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

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JUN 30 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Dantobrom S. EPA ID No. 38906-13 and Dantobrom P
EPA ID No. 38906-15
Caswell No 114A/306/309C; Project No(s) 2338/2339
366D 568E

From: Joycelyn E. Stewart, Ph.D. *JP 5/16/87*
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Hazard Evaluation Division

To: Jeffrey Kempter/Barbara Pringle, PM# 32
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Thru: Albin B. Kocialski, Ph.D.
Supervisory Pharmacologist
Toxicology Branch (TS-769C)
Hazard Evaluation Division

ABK 6/29/87
WWS 6/30/87

Registrant: Glyco Inc.
Norwalk, Connecticut 06856-5100

Action Requested: Review primary eye irritation, primary dermal irritation, and distribution studies in rabbits of 5 ethyl, 5 methylhydantoin (EMH) and 5,5,dimethylhydantoin (DMH) the organic moieties of Dantobrom. Dantobrom consists of: of : bromochlorodimethylhydantoin 60%;dichlorodimethylhydantoin 27.4%; and dichloroethylmethylhydantoin 10.6%. The sponsor is seeking registration of Dantobrom P and Dantobrom S as disinfectants for swimming pools and spas. Dantobrom P and Dantobrom S are identical in composition.

Recommendations:

The primary eye irritation studies on 5,5, dimethylhydantoin and 5 ethyl,5 methylhydantoin are core classified Minimum. The toxicity category for each of these studies is III. The primary dermal irritation studies on 5,5 dimethylhydantoin and 5 ethyl,5 methylhydantoin are also core classified Minimum, with toxicity category IV. These studies are adequate for labelling purposes.

The metabolism studies on 5,5, dimethylhydantoin and 5 ethyl,5 methylhydantoin were Unacceptable to support registration of the compounds because they were deficient in the following respects: the radiochemical purity and specific activity of the test compounds were not reported; individual animal weights were not reported; except for the repeat section of the EMH study in which all females received the same dose regardless of weight, the individual doses administered were not reported; inadequate numbers of animals were used in the studies; data were reported as cpm without correction for background counts, quenching or counting efficiency rather than as ^{14}C equivalents of parent compound. In addition, the TLC plates used to identify the metabolites seemed to have been contaminated.

DATA EVALUATION RECORD

005971

1. Subject: Primary Eye Irritation Evaluation of
5-Ethyl-5-methylhydantoin (EMH) - Rabbit
2. Test Material: 5-Ethyl-5-methylhydantoin, white
crystalline test material - purity 19.7%
N theory, 19.5% N assay. Lot/Batch
No. 1083:31
3. EPA File No.: 38906-13
4. Accession No.: 263899
5. Sponsor: Glyco, Inc.
P.O. Box 3187
Williamsport, PA 11701
6. Testing Facility: Findley Research, Inc.
420 Airport Road
Fall River, MA 02720
7. Report No./Date: FRI Study No. T86M0026G; April 18, 1986
8. Authors: Salinas, Julio A., Study Director
9. Toxicity Category: III
10. Classification: Core Minimum Data.

11. Materials and Methods:

The test material was pulverized using a mortar and pestle prior to eye administration. One hundred mg of pulverized test material (5-ethyl-5-methylhydantoin) was placed on the lower eyelid of three male and three female New Zealand White rabbits by gently pulling the eyelid away from the eyeball. Upper and lower lids were gently held together for approximately 1 second following test substance addition. Left eyes remained untreated and served as controls.

Eyes were examined at 1, 24, 48, and 72 hours and 7 days posttreatment, according to the method of Draize. Body weights were recorded prior to testing and at study termination, 7 days after test exposures.

12. Results:

The tester did not follow the Draize numerical scoring system.

Cornea - Only the degree of intensity of lesions was recorded.

Iris - The correct observations were made.

Conjunctivae - Scoring for conjunctival discharge was omitted.

