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

MAR 30 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 006836-00115, Metabolism Studies with Dimethylhydantoin in Rats

Tox. Chem. No.: 114A,30L,30?C
Project No.: 2-1174 3662,56??
Record No. : S410419

FROM: Melba S. Morrow, D.V.M. MSM 3/25/92 MM 3/25/92
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Health Effects Division (H7509C)

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Registration Division (H7505C)

THRU: Joycelyn E. Stewart, Ph.D. JS 3/25/92 KB 3/27/92
Acting Section Head, Review Section II
Toxicology Branch I
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CONCLUSIONS:

Based on the results of the studies conducted with Dimethylhydantoin (DMH) and 5-ethyl,5-methylhydantoin (MEH), both compounds were rapidly absorbed and excreted unchanged primarily in the urine. For DMH, most of the radioactivity was recovered in the first 12 hours. For MEH, most of the radioactivity was recovered in the first 12 to 24 hours. Neither the sex of the animal nor the route of administration had an effect on the excretion or bioaccumulation of either of the test compounds.

Both studies that were designed to determine the metabolites of DMH and MEH in the urine are classified as supplementary. The sponsor needs to supply analytical data to demonstrate that the HPLC methodology was validated to confirm the identity of the metabolites in the urine samples. The studies may be upgraded upon receipt and evaluation of the requested data.

The studies that were conducted to address the metabolism of DMH and MEH are both acceptable but do not meet the guideline requirements for metabolism studies without the additional information that was obtained from the studies conducted to

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identify the urinary metabolites.

Copies of the DERs are attached for your reference.

Reviewed by: Melba S. Morrow, D.V.M. 3/24/92
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Paul Chin, Ph.D. 3/24/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Rats

GUIDELINE #: 85-1

TOX. CHEM. #: 114A

MRID #: 421739-01

TEST MATERIAL: Dimethylhydantoin

SYNONYMS: Dantobrom, DMH

STUDY NUMBERS: P01982

SPONSOR: Lonza, Inc.
Fair Lawn, N.J.

TESTING FACILITY: Biological Test Center
Irvine, California

TITLE OF REPORT: Absorption, Distribution, Metabolism and Excretion (ADME) Studies of 5,5-Dimethylhydantoin in the Rat

AUTHORS: Sami Selim, Ph.D.

REPORT ISSUED: November 17, 1991

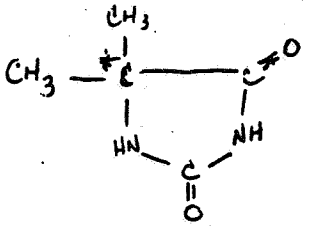
CONCLUSIONS: Under the conditions of this study, DMH was rapidly absorbed and was excreted primarily in the urine. Most of the radioactivity was recovered in the first 12 hours in both sexes and there was very little accumulation in the tissues.

CLASSIFICATION: Acceptable. This study alone does not satisfy the guideline requirements for a metabolism; however, an additional study was conducted to identify the metabolites of DMH in the urine.

TOX. CATEGORY: N/A

MATERIALS:

^{14}C Radiolabeled DMH with a ^{14}C in the ring 5 position and with a purity of 98.8% and non radiolabeled DMH with a purity of 99.5% were the test materials. The structure of the compound and the position of the label are shown below:



Both compounds were white crystalline material with a slight odor. Radiolabeled DMH had a specific activity of 26.3 mCi/mmol.

The test animals were 20 male and 20 female Crl:CDBR rats. The animals were approximately 7 weeks when the test materials were administered.

METHODS:

All test animals were subjected to a two week acclimation period during which they were weighed, identified and examined for general health. The animals were individually housed in an environment which allowed for a 12 hour light /dark cycle and in which the temperature and relative humidity were maintained at 63 to 71° F and 25 to 72%, respectively. Food and water were provided ad libitum.

