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WASHINGTON, D.C. 20460

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SEP 23 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Five Dermal Sensitization Studies with Diuron, Krovar I and II, and Diurex

TO: Carol Peterson/Walter Waldrop PM-71
RD (H-7508W)

FROM: David S. Liem, Ph.D. David Liem 9/4/92
Section II, Toxicology Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 9/8/92
Section II, Toxicology Branch II/HED (7509C)
and
Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II/HED (H7509C) Marcia van Gemert 9/8/92

MRID#: 402288-04, and 409840-04

TRID#: 4700-140-26, 460094-019, and 460075-16

DP Barcode#: D169284 Caswell No.: 410 HED Project No.: 1-2600

ACTION REQUESTED

To review the following Dermal Sensitization Studies with Diuron, Krovar I and II, and Diurex:

1. Guinea Pig Maximization Test Using Diuron (EPA Reg# 12020-1) INTOX Sample #585 (TRID# 4700-140-26)

2. Dermal Sensitization Study with IN-14,740-146 in Guinea Pigs (MRID# 402288-04)

3. Guinea Pig Sensitization Study (Buehler) (TRID# 460094-019)

4. Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN-M2574-25 in Guinea Pigs (MRID# 409840-04)

5. Dermal Sensitization Study in Guinea Pigs (TRID# 460075-16)

CONCLUSIONS

1. Guinea Pig Maximization Test Using Diuron (EPA Reg# 12020-1) INTOX Sample #585 (TRID# 4700-140-26)

TEST MATERIAL: Diuron batch# 294537026

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl

SPONSOR: Griffin Corporation, Valdosta, GA 31601

CONCLUSIONS: The topical application of 100 mg of diuron (no. 12020-1; 97% purity) over a 4 CM^2 skin area, following the second induction, did not cause increased erythema, edema, or any indication of skin irritation in guinea pigs. Therefore, topical application of diuron with a 97% purity did not induce sensitization in guinea pigs under the conditions of this study. This study is currently classified as core-supplementary, because the positive control data were not provided. The sponsor should submit positive control data for the same strain tested during the same time frame as this study.

Classification: Core-supplementary. This study is upgradable after satisfactory review of the requested positive control data.

2. Dermal Sensitization Study with IN-14,740-146 in Guinea Pigs (MRID# 402288-04)

TEST MATERIAL: Diuron (IN-14,760-146; 99% pure)

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl

CONCLUSIONS: Based on the results of the study, exposure to challenge dose of diuron (IN-14,170-146; 99% pure) of up to 80% concentration, following two weeks of induction did not cause increased erythema, edema, or any other indication of skin irritation in guinea pigs. Therefore, diuron is not considered a sensitizing agent in guinea pigs.

Classification: Core-minimum. This study satisfies USEPA's guideline 81-6 requirements for a dermal sensitization study in guinea pigs.

3. Guinea Pig Sensitization Study (Buehler) (TRID# 460094-019)

TEST MATERIAL: Diurex Tech (Diuron); purity was not specified

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron)

CONCLUSIONS: Based on the results of the study, exposure to challenge dose of diurex Tech (diuron), following two weeks of induction did not appear to cause increased erythema, edema, or any other dermal irritation effect in guinea pigs. Based on the results of the study, Diurex Tech appears to be a nonsensitizer in guinea pigs. This study is currently classified as core-supplementary because the purity of Diurex Tech (diuron) was not specified, the initial and terminal body weights of the test and positive control animals were not recorded, no negative control tests (vehicle and test article) were conducted, and also it was not clear when the positive control test was conducted. The registrant must provide the purity of the test article and the interval in which the positive controls were dosed. This study may be upgraded, after satisfactory review of the requested information.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

4. Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN-M2574-25 in Guinea Pigs (TRID# 409840-04)

TEST MATERIAL: Krovar I DF (IN-M2574-25) - A mixture of 80% of two active ingredients (50% diuron and 50% bromacil)

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron) and 5-bromo-6-methyl-3-(1-methylpropyl)-2,4-(1M,3H)-pyrimidinedione (bromacil); Pro-Serve (tradename)

CONCLUSIONS: Based on the results of the study, exposure to challenge dose of Krovar I DF as received (IN-M2574-25), a mixture of 80% of two active ingredients (50% diuron and 50% bromacil), following two weeks of induction did not cause increased erythema, edema, or any other skin irritation effect in guinea pigs. Krovar I DF (IN-M2574-25) is not considered a sensitizing agent in guinea pigs.

This study is currently classified as core-supplementary because the purity of both active ingredients, diuron and bromacil, was not specified, the physical form of Krovar was not clear, and the percent of technical impurities was not clear. The registrant should also give a detailed description of the test material used in this study. This study is upgradable, after satisfactory review of the requested information.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

5. Dermal Sensitization Study in Guinea Pigs (TRID# 460075-16)

TEST MATERIAL: A mixture of Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron) and 5-bromo-6-methyl-3-(1-methylpropyl)-2,4-(1M,3H)-pyrimidinedione (bromacil); Haskell # 15,600; INM-2574.

SYNONYM: Eighty percent of Krovar II DF (a mixture of two active ingredients: 53% diuron and 27% bromacil) and 20% inerts

CONCLUSIONS: Based on the data and method as presented in the study report, the result of this study could not be evaluated because no positive control was conducted. Furthermore, the following information was not provided: the method used for the dermal sensitization test, sex and strain of the test animals, purity of the two active ingredients (diuron and bromacil), initial and terminal body weights of test animals, and the environmental parameters of the study room. This study is presently classified as core-supplementary. It may be upgradable if the registrant provides the following:

- o positive control data from a study (same sex and strain) conducted at that facility during the same time frame; and
- o the purity of the two active ingredients of the test materials.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

DERs of the above five dermal sensitization studies are attached.

Reviewer: David S. Liem, Ph.D. David Liem 9/3/92
Toxicology Branch II, Section II
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 9/3/92
Toxicology Branch II, Section II

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization Study Guideline: 81-6

HED PROJECT NO.: 1-2600 CASWELL NO.: 410 TRID NO.:4700140-26

TEST MATERIAL: Diuron batch# 294537026

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl

STUDY NUMBERS: GRF-AT-009

SPONSOR: Griffin Corporation, Valdosta, GA 31601

TESTING FACILITY: INTOX Laboratories, P.O. Box 350
Redfield, Arkansas 72132

TITLE OF REPORT: Guinea Pig Maximization Test Using Diuron (EPA Reg# 12020-1) INTOX Sample #585

AUTHOR: Michael J. Schulz

REPORT ISSUED: June 1, 1985

CONCLUSIONS:

The topical application of 100 mg of diuron (no. 12020-1; 97% purity) over a 4 CM^2 skin area, following the second induction, did not cause increased erythema, edema, or any indication of skin irritation in guinea pigs. Therefore, topical application of diuron with a 97% purity did not induce sensitization in guinea pigs under the conditions of this study. This study is currently classified as core-supplementary, because the positive control data were not provided. The sponsor should submit positive control data for the same strain tested during the same time frame as the study.