Attempts to compile ocular lesion scores were not made; thus it was impossible to determine a Primary Ocular Index.

A slight corneal opacity was observed in one rabbit at 24 hours and in two rabbits at 48 hours. By 72 hours, all corneal opacity was absent.

A slight conjunctival redness was observed in five of six rabbits at 1 hour which persisted to the 24-, 48-, and 72-hour observation periods. All conjunctival redness was absent at the 7-day observation.

Slight conjunctival chemosis was also apparent in five of six rabbits at the 1-hour and 24-hour observation periods, in three of six rabbits at 48 hours, and in two of six rabbits at 72 hours, and was absent at the 7-day observation.

Four of six rabbits showed a slight fluorescein intensity when tested at 48 hours, and one of six rabbits showed a similar fluorescein test response at 72 hours. At 7 days, no fluorescein-positive test was noted. A slight fluorescein response area was noted for the rabbits described as showing fluorescein intensity above.

13. Conclusions:

The ocular lesion scoring method employed did not permit an overall PI score by the Draize method; however, sufficient data were generated to evaluate the test material eye irritation potential.

14. Toxicity Category: III

15. Classification: Core Minimum Data.

DATA EVALUATION RECORD

005971

1. Subject: Primary Dermal Irritation Evaluation of
5-Ethyl-5-methylhydantoin (EMH) - Rabbit
2. Test Material: 5-Ethyl-5-methylhydantoin (EMH), 19.7%
N theory, 19.6% N assay - purity - white
crystals. Lot/Batch No. 1083:46
3. EPA File No.: 38906-13
4. Accession No.: 263899
5. Sponsor: Glyco, Inc.
P.O. Box 3187
Williamsport, PA 11701
6. Testing Facility: Findley Research, Inc.
420 Airport Road
Fall River, MA 02720
7. Report No./Date: FRI Study No. T86M0074G; April 17, 1986
8. Authors: Salinas, Julio A., Study Director
9. Toxicity Category: IV.
10. Classification: Core Minimum Data.

11. Materials and Methods:

The crystalline test article was pulverized and 0.5 g aliquots were weighed onto 5 x 5 cm pieces of sterile gauze. The test protocol states that the test article was applied to a 2.5 cm² clipped site on the left dorsal side of each of six New Zealand White rabbits and moistened to ensure good skin contact. A one-half g powdered sample was spread on each skin test site and covered with 5 x 5 cm gauze patches, which were held in place with nonirritating tape.

Gauze patches held in place on untreated clipped right dorsal flanks served as controls. Occlusive wrappings secured treated and control skin sites during the four exposure periods.

At the end of the 4-hour exposure, all patches were removed, residual test chemical was removed with sterile water, and the animals were examined for signs of erythema and edema. Treatment sites were scored according to the Draize system at 30 to 60 minutes, 24, 48, and 72 hours following patch removal.

12. Results:

All animals gained weight during the observation period.

No erythema, edema, or eschar formation occurred in any of the test animals at any of the observation periods, including the 72-hour period.

13. Conclusions: 5-Ethyl-5-methylhydantoin was not a skin irritant.

14. Toxicity Category: IV.

15. Classification: Core Minimum Data.

DATA EVALUATION RECORD

005971

1. Subject: Primary Eye Irritation Study Using 5,5-Dimethylhydantoin - Rabbit
2. Test Material: 5,5-Dimethylhydantoin - purity 21.9% N theoretical, 21.9% N assayed - white crystals. Lot/Batch No. 1083:45
3. EPA File No.: 38906-15
4. Accession No.: 263899
5. Sponsor: Glyco, Inc.
P.O. Box 3187
Williamsport, PA 11701
6. Testing Facility: Findley Research, Inc.
420 Airport Road
Fall River, MA 02720
7. Report No./Date: FRI Study No. T86M0027G; April 18, 1986
8. Authors: Salinas, Julio A., Study Director
9. Toxicity Category: III.
10. Classification: Core Minimum Data.

