UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 006724
WASHINGTON, D.C. 20460

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JUN 2 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Dantobrom S and Dantobrom P (6836-116 and 6836-115)
EPA ID #'s ~~38906~~-13 and ~~38906-15~~
Acc. Nos. 403313-01 and 403313-07

To: Jeffrey Kempter/Ruth Douglas PM # 32
Disinfectants Branch
Registration Division (TS-767C)

Cas. No. 114A
306
568E
366D

From: Joycelyn E. Stewart, Ph.D. 5/24/88
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Thru: Albin B. Kocialski, Ph.D.
Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 5/26/88
6/2/88

Registrant: Lonza Inc.
Fair Lawn, N.J. 07410

Lonza supplied additional data in response to Toxicology Branch's memorandum dated 5/14/87. These data consisted of: information on the composition and purity of the test compounds EMH and DMH used in the delayed hypersensitivity study, the rat teratology study, the rabbit teratology study, the 90 rat gavage study, responses to the request for indivual animal data for the 90 day study, historical control data for the rabbit teratology study, and to the review of the distribution study in rabbits.

This memorandum addresses issues outstanding in Toxicology Branch's memorandum dated 3/16/88, and consists of the comments relating to the distribution study using ^{14}C-EMH and $_{14}$C-DMH in rabbits.

The original distribution studies (Accession #'s 26025 and 265036) and the supplemental submissions 403314-06 and 403314-07 were reviewed by EPA contractors, whose comments are located in the Conclusion section of the review. Based on the deficiencies cited in the review, Toxicology Branch cannot accept these studies as fulfilling the requirements for metabolism data.

The metabolism study will need to be repeated if the Agency determines that additional information is necessary to clarify unusual effects in chronic or reproductive studies as required in the Data Call-In Notice for Subchronic and Chronic Toxicological Data for Antimicrobial Pesticide Active Ingredients.

EPA: 68-02-4225
DYNAMAC No. 339-C
May 9, 1988

DATA EVALUATION RECORD

5,5-DIMETHYLHYDANTOIN

Metabolism in Rabbits

STUDY IDENTIFICATION: Rogler, D. L. Supplemental Submission - 158.135-Distribution study of 5,5'-dimethylhydantoin [5-^{14}C] in New Zealand White rabbits. (Unpublished report prepared by Lonza, Inc., location: unknown; dated September 30, 1987.) Accession Nos. 265025 and 265036. Supplemental submission for the following study:

Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5,5'-dimethylhydantoin [5-^{14}C] ($C_5H_8N_2O_2$) in New Zealand White rabbits. (Unpublished report No. MRI-685-GC-86-50 prepared by EG&G Mason Research Institute, Worchester, MA, for Glyco, Inc., Williamsport, PA; dated July 30, 1986.) Accession Nos. 265025 and 265036.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Sharon Ambrose for
Date: May 10, 1988

1. CHEMICAL: 5,5'-Dimethylhydantoin (DMH).

2. TEST MATERIAL: 5,5'-[5-^{14}C]Dimethylhydantoin ([^{14}C]DMH) was supplied by New England Nuclear Products. The specific activity of the compound was 29.8 mCi/mmol and the radiochemical purity was 97.5 percent.

3. STUDY/ACTION TYPE: Metabolism in rabbits.

4. STUDY IDENTIFICATION: Rogler, D. L. Supplemental Submission - 158.135-Distribution study of 5,5'-dimethylhydantoin [5-^{14}C] in New Zealand White rabbits. (Unpublished report prepared by Lonza, Inc., location: unknown; dated September 30, 1987.) Accession Nos. 265025 and 265036. Supplemental submission for the following study:

Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5,5'-dimethylhydantoin [5-^{14}C] ($C_5H_8N_2O_2$) in New Zealand White rabbits. (Unpublished report No. MRI-685-GC-86-50 prepared by EG&G Mason Research Institute, Worchester, MA, for Glyco, Inc., Williamsport, PA; dated July 30, 1986.) Accession Nos. 265025 and 265036.

5. REVIEWED BY:

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

Signature: Nicolas P. Hajjar
Date: May 10, 1988

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: May 10, 1988

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

Joycelyn Stewart, Ph.D.
EPA Reviewer

Signature: Joycelyn Stewart
Date: 10/24/88

Albin B. Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 5/25/88

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7. CONCLUSIONS:

A. Following the administration of 5,5'-[5-^{14}C]dimethylhydantoin ([^{14}C]DMH) to male and female rabbits, most of the radioactivity was eliminated in the urine. Radioactivity in blood peaked 3 to 6 hours after dosing and thereafter decreased biphasically. The half-life for the initial rapid phase was about 7 to 8 hours. Radioactive residue levels in tissues were low. However, a quantitative evaluation of the data, differences between sexes, and chromatographic analysis for urine could not be made.

B. This study provides supplementary data on the metabolism of DMH, but does not fulfill EPA's requirements for registration.

