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

DEC 10 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Submission of Toxicology Data In Response to Testing Requirements of the 2,4-D Registration Standard for Derivatives of 2,4-D.

FROM: Jess Rowland, Toxicologist Jess Rowland 11/28/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Bert Baker
Product Manager (74)
Registration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 11/29/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief
Toxicology Branch II (HFAS) Marcia van Gemert 11/29/90
Health Effects Division (H7509C)

ACTION REQUESTED: Evaluate three 21-day dermal toxicity studies with derivatives of 2,4-D in support of regulatory action.

STUDY IDENTIFICATION: EPA I.D. No.:0073 Record No.: 261,250
Registrant: DowElanco HED Project No.: 0-0696

I. 2,4-Dichlorophenoxyacetic acid butoxyethyl ester: 21-day Dermal toxicity study in New Zealand White Rabbits. MRID No. 414079-01.

II. 2,4-D triisopropanolamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. MRID No. 414079-02.

III. 2,4-D isopropylamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. MRID No.414079-03.

RESPONSE: A separate Data Evaluation Report (DER) for each of the above referenced studies are attached. All three studies satisfy the toxicology guideline requirements (82-2), and are acceptable for regulatory purposes. A summary of each study is provided below:

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I. 2,4-Dichlorophenoxyacetic acid butoxyethyl ester: 21-day Dermal toxicity study in New Zealand White Rabbits. MRID No. 414079-01.

Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-Dichlorophenoxyacetic acid butoxyethyl ester (2,4-D BEE) in corn oil at 0, 50, 150 or 500 mg/kg/day. Dermal irritation observed at the site of application of various animals in all dose groups, including the controls, included erythema, edema and scaling. Histopathology revealed slight fibrosis, epithelial hyperplasia and acute inflammation at the treated skin of some males and females from all dose groups, including the control. There was no evidence of systemic toxicity; 2,4-D BEE had no adverse effects on survival, body weight gain, clinical signs, hematology and clinical chemistry parameters, absolute and relative organ weights, gross pathology, or histopathology.

NOEL= 500 mg/kg/day (HDT) for dermal irritation and systemic toxicity.

CORE CLASSIFICATION: Minimum; satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

II. 2,4-D triisopropanolamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. MRID No. 414079-02.

Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D triisopropanolamine salt (2,4-D TIPA) in distilled water at 0, 100, 350 or 1000 mg/kg/day. Dermal irritation was limited to very slight, transient erythema at the application site. Histologically they were characterized as very slight or slight inflammatory reaction of the dermis seen in two animals at 100 mg/kg/day, four at 350 mg/kg/day, and five at 1000 mg/kg/day. The inflammatory reaction was confined to the superficial dermis; no epidermal lesions were seen. 2,4-D TIPA did not induced systemic toxicity; no treatment-related effects were observed on survival, body weight gain, clinical signs, organ weights, hematology and clinical chemistry, organ weights, gross or histopathology.

NOEL= 1000 mg/kg/day (HDT) for dermal irritation and systemic toxicity.

CORE CLASSIFICATION: Minimum; satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

III. 2,4-D isopropylamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits. MRID No.414079-03.

Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D isospropylamine salt (2,4-D IPA) in distilled water at 0, 50, 125 or 350 mg/kg/day. Treatment caused dose-dependent dermal irritation manifested by slight, transient erythema at 50 mg/kg/day, slight erythema and scaling at 125 mg/kg/day, and slight to moderate erythema at 350 mg/kg/day. Histologically dermal lesions were characterized as focal or multifocal irritative effects, namely, inflammation and epidermal hyperplasia; lesions were seen only at the 125 and 350 mg/kg/day levels. No histopathological dermal lesions were seen at the 50 mg/kg/day. 2,4-D IPA did not induce systemic toxicity which was evident by the lack of effects on survival, body weight gain, clinical signs, hematology or clinical chemistry parameters, organ weights, gross, or histopathology.