Classification: Core-supplementary. This study is upgradable after satisfactory review of the requested positive control data.

STUDY TITLE: Guinea Pig Maximization Test Using Diuron (EPA Reg# 12020-1) INTOX Sample #585

Objectives: This study was designed to evaluate the dermal sensitization potential of Diuron in guinea pigs.

Test Materials: Diuron (N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea); purity 97%; white powder; solubility is 42 mg/L in water at 25°C; storage condition and pH of the test material were not provided in the study report.

Test Animals: Albino guinea pigs (Duncan Hartley strain) obtained from Hazelton/Dutchland, Denver were acclimated for at least 7 days prior to study initiation, and were housed in individual screened-bottom cages in a temperature-controlled room. Fifteen (313 - 348 gm at dosing) out of the 21 animals received were used.

Feed and Water: Test animals were provided with Purina Certified Guinea Pig Chow #5025 and water ad libitum.

Environmental Parameters: Test animals were kept in a controlled environment; a 12-hour light/dark cycle; room temperature (69°-74° F); relative humidity (57%-70%).

Body Weights: Individual body weights of all animals were recorded at the start and termination of the study.

PROCEDURES

A total of 15 female guinea pigs (10 treated and 5 vehicle control). The dorsal area (4 cm X 6 cm) was clipped to provide an exposure area. The dermal sensitization study using the Maximization Test Method (reference not given) is summarized below.

The study consisted of 3 phases:

o Initial Insult

Three pairs of intradermal injections were made on the dorsal skin exposure area.

- The first pair of injections (one on each side of the spinal column, 3.5 cm apart) was a 1:1 emulsion of Freund's Complete Adjuvant in sterile physiological saline (control and treated).
- The second pair of injections was the test material suspended in sterile physiological saline (20 mg/ml) for treated animals and only physiological saline for the control animals.
- The third pair of injections was a 1:1 emulsion of the test material suspended in sterile physiological saline (20 mg/ml) and Freund's Complete Adjuvant for treated animals and a 1:1 emulsion of Freund's adjuvant and sterile physiological saline for the control animals.

All three pairs of injections were confined to an area of approximately 2 cm by 4 cm within the 4 cm by 6 cm exposure area. The volume of administration was 0.1 ml for each injection.

o Second Insult*
The second insult treatment occurred 7 days after the initial insult. Twenty-four hours prior to second insult, the intradermal injection sites were graded for erythema and edema. Since the test article in saline was non-irritating, the exposure sites of the treated and control animals were massaged with a 10.0 w/w mixture of sodium lauryl sulfate in petrolatum.
Twenty-four hours later, the undiluted test article (250 mg) was applied and massaged onto the treated animals. Physiological saline was applied and massaged onto the control animals.

o Challenge Treatment
Two weeks after the 2nd insult, the test article (±100 mg/animal) was applied and massaged onto the right flank exposure area of the treated animals and a patch was wrapped over it. After 24 hours, the patch and wrappings were removed and the challenge sites were evaluated and graded 3 hours later. At 48 hours, the skin was evaluated and graded again.

* Prior to the second insult exposure, the primary irritancy of the test article was determined; the entire back of 4 naive animals was exposed up to 125 mg of the test article for 24 hours and skin responses were graded at 28 and 52 hours following the initial exposure. The highest non-irritating concentration which was approximately 100-125 mg/4cm^2 skin area was used for the second insult and the challenge treatments.

Skin Evaluation

Animals were considered sensitized when scores for erythema and edema after the challenge treatment were greater than zero. The sensitization potency is based on the percentage of animals sensitized as follows:

Grade	% Number of Animals Sensitized	Rating
0	0	Non-sensitizer
I	1 - 8	Weak Sensitizer
II	9 - 28	Mild Sensitizer
III	29 - 64	Moderate Sensitizer
IV	65 - 80	Strong Sensitizer
V	81 -100	Extreme Sensitizer

Skin responses were scored according to the grading scale presented in Appendix A.

RESULTS AND DISCUSSIONS

All animals survived to termination of the study. All animals gained weight during the study and the body weights were comparable among all groups (p. 13 of the study report)

A. Initial Insult

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The skin responses of the initial insult are presented in Appendix B.

- **Site 1: First Pair of Injections**
  Freund's in saline for both Control and Treated Groups
  These treatments caused very slight to severe erythema and very slight to severe edema for the majority of the control as well as the treated animals. One animal (no. 6916) of the treated group did not show any skin response.

- **Site 2: Second Pair of Injections**
  Saline for Control Animals; Test Article in Saline for Treated Animals
  No skin response was evident in the control and in the treated animals.

- **Site 3: Third Pair of Injections**
  Freund's in Saline for Controls; Freund's in Saline and Test Article for Treated Animals
  The control group exhibited very slight or severe erythema and very slight or severe edema. Only one control animal (no. 6911) did not show any erythema on one side.
  Injections of Freund's, saline, and test article to treated animals produced very slight to severe erythema and very slight and severe edema in the majority of the animals. Treated animal no. 6916 showed no erythema nor edema at one site, treated animal no. 6918 showed no edema at one site, and treated animal no. 6921 showed no erythema at two sites and no edema at one site.

B. Challenge Treatment

No skin responses were evident in both the control and treated animals at 24- and 48-hour evaluations following the challenge treatments (see Appendix C).

CONCLUSIONS

The topical application of 100 mg of diuron (no. 12020-1; 97% purity) over a 4 CM^2 skin area, following the second induction, did not cause increased erythema, edema, or any indication of skin irritation in guinea pigs. Therefore, topical application of diuron with a 97% purity did not induce sensitization in guinea pigs under the conditions of this study. This study is currently classified as core-supplementary, because the positive control data were not provided. The sponsor should submit positive control data for the same strain tested during the same time frame as the study.

Classification: Core-supplementary. This study is upgradable after satisfactory review of the requested positive control data.

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APPENDIX A
Skin Response Grading Scale for Guinea Pigs Exposed to Diuron
(copied from p. 18 of the study report)

Erythema and Eschar Formation

No erythema.....0

Very slight erythema (barely perceptible).....1

Well defined erythema.....2

Moderate to severe erythema.....3

Severe erythema (beet redness) to slight eschar formation.....4

Edema Formation

No edema.....0

Very slight edema (barely perceptible).....1

Slight edema (edges of area well defined by definite raising).....2

Moderate edema (raised approximately 1 millimeter).....3

Severe edema (raised more than 1 millimeter and extending beyond the area of exposure).....4

APPENDIX B

Skin Responses Observed in Guinea Pigs Following Intradermal Injections Using Various Combinations of Freund's, Test Article, and Saline Suspensions to the Control and Treated Groups During the Induction Period (copied from p. 19 of the study report)