Prior to the initiation of this study, a preliminary study was conducted to determine whether labeled CO_2 would be expired by the test animals. The study did not reveal that this would occur.

Preparation of Dosing Solutions:

Solutions of radiolabeled DMH were prepared by mixing a weighed amount of nonlabeled DMH with 14C-DMH then adding the mixture to sterile water. For the preparation of the non-radiolabeled oral solution, non-labeled compound was mixed with sterile water. Two different batches of the non-labeled material were prepared to allow for dosing on days 1 through 7 and for days 8 through 14.

IV solutions were prepared by mixing appropriate amounts of radiolabeled and non-radiolabeled DMH with normal saline solution. This material was injected into the jugular.

Dose Groups

This study was conducted in four parts and animals were randomly assigned to one of the following treatment groups:

Group	Dose (mg/kg)	Volume (mL/kg)	Route
1	100	5.0	oral
2	100	5.0	oral
3	1000	10.0	oral
4	100	2.5	IV

Animals in groups 1, 3 and 4 received single doses. Group 2 animals received repeat doses of non-radiolabeled DMH at a rate of 100 mg/kg for days 1 thru 7 and at a rate of 80 mg/kg for days 8 thru 14. These oral treatments were followed by a single oral dose of labeled DMH.

Sample Collection:

Urine, feces and washings from the urine/feces separators were collected at the following hourly intervals 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144 and 144-168. Samples were weighed at the time of collection and stored at -15°C until they were analyzed.

Animals were anesthetized with Ketamine and xylazine and euthanized by exsanguination. A 5 mL blood sample was collected from each animal and duplicate aliquots of 100 uL each were transferred to combustion cones.

The following tissues were collected : gastrointestinal tract and its contents, bone, brain, fat, gonads, heart, kidneys, liver, lungs, muscle, spleen, pancreas, skin, hair and residual carcass. All tissues were weighed and were then stored at -15° C until a radioassay was conducted.

Sample Preparation:

Duplicate aliquots of urine, plasma and cage washings were transferred to liquid scintillation vials and one mL of distilled water was added to facilitate the analysis of the 100 uL urine samples at 0-4, 4-8 and 8-12 hour sample collection times. The feces were homogenized with 5X distilled water and duplicate aliquots of approximately 200 mg of the homogenate were transferred to combustion cones.

Tissues and organs that were greater than 0.5 g were homogenized with 5 to 10X distilled water and duplicate aliquots of

approximately 1 mL of the homogenate were transferred to combustion cones. Carcasses were pulverized and a 5 g homogeneous aliquot was homogenized with 5 to 10 X of distilled water prior to processing for combustion. Gonads, muscle, fat, spleen, hair and bones and duplicate 100 uL aliquots of blood were combusted directly.

Calculations:

The background for urine, cage rinses and plasma was determined by counting a number of liquid scintillation vials containing only Insta-Gel and then averaging the values. The background for combustion samples for tissues and feces was calculated by combusting blank samples. Background was subtracted from all samples.

The following formulas were used in the calculation of ppm equivalents of DMH and in determining the percent of the total dose of DMH:

$$\text{ppm} = \frac{\text{net DPM/g of treatment sample}}{\text{specific activity of active ingredient (DPM/ug)}}$$

$$\%\text{ of dose} = \frac{\text{radioactivity of sample}}{\text{Total radioactivity administered}} \times 100$$

STATISTICS:

Mean and standard deviations were used to characterize data for radioactivity measurement, concentration and body weights.

QUALITY ASSURANCE:

A statement of Quality Assurance dated November 14,1991 was included in the submission along with a statement of compliance with GLPs dated November 19,1991.

RESULTS:

All animals survived the study in each of the four sub-groups.