NOEL= 50 mg/kg/day for dermal irritation; 350 mg/kg/day (HDT) for systemic toxicity.

LOEL= 125 mg/kg/day for dermal irritation.

CORE CLASSIFICATION: Minimum; satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 4/28/90
Toxicology Branch II, Section II (H7509C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 4/30/90
Toxicology Branch II, Section II (H7509C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

MRID NO. 414679-01 CASWELL NO.: 315AI

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE)

SYNONYMS: 2,4-D BEE, 2,4-D butoxyethylester

STUDY NUMBER: K-007722-008

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-Dichlorophenoxyacetic acid butoxyethyl ester: 21-Day Dermal Toxicity Study in New Zealand White Rabbits

AUTHORS: M.J. Misell, L. Atkin and J. W. Crissman

REPORT ISSUED: February 21, 1990

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE) in corn oil at 0, 50, 150 or 500 mg/kg/day. Dermal irritation observed at the site of application of various animals in all dose groups, including the controls, was characterized by erythema, edema and scaling. Histopathology revealed slight fibrosis, epithelial hyperplasia and acute inflammation at the treated skin of some males and females from all dose groups, including the corn oil control. There was no evidence of systemic toxicity; no treatment-related effects were observed on survival, clinical signs of toxicity, body weight gain, hematology and clinical chemistry, organ weights, gross pathology or histopathology. Under the conditions of this study, a no-observed-effect-level (NOEL) of 500 mg/kg/day was established for the dermal irritation and systemic toxicity of 2,4-D BEE in rabbits.

GUIDELINE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-Dichlorophenoxyacetic acid, butoxyethyl ester (2,4-D BEE).

II. MATERIALS AND METHODS

1. Test Material: 2,4-Dichlorophenoxyacetic Acid, butoxyethyl ester (2,4-D BEE)

Lot No.	AGR 276426
ID No.:	K-007722-008
Description:	Dark Amber
Purity:	94.6%
Homogeneity:	stability and homogensity of 2,4-D BEE dose solutions was determined concurrently with this study

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Feed:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D BEE following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received four dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose level of 2024 mg 2,4-D BEE/kg/day or 1300 mg/kg/day 2,4-D acid equivalent. Owing to irritation at this level, the material was diluted 1:3 with corn oil and applied to another rabbit at a rate of 1.7 ml/kg which corresponded to a dose of 562 mg 2,4 D BEE or 361 mg/kg 2,4 D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 50, 150, and 500 mg/kg/day. The test material was diluted in corn oil, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). A control group received corn oil (1.7 ml/kg) only.

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Barlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way ANOVA's with Bonferroni's Correction.

III. RESULTS

(i). Probe study

In the probe study, when tested at 2024 mg/kg/day, irritation and moderate to severe erythema, and well defined edema and moderate to severe fissuring was observed at the site of application. When diluted to 1:3 with corn oil, and applied at 562 mg/kg/day, well defined erythema was seen at the test site. No systemic toxicity was observed. Based on these results, 50, 100, or 500 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Signs of localized reaction to treatment consisted of dermal irritation at the site of application in various animals in all dose groups including the corn oil control. Two male and two female controls had slight, transient edema and/or scaling. One female control had very slight erythema at the test site. At the 50 mg/kg/day level, 4 males and 3 females showed very slight erythema and edema, and one female had slight scaling at the test site. All animals at the 150 mg/kg /day level developed slight erythema and edema, and 4 females had slight scaling at the test site. Well defined erythema and edema were present in 5 males and 4 females at the high-dose (500 mg/kg/day). In addition, slight scaling at the test site was observed in both sexes of rabbits at the high dose.

