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

MAY 27 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Command®: Review of Six Toxicity Studies on Formulation of Command®/Treflan®

Caswell No.: 463D
EPA Acce. NO.: 402796-01
402796-02

Project No.: 8-0624
EPA ID No.: 1471-157
279-3074

TO: R. Taylor/J. Yowell, PM (25)
Registration Division (TS-767c)

FROM: Whang Phang, Ph.D.
Pharmacologist
Toxicology Branch/HED (TS-769c)

THROUGH: Marcia van Gemert, Ph.D.
Head, Section III
and
William Burnam
Deputy Branch Chief
Toxicology Branch/HED (TS-769c)

The registrant, Elanco Products Co., submitted several acute and 21-day dermal toxicity studies which were conducted on a formulation containing Command® and Treflan®. These studies have been reviewed, and the data evaluation report of each study is attached, and the conclusion for each study is summarized as follows:

1. Acute Oral Toxicity (Rats)

Groups of rats (5/sex) were gavaged with a formulation of Command® and Treflan® at a dose of either 540 mg/kg or 5400 mg/kg. ALL the high dose animals died within 24 hours of dosing, but no death occurred in the low dose animals. This study used only 2 doses which did not yield sufficient information for establishing an LD_{50} value for the acute oral toxicity of the test article. This study is classified as Supplementary. However, the test agent could be placed in Category III for acute oral toxicity.

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2. Acute Dermal Irritation and Toxicity (Rabbits)

This study was intended to be used to fulfill the requirements for acute dermal toxicity and primary dermal irritation studies. For acute dermal toxicity, only one dose (5400 mg/kg) was tested. It produced no overt toxicity and was over the limit dose level. Under these conditions, the Agency's Guidelines for dermal toxicity study stated that a full study using a minimum of three dose levels might not be necessary. This study was considered as Minimum, and the toxicity category for the test article with respect to acute dermal toxicity was IV.

The data of the dermal irritation segment of the study indicated that the test article caused slight dermal irritation. This part of the study was considered as Minimum, and the toxicity category for dermal irritation was III.

3. Acute Eye Irritation (Rabbits)

The reported data indicated that the test article was an eye irritant causing moderate iritis, corneal dullness, slight to moderate conjunctivitis. The toxicity category for eye irritation for this article is II. The study is considered as Minimum.

4. Acute Inhalation (Rats)

Groups of Fischer 344 rats (10/sex/dose) were exposed by inhalation to the test article at analytical or gravimetric concentrations of 4.74 and 0.66 mg/L. In high dose animals, 5/10 males and 7/10 females died; no death occurred among the low dose rats.

The report is lacking data on clinical observations and gross pathology. Not enough doses were tested to allow accurate calculation of a LC_{50} value.

This study is considered as Supplementary. Attempts should be made to obtain greater percentage of the particles whose sizes are smaller than 1.5 micron when this study is repeated

5. 21-Dermal Toxicity (Rabbits)

Groups of New Zealand white rabbits (5/sex/dose) were dermally applied a formulation of TREFLAN®/COMMAND® at concentrations of 5.0, 10.0, and 20.0% for 6 hours/day and for 21 days; the applied concentrations were equivalent to 54, 108, and 216 mg/kg. One group

006733

(5/sex) of control and a high dose group (5/sex) were maintained for an additional 14 days after termination of treatment.

The results showed that in all treated animals, a formulation of TREFLAN®/COMMAND® caused a dose-related increase in dermal irritation which was characterized by slight to severe erythema and slight to moderate edema. Therefore, LEL for skin irritation was 54 mg/kg (LDT). No Systemic toxicity was observed, and NOEL for systemic toxicity was 216 mg/kg (HDT).

This study is classified as Minimum.

Reviewed By: Whang Phang, Ph.D. 5/4/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769C)

006733

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity (Rats)

EPA ACCESSION No.: 402796-02 TOX. CHEM. No.: 463D

EPA ID No.: 1471-157/279-3074 RECORD No.: 205844/ 205842

CHEMICAL: TREFLAN/COMMAND PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 lbs of TREFLAN and 2.25 lbs of COMMAND® per gallon

TESTING FACILITY: Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, Indiana

CITATION: Negilski, D. S., Brown, G. E., and Markey, T.F. The Acute Oral Toxicity of TREFLAN®/ COMMAND® PREPACK E.C., an Emulsifiable Concentrate Formulation (FN 5227) Containing 3 Pounds of Treflan and 2.25 Pounds of Command per Gallon, Administered Orally to The Fischer 344/NHsd Rat. Lilly Research Laboratories. Study No.: R-O-165-86 and R-O-166-86. Nov 6, 1986. Submitted by Elanco Products Co.

CONCLUSION:

Groups of rats (5/sex) were gavaged with either 540 mg/kg or 5400 mg/kg. ALL the high dose animals died within 24 hours of dosing, but no death occurred in the low dose animals. This study used only 2 doses which did not yield sufficient information for establishing an LD_{50} value for the acute oral toxicity of the test article.

This study is classified as Supplementary; however, the test agent could be placed in Category III for acute oral toxicity.

METHODS AND MATERIALS:

F344/NHsd rats were obtained from Harlan Sprague Dawley Inc., Indiana. These rats were 8 to 9 weeks old and weighed approximately 185 gm and 137 gm for males and females, respectively. Animals (5/sex) were randomly assigned to 2 dose groups, 0.5 ml/kg (540 mg/kg) or 5.0 ml/kg (5400 mg/kg).