Single Oral Low Dose Group:

The mean amount of DMH administered to this group of rats was 103.73 mg/kg and 104.2 mg/kg for males and females, respectively. The radioactivity in males was 25.9 uCi and 16.4 uCi in females. during the first 12 hours, more than 90% of the total dose of DMH was excreted in the urine of both sexes. The next greatest portion of radioactive test material was recovered from the feces (0.74% in males and 0.72% in females during 7 days following the administration of the test material).

Residual levels of DMH were present in the hair, with males having a higher mean (0.968 ppm) than females (0.44). Detectable levels of DMH were also present in the carcasses of both sexes and in the fat of one male.

Single Oral High Dose Group:

In males in this group, the mean amount of DMH administered was 1036 mg/kg with 23.8 uCi radic ctivity. One animal had urine recovery that was lower than expected and was not considered in the calculation of the mean value of 90.5% for urine radioactivity. Mean radioactivity in the feces was 1.0% during the 7 days following the administration of the test material.

In females, the specific activity was 15.5 uCi and the mean dose of DMH was 1043 mg/kg. Urinary excretion of the radioactive test material was 93.37% and 0.76% was excreted in the feces during the 7 days following the adminstration of the test material. Most of the test material was recovered in the first 12 hours of the study.

As in the single low dose group, the detectable levels of DMH in the hair was higher for males (mean value 7.497 ppm) than for females (mean 3.474 ppm). Detectable levels were higher in male carcasses (1.723 ppm) than in female carcasses (0.178 ppm, only detectable in two animals).

Repeated Oral Low Dose Group

DMH was administered to males in this group at a mean dose of 103.59 mg/kg and to females at a mean dose of 104.61 mg/kg. The activity of the test material was 24.6 uCi and 16.3 uCi for males and females, respectively. More than 95% of the radioactive test material was recovered in the urine of both sexes of animals during the first 12 hours. Fecal recovery was 0.88% in males and 1.37% in females during the 7 days which followed the administration of the test material.

In this group, the detectable levels of DMH were higher in the carcasses of females (0.130 ppm) than males (0.017). However, males had higher levels of the test material in their hair, with a mean value of 2.68 ppm being detected vs 0.57 ppm in females.

Single Intravenous Low Dose

Radioactive DMH with a specific activity of 24.8 uCi was administered to male rats at a dose of 105.06 mg/kg. The compound was administered to females at a dose of 106.35 with a specific activity of 16.3 uCi. Urinary excretion accounted for the recovery of 95.34% in males and 93.8% in females. Fecal

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recovery was 1.23% and 0.66% in males and females, respectively during the 7 days following the administration of the test material.

As in the other three groups, males had higher mean detectable levels of DMH in the hair (1.303 ppm) when compared to mean values for females (0.753 ppm). Detectable levels of DMH in the carcasses of females (0.050 ppm) was less than half of the detectable level in males (0.131 ppm).

See Table I for information on the total recovery of radioactivity in urine, feces and carcass/tissues.

DISCUSSION:

Based on the results of this study, DMH was rapidly absorbed from the gastrointestinal tract and was rapidly excreted from the body primarily, in the urine. Most of the radioactivity was recovered in the urine during the first 12 hours and very small amounts of the compound were detected in the tissues following sacrifice. The sex of the animal did not appear to affect the excretion of the test material; however, males had more accumulation of the test material in the hair when compared to females. Additionally, the dosing regimen did not appear to affect the excretion or the accumulation of the test material.

This study is acceptable; however, it does not satisfy the guideline requirements for a metabolism when considered alone. An additional study was conducted to identify any major metabolites of DMH. A review of this study is included as an addendum to this DER.

