conjunctivae							
Redness	1/1	1/1	1/1	1/1	1/1	0/1	0/1
Chemosis	1/1	1/1	1/1	1/1	0/1	0/1	0/1
Discharge	1/1	1/1	1/1	1/1	1/1	0/1	0/1

From 1 hour through 24 hrs., easily discernible translucent areas of opacity w/sloughing of cornea, iritis, diffuse crimson red conjunctivae, obvious swelling w/partial eversion of lids, & discharge w/moistening of lids/hairs and considerable area around eye. At 48 hrs., scattered diffuse opacity, iritis, diffuse crimson red conjunctivae w/white area over the nictitating membrane, obvious swelling w/partial eversion of lids, & discharge w/moistening of lids/hairs and considerable area around eye. By 72 hrs., easily discernible translucent areas of opacity, iritis, diffuse crimson red conjunctivae w/white area over the nictitating membrane, obvious swelling w/partial eversion of lids, & discharge w/moistening of lids/hairs and considerable area around eye. On day 7, scattered diffuse opacity w/vascularisation along bottom edge of cornea, white area over the nictitating membrane, & discharge w/moistening of lids/hairs just adjacent to lids. On day 14 through day 21, vascularisation along bottom edge of cornea.

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

Product Manager: John Hebert, 07
MRID No.: 471358-06

Reviewer: Marianne Lewis
Study Completion Date: 7/24/95
Report No.: SPL 799/3

Testing Facility: Safepharm Laboratories, Ltd.
Author: D. Allen

Quality Assurance (40 CFR §160.12): Included
Test Material: Bitrex, white granules

Dosage: 0.5 g (ground prior to application)
Species: New Zealand albino rabbit
Age: young adult
Sex: 3 males, 3 females
Weight: males = 2.63 – 2.71 kg; females = 2.23 – 2.52 kg
Source: David Percival Ltd.

Summary:

1. **Toxicity Category:** III **PII** = 2.8

2. **Classification:** Acceptable

Procedure (Deviations From §81-5): none

Results: Twenty four hours prior to application of the test material the dorsal/flank area was clipped free of hair. The test material was ground prior to application. The test material was moistened with 0.5 mL of distilled water and introduced under a 2.5 x 2.5 cm gauze patch which was secured with surgical adhesive tape. The patches and trunks were then wrapped in an elasticated corset (Tubigrip). After 4 hours the pads and corsets were removed and the test sites were wiped with cotton wool soaked in 74% Industrial Methylated Spirits.

At 1 hr., 5/6 very slight erythema, 2/6 very slight edema, & 2/6 slight edema. At 24 hrs., 1/6 very slight erythema, 5/6 well defined erythema, 1/6 very slight edema, 3/6 slight edema, & 1/6 moderate edema. By 48 hrs., 3/6 very slight erythema, 3/6 well defined erythema, 2/6 very slight edema, & 3/6 slight edema. At 72 hrs., 2/6 very slight erythema, 3/6 well defined erythema, 3/6 very slight edema, & 1/6 slight edema. On day 7, 5/6 exhibited desquamation at test site. Study ended on day 7.

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

Product Manager: John Hebert, 07
MRID No.: 471358-07

Reviewer: Marianne Lewis
Study Completion Date: 7/24/95
Report No.: SPL 799/5

Testing Facility: Safepharm Laboratories, Ltd.
Author: D. Allen

Quality Assurance (40 CFR §160.12): Included

Test Material: Bitrex, white granules

Positive Control Material: 2-Mercaptobenzothiazole (historical 11/94 #39/106)

Species: Dunkin Hartley guinea pig
Weight: females = 336 – 449 g
Age: young adult
Source: David Hall Limited

Method: Buehler

Summary:

1. **This Product is a** non sensitizer

2. **Classification:** Acceptable

Procedure (Deviation From §81-6): none

Procedure: A group of animals were used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The HNIC for the test substance was determined to be 75% w/w in distilled.

The test animals were induced with 0.5 mL of 75% w/w test material in distilled water once a week for three weeks under an absorbent lint (15 mm x 35 mm) held in place by strip of surgical adhesive tape (Blenderm) and covered with an overlapping length of aluminum foil overwrapped with Elastoplast. Twenty four hours after each induction dose the animals were scored for irritation. Two weeks after the last induction dose, 0.5 mL of 75% w/w of test material in distilled water was used to challenge the test animals (the lab also utilized a 0.5 mL of 50% w/w test material in distilled water to ensure max. nonirritant concentration was used). Twenty four and 48 hours after the challenge the animals were evaluated for sensitization.

A group of ten animals were used as naive controls. These animals received only the challenge doses of the test material.

Results: Incidents of fur loss were noted in 5/20 test material induced animals and 3/10 of naïve control group animals.

Twenty four hours after the first induction dose for the test material-induced animals, no irritation was seen. After the second induction dose, 3/20 exhibited very faint erythema. After the third induction dose, 2/20 exhibited very faint erythema at the test site.

Twenty four hours after challenge, none of test material-induced animals exhibited any dermal irritation.

At 24 hours, in the naive control group of animals challenged with the test material, none exhibited any dermal irritation.