Scores of Intradermal Injection Sites

UIN	Sex	Group	ERYTHEMA / EDEMA 1 Left	1 Right	2 Left	2 Right	3 Left	3 Right
6907	F	Control	2/3	3/3	0/0	0/0	1/1	4/4
6908	F	Control	4/3	1/1	0/0	0/0	4/3	1/3
6909	F	Control	4/3	2/3	0/0	0/0	4/3	1/2
6910	F	Control	1/1	1/1	0/0	0/0	4/2	1/1
6911	F	Control	1/1	3/3	0/0	0/0	4/4	0/1
6912	F	Treated	4/4	4/4	0/0	0/0	1/1	2/2
6913	F	Treated	3/2	3/2	0/0	0/0	4/3	4/3
6914	F	Treated	3/2	4/2	0/0	0/0	4/4	4/3
6915	F	Treated	4/3	3/3	0/0	0/0	4/4	4/4
6916	F	Treated	0/0	0/0	0/0	0/0	4/4	0/0
6917	F	Treated	4/2	3/4	0/0	0/0	1/2	3/4
6918	F	Treated	1/1	1/3	0/0	0/0	1/2	1/0
6919	F	Treated	1/1	4/2	0/0	0/0	4/4	4/3
6920	F	Treated	4/3	4/4	0/0	0/0	4/4	4/4
6921	F	Treated	3/3	4/4	0/0	0/0	0/0	0/2

Site 1 = Freunds and Saline for Controls and Treated

Site 2 = Saline for Controls, Test Article and Saline for Treated

Site 3 = Freunds and Saline for Controls; Freunds, Saline and Test Article for Treated

APPENDIX C
Skin Responses Observed at 24 and 48 Hours Following Challenge Treatment with Diuron (25 mg/cm^2)
(copied from p. 20 of the study report)

UIN	Sex	Group	Erythema/Edema 24 Hours 5/31/85	Erythema/Edema 48 Hours 6/1/85
6907	F	Control	0/0	0/0
6908	F	Control	0/0	0/0
6909	F	Control	0/0	0/0
6910	F	Control	0/0	0/0
6911	F	Control	0/0	0/0
6912	F	Treated	0/0	0/0
6913	F	Treated	0/0	0/0
6914	F	Treated	0/0	0/0
6915	F	Treated	0/0	0/0
6916	F	Treated	0/0	0/0
6917	F	Treated	0/0	0/0
6918	F	Treated	0/0	0/0
6919	F	Treated	0/0	0/0
6920	F	Treated	0/0	0/0
6921	F	Treated	0/0	0/0

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Reviewer: David S. Liem, Ph.D. David Liem 7/30/92
Toxicology Branch II, Section II
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 7/30/92
Toxicology Branch II, Section II

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization Study Guideline: 81-6

HED PROJECT NO.: 1-2600 CASWELL NO.: 410 MRID NO.: 402288-04

TEST MATERIAL: Diuron (IN-14,760-146)

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl

STUDY NUMBERS: HLR 640-86

SPONSOR: Dupont de Nemours and Co., Agricultural Product Division, Wilmington, DE

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714

TITLE OF REPORT: Dermal Sensitization Study with IN-14,740-146 in Guinea Pigs

AUTHOR: John E. Henry

REPORT ISSUED: March 16, 1987

CONCLUSIONS:

Based on the results of the study, exposure to challenge dose of diuron (IN-14,170-146; 99% pure) of up to 80% concentration, following two weeks of induction did not cause increased erythema, edema, or any other indication of skin irritation in guinea pigs. Therefore, diuron is not considered a sensitizing agent in guinea pigs.

Classification: Core-minimum. This study satisfies USEPA's guideline 81-6 requirements for a dermal sensitization study in guinea pigs.

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STUDY TITLE: Dermal Sensitization Study with IN-14,740-146 in Guinea Pigs

Objectives: This study was designed to evaluate the dermal sensitization potential of Diuron in guinea pigs.

Test Materials: Diuron (N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea); purity 99%; tan solid; storage condition and pH of the test material were not provided in the study report. Diuron was assumed stable under the conditions of administration.

Test Animals: Albino guinea pigs (Duncan Hartley strain) obtained from Charles River Breeding Laboratories, New York were acclimated for at least 7 days prior to study initiation, and were housed in individual screened-bottom cages in a temperature-controlled room.

Feed and Water: Test animals were provided with Purina Certified Guinea Pig Chow #5026 and water ad libitum.

Environmental Parameters: Test animals were kept in a controlled environment; a 12 hours light/dark cycle; room temperature (23° ± 2°); relative humidity (50% ± 10%).

Body Weights: Individual body weights of all animals were recorded at the start and termination of the study.

PROCEDURES

A. To estimate the primary irritation potential of the compound, a rangefinding test was conducted on 3 males weighing from 328 to 355 grams. Aliquots (0.05 ml) of 80%, 40%, 20%, and 10% (wt/vol) suspensions of diuron in dimethyl phthalate were applied and lightly rubbed into separate test sites on the shaved intact skin of each animal's back. Dermal irritation responses were scored at 24 and 48 hours after treatment.

B. The main study (method was not cited) consisted of 3 phases:

- o A Primary Irritation Phase
  Ten guinea pigs (344-428 gms) were used. Aliquots (0.05 ml) of 80% and 8% (wt/vol) suspensions of diuron in dimethyl phthalate were applied and lightly rubbed into separate test sites on the shaved intact skin of each animal's back.
  Ten unexposed positive controls (422-520 gms) were treated by applying and lightly rubbing 0.05 ml of 30% and 3% (wt/vol) suspensions of p-phenylenediamine in acetone: dimethyl phthalate (1:9 ratio) onto separate sites of shaved intact shoulder skin of each animal.
  Ten unexposed guinea pigs (337-389 gms) were assigned as concurrent controls during the challenge phase.

- o Induction Phase (2 days after the primary dermal treatment)
  The same ten animals exposed in the primary irritation phase received four sacral intradermal injections (once a week) of 0.1 ml of a 1.0% (wt/vol) suspension of diuron in dimethyl-phthalate.
  The same ten positive controls treated in the primary phase also received four sacral intradermal injections (once a week) of 0.1 ml of a 1.0% (wt/vol) suspension of p-phenylene-diamine in acetone:dimethyl phthalate (1:9 ratio).

- o A Challenge Phase (after a 2-week rest period)
  The ten animals exposed in the primary irritation and in the induction phases were challenged for sensitization by applying and lightly rubbing 0.05 ml of 80% and 8% (wt/vol) suspensions of diuron in dimethyl phthalate onto separate test sites on the shaved intact skin of each animal's back.
  The ten unexposed guinea pigs (assigned as concurrent controls) now received the same topical treatment as the ten exposed animals noted above.
  The ten exposed positive control animals were challenged for sensitization by applying and lightly rubbing 0.05 ml of 30% and 3% (wt/vol) suspensions of p-phenylenediamine in acetone:dimethyl phthalate (1:9 ratio) onto separate test sites on the shaved intact skin of each animal's back.

Scoring

After each phase of the main dermal irritation study (24 and 48 hours after the primary irritation and challenge phases and 24 hours after the induction phase), skin responses were scored using the following scoring system:

- No Erythema or Edema	0
- Mild Erythema	1
- Moderate Erythema	2
- Strong Erythema	3
- Erythema and Edema	4
- Necrosis or Vesicles	5

The body weights were recorded weekly. The initial and the final body weights were reported in the study report.