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TABLE I
Mean Total Recovery of Radioactivity
During the 7 days Following the Administrati
Test Material
(% of Administered Dose of DMH)

Males

Group	Urine	Feces	Carcass/Tissues	Total ^{14}C
Oral (LD)	94.18	0.74	0.03	94.96
Oral (HD)	90.50	1.00	0.16	91.65
Repeat Oral	95.68	0.88	0.01	96.57
Single IV	95.34	1.23	0.12	96.69

Females

Group	Urine	Feces	Carcass/Tissues	Total ^{14}C
Oral (LD)	93.34	0.72	0.03	94.09
Oral (HD)	93.37	0.76	0.01	94.14
Repeat Oral	96.25	1.37	0.11	97.73
Single IV	93.80	0.66	0.04	94.51

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Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Paul Chin, Ph.D. 3/25/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

GUIDELINE #: 85-1

TOX. CHEM. #: 114A

MRID #: 421238-01 (Addendum to 421739-01)

TEST MATERIAL: ^{14}C DMH

SYNONYMS: Dantobrom, Dimeth ydantoin

STUDY NUMBERS: PO1982 - Addendum

SPONSOR: Lonza, Inc.
Fair Lawn, N.J.

TESTING FACILITY: Biological Test Center
Irvine, California

TITLE OF REPORT: Absorption, Distribution, Metabolism and Excretion (ADME) Studies of 5, 5, Dimethylhydantoin in the Rat

AUTHORS: Sami Selim, Ph.D.

REPORT ISSUED: November 15, 1991

CONCLUSIONS: Unmetabolized ^{14}C-DMH recovered in the urine represented more than 90% of the administered dose of DMH.

CLASSIFICATION: Supplementary. The sponsor needs to supply analytical data to demonstrate that the HPLC methodology was validated to confirm the identity of the metabolites in the urine samples. This study may be upgraded upon receipt and evaluation of the requested data.

TOX. CATEGORY: N/A

MATERIALS:

Ten Crl:CDBR rats (5 males and 5 females) were used in this portion of the study for qualitative analysis. Animals were approximately 5 weeks of age at arrival. 14C DMH was the test material and was administered at a single oral dose of 1000 mg/kg. A constant volume of 10 mL/kg body weight was administered to achieve the desired dose.

METHODS:

Dosing solutions were prepared by mixing radiolabeled DMH in sterile water. The specific activity of the test material was 8.25 uCi/g. Urine and feces were collected at the following intervals following the administration of the test compound: 0-6, 6-24, 24-48 and 48-72 hours. Metabolites were isolated by HPLC using samples collected at 0 to 6 hours and 6 to 24 hours. Twenty-five microliters of urine were collected from male rats and 15 uL of urine were collected from females for analysis.

A Walker Model 510 HPLC was used to analyze the samples. The HPLC was equipped with a Beckman Ultrasphere ODS which had an ambient column temperature. The mobile phase was conducted using acetonitrile and distilled, deionized water. The flow rate in the column was 1 mL/min. To calculate the percent of the administered dose of DMH in a specific sample, the following formula was used:

$$\% \text{ Dose} = \frac{\text{Total } \% \text{ excreted in urine}}{100} \times \% \text{ radioactivity in urine as DMH}$$

HPLC was also used to quantify individual residues in 20% of the urine samples collected in the main ADME study.

QUALITY ASSURANCE: A statement of Quality Assurance dated November 27, 1991 was included in the report.

RESULTS:

Unmetabolized ^{14}C-DMH was the only residue found in the urine of both sexes of rats. In both sexes, the unmetabolized DMH represented more than 90% of the administered dose. See Table I for information on DMH r[illegible] urine.

DISCUSSION:

This study is supple[illegible]ary and does not satisfy the requirements for a metabolism stu[illegible]en conside[illegible]e. The registrant needs to supply analy[illegible]at the HPLC method that was used was validat[illegible]ation is received and reviewed, the study m[illegible]ceptable.

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TABLE I
Urine Residues of DMH

Males Dose Group	% 14C Residues in Urine as DMH	% of Administered Dose in Urine as DMH
Oral Single (LD)	97.5	91.8
Single Oral (HD)	100.0	90.5
Multiple Oral	97.8	93.6
Single IV	98.1	93.6
Females		
Single Oral (LD)	98.5	91.9
Single Oral (HD)	98.7	92.2
Multiple Oral	97.1	93.4
Single IV	99.0	92.9

Table extracted from data presented on page 16 of Addendum report.