4. Body Weight: Treatment had no adverse effect on body weight; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen in mean hematology or clinical chemistry parameters in treated groups when compared to appropriate corresponding control group values.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights. The statistically significant increase observed in absolute liver weights of both sexes of rabbits at the 100 mg/kg/day was not considered to be toxicologically significant since the increase was not dose-dependent, there was no effect on relative liver weights, and histopathology showed no evidence of liver damage.

7. Gross Pathology: Treatment-related gross pathological changes were limited to the very slight to slight dermal thickening seen in male rabbits at the mid-and high-dose groups. Slight erythema, scabs and scales were observed in an incidence unrelated to exposure. The authors stated that the apparent discrepancy between gross pathological observations and dermal observations (dermal scoring) was most likely due to exsanguination of the animals and the subsequent blood loss which occurs.

8. Histopathology: There was no evidence of pathological changes attributable to 2,4-D BEE. Lesions observed at the application site were acute inflammation, epithelial hyperplasia and fibrosis; these lesions were seen in the control and treated groups and showed neither a dose response nor trend (Table 1). Histopathology of the liver, kidneys from high dose animals revealed no evidence of systemic toxicity.

XV. DISCUSSION

In a 21-day dermal toxicity study, 15 repeated dermal applications of 2,4-D BEE at 0, 50, 150, or 500 mg/kg/day produced dermal irritation (at the application site) characterized by erythema, edema, and scaling. Histologically, the dermal lesions were diagnosed as slight fibrosis, epithelial hyperplasia and acute inflammation. These lesions were seen in some males and some females in all test groups including the corn oil control. Since the dermal lesions were seen both in the control and treated groups and showed no dose-response the dermal lesions were not considered to be compound-induced. 2,4-D BEE did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathology.

V. CONCLUSION

Under the conditions of this study, a NOEL of 500 mg/kg/day is established for both dermal irritation and systemic toxicity of 2,4-D BEE.

VI. CORE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

Table 1. DERMAL LESIONS AT APPLICATION SITE IN RABBITS RECEIVING 15-DERMAL APPLICATIONS OF 2,4-D BEE.

Sex	Males				Females			
Dose (mg/kg/day)	0	50	150	500	0	50	150	500
No. Examined	5	5	5	5	5	5	5	5
Dermal lesions								
Fibrosis, very slight	1	0	0	0	2	2	1	0
Fibrosis, slight	0	0	0	0	0	0	1	0
Hyperplasia, epithelial								
very slight	3	5	5	5	4	2	3	4
slight	2	0	0	0	1	2	2	0
Inflammation acute								
very slight	1	5	2	4	3	1	1	5
slight	0	0	1	1	0	2	2	0
moderate	0	0	1	0	0	1	1	0
severe	1	0	1	0	2	1	1	0

Data are the numbe of animals with specified observations.

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 11/28/90
Toxicology Branch II, Section II (H7059C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 11/29/90
Toxicology Branch II, Section II (H7059C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

EPID NO. 414079-02 CASWELL NO.: 315U

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid, triisopropanolamine salt

SYNONYMS: 2,4-D triisopropanolamine salt; (2,4-D TIPA)

STUDY NUMBER: K-006866-004

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-D Triisopropanolamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits.

AUTHORS: M.J. Misell, L. Atkin, K.T. Haut and K.E. Stebbins

REPORT ISSUED: December 8, 1989

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D Triisopropanolamine salt (2,4-D TIPA) in distilled water at 0, 100, 350 or 1000 mg/kg/day. Dermal irritation was limited to very slight, transient erythema at the application site. Histologically they were characterised as very slight or slight inflammatory reaction of the dermis; inflammation was present in two low-dose, four mid-dose, and five high-dose animals. The inflammatory reaction was confined to the superficial dermis; no epidermal lesions were seen. There was no evidence of systemic toxicity; no treatment-related effects were seen on survival, clinical signs, body weight gain, hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology. Under the conditions of this study, a no-observed-effect-level (NOEL) of 1000 mg/kg/day was established for the dermal irritation and systemic toxicity of 2,4-D TIPA in rabbits.