RESULTS AND CONCLUSIONS

All animals survived to termination of the study. All animals gained weight during the study and the body weights were comparable among all groups.

A. Rangefinding Study

Diuron did not produce any dermal irritation when topically applied on guinea pigs at concentration as high as 80%.

B. Main Dermal Irritation Study

o Primary Irritation Phase

Topical application of diuron did not produce skin irritation in guinea pigs at 80% and 8% concentrations. No dermal irritation was present in the positive control guinea pigs at 30% and 3% concentrations of p-phenylenediamine in acetone:dimethyl phthalate (1:9 ratio).

o Induction Phase (Appendix A)

After the induction phase, the test animals exhibited strong erythema (score:3) or erythema and edema (score:4) with blanching (B) after each injection. Mild erythema (score:1) to erythema and edema with blanching (score:4B), and/or necrotic centers were noted in the positive control animals.

o Challenge Phase (Appendix B)

Diuron produced mild erythema (score:1) in three of the ten test guinea pigs at the 80% concentration sites at 24 and 48 hours after treatment. No dermal irritation was observed in the 8% concentration sites or in the concurrent control guinea pigs. These data indicate a negative response for dermal sensitization in guinea pigs challenged with diuron.

In the positive control group, p-phenylenediamine produced mild erythema (score:1) to erythema and edema (score:4) at the 30% concentration sites during the challenge phase. Based on the primary dermal irritation scores, nine of the ten positive controls had significant dermal score increases after the challenge phase.

CONCLUSIONS

Based on the results of the study, exposure to challenge dose of diuron (IN-14,170-146; 99% purity) of up to 80% concentration, following two weeks of induction did not cause increased erythema, edema, or any indication of skin irritation in guinea pigs. Therefore, diuron is not considered a sensitizing agent in guinea pigs.

Classification: Core-minimum. This study satisfies USEPA's guideline 81-6 requirements for a dermal sensitization study in guinea pigs.

APPENDIX A

Skin Responses Observed in Guinea Pigs Following Intradermal Injections of Diuron and in Positive Control Guinea Pigs Following Intradermal Injections of p-phenylenediamine (copied from p. 15-16 of the study report)

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS
FOLLOWING INTRADERMAL INJECTIONS OF IN-14,740-146

GUINEA PIG NUMBER	ID1 (LEFT)	ID2 (RIGHT)	ID3 (LEFT)	ID4 (RIGHT)
58653	3B	4B	3B	4B
58654	3B	4B	3B	4B
58655	3B	4B	4B	4B
58656	3B	4B	4B	4B
58657	3B	4B	3B	4B
58659	3B	4B	4B	4B
58660	3B	4B	3B	4B
58661	3B	4B	4B	4B
58662	3B	4B	4B	4B
58663	3B	4B	4B	4B

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL
GUINEA PIGS FOLLOWING INTRADERMAL INJECTIONS OF p-PHENYLENDIAMINE

GUINEA PIG NUMBER	ID1 (LEFT)	ID2 (RIGHT)	ID3 (LEFT)	ID4 (RIGHT)
58803	4B,NC	4B	2B	2B
58804	3B	4B	2B	2B
58805	4B	4B	2B	3B
58806	3B	4B	2B	2B
58807	2B	4B	2B	2B
58808	3B	4B	2B	2B
58809	2B	4B	2B	2B
58810	2B	4B	2B	2B
58811	2B	3B	1B,NC	2B
58812	1B	3B,NC	2B,NC	2B,NC

B = Blanching NC = Necrotic center

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APPENDIX B

Skin Responses Observed Following Topical Application of Diuron in Test and Concurrent Control Guinea Pigs, and Application of p-phenylenediamine in Positive Control Guinea Pigs After the Challenge Phase (copied from p.17-18 of the study report)

SKIN RESPONSES OBSERVED IN CONCURRENT CONTROL AND TEST GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF IN-14740-146

CONTROL GUINEA PIG NUMBER	LEFT FRONT 80% 24 hr	LEFT FRONT 80% 48 hr	RIGHT FRONT 8% 24 hr	RIGHT FRONT 8% 48 hr	TEST GUINEA PIG NUMBER	LEFT FRONT 30% 24 hr	LEFT FRONT 30% 48 hr	RIGHT FRONT 8% 24 hr	RIGHT FRONT 8% 48 hr
58643	0	0	0	0	58653	0	0	0	0
58644	0	0	0	0	58654	0	0	0	0
58645	0	0	0	0	58655	0	0	0	0
58646	0	0	0	0	58656	1	1	0	0
58647	0	0	0	0	58657	1	1	0	0
58648	0	0	0	0	58659	1	1	0	0
58649	0	0	0	0	58660	0	0	0	0
58650	0	0	0	0	58661	0	0	0	0
58651	0	0	0	0	58662	0	0	0	0
58652	0	0	0	0	58663	0	0	0	0

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF p-PHENYLENDIAMINE

CONTROL GUINEA PIG NUMBER	LEFT FRONT 30% 24 hr	LEFT FRONT 30% 48 hr	RIGHT FRONT 3% 24 hr	RIGHT FRONT 3% 48 hr
58803	2	4	2	1
58804	0	2	0	1
58805	4	4	2	1
58806	3	3	3	2
58807	2	3	2	2
58808	3	3	2	2
58809	1	2	0	0
58810	2	2	0	0
58811	1	2	1	1
58812	1	0	1	0

Reviewer: David S. Liem, Ph.D. David Liem 8/7/92
Toxicology Branch II, Section II
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 8/7/92
Toxicology Branch II, Section II

DATA EVALUATION REPORT 009738

STUDY TYPE: Dermal Sensitization Study Guideline: 81-6

HED PROJECT NO.: 1-2600 CASWELL NO.: 410 TRID NO.: 460094-019

TEST MATERIAL: Diurex Tech (Diuron)

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron)

STUDY NUMBERS: 1222F

SPONSOR: Makhteshim-Agan (America) Inc., 2 Park Ave, N.Y., N.Y.

TESTING FACILITY: Cosmopolitan Safety Evaluation (C.S.E.), Inc.
P.O. Box 71. Lafayette, N.J. 07848

TITLE OF REPORT: Guinea Pig Sensitization Study (Buehler)

AUTHOR: Gerald Rosenfeld

REPORT ISSUED: August 17, 1988

CONCLUSIONS: Based on the results of the study, exposure to challenge dose of diurex Tech (diuron), following two weeks of induction did not appear to cause increased erythema, edema, or any other dermal irritation effect in guinea pigs. Based on the results of the study, Diurex Tech appears to be a nonsensitizer in guinea pigs.

This study is currently classified as core-supplementary because the purity of Diurex Tech (diuron) was not specified, the initial and terminal body weights of the test and positive control animals were not recorded, no negative control tests (vehicle and test article) were conducted, and also it was not clear when the positive control test was conducted. The registrant must provide the purity of the test article and the interval in which the positive controls were dosed. This study may be upgraded, after satisfactory review of the requested information.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

STUDY TITLE: Guinea Pig Sensitization Study (Buehler)

Objectives: This study was designed to evaluate the dermal sensitization potential of Diurex technical (diuron).