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Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Paul Chin, Ph.D. 3/24/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Rats

GUIDELINE #: 85-1

TOX. CHEM. #: 114A

MRID #: 421739-02

TEST MATERIAL: 5-ethyl-5 methylhydantoin

SYNONYMS: MEH

STUDY NUMBERS: P02000

SPONSOR: Lonza, Inc.
Fair Lawn, N.J.

TESTING FACILITY: Biological Test Center
Irvine, California

TITLE OF REPORT: Absorption, Distribution, Metabolism and Excretion (ADME) Studies of 5-ethyl,5-Methylhydantoin in the Rat

AUTHORS: Sami Selim, Ph.D.

REPORT ISSUED: November 15, 1991

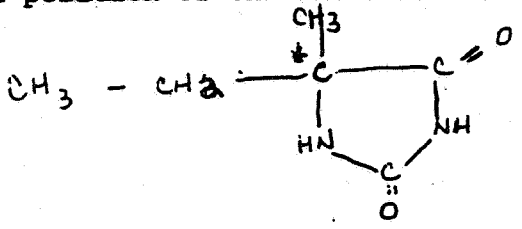
CONCLUSIONS: Under the conditions of this study, MEH was rapidly absorbed and was excreted primarily in the urine. Most of the radioactivity was recovered in the first 24 hours in both sexes and there was very little accumulation in the tissues.

CLASSIFICATION: Acceptable. This study alone does not satisfy the guideline requirements for a metabolism study; however, an additional study was conducted to identify the metabolites of MEH that are present in the urine.

TOX. CATEGORY: N/A

MATERIALS:

^{14}C Radiolabeled MEH with a purity of 99.2% and non radiolabeled MEH with the ^{14}C ring in the ring 5 position and with a purity of 99.5% were the test materials. The structure of the compound and the position of the label are shown below:



Both compounds were white crystalline material with a slight odor. Radiolabeled MEH had a specific activity of 24.6 mCi/mmcl.

The test animals were 20 male and 20 female Crl:CDBR rats. The animals were approximately 7 weeks when the test materials were administered.

METHODS:

All test animals were subjected to a two week acclimation period during which they were weighed, identified and examined for general health. The animals were individually housed in an environment which allowed for a 12 hour light /dark cycle and in which the temperature and relative humidity were maintained at 65 to 69° F and 50 to 69%, respectively. Food and water were provided ad libitum, except during the 18 hours prior to the administration of the test material.

Prior to the initiation of this study, a preliminary study was conducted to determine whether labeled CO_2 would be expired by the test animals following oral administration of the test material. The study did not reveal that this would occur.

Preparation of Dosing Solutions:

Solutions of radiolabeled MEH were prepared by mixing a weighed amount of nonlabeled MEH with ^{14}C-MEH then adding the mixture to 200 mL of sterile water. For the preparation of the non-radiolabeled oral solution, non-labeled compound was mixed with sterile water.

IV solutions were prepared by mixing appropriate amounts of radiolabeled MEH with normal saline solution. This material was injected into the jugular vein.

Dose Groups

This study was conducted in four parts and animals were randomly assigned to one of the following treatment groups:

Group	Dose (mg/kg)	Volume (mL/kg)	Route
1	100	5.0	oral
2	100	5.0	oral
3	1000	10.0	oral
4	100	2.5	IV

Animals in groups 1, 3 and 4 received single doses. Group 2 animals received repeat doses of non-radiolabeled MEH at a rate of 100 mg/kg for approximately 2 weeks. These oral treatments were followed by a single oral dose of labeled MEH.

Sample Collection:

Urine, feces and washings from the urine/feces separators were collected at the following hourly intervals 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144 and 144-168. Urine was freezed trapped to avoid oxidation, evaporation or bacterial degradation. Samples were weighed at the time of collection and stored at -15°C until they were analyzed.