CORE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-D Triisopropanolamine salt (2,4-D TIPA).

II. MATERIALS AND METHODS

1. Test Material: 2,4-D Triisopropanolamine salt (2,4-D TIPA)

Lot No.	AGR 276428
ID No.:	K-008866-004
Description:	Amber
Purity:	72.2%
Homogeneity:	stability and homogeneity of 2,4-D TIPA dose solutions was determined concurrently with the study.

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Feed:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D TIPA following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received eight dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose level of 1474 mg 2,4-D TIPA/kg/day or 815 mg/kg/day 2,4-D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 100, 350, or 1000 mg/kg/day. The test material was diluted in distilled water, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). A control group was dosed with distilled water at a rate of 1.7 ml/kg; this control group was also used for the 21-day dermal toxicity study with 2,4-D isopropylamine salt (MRID No. 414078-03).

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen (BUN)	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Bartlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way ANOVA's with Bonferroni's Correction.

XII. RESULTS

(i). Probe study

Dermal application of 1474 mg 2,4-D TIPA/kg/day caused very slight erythema at the dermal site after the first application; there were no other signs of dermal irritation at any time, and there were no signs of systemic toxicity. Based on these results, 100, 350 and 1000 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Localized dermal irritation at the application site was limited to very slight, transient erythema observed in five animals at the 100 mg/kg/day group, two animals at the 350 mg/kg/day groups, and three animals at the 1000 mg/kg/day group during days 1 through 6. After day six, there were no signs of dermal irritation at any dose level.

4. Body Weight: No adverse effects were seen on body weight in either sex at any dose level; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen on hematologic parameters. Sporadic, statistically significant differences were seen in a few clinical chemistry parameters (TBILI, ALT, BUN, TP) in all treated groups when compared to appropriate corresponding control group values. However, since the values were within the range of normal healthy rabbits, and there was no evidence of dose-response, these differences were not considered to be toxicologically significant.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights for male or female rabbits.

7. Gross Pathology: No treatment-related gross pathological changes were seen.

8. Histopathology: Histopathology revealed very slight, multifocal inflammation of the dermis at the test site in 1/5 males and 1/5 females at 100 mg/kg/day; 1/5 males and 3/5 females at 350 mg/kg/day; and 3/5 males and 1/5 females at 1000 mg/kg/day; in addition, 1 female at the high-dose exhibited slight, multifocal inflammation at the treatment area. The inflammatory lesions were confined to the superficial dermis, and no epidermal lesions were seen. Histopathology of the liver and kidneys from high dose animals revealed no evidence of systemic toxicity.

IV. DISCUSSION

In a 21-day dermal toxicity study, 15 repeated dermal applications of 2,4-D TIPA at 0, 100, 350 or 1000 mg/kg/day produced no severe dermal irritation. Localized signs at the application site were limited to very slight, transient erythema in a few animals up to treatment day 6. The application site of all treated rabbits was within normal limits during the remainder of the study. Histologically, the dermal lesions were diagnosed as very slight or slight, multifocal inflammatory reactions. These lesions were seen in 2 low dose, 4 intermediate dose, and 5 high dose rabbits. Some animals in all three treatment groups had no lesions at the application site. 2,4-D TIPA did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathology.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1000 mg/kg/day is established for both dermal irritation and systemic toxicity of 2,4-D BEE.

VI. CORE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 11/28/90
Toxicology Branch II, Section II (H7059C)

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 11/29/90
Toxicology Branch II, Section II (H7059C)

DATA EVALUATION REPORT

STUDY: 21-Day Dermal, Rabbit

MRID NO. 414079-03 CASWELL NO.: 315AE

TEST MATERIAL: 2,4-Dichlorophenoxyacetic Acid, Isopropylamine salt

SYNONYMS: 2,4-D Isopropylamine salt; (2,4-D IPA)

STUDY NUMBER: M-004725-004

SPONSOR: DowElanco

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company
Midland, MI

TITLE OF REPORT: 2,4-D Isopropylamine salt: 21-Day Dermal Toxicity Study in New Zealand White Rabbits.