Items 8 thru 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. It was reported that a stock solution of [^{14}C]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 µCi/kg. The amounts given to each rabbit were presented as counts per minute (cpm)/rabbit. The rationale for dose selection was not reported.

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

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[1]Only items appropriate to this DER have been included.

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). The counting efficiency of the LSC was reported to be 51 percent. Background counts were automatically subtracted and results were reported as 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 of the 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 (361.13×10^6 cpm). This value accounts for approximately 90.8 percent of the dose, assuming that the counting efficiency of the LSC is constant 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. Radiolabeled [^{14}C] residues in tissues were very low, accounting for less than 0.01 percent of the dose in males and females. Radiolabeled [^{14}C] residues in the carcasses were not reported (Table 1).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

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:

Although the supplemental submission provides information on the specific activity of the test material and background counts and efficiency of the LSC used, there are still five major deficiencies in this study which renders it inadequate to support registration of the compound. (1) The combustion of up to 0.35 g of, e.g., adipose tissue and/or GI tract, will invariably lead to incomplete combustion and/or quenching; it is not clear from the submission whether that was taken into account, using one of many available methods. (2) Only three animals/sex were used, whereas EPA guidelines require at least five animals/sex. (3) The data for females are inadequate; the total recovery in the first experiment was very low for two of the three animals, whereas in the second experiment one of the three females died 1 hour after dosing. Furthermore, radioactivity was assayed in the urine only of the two females that survived. These two experiments cannot complement each other because neither one is a complete study. Moreover, the data cannot be used to evaluate differences between sexes, if any. (4) The TLC solvent used was inadequate. The author's statement that "the apparent multiple spots in reference samples on these plates was a tailing as a consequence of the laboratory's error in its selection of solvent system" further supports the fact that the data presented cannot be adequately evaluated. (5) Finally, the data in this report were very poorly presented, and in some instances it was difficult to determine what was actually done. Although this submission clarifies some of the questions raised in the original review, many more questions remain (see item 8 in the original DER.) For example, data for [^{14}C] levels in blood are best presented in a figure in order to determine the half-lives for elimination. In addition, it is not clear what the weight of the rabbits in the repeat experiment was; the tables indicate 300g, whereas the calculation indicates animal weights of about 2 kg.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Dynamac DER No. 252-A.

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

Tissue	Radioactivity (cpm)/g (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	5850	5186

[a]Values are the means for three rabbits.

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EPA: 68-02-4225
DYNAMAC No. 339-B
May 9, 1988

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

5-METHYL-5-ETHYLHYDANTOIN

Metabolism in Rabbits

STUDY IDENTIFICATION: Rogler, D. L. Supplemental Submission--158.135--Distribution study of 5-methyl-5-ethylhydantoin [5-^{14}C] in New Zealand White rabbits. (Unpublished report prepared by Lonza, Inc.; dated September 3, 1987.) Accession Nos. 265026 and 265038. Supplemental submission for the following study:

Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5-methyl-5-ethylhydantoin [5-^{14}C] ($C_6H_{10}N_2O_2$) in New Zealand White rabbits. (Unpublished report No. MRI-702-GC-86-51 prepared by EG&G Mason Research Institute, Worcester, MA, for Glyco, Inc., Williamsport, PA; dated July 30, 1986.) Accession No. 265026.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Shawn Ambrose for

Date: May 10, 1988

1. CHEMICAL: 5-Methyl-5-ethylhydantoin.

2. TEST MATERIAL: [5-^{14}C]5-methyl-5-ethylhydantoin ([^{14}C]EMH) was supplied by New England Nuclear Products. The specific activity of the test material was 25.9 mCi/mmol and the radiopurity was 98 percent.

3. STUDY/ACTION TYPE: Metabolism in rabbits.

4. STUDY IDENTIFICATION: Rogler, D. L. Supplemental Submission--158.135--Distribution study of 5-methyl-5-ethylhydantoin [5-^{14}C] in New Zealand White rabbits. (Unpublished report prepared by Lonza, Inc.; dated September 3, 1987.) Accession Nos. 265026 and 265038. Supplemental submission for the following study:

Esber, H. J., Lilja, H. S., Niemi, S. M., and Zavorskas, P. A. Distribution study of 5-methyl-5-ethylhydantoin [5-^{14}C] ($C_6H_{10}N_2O_2$) in New Zealand White rabbits. (Unpublished report No. MRI-702-GC-86-51 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: May 10, 1988

William McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: May 10, 1988

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: _____
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Joycelyn Stewart, Ph.D.
EPA Reviewer

Signature: Joycelyn Stewart
Date: 5/24/88

Albin B. Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 5/25/88

7. CONCLUSIONS:

A. Following the administration of [^{14}C]EMH to male and female rabbits, most of the radioactivity is eliminated in the urine. Radioactivity in blood peaked at 3 hours after dosing and decreased biphasically thereafter. The half-life for the initial rapid phase was about 8 to 10 hours. Radioactivity found in tissues was low. However, a quantitative evaluation of the data, differences between sexes, and chromatographic analysis for urine could not be made.