Test Materials: Diurex Tech is labeled as DIUREX Tech (diuron); white powder; purity, storage condition and stability were not specified. For this study a 0.5 g of Diurex was made into a paste with 0.4 ml paraffin oil. Purity and source of vehicle were not specified.

Positive Control Test Material: p-phenylenediamine; source and purity were not specified.

Test Animals: Ten young albino guinea pigs males weighing 300 to 500 grams were obtained from Summit View Farm, NJ were housed in individual screened-bottom cages in a temperature-controlled room. The test animals were acclimated for five days.

Feed and Water: Test animals were provided with Agway Guinea Pig Feed and water ad libitum.

Environmental Parameters: As per Guide for the Care and Use of Laboratory Animals (DHEW #78-23, 1978).

Body Weights: Individual body weights of all animals were recorded at the start and at termination of the study.

PROCEDURES

The dermal sensitization study, described in the study report as the Buehler's Method[a], consisted of two phases:

o Induction Phase

Ten males guinea pigs were used. A 0.5 g Diurex as a paste was applied onto the right side of the shaved intact skin of each animal's back under a 20X20 mm Webril cloth patch. This patch was wrapped with a plastic film and secured with an adhesive bandage. After 6 hours the patch and the wrapping were removed and the test sites were washed with warm water. This induction procedure was conducted once a week for 3 consecutive weeks. Skin responses were scored at 24 and 48 hours after treatment.

o Challenge Phase (After a 2-week rest period)
Two weeks after the last induction treatment, the same ten exposed animals were challenged for sensitization by applying 0.5 g of Diurex paste onto the right side (previously exposed) and on the left unexposed side of the shaved intact skin of each test animal's back under a 20 X 20 mm Webril cloth patch. This

patch was then wrapped with a plastic film and secured with an adhesive bandage. After 6 hours the patch and the wrapping were removed and the test sites were washed with warm water. Skin responses were scored at 24 and 48 hours after treatment.

It was noted on p. 8 of the study report that "periodically a known sensitizer is tested under same conditions".

Negative control tests with either the vehicle and test article (diurex) were not conducted.

Evaluation and Scoring

The individual and mean scores at the previously treated (right side) and the left side at 24 and 48 hours following the challenge phase were compared to the average individual and group mean scores following the induction period. The skin irritation response was considered positive if the score following the challenge phase was higher than the average score during induction phase and also met the following criteria:

- o The erythema score was greater than one grade;
- o the edema score was greater than one grade; and
- o the erythema diameter size increased by more than 1 cm.

After each phase of treatment, skin responses were scored using the standard Draize scoring system[b] as follows:

a. Erythema and Eschar Formation

- No Erythema 0
- Slight Erythema (barely perceptible) 1
- Well-defined Erythema..... 2
- Moderate to Severe Erythema..... 3
- Severe Erythema to slight eschar formation..... 4

b. Edema Formation

- No edema... 0
- Slight Edema (barely perceptible)... 1
- Well-defined Edema..... 2
- Moderate Edema (raised $\pm$ 1 mm)..... 3
- Severe Edema (raised > 1 mm)..... 4

[a] Buehler, E.V. Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Derma., 91:171-175, 1965.

[b] Draize, J.H. Appraisal of the Safety of Chemical in Foods, Drugs and Cosmetics. Assoc. Food and Drug Official, 1959.

RESULTS AND DISCUSSIONS

A rangefinding study was not conducted to determine the dosages used for the main dermal sensitization study.

Based on the individual data presented, all animals survived to termination of the study. The initial and terminal body weight data of the test animals were not provided in the study report.

A. Dermal Sensitization Study (Buehler's Method)

o Induction Phase

Topical application of Diurex made into a paste with paraffin oil produced erythema (grade 1) in three male guinea pigs at the 24 hours evaluation after treatment. None of the animals exhibited dermal response after the 2nd and 3rd induction treatments (Appendices IA and IB of the study report).

o Challenge Phase

During the challenge phase, erythema (grade 1) was noted in three animal on the previously exposed site (right site) and in one animal on the previously unexposed site (left site) at 24 hours after treatment. At the 48 hour evaluation, erythema (grade 1) was noted on the left site of the shaved back of one animal.

B. Positive and Negative Controls

It was noted on p. 8 of the study report that "periodically a known sensitizer is tested under same conditions" and served as positive controls. It is not clear whether this positive control test was conducted concurrently. Also the body weights, sex, and the test animal strain used were not provided. Individual scores for positive controls were not provided but the mean scores of the positive controls were presented on p. 10 of the study report, and copied in the attached Appendix A. Negative control tests with both the vehicle and test article were not conducted.

C. Evaluation of the Test Results

The mean scores of topical application of diurex (in paraffin oil) on guinea pigs following induction and challenge phases as compared to the positive controls treated with p-phenylenediamine in saline are presented in Appendix A. The mean scores following the challenge were not substantially higher than the mean scores following the induction phase for the test animals. The positive controls showed a strong positive sensitizing response. Based on these results presented and the criteria set in this study report, diurex Tech (diuron) appears to be a nonsensitizer in guinea pigs.

CONCLUSIONS

Based on the results of the study, exposure to challenge dose of diurex Tech (diuron), following two weeks of induction did not appear to cause increased erythema, edema, or any other dermal irritation effect in guinea pigs. Based on the results of the study, Diurex Tech appear to be a nonsensitizer in guinea pigs.

This study is currently classified as core-supplementary because the purity of Diurex Tech (diuron) was not specified, the initial and terminal body weights of the test and positive control animals were not recorded, negative control tests (vehicle and test article) were not conducted, and it was not clear when the positive control test was conducted. The registrant must provide the purity of the test article and the interval in which the positive controls were dosed. This study may be upgraded, after satisfactory review of the requested information.

Classification: Core-supplementary. This study does not satisfy USEPA's guideline 81-6 requirements for a dermal sensitization study in guinea pigs. It is upgradable after satisfactory review of the requested information.