11. Materials and Methods:

White crystalline test material was pulverized using a mortar and pestle. One-tenth g of pulverized material was placed on the lower, everted right eye lid of six New Zealand White rabbits. Upper and lower eyelids were gently held together for 1 second and released. Untreated left eyes served as controls. Animals weighed from 2.68 to 4.30 kg at experiment initiation.

Rabbit eyes were examined at 1, 24, 48, and 72 hours and 7 days posttreatment for signs of irritation. Eye irritation was scored according to the procedure described by TSCA Test Guidelines, which is an adaptation of the procedure described by Draize.

A fluorescein test was conducted at the 48-, 72-, and 7-day observation period. Animals were weighed at the end of the observation period.

12. Results:

No signs of clinical toxicity were detected (other than eye examinations).

All but one animal gained weight during the observation period.

All left (control) eyes were normal.

No corneal involvement was observed.

No iris damage or irritation occurred.

The only eye irritation effects were conjunctival redness (1 on a scale from 1 to 3), beginning at 1 hour and continuing through 72 hours posttreatment. Also, conjunctival chemosis occurred, beginning at 1 hour and continuing through 72 hours posttreatment. Chemosis scoring was two rabbits with a score of 2 at 1 hour, four rabbits with a score of 1 at 1 hour (1-4 scale), three animals with a score of 1 at 4 and 48 hours, one rabbit scored 2 and 1 animal scored 1 at 72 hours.

Four animals were scored 1.0 (1-4 scale) at the 48-hour fluorescein examination, and three were scored 1.0 at the 72-hour examination.

13. Conclusions:

The ocular lesion scoring method employed did not permit determination of an overall PI score by the Draize method; however, sufficient data were generated to evaluate the test material eye irritation potential.

Thus, 5,5-Dimethylhydantoin was shown to be a mild eye irritant.

14. Toxicity Category: III.15. Classification: Core Minimum Data.

DATA EVALUATION RECORD

005971

1. Subject: Primary Dermal Irritation Evaluation of 5,5-Dimethylhydantoin - Rabbit
2. Test Material: 5,5-Dimethylhydantoin (DMH) - purity 21.9% N theory, 21.9% N assay - white crystals.
Lot/Batch No. 1083:45
3. EPA File No.: 38906-15
4. Accession No.: 263899
5. Sponsor: Glyco, Inc.
P.O. Box 3187
Williamsport, PA 11701
6. Testing Facility: Findley Research, Inc.
420 Airport Road
Fall River, MA 02720
7. Report No./Date: FRI Study No. T86M0075G; April 17, 1986
8. Authors: Salinas, Julio A., Study Director
9. Toxicity Category: IV.
10. Classification: Core Minimum Data.

11. Materials and Methods:

White crystalline test material was pulverized. One-half g aliquots of test material were weighed onto 5 x 5 sq cm pieces of gauze and moistened prior to placing on clipped left dorsal skin sites of each of six New Zealand White rabbits, and were held in place with tape. Similar, nontreated gauze patches were placed on clipped right dorsal skin sites and held with tape to serve as controls.

Treated animals were then examined for erythema and edema and scored according to the method of Draize within 30 to 60 minutes and at 24, 48, and 72 hours following removal of all test site covering materials. Treated sites were washed with sterile water prior to scoring for irritation.

12. Results:

All animals gained weight during the observation period.

No erythema and no edema were noted at any of the observation periods. No evidence of any signs of clinical illness was observed.

13. Conclusions:

5,5-Dimethylhydantoin was not a skin irritant as determined by the present experiment.