B. This study provides supplementary data on the metabolism of EMH, but does not fulfill EPA's requirements for registration.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. It was reported that a stock solution of 2 mCi/mL [^{14}C]EMH, was diluted to 0.1 mCi/mL with water and administered to the rabbits by gavage at a dose of 100 µCi/kg. The amounts given to each rabbit were presented as counts per minute (cpm)/rabbit. The rationale for dose selection was not reported.

2. Three male and three female New Zealand White rabbits were obtained from Millbrook Farm, Amherst, MA. The animals weighed 1.906 to 3.376 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 after 72 hours. 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 and then sacrificed; the following organs were removed and weighed: testes, ovaries, pancreas, spleen, heart, lung, urinary bladder, kidney, liver, brain, stomach, and gastrointestinal tract.

4. All samples (0.2 mL of blood, 0.2 mL of urine, and about 200 mg of feces or tissue) were oxidized in a Packard Oxidizer

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[1]Only items appropriate to this DER have been included.

(Model B306) and radioassayed by a Packard liquid scintillation counter (LSC) (Model 4430). The counting efficiency of the LSC was reported to be 51 percent. Background counts were automatically subtracted and results were reported as cpm. Urine and blood samples were spotted on silica G plates and the thin layer chromatograms were developed in chloroform: ethyl acetate:ethanol (8:1:1, v:v:v). Radiolabeled compounds were visualized by autoradiography.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Seventy-two hours after dosing, most of the administered radioactivity was eliminated in the urine of male and female rabbits (223×10^6 and 386×10^6 cpm, respectively). These values accounted for approximately 96.7 and 100.7 percent of the dose in males and females, respectively, assuming that the counting efficiency of the LSC is constant for the [^{14}C] administered and the [^{14}C] in urine. In feces, only 3.01×10^6 and 5.19×10^6 cpm or 1.3 and 1.8 percent of the dose (using the same assumptions) were eliminated in the feces of male and female rabbits, respectively. No radiolabeled metabolites were detected in urine by thin layer chromatography (TLC) and autoradiography. Only unchanged parent compound was found. Approximately 1.0 and 0.3 percent of the dose were found in the cage wash of male and female rabbits, respectively.

B. Radiolabel [^{14}C] levels in blood were highest 3 hours after dosing and decreased biphasically, with the initial rapid phase having a half-life of about 8 to 10 hours.

C. Radiolabel [^{14}C] residues in tissues were very low, accounting for less than 0.01 percent of the dose in males and females. Radiolabel [^{14}C] residues in the carcasses were not reported (Table 1).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The primary route of detoxification occurred via urinary excretion, with an estimated half-life in the "test population" of 8 to 10 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.

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

Tissue	Radioactivity (cpm)/g (mL)[a] Males	Females
Blood	247	287
Liver	242	364
Kidneys	188	260
Gonads	234	456
Heart	230	223
Lungs	208	217
Spleen	208	365
Pancreas	132	228
Brain	173	199
Muscle	199	233
Bone marrow	270	205
Bone	236	266
Adipose tissue	183	143
Gastrointestinal tract	207	247
Skin	187	273
Stomach	234	309
Bladder	241	336
Total	3,619	4,611

[a]Values are means for three rabbits.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Although the supplemental submission provides information on the specific activity and radiopurity of the test material and background counts and efficiency of the LSC used, there are still five major deficiencies in this study which renders it inadequate to support registration of the compound. (1) The combustion of up to 0.35 g of, e.g., adipose tissue and/or gastrointestinal tract will invariably lead to incomplete combustion and/or quenching; it is not clear from the submission whether that was taken into account, using one of many available methods. (2) Only three animals/sex were used, whereas EPA guidelines require at least five animals/sex. (3) The total [^{14}C] recoveries in males (98.6, 100.9, and 96.8 percent) and females (107.0, 96.3, and 103.1 percent) are relatively high, even though urine and fecal samples collected at 48 to 72 hours still contained substantial radioactivity and [^{14}C] in carcasses was not determined (see also initial DER, Dynamac No. 252-B, Appendix A). Thus, there may have been problems with the calculations used and the experiment should have been extended beyond 72 hours. (4) The TLC solvent used was inadequate. The author's statement that "the apparent multiple spots in reference samples on these plates was a tailing as a consequence of the laboratory's error in its selection of solvent system..." further supports the fact that the data presented cannot be adequately evaluated. (5) Finally, the data in this report were very poorly presented and in some instances it was quite difficult to determine what was actually done. Although this submission clarifies some of the questions raised in the original review, many more questions remain (see item 8 in original DER). For example, data for [^{14}C] levels in blood are best presented in a figure in order to determine the half-lives for elimination. In addition, the rationale for dose selection was not presented; the results are presented in cpm and mean value and standard deviations for individual animals were not reported.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Dynamac DER No. 252-B.