Animals were anesthetized with Ketamine and xylazine and euthanized by exsanguination seven days after the administration of labeled MEH. A 5 mL blood sample was collected from each animal and duplicate aliquots of 100 uL each were transferred to combustion cones.

The following tissues were collected : gastrointestinal tract and its contents, bone, brain, fat, gonads, heart, kidneys, liver, lungs, muscle, spleen, pancreas, skin, hair and residual carcass. All tissues were weighed and were then stored at -15° C until a radioassay was conducted. The remaining blood was centrifuged to separate plasma from cells.

Sample Preparation:

Duplicate aliquots of urine, plasma and cage washings were transferred to liquid scintillation vials and one mL of distilled water was added to facilitate the analysis of the 100 uL urine samples at 0-4, 4-8 and 8-12 hour sample collection times. The feces were homogenized with 5X distilled water and duplicate aliquots of approximately 200 mg of the homogenate were transferred to combustion cones.

Tisssues and organs that were greater than 0.5 g were homogenized with 5 to 10X distilled water and duplicate aliquots of approximately 1 mL of the homogenate were transferred to combustion cones. Carcasses were pulverized and a 5 g homogeneous aliquot was homogenized with 5 to 10 X of distilled water prior to processing for combustion. Gonads, muscle, fat, spleen, hair and bones and duplicate 100 uL aliquots of blood were combusted directly.

Insta-Gel was added to the urine, plasma and cage washings at a rate of 10 mL and the samples were counted in a Beckman Liquid Scintillation Counter. A Harvey Sample Oxidizer was used in determining combustion.

Calculations:

The background for urine, cage rinses and plasma was determined by counting a number of liquid scintillation vials containing only Insta-Gel and then averaging the values. The background for combustion samples for tissues and feces was calculated by combusting blank samples. Background was subtracted from all samples.

The following formulas were used in the calculation of ppm equivalents of MEH and in determining the percent of the total dose of MEH:

$$\text{ppm} = \frac{\text{net DPM/g of treatment sample}}{\text{specific activity of active ingredient (MEH/ug)}}$$

$$\%\ \text{of dose} = \frac{\text{radioactivity of sample}}{\text{Total radioactivity administered}} \times 100$$

STATISTICS:

Mean and standard deviations were used to characterize data for radioactivity measurement, concentration and body weights.

QUALITY ASSURANCE:

A statement of Quality Assurance dated November 20,1991 was included in the submission along with a statement of compliance with GLPs dated November 15,1991.

RESULTS:

All animals survived the study in each of the four sub-groups. Additionally, no signs of toxicity were observed.

Single Oral Low Dose Group:

The mean amount of MEH administered to this group of rats was 99.86 mg/kg and 99.88 mg/kg for males and females, respectively. The radioactivity in males was 15.9 uCi and 10.4 uCi in females. during the first 24 hours, more than 90% of the total dose of MEH was excreted in the urine of both sexes. The next greatest portion of radicactive test material was recovered from the feces (0.78% in males and 1.68% in females during the 7 days following the administration of the test material).

The mean residue levels in female hair samples was 1.197 ppm and in female carcasses, the mean radioactivity was 0.48%. In males, the mean residue levels in the hair were lower (0.945 ppm) than those reported for females and the radioactivity in the carcass was 0.124 ppm or 0.11% of the radioactive dose of MEH.

Single Oral High Dose Group:

In males in this group, the mean amount of MEH administered was 1016 mg/kg with 21.2 uCi radioactivity. The mean value for urine radioactivity was 101.82% and the mean radioactivity in the feces was 1.14% during the 7 days following the administration of the test material. Mean residual radioactivity in the carcass was 1.734 ppm and the mean residue levels in the hair was 13.404 ppm.