APPENDIX A

Summary Mean Skin Irritation Scores of Topical Application of Diurex Following Induction and Challenge Phases and Summary Mean Scores of Positive Control Guinea Pigs Treated Topically with p-phenylenediamine (copied from p. 10 of the study report)

	ERYTHEMA + EDEMA			
	24 Hours		48 Hours	
	Induction	Challenge	Induction	Challenge
Test Article	0.10 ± 0.3	0.20 ± 0.4	0.00 ± 0.0	0.05 ± 0.2
Positive Control	0.87 ± 0.8	2.50 ± 0.7	0.63 ± 0.6	2.15 ± 0.4

	DIAMETER (cm)			
	24 Hours		48 Hours	
	Induction	Challenge	Induction	Challenge
Test Article	0.18 ± 0.6	0.40 ± 0.8	0.00 ± 0.0	0.11 ± 0.5
Positive Control	1.05 ± 0.8	3.41 ± 0.3	0.96 ± 0.9	3.53 ± 0.3

009738

Reviewer: David S. Liem, Ph.D. David Liem 8/4/92
Toxicology Branch II, Section II
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel
Toxicology Branch II, Section II
8/4/92

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization Study Guideline: 81-6

HED PROJECT NO.: 1-2600 CASWELL NO.: 410 MRID NO.: 409840-04

TEST MATERIAL: Krovar I DF (IN-M2574-25) - A mixture of 80% of two active ingredients (50% diuron and 50% bromacil)

SYNONYMS: Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron) and 5-bromo-6-methyl-3-(1-methylpropyl)-2,4-(1M,3H)-pyrimidinedione (bromacil); Pro-Serve (tradename)

STUDY NUMBERS: HLR 533-88

SPONSOR: Dupont de Nemours and Co., Agricultural Product Division, Wilmington, DE

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19714

TITLE OF REPORT: Closed- Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN-M2574-25 in Guinea Pigs

AUTHOR: William G. Brock

REPORT ISSUED: August 17, 1988

CONCLUSIONS:

Based on the results of the study, exposure to challenge dose of Krovar I DF as received (IN-M2574-25), a mixture of 80% of two active ingredients (50% diuron and 50% bromacil), following two weeks of induction did not cause increased erythema, edema, or any other skin irritation effect in guinea pigs. Krovar I DF (IN-M2574-25) is not considered a sensitizing agent in guinea pigs.

This study is currently classified as core-supplementary because the purity of both active ingredients, diuron and bromacil, was not specified, the physical form of Krovar was not clear (see p. 4 nad 9), and the percent of technical impurities noted on p. 4 was not clear. The registrant should also give a detailed description of the test material used in this study. This study is upgradable, after satisfactory review of the requested information.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

STUDY TITLE: Closed- Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN-M2574-25 in Guinea Pigs

Objectives: This study was designed to evaluate the dermal sensitization potential of Krovar (a mixture of 50% diuron and 50% bromacil) in guinea pigs.

Test Materials: Krovar (N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea); 80% active ingredient (1:1 ratio of diuron and bromacil) and 20% inert ingredient; 5.5% technical impurities (not specified ?); purity of diuron and bromacil were not specified; tan solid; storage condition and pH (if liquid) of the test material were not provided in the study report. Krovar was assumed to be stable under the conditions of administration.

Positive Control Test Material: 1-chloro-2-4-dinitrobenzene with a purity of 98%; lot# Ai1G; source: Eastman-Kodak Co. The test material was assumed to be stable under the conditions of administration.

Test Animals: Albino guinea pigs (Duncan Hartley strain) obtained from Charles River Breeding Laboratories, New York were acclimated for at least 7 days prior to study initiation, and were housed in individual screened-bottom cages in a temperature-controlled room.

Feed and Water: Test animals were provided with Purina Certified Guinea Pig Chow #5026 and water ad libitum.

Environmental Parameters: Test animals were kept in a controlled environment; a 12 hours light/dark cycle; room temperature ($23^{\circ} \pm 2^{\circ}$); relative humidity (50%$\pm$ 10%).

Body Weights: Individual body weights of all animals were recorded at the start and at termination of the study.

PROCEDURES

A. To estimate the primary irritation potential of the compound, a rangefinding study was conducted on 2 males and 2 females weighing from 373 to 435 grams. Aliquots of 0.4 ml (equivalent to 0.2203 gm) of 60%, 30%, and 15% (Wt/vol) suspensions of the test material in distilled water were applied onto the shaved intact skin of each animal's back under a water-moistened patch (25 mm Hill Top Chamber Delivery System). This patch was wrapped with a plastic wrap and secured with an adhesive bandage. After six hours the patch and the wrapping were removed and then the test sites were washed with warm water. Dermal irritation responses were scored about 24 hours after treatment.

B. The main dermal sensitization study (Buehler's Method) consisted of two phases:

- o Induction Phase
  Five males and 5 females (434-571 gms) were used. Krovar (0.4 ml?) as received was applied onto the shaved intact skin of each animal's back under a water-moistened patch (25 mm Hill Top Chamber Delivery System). This patch was wrapped with a plastic wrap and secured with an adhesive bandage. After 6 hours the patch and the wrapping were removed and the test sites were washed with warm water. This induction procedure was conducted once a week for 3 consecutive weeks.

  Following the same procedure described above, 5 positive control guinea pigs (3♂ and 2♀) weighing from 447 to 620 grams, were treated with a 0.4 ml suspension of 0.2% DNCB (1-chloro-2-4-dinitrobenzene) in 80% ethanol in water Skin responses were scored at 24 and 48 hours after treatment.

- o Challenge Phase (After a 2-week rest period)
  Two weeks after the last induction treatment, the same ten exposed animals were challenged for sensitization by applying 0.4 ml(?) of Krovar as received onto the shaved intact skin of each animal's back under a water-moistened patch (25 mm Hill Top Chamber Delivery System). This patch was wrapped with a plastic wrap and secured with an adhesive bandage. After 6 hours, the patch and the wrapping were removed and the test sites were washed with warm water.

  The five positive control guinea pigs (3♂ and 2♀) were challenged for sensitization by applying 0.4 ml suspension of 0.2% DNCB in 80% ethanol in water.

  Two groups of five unexposed guinea pigs (2 ♂ and 3♀), weighing from 404 to 609 gms, assigned as negative controls were treated with 0.4 ml(?) of Krovar as received and 0.1% DNCB (in 80% ethanol in water), respectively, following the same challenge procedure noted above.

  Twenty-three hours after treatment, a depilatory was placed on the test sites for 30 minutes, then washed with warm water to remove the depilatory. Irritation responses were scored 2 hours after depilation and again 48 hours after treatment.

Evaluation and Scoring

The incidence of sensitization is defined as the number of animals sensitized by the test materials divided by the total number of animals in that group. The severity of the skin irritation response is the sum of test scores divided by the total number of animals. Evaluations were conducted at 24 and 48 hours after treatment.

After each phase of treatments, skin responses were scored using the following scoring system:

- No Erythema or Edema..... 0
- Slight Erythema (usually nonconfluent)..... 1
- Mild (well defined) Erythema (usually confluent... 2
- Moderate Erythema..... 3
- Severe Erythema, with or without Edema, necrosis or eschar formation..... 4

Body weights were recorded weekly. The initial and the final body weights were reported in the study report.

RESULTS AND CONCLUSIONS

All animals survived to termination of the study. All animals gained weight during the study and the body weights were comparable among all groups.

A. Rangefinding Study

Krovar did not produce any dermal irritation when topically applied on guinea pigs at concentration as high as 60%.

B. Main Dermal Irritation Study (Buehler's Method)

o Induction Phase (Appendix A)

Topical application of Krovar as received did not produce any dermal irritation in guinea pigs. Slight or mild erythema was observed in three positive controls after the first induction treatment. All positive controls had mild or severe erythema with superficial necrosis after the 2nd and 3rd induction treatments.

o Challenge Phase (Appendix B)

During the challenge phase, one test animal exhibited severe erythema, but this was considered to be due to mechanical injury during the removal of the patch. Another test animal exhibited a slight erythema at the 48 hours evaluation.