AUTHORS: M.J. Mizell, L. Atkin, K.T. Haut and K.E. Stebbins

REPORT ISSUED: February 20,1990

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Groups of five male and five female New Zealand White rabbits received 15 six hour dermal applications of 2,4-D Isopropylamine salt (2,4-D IPA) in distilled water at 0, 50, 125 or 350 mg/kg body weight/day. Dose-dependent dermal irritation observed were slight, transient erythema at the low-dose, slight erythema and scaling at the mid-dose, and slight to moderate erythema and scaling at the high-dose. Histologically, dermal lesions characterized as focal or multifocal irritative effects (inflammation and epidermal hyperplasia) were seen only at the 125 and 350 mg/kg/day groups; no histopathological dermal lesions were seen at 50 mg/kg/day group. There was no evidence of systemic toxicity; no treatment-related effects were seen on survival, clinical signs, body weight gain, hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology of kidneys and liver. Under the conditions of this study, the no-observed-effect-levels (NOELs) established were 50 mg/kg/day for dermal irritation and 350 mg/kg /day for the systemic toxicity of 2,4-D TIPA in rabbits. The lowest-observed-effect-level (LOEL) for dermal irritation was 125 mg/kg/day.

CORE CLASSIFICATION: Minimum; this study satisfies the guideline requirements (82-2) for a 21-day dermal toxicity study.

I. INTRODUCTION

This Data Evaluation Report (DER) summarizes the results of a 21-day dermal toxicity study in rabbits with 2,4-D Isopropylamine salt (2,4-D IPA).

II. MATERIALS AND METHODS

1. Test Material: 2,4-D Isopropylamine salt (2,4-D IPA)

Lot No.	AGR 276461
ID No.:	M-00475-004
Description:	Amber
Purity:	50.2%
Homogeneity:	Stability and homogeneity of 2,4-D IPA dose solutions was determined concurrently with the study.

2. Test Animals:

Species:	Rabbit
Strain:	New Zealand White
Age:	Five Months
Weight:	not provided
Source:	Hazleton Research Products Inc., Denver Pa.
Housing:	In individual cages
Food:	Purina Certified Chow #5322
Water:	ad libitum

3. Experimental Design

(i). A probe study was conducted to evaluate the dermal irritation potential of undiluted 2,4-D IPA following repeated application to aid in determining dose levels for the 21-day study. One male rabbit received three dermal applications of the undiluted test material at a rate of 1.7 ml/kg. This corresponded to a dose of approximately 1008 mg 2,4-D IPA/kg/day or 788 mg/kg/day 2,4-D acid equivalent. Owing to irritation observed at this level, the material was diluted 1:3 with distilled water and applied to another rabbit at a rate of 1.7 ml/kg which corresponded to a dose of 301 mg 2,4-D IPA/kg/day or 235 mg 2,4-D acid equivalent.

(ii). Based on the results of the probe study, the dose levels selected for the 21-day study were 50, 125 or 350 mg/kg/day. The test material was diluted in distilled water, and the dosing volume was 1.7 ml/kg. Groups of five male and five female rabbits received a total of 15 dermal applications during a 21-day interval (weekends and holidays excluded). Since this study was conducted concurrent with the 21-day study with 2,4-D Triisopropanolamine salt (MRID No. 414079-02), one control group dosed with distilled water at a rate of 1.7 ml/kg was employed for both studies.