In females, the specific activity was 13.3 uCi and the mean dose of MEH was 1048.85 mg/kg. Urinary excretion of the radioactive test material was 97.53% and 1.26% was excreted in the feces during the 7 days following the administration of the test material. Most of the test material was recovered in the first 12 hours of the study. In females, the detectable levels of MEH in the hair and carcass were 8.106 ppm and 3.112 ppm, respectively.

Repeated Oral Low Dose Group

MEH was administered for 14 days to males in this group at a mean dose of 100.86 mg/kg and for 15 days to females at a mean dose of 100.97 mg/kg. Females received an extra day of treatment because they were not fasted on one occasion prior to the administration of the test material. The radioactivity of the test material was 15.3 uCi and 10.4 uCi for males and females, respectively. More than 95% of the radioactive test material was recovered in the urine of male rats during the first 24 hours. Urine recovery in females was 91.7% during the 7 days following the administration of the test material, with most of the radioactivity also being recovered in the first 24 hours. Fecal recovery was 1.95% in males and 2.33% in females during the 7 days following the administration of the test material.

In this group, the detectable levels of MEH were present in all tissues in females except the ovaries, fat and bone. Highest residue levels were reported in the hair (1.694 ppm), carcass (0.333 ppm) and the skin (0.118 ppm). When these residues were considered together they accounted for 0.28% of the total dose of MEH.

In males, 1.549 ppm was detected in the hair, 0.195 ppm was detected in the carcass and 0.178 ppm was detected in the plasma.

Single Intravenous Low Dose

Radioactive MEH with a specific activity of 16.8 uCi was administered to male rats at a dose of 102.06 mg/kg. The compound was administered to females at a dose of 100.97 with a specific activity of 11.0 uCi. Urinary excretion accounted for the recovery of more than 95% in both males and females and fecal recovery was 1.47% and 0.71% in males and females, respectively during the 7 days following the administration of the test material.

Males in this group had higher mean detectable residues in the hair and carcasses when compared to females. In males, the detectable residues in the hair and carcass were 1.341 ppm and 0.555 ppm, respectively. Carcass residues accounted for 0.47% of the total amount of MEH administered. In females, the the mean residue levels in hair were 0.689 ppm and the mean residue levels were 0.124 ppm. The carcass residues accounted for 0.11% of the total amount of MEH administered.

See Table I for information on the total recovery of radioactivity in urine feces and carcass/tissues.

DISCUSSION:

Based on the results of this study, MEH was rapidly absorbed from the gastrointestinal tract and was rapidly excreted from the body primarily, in the urine. Most of the radioactivity was recovered in the urine during the first 12 to 24 hours. Very small amounts of the compound were detected in the tissues following sacrifice. The sex of the animal did not appear to affect the excretion of the test material. Additionally, the dosing regimen did not appear to affect the excretion or the accumulation of the test material.

This study is acceptable but does not satisfy the guideline requirements for a metabolism study when considered alone. An additional study was conducted to identify any major metabolites of MEH. A review of this study is included as an addendum to this DER.

TABLE I
Mean Total Recovery of Radioactivity
During the 7 Days Following the Administration of the
Test Material
(% of Administered Dose of MEH)

Males

Group	Urine	Feces	Carcass/Tissues	Total ^{14}C
Oral (LD)	97.45	0.78	0.11	98.33
Oral (HD)	101.82	1.14	0.15	103.10
Repeat Oral	95.35	1.95	0.17	97.47
Single IV	96.36	1.47	0.47	98.67

Females

Group	Urine	Feces	Carcass/Tissues	Total ^{14}C
Oral (LD)	93.67	1.68	0.22	95.57
Oral (HD)	97.53	1.26	0.25	99.04
Repeat Oral	91.70	2.33	0.28	94.31
Single IV	97.39	0.71	0.11	98.21

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Secondary Reviewer: Paul Chin, Ph.D. Paul Chin 3/25/92
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism

GUIDELINE #: 85-1

TOX. CHEM. #: 114A

MRID #: 421238-02 (Addendum to 421739-02)

TEST MATERIAL: ^{14}C MEH

SYNONYMS: Dantobrom, Dimethylhydantoin

STUDY NUMBERS: PO2000 - Addendum

SPONSOR: Lonza, Inc.
Fair Lawn, N.J.