In the positive control group, 3 mild and 2 moderate erythema were observed at the 24 hours evaluation, and 1 slight and 4 mild erythema at the 48-hour evaluation. Based on the incidence and severity response data, positive dermal irritation effects were noted in the positive control guinea pigs at 24 and 48 hours evaluations.

No dermal irritation was observed in the negative controls treated with Krovar. Only one negative control treated with 0.1% DNCB exhibited slight erythema. No significant increase of dermal irritation was noted in any animal.

CONCLUSIONS

Based on the results of the study, exposure to challenge dose of Krovar I DF as received (IN-M2574-25), a mixture of 80% of two active ingredients (50% diuron and 50% bromacil), following two weeks of induction did not cause increased erythema, edema, or any other dermal irritation effect in guinea pigs. Krovar I DF (IN-M2574-25) is not considered a sensitizing agent in guinea pigs.

This study is currently classified as core-supplementary because the purity of both active ingredients, diuron and bromacil, was not specified, the physical form of Krovar was not clear (see p. 4 nad 9), and the percent of technical impurities noted on p. 4 was not clear. The registrant should also give a detailed description of the test material used in this study. This study is upgradable, after satisfactory review of the requested information.

Classification: Core-supplementary. This study does not satisfy USEPA's guideline 81-6 requirements for a dermal sensitization study in guinea pigs. It is upgradable after satisfactory review of the requested information.

APPENDIX A

Skin Responses Observed in Guinea Pigs Following Close-Patch Topical Exposure to Krovar and Positive Control Guinea Pigs Following Close-Patch Topical Exposure to 0.2% DCNB (copied from p. 13-14 of the study report)

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS FOLLOWING CLOSED-PATCH TOPICAL EXPOSURE TO IN M2574-25

GUINEA PIG NUMBER	Induction #1		Induction #2		Induction #3	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
52640	0	0	0	0	0	0
62641	0	0	0	0	0	0
62642	0	0	0	0	0	0
62643	0	0	0	0	0	0
62644	0	0	0	0	0	0
62685	0	0	0	0	0	0
62686	0	0	0	0	0	0
62687	0	0	0	0	0	0
62688	0	0	0	0	0	0
62689	0	0	0	0	0	0

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL GUINEA PIGS FOLLOWING CLOSED-PATCH TOPICAL EXPOSURE TO 0.2% DNCB

GUINEA PIG NUMBER	Induction #1		Induction #2		Induction #3	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
62645	0	1	4SN	4SN	4SN	4SN
62646	1	1	4SN	4SN	4SN	4SN
62647	0	1	4SN	4SN	4SN	4SN
62690	0	2	2	4SN	2	2
62691	0	0	2	2	2	2

SN = Superficial Necrosis

APPENDIX B
Summary of Skin Responses, and the Incidence and Severity Scores of the Test, Positive, and Negative Control Animals After the Challenge Phase (copied from p. 11 and 18 of the study report)

Summary of Skin Responses

Challenge Phase

Response	Test		Negative Control				Positive Control	
			IN M2574-25		DNCB			
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
No reaction	9/10	8/10	5/5	5/5	4/5	4/5	0/5	0/5
Slight erythema	0/10	1/10	0/5	0/5	1/5	1/5	0/5	1/5
Mild erythema	0/10	0/10	0/5	0/5	0/5	0/5	3/5	4/5
Moderate erythema	0/10	0/10	0/5	0/5	0/5	0/5	2/5	0/5
Severe erythema	1/10	1/10	0/5	0/5	0/5	0/5	0/5	0/5

INCIDENCE AND SEVERITY OF RESPONSES AT CHALLENGE[a]

Test Article	24 hr		48 hr	
	Incidence	Severity	Incidence	Severity
Test Group Animals (IN M2574-25)	0/10	0[b]	2/10	0.1[b]
Negative Control Animals (IN M2574-25)	0/5	0	0/5	0
Negative Control Animals (0.1% DNCB)	0/5	0.2	0/5	0.2
Positive Control Animals (0.1% DNCB)	5/5	2.4	4/5	1.8

[a] The incidence of sensitization is defined as the number of animals in each group sensitized to the test material divided by the total number of animals tested in that group. Severity of the irritation response is the sum of the test scores in each group divided by the total number of animals tested in that group for both the 24- and 48-hour evaluations.

[b] This value does not include the guinea pig that exhibited the severe erythema that was considered to be related to removal of the patch.

009738

Reviewer: David S. Liem, Ph.D. David Liem 8/10/92
Toxicology Branch II, Section II
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 8/10/92
Toxicology Branch II, Section II

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization Study Guideline: 81-6

HED PROJECT NO.: 1-2600 CASWELL NO.: 410 TRID NO.: 460075-16

TEST MATERIAL: A mixture of Urea, N'-(3,4-dichlorophenyl)-N,N-dimethyl (diuron) and 5-bromo-6-methyl-3-(1-methylpropyl)-2,4-(1M,3H)-pyrimidinedione (bromacil); Haskell # 15,600; INM-2574.

SYNONYMS: Eighty percent of Krovar. II DF (a mixture of two active ingredients: 53% diuron and 27% bromacil) and 20% inerts

STUDY NUMBERS: HLR 201-797

SPONSOR: Dupont de Nemours and Co., Agricultural Product Division, Wilmington, DE

TESTING FACILITY: Hazelton Laboratories America, Vienna, VA

TITLE OF REPORT: Dermal Sensitization Study in Guinea Pigs

AUTHOR: James L. Gargus (Study Director)

REPORT ISSUED: March 5, 1985

CONCLUSIONS: Based on the data and method as presented in the study report, the result of this study could not be evaluated because no positive control was conducted. Furthermore, the following information was not provided: the method used for the dermal sensitization test, sex and strain of the test animals, purity of the two active ingredients (diuron and bromacil), initial and terminal body weights of test animals, and the environmental parameters of the study room. This study is presently classified as core-supplementary. It may be upgradable if the registrant provides the following:

- o positive control data from a study (same sex and strain) conducted at that facility during the same time frame; and
- o the purity of the two active ingredients of the test materials.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

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STUDY TITLE: Dermal Sensitization Study in Guinea Pigs

Objectives: This study was designed to evaluate the dermal sensitization potential of INM-2574 (Eighty percent of Krovar II DF, a mixture of two active ingredients: 53% diuron and 27% bromacil) in guinea pigs.

Test Materials: INM-2574; Haskell # 15,600; white powder; Krovar II DF - A mixture of 80% of two active ingredients (53% diuron and 27% bromacil) and 20% inert; stability was not specified; stored at room temperature.
Saline (0.9% Sodium chloride injection, USP, Travenol Laboratories) a clear colorless liquid, was used as the vehicle.

Test Animals: Twenty three albino guinea pigs (Hartley strain) obtained from Hazelton Dutchland Inc, Denver, PA, were acclimated for at least 7 days prior to study initiation, and were housed in individual screened-bottom cages.