4. Treatment Procedure

Animals were acclimated for at least 14 days prior to the initiation of the study. All rabbits were acclimated to an elastic jacket (used to hold the test material and dressing in dermal contact) for at least four days prior to the first application. An area, approximately 10 x 15 cm on the back of each rabbit was clipped free of fur prior to the initiation of the study and as necessary thereafter. A dressing consisting of absorbent gauze and non-absorbent cotton was used to hold the test material in dermal contact. The jacket and dressing were removed approximately six hours after application and the test site was wiped with a water-dampened disposable towel to remove any residual test material.

5. Experimental Procedures

Parameter	Time measured
General appearance	Daily
Dermal observations	Daily
Body weight	Weekly
Hematology	At termination
Hematocrit (HCT)	
Hemoglobin (HGB)	
Leukocyte count (WBC)	
Erythrocyte Count (RBC)	
Platelets (PLAT)	
Leukocyte Differential count	
Cellular morphology	
Clinical chemistry:	At termination
Calcium	
Chloride	
Phosphorous	
Potassium	
Sodium	
Alkaline Phosphatase (AP)	
Serum Alanine aminotransferase (SGPT)	
Serum Aspartate Aminotransferase (SGOT)	
Albumin (ALB)	
Blood creatinine	
Blood Urea Nitrogen	
Globulin (GLOB)	
Glucose (GLCU)	
Total bilirubin (TBILI)	
Total protein	
Albumin/globulin ratio	

6. Termination

At termination, body weights were obtained, and all animals were subjected to a complete gross necropsy. Liver, kidney and testes were weighed, and organ weight to final weight ratios were calculated on all animals. Representative samples of all tissues/organs were preserved in 10% formalin.

7. Histopathology

Histologic evaluation of normal and treated skin, liver, kidney and any masses or lesions were made on all control and high-dose animals. Histopathologic examination was extended to lower dose animals and consisted of skin from the application site and skin from an adjacent site, as well as any grossly visible lesions.

8. Statistical Analyses

Body weights, absolute and relative organ weights, hematology and clinical chemistry data were analyzed by Bartlett's test for equality of variances. If the Barlett's test rejects the equality of variances, the parameter was flagged for careful evaluation of results. Body weights were then analyzed by a three-way repeated measures ANOVA, hematology and clinical chemistry parameters, terminal body weights, organ weights (absolute and relative) were evaluated by a two-way analysis of variance. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using one-way Bonferroni's Correction.

XII. RESULTS

(i). Probe study

In the probe study, when tested at 1008 mg/kg/day, slight erythema and slight edema, as well as necrosis was seen at the treatment site. When diluted to 1:3 with distilled water, and applied at 301 mg/kg/day, slight erythema was present in the treated area. No systemic toxicity was observed at either level. Based on these results, 50, 125, and 350 mg/kg was selected as the dose levels for the 21-day study.

(ii). 21-Day Study

1. Survival: No mortality occurred during the study

2. Clinical Signs: No treatment-related clinical signs of toxicity were observed.

3. Dermal Observations: Dose-dependent dermal irritation observed at the application site was characterized as slight, transient erythema at the low-dose (50 mg/kg/day), slight erythema and scaling at the mid-dose (125 mg/kg/day), and slight to moderate erythema at the high-dose (350 mg/kg/day). In addition, three males and one female at the high dose had scabs/crusts and scattered necrosis at the application site. No dermal irritation was seen in rabbits receiving distilled water only. One male rabbit at the high dose was not treated after the second application due to clipper abrasions which became necrotic and edematous.

4. Body Weight: No adverse effect on mean body weight was seen in either sex at any dose level; all animals gained weight or were near their pretreatment body weights at termination.

5. Clinical Pathology: No biologically or statistically significant changes were seen in hematology parameters. A statistically significant difference between high dose and control phosphorous levels was attributed to the normal variability of this parameter in rabbits and was considered to be of no toxicological significance.

6. Absolute and Relative Organ Weights: No treatment-related effects were noted on absolute or relative organ weights for male or female rabbits.