14. Toxicity Category: IV.15. Classification: Core Minimum Data.

89867:Woodrow:HED-07:KENCO:2/27/87:3/11/87:de:lf:dej:JH:BJP
R:89877:Woodrow:HED-07:KENCO:3/18/87:5/30/87:NeeCee:dsq:dej

DATA EVALUATION RECORD

5,5-DIMETHYLHYDANTOIN

Metabolism in Rabbits

STUDY IDENTIFICATION: Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5,5'-dimethylhydantoin [$5-^{14}\text{C}$] ($\text{C}_5\text{H}_8\text{N}_2\text{O}_2$) in New Zealand White rabbits. (Unpublished report No. MRI-685-GC-86-50 prepared by EG&G Mason Research Institute, Worcester, MA, for Glyco, Inc., Williamsport, PA; dated July 30, 1986.) Accession No. 265025/265036

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: _____

I. Cecil Felkner
5-9-87

1. CHEMICAL: 5,5'-Dimethylhydantoin (DMH).
2. TEST MATERIAL: 5,5'-[5-¹⁴C]Dimethylhydantoin ([¹⁴C]DMH) was supplied by New England Nuclear Products. The specific activity of the compound was not reported. The radiochemical purity was 97.5 percent.
3. STUDY/ACTION TYPE: Metabolism in rabbits.
4. STUDY IDENTIFICATION: Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5,5'-dimethylhydantoin [5-¹⁴C] (C₅H₈N₂O₂) in New Zealand White rabbits. (Unpublished report No. MRI-685-GC-86-50 prepared by EG&G Mason Research Institute, Worcester, MA, for Glyco, Inc., Williamsport, PA; dated July 30, 1986.) Accession No. 265026.

5. REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: *Nicolas P. Hajjar*
Date: 5/9/87

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6. APPROVED BY:

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Date: 5/11/87

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EPA Section Head

Signature: *A. Kocalski*
Date: 6/29/87

7. CONCLUSIONS:

- A. Following the administration of 5,5'-[5-¹⁴C]dimethylhydantoin ([¹⁴C]DMH) to male and female rabbits, most of the radioactivity was eliminated in the urine. However, several deficiencies in the study render it unacceptable. These deficiencies are listed in Section B (Recommendations) and Section 14 (Reviews Discussion and Interpretation of study Results).
- B. This study is unacceptable.

8. RECOMMENDATIONS:

All data should be presented as [¹⁴C] equivalents of the parent compound and the mean values with standard deviations listed in tabular form. Data for [¹⁴C] levels in blood are best presented in a figure to show that elimination is biphasic. The specific activity of the test material, individual animal weights, and dosages should be presented. The metabolism study in females is unacceptable and should be repeated. Dosing should be proportional to the individual animal weight, i.e., mg/kg basis. Subdivision F Guidelines recommend 5 animals/sex/dose.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. It was reported that a stock solution of [¹⁴C]DMH, 1.4 mCi/mL, was diluted to 0.1 mCi/mL with water and administered to the rabbits by gavage at a dose of 100 μ Ci/kg. The amounts given to each rabbit were presented as cpm/rabbit.
2. Three male and three female New Zealand White rabbits were obtained from Millbrook Farm, Amherst, MA. The animals weighed 2.182 to 2.618 kg at study initiation. The age and individual weights were not reported. The animals were acclimated to laboratory conditions for 7 days and fasted overnight prior to dosing.
3. Following dosing, each rabbit was placed in a stainless steel metabolism cage until sacrifice at hours 72. Blood, urine, and feces were collected, when present, at 3, 6, 9, 12, 24, 36, 48, and 72 hours after treatment. The animals were weighed, then sacrificed, and the following organs were removed and weighed: testes, ovaries, pancreas, spleen, heart, lung, urinary bladder, kidney, liver, brain, stomach,

¹Only items appropriate to this DER have been included.

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femur and gastrointestinal tract. In addition, samples of muscle tissue, bone marrow, bone, adipose tissue, and skin were collected for radioassay.