TESTING FACILITY: Biological Test Center
Irvine, California

TITLE OF REPORT: Absorption, Distribution, Metabolism and Excretion (ADME) Studies of 5 Ethyl, 5-Methylhydantoin in the Rat

AUTHORS: Sami Selim, Ph.D.

REPORT ISSUED: November 15, 1991

CONCLUSIONS: Unmetabolized ^{14}C-MEH recovered in the urine represented more than 89% of the administered dose of MEH.

CLASSIFICATION: Supplementary. The sponsor needs to supply analytical data to demonstrate that the HPLC methodology was validated to confirm the identity of the metabolite in the urine samples. This study may be upgraded upon receipt and evaluation of the requested data.

TOX. CATEGORY: N/A

MATERIALS:

Ten Crl:CDBR rats (5 males and 5 females) were used in this portion of the study for qualitative analysis. Animals were approximately 5 weeks of age at arrival. ^{14}C MEH was the test material and was administered at a single oral dose of 100 mg/kg. A constant volume of 5 mL/kg body weight was administered to achieve the desired dose.

METHODS:

Dosing solutions were prepared by mixing radiolabeled MEH in sterile water. The specific activity of the dosing solution was 11.1 uCi/g. Urine and feces were collected at the following intervals following the administration of the test compound: 0-6, 6-24, 24-48 and 48-72 hours. Metabolites were isolated by HPLC using samples collected at 0 to 6 hours and 6 to 24 hours. Twenty-five microliters of urine were collected from male rats and 17 uL of urine were collected from females for analysis.

A Walker Model 510 HPLC was used to analyze the samples. The HPLC was equipped with a Beckman Ultrasphere ODS which had an ambient column temperature. The mobile phase was conducted using acetonitrile and distilled, deionized water. The flow rate in the column was 1 mL/min. To calculate the percent of the administered dose of MEH in a specific sample, the following formula was used:

$$\% \text{ Dose} = \frac{\text{Total \% excreted in urine}}{100} \times \% \text{ radioactivity in urine as MEH}$$

HPLC was also used to quantify individual residues in 20% of the urine samples collected in the main ADME study.

QUALITY ASSURANCE: A statement of Quality Assurance dated November 27, 1991 was included in the report.

RESULTS:

Unmetabolized ^{14}C-MEH was the only residue found in the urine of both sexes of rats. In both sexes, the unmetabolized MEH represented more than 89% of the administered dose. See Table I for information on MEH residues in the urine.

DISCUSSION:

The results obtained in the other study in which Dimethylhydantoin (DMH) was the test material were similar to the results obtained in this study. Both compounds were unmetabolized and were rapidly excreted in the urine. Neither sex of the animal nor route of administration had any affect on the absorption, distribution, metabolism or excretion of either MEH or DMH.

This study is supplementary and does not satisfy the requirements for a metabolism study when considered alone. The registrant needs to supply analytical data to show that the HPLC methodology that was used was validated for this study. Once this information is received and reviewed, the study may be upgraded.

TABLE I
Urine Residues of MEH

Males Dose Group	% ^{14}C Residues in Urine as MEH	% of Administered Dose in Urine as MEH
Oral Single (LD)	96.3	93.9
Single Oral (HD)	93.7	95.4
Multiple Oral	95.9	91.4
Single IV	97.8	94.6
Females		
Single Oral (LD)	96.9	90.8
Single Oral (HD)	94.6	92.3
Multiple Oral	97.9	89.8
Single IV	98.6	96.0

Table extracted from data presented on page 16 of Addendum report.

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