7. Gross Pathology: No treatment-related gross pathological alterations of the skin were seen in rabbits receiving distilled water or the low-dose (50 mg/kg/day). In rabbits at the 125 and 350 mg/kg/day dose levels, dermal lesions confined to the treatment area consisted of one, or a combination of more than one, of the following: thickened skin, scales, scabs, and hyperemia. The cutaneous lesions were dose-dependent; 4/5 males and 2/5 females at the 125 mg/kg/day group and 5/5 and 4/5 females at the 350 mg/kg/day group. No other treatment-related gross pathological changes were seen at any level.

8. Histopathology: No histopathological lesions were seen in the skin of rabbits receiving the vehicle or the 50 mg 2,4-D IPA/kg/day. Dermal lesions observed at the 125 mg/kg/day group included slight multifocal dermal inflammation, multifocal hyperkeratosis, focal keratosis, multifocal parakeratosis, and focal parakeratosis. Cutaneous lesions at the 350 mg/kg/day included very slight multifocal dermal inflammation, very slight to moderate epidermal hyperplasia, multifocal parakeratosis, multifocal hyperkeratosis, moderate focal suppurative epidermal inflammation, very slight to moderate dermal fibrosis (Table 21). Histopathology of the liver and kidneys from high dose animals revealed no evidence of systemic toxicity.

XV. DISCUSSION

In a 21-day dermal toxicity study, rabbits received 15 repeated dermal applications of 2,4-D IPA at 0, 50, 125 or 350 mg/kg/day. Dose-dependent dermal irritation ranged from slight, transient erythema at the 50 mg/kg/day group to moderate erythema, edema, and scaling at the 350 mg/kg/day group. No gross or histological dermal lesions were seen in rabbits at the low-dose. However, at the mid- (125 mg/kg/day) and high-dose (350 mg/kg/day), histopathology revealed focal or multifocal irritative effects at the dermal site. The dermal lesions consisted of inflammation and epidermal hyperplasia; the majority of these lesions were graded as very slight or slight. One female rabbit at the high-dose exhibited moderate suppurative inflammation of the epidermis, moderate epidermal hyperplasia, and moderate dermal fibrosis. 2,4-D IPA did not induce adverse effects on survival, body weight, hematology and clinical chemistry parameters, absolute and relative organ weights, or gross or histopathological changes in the liver and kidneys.

V. CONCLUSION

Under the conditions of this study, the NOELs established were 50 mg/kg/day for dermal irritation and 350 mg/kg/day for systemic toxicity. The LOEL was 125 mg/kg/day for dermal irritation.

VI. GOOD LABORATORY PRACTICE CLASSIFICATION

Minimum; this study satisfies the guideline requirements (82-2) for a dermal toxicity study.

Table 1. Dermal Lesions At Application Site in Rabbits Receiving 15-Dermal Applications of 2,4-D Isopropylamine salt (2,4-D IPA).

Sex	Males				Females			
Dose (mg/kg/day)	0	50	125	350	0	50	125	350
No. Examined	5	5	5	5	5	5	5	5
Dermal lesions								
Normal	5	5	1	0	5	5	2	1
Fibrosis, dermis, focal	0	0	0	0	0	0	0	1
Fibrosis, dermis, multifocal	0	0	0	0	0	0	0	1
Hyperkertosis, epidermis focal	0	0	1	0	0	0	0	0
multifocal	0	0	2	4	0	0	1	2
Hyperplasia, epidermis, multifocal: very slight	0	0	0	2	0	0	1	1
slight	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Hyperplasia, epidermis, diffuse	0	0	0	0	0	0	0	1
Inflammation multifocal very slight	0	0	2	3	0	0	2	2
slight	0	0	0	0	0	0	1	2
Inflammation suppurative, Epidermis, focal, moderate	0	0	0	0	0	0	0	1
Parakeratosis, epidermis focal	0	0	1	0	0	0	0	0
multifocal	0	0	2	3	0	0	1	3

Data are number of animals with the specified observations.