4. All samples (0.2 mL of blood, 0.2 mL of urine, and about 200 to 350 mg of feces or tissue) were oxidized in a Packard Oxidizer (Model B306) and radioassayed by a Packard liquid scintillation counter (LSC Model 4430). Results were reported as counts per minute (cpm). Urine and blood samples were spotted on silica G plates and the thin layer chromatograms were developed in chloroform:ethylacetate:ethanol (8:1:1, v:v:v). Radiolabeled compounds were visualized by autoradiography.
5. Due to low recoveries of [^{14}C] in the urine of two to three female rabbits, a second experiment was conducted. Three females weighing 1.796 to 1.812 kg were given 30 μCi of the test material in a single oral dose. Urine and feces were collected at the same intervals mentioned above and radioassayed.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

- A. Seventy-two hours after dosing male rabbits, most of the administered radioactivity was eliminated in the urine (361.13 cpm). These values account for approximately 90.8 percent of the dose, assuming that the efficiency of the LSC is constant and that quenching, counting, and background cpm are the same for the [^{14}C] administered and the [^{14}C] in urine. In feces, only 6.93×10^6 cpm or 1.7 percent of the dose (using the same assumptions) were eliminated in the feces of male rabbits.

A similar elimination pattern was observed in one of the three females. However, total recovery of [^{14}C] for the other two females was only 19 and 38.8 percent. The lower recoveries appeared to be due to "incomplete urine collections." Consequently, a second group of females was dosed with approximately 5×10^7 cpm (30 μCi) and urine was collected and radioassayed. Elimination in the feces and tissue residues were not determined. One of the three rabbits died about 1 hour after dosing as a result of inadvertently administering the test material into the lung. In the remaining females, 85.8 and 93.1 percent of the dose was recovered in the urine.

No radiolabeled metabolites were detected in urine following thin layer chromatography (TLC) and autoradiography. Only unchanged parent compound was found in both the original and repeat experiments.

- B. [^{14}C] levels in blood were highest 3 to 6 hours after dosing and decreased biphasically, with the initial rapid phase having a half-life of about 7 to 8 hours.
- C. [^{14}C] residues in tissues were very low, accounting for less than 0.01 percent of the dose in males and females. [^{14}C] residues in the carcasses were not reported (Table 1).

TABLE 1. [^{14}C] Distribution in Tissues of Rabbits 72 Hours After Oral Administration of [^{14}C]EMH

Tissue	Radioactivity (cpm/g or cpm/ml) ^a	
	Males	Females
Blood	297	269
Liver	374	460
Kidneys	300	279
Gonads	251	616
Heart	202	277
Lungs	310	293
Spleen	344	295
Pancreas	325	300
Brain	216	190
Muscle	284	239
Bone marrow	197	196
Bone	306	293
Adipose tissue	261	136
Gastrointestinal tract	283	268
Skin	242	337
Stomach	322	268
Bladder	1339	470

Total	5,850	5,186

^aValues are the means for three rabbits.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

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- A. The primary route of detoxification occurred via urinary excretion, with an estimated half-life in the "test population" of 7 to 8 hours. Test materials were excreted essentially unchanged and no evidence of cumulative body burdens was present. No organ was consistently found to have a concentrated level of [^{14}C] activity 72 hours after dose administration. No sex differences were observed.
- B. A quality assurance statement was signed and dated July 21, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study has the same major deficiencies noted in another study on 5-methyl-5-ethylhydantoin metabolism (MRI-702-GC-86-51) and is also unacceptable. The specific activity of DMH and the individual animal weights and dosages were not reported. The range of body weights presented in the report is different from that in the protocol. All data were reported as cpm without any apparent corrections for background counts, quenching, counting efficiency, etc.; these factors should have been taken into consideration and the data reported as [^{14}C] equivalents of parent compound.

Although the animals were killed after 72 hours, both urine and fecal samples collected at 48 to 72 hours contained substantial amounts of radioactivity. TLC plates indicated streaking of the plasma and urine samples and the presence of some impurities/metabolites, especially in the blood. Thus, the TLC method may have been inadequate.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 28-33.