Feed and Water: Test animals were provided with Purina Guinea Pig Chow #5026 and water ad libitum.

Environmental Parameters: The light/dark cycle, room temperature, and relative humidity were not specified.

Body Weights: The initial and terminal body weights of test animals were not provided in the study report.

PROCEDURES

A. To estimate the primary irritation potential of the compound, a range-finding test was conducted on 3 animals. Aliquots (0.05 ml) of 75%, 50%, and 75% (wt/vol) of test article in saline were applied on the shaved intact skin of each animal's back. Dermal responses were scored according to the Draize[b] System at 24 and 48 hours after treatment. Based in the results of this study, the concentrations used for the main dermal study were determined.

B. The main study (method was not cited).
Twenty guinea pigs (weight and sex were not specified) were used. They were grouped into two groups of ten, the test and the control groups. This main study consisted of 3 phases:

- Primary Irritation Phase
  Ten test guinea pigs were used. Aliquots (0.05 ml) of 75% and 7.5% (wt/vol) suspensions of Krovar II in saline were applied onto the shaved intact skin of each animal's back. Dermal responses were scored in order to compare them with the challenge scores.

Ten unexposed negative controls were treated by applying saline (vehicle) onto separate sites of shaved intact shoulder skin of each animal.

- o Induction Phase
  The same ten test animals exposed in the primary irritation phase received four sacral intradermal injections (once a week) of 0.5 ml of Krovar II suspension in 1% saline. The same ten negative vehicle controls treated in the primary phase also received four sacral intradermal saline (vehicle) injections, once a week.

- o Challenge Phase (after a 2-week rest period)
  The ten animals exposed in the primary irritation and in the induction phases were challenged for sensitization by applying 0.05 ml of 75% and 7% (wt/vol) suspensions of Krovar II on the shaved intact skin of each animal's back.
  The ten negative vehicle controls were challenged for sensitization by applying saline on the shaved intact skin of each animal's back.
  The challenge phase was repeated one week later using the same method and concentration.

C. Scoring

At 24 and 48 hours after each application in the range-finding, primary irritation, and challenge phases, skin responses were scored using the standard Draize Scoring System[b]. After each induction phase treatment, the test sites were observed for necrosis and erythema after 24 hours of treatment only. The Draize Scoring System is as follows:

a. Erythema and Eschar Formation
- No Erythema 0
- Slight Erythema (barely perceptible) 1
- Well-defined Erythema..... 2
- Moderate to Severe Erythema..... 3
- Severe Erythema to slight eschar formation..... 4

b. Edema Formation
- No edema... 0
- Slight Edema (barely perceptible)... 1
- Well-defined Edema..... 2
- Moderate Edema (raised ± 1 mm)..... 3
- Severe Edema (raised > 1 mm)..... 4

[b] Draize, J.H. Appraisal of the Safety of Chemical in Foods, Drugs and Cosmetics. Assoc. Food and Drug Official, 1959.

RESULTS AND CONCLUSIONS

All animals survived to termination of the study.

A. Range-finding Study

Krovar II did not produce any dermal irritation when topically applied on guinea pigs at concentration as high as 75%.

B. Main Dermal Irritation Study

o Primary Irritation Phase

Topical application of Krovar II did produce a slight erythema in two guinea pigs at 75% concentrations at 24 hours post-dose. No dermal irritation was present in the negative controls (Tables 1 of the study report).

o Induction Phase (Appendix A)

Twenty-four hours after each injection during the induction phase, all test animals exhibited slight to mild erythema. The vehicle control animals did not exhibit any dermal response after intradermal injection with saline (vehicle) solution (Appendix A).

o Challenge Phase (Appendix B)

Krovar II produced slight erythema in one of the ten test guinea pigs at the 75% concentration at 24 hours after the first challenge. No dermal response was observed in any test animals after the second challenge. The negative (vehicle) controls did not show any dermal responses after the two challenges.

CONCLUSIONS

Based on the data and method as presented in the study report, the result of this study could not be evaluated because no positive control was conducted. Furthermore, the following information was not provided: the method used for this skin sensitization test, sex and strain of the test animals, purity of the two active ingredients (diuron and bromacil), initial and terminal body weights of test animals, and the environmental parameters of the study room. This study is now classified as core-supplementary. It may be upgradable if the registrant provides the following:

- o positive control data from a study (same sex and strain) conducted at that facility during the same time frame; and
- o the purity of the two active ingredients of the test materials.

Classification: Core-supplementary. It is upgradable after satisfactory review of the requested information.

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APPENDIX A

Skin Responses Observed in Test Animals During the Induction Period Following Intradermal Injections with Krovar II and in Negative (Vehicle) Controls Following Injection with Saline Solution (p. 11-13 of the study report)

009738

Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study of Hoechst No. 15,680 in Guinea Pigs
Induction Phase - Test Animals

Animal Number	Week 1 - Left Side - Injection 1		Week 2 - Right Side - Injection 2	
	Erythema	Necrosis	Erythema	Necrosis
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0

Animal Number	Week 3 - Left Side - Injection 3		Week 4 - Right Side - Injection 4	
	Erythema	Necrosis	Erythema	Necrosis
[illegible]	2	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	2	0
[illegible]	1	0	1	0
[illegible]	2	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	1	0	1	0
[illegible]	2	0	2	0

Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study of Hoechst No. 15,680 in Guinea Pigs
Induction Phase - Control Animals

Animal Number	Week 1 - Left Side - Injection 1		Week 2 - Right Side - Injection 2	
	Erythema	Necrosis	Erythema	Necrosis
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0

Animal Number	Week 3 - Left Side - Injection 3		Week 4 - Right Side - Injection 4	
	Erythema	Necrosis	Erythema	Necrosis
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0
[illegible]	0	0	0	0

APPENDIX B

Skin Responses Observed Following the First and the Second Challenge of Krovar II in the Test Animals (copied from p.15 and 17 of the study report)

Primary Irritation and Sensitization Study of Haskell No. 15,[illegible] in Guinea Pigs
First Challenge Phase - Test Animals

Animal Number	Site Number	Dose Level %	24 Hours Erythema	24 Hours Necrosis	48 Hours Erythema	48 Hours Necrosis
MB7039	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7040	1	7.5	0	0	0	0
	2	75	1	0	0	0
MB7041	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7042	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7043	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7044	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7045	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7046	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7047	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7048	1	7.5	0	0	0	0
	2	75	0	0	0	0

Primary Irritation and Sensitization Study of Haskell No. 15,[illegible] in Guinea Pigs
Second Challenge Phase - Test Animals

Animal Number	Site Number	Dose Level %	24 Hours Erythema	24 Hours Necrosis	48 Hours Erythema	48 Hours Necrosis
MB7039	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7040	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7041	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7042	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7043	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7044	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7045	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7046	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7047	1	7.5	0	0	0	0
	2	75	0	0	0	0
MB7048	1	7.5	0	0	0	0
	2	75	0	0	0	0