The test article was undiluted emulsifiable formulation containing 3 lbs of TREFLAN and 2.25 lbs of Command per gallon of formulation. TREFLAN or trifluralin is chemically identified as 2,6-dinitro-N,N-di-n-propyl-a,a,atrifluoro-p-toluidine; Command, 2-(2-chlorophenyl)methyl4,4-dimethyl-3-isoxazolidinone.

The animals were fasted for at least 16 hours prior to treatment, and they were administered the test chemical by gavage.

006733

After treatment the animals were observed for signs of toxicity at approximately 1 hour intervals for the first 6 hours, then daily for the next 14 days. Body weights were recorded, and gross pathology was conducted after sacrifice.

RESULTS:

All rats which received 5400 mg/kg of the formulation died within 24 hours after dosing. There were no death among the 540 mg/kg animals.

Clinical signs in 540 mg/kg animals were limited to leg weakness which disappeared within 24 hours. For 5400 mg/kg rats, signs of toxicity included lethargy, ataxia, clear ocular discharge, and diarrhea.

The 540 mg/kg rats gained weight through out the length of the study. Nc specific lesions were observed in 5400 mg/kg animal which died during the study.

DISCUSSION:

Group of 5 rats/sex were gavaged with either 540 mg/kg or 5400 mg/kg of the test article. The high dose animals died within 24 hours of dosing, but no death was observed in low dose animals. The author of the report concluded that LD_{50} was between 540 mg/kg and 5400 mg/kg. However, This study used only 2 doses, and results did not provide sufficient information for establishing a LD_{50} value for the acute oral toxicity of this test article.

The study is classified as Supplementary.

Reviewed by: Whang Phang, Ph.D. 5/18/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 5/19/88
Section III, Tox. Branch (TS-769C)

006733

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity Study (Rats)

EPA ACCESSION No.: 402796-01 TOX. CHEM. No.: 463D

EPA ID No.: 1471-157/.79-3074 RECORD No.: 205844/ 205842

CHEMICAL: TREFLAN/COMMAND PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 lbs of TREFLAN and 2.25 lbs of COMMAND® per gallon (By weight, TREFLAN, 34.55%; COMMAND, 26.24%)

TESTING FACILITY: Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, Indiana

CITATION: Adams, E.R., Fisher, L.F., and Torrence, T.L. Subchronic (21 day) dermal toxicity study in New Zealand white rabbits with TREFLAN®/COMMAND® PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 pounds of Treflan and 2.25 pounds of Command per gallon. Study No. B05786; Lilly Research Laboratories. June 24, 1987. Submitted by Elanco Products Co. on July 29, 1987.

CONCLUSION:

Groups of New Zealand white rabbits (5/sex/dose) were dermally applied a formulation of TREFLAN/COMMAND at concentrations of 5.0 10.0, and 20.0% for 6 hours/day and for 21 days; the applied concentrations were equivalent to 54, 108, and 216 mg/kg. One group (5/sex) of control and a high dose group (5/sex) were maintained for an additional 14 days after termination of treatment.

The results showed that in all treated animals, a formulation of TREFLAN/COMMAND caused a dose-related increase in dermal irritation which was characterized by slight to severe erythema and slight to moderate edema. Therefore, LEL for skin irritation was 54 mg/kg (LDT). No Systemic toxicity was observed, and NOEL for systemic toxicity was 216 mg/kg (HDT).

This study is classified as Minimum.

METHODS AND MATERIALS:

A brief summary of the experimental procedures are discussed below, and the details are presented in the Appendix A.

Groups of New Zealand white rabbits (5/sex) were obtained from Lesser's Rabbitry, Wisconsin. These rabbits were 12 to 18 weeks of age and weighed approximately 2.72 and 2.64 kg for males and females, respectively.

The test article was an emulsifiable formulation containing 3 lbs of TREFLAN and 2.25 lbs of Command per gallon of formulation. TREFLAN or trifluralin is chemically identified as 2,6-dinitro-N,N-di-n-propyl-a,a,a-trifluoro-p-toluidine; Command, 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone. The emulsifiable formulation, with a density of 1.08 g/ml, was diluted with water to yield dosing solutions containing 5, 10, and 20% of the test article.

The animals were randomly assigned to the following dosage groups:

Group No.	Dose		21 Day Treatment Group		Satellite Group*	
	%	mg/kg	M	F	M	F
1 (Cont)	0	0	5	5		
2 (Low)	5%	54	5	5		
3 (Mid)	10%	108	5	5		
4 (High)	20%	216	5	5		
Satellite Reversibility Groups						
0 (Cont)	0	0			6†	4†
5 (High)	20	216			5	5

* Groups of rabbits were placed in the study for 35 days of which the treated animals received the test article for 21 days; then the animals were observed for an additional 14 days to study any reversible effects.

† One of the animals in this control group was wrongly sexed.

The back of each rabbit was shaved, and 1.0 ml of dosing solution/kg body weight was applied onto the shaved area. However, the report failed to specify the size of the application site except to say that the application site was 10% of the body surface. The application site was covered with a nonocclusive dressing and an elastic sleeve for 6 hours/day, after which time the treatment site was rinsed with warm water. A collar was placed on each animal to prevent ingestion of the test article. The animals

were treated for 21 consecutive days. For animals in the satellite groups, additional 14 days of observations were carried out. For control animals, a damp gauze dressing was placed on the application site, and other treatments were similar to those of the treated animals.

Animals were weighed at the initiation of the study and weekly thereafter. All rabbits were examined daily for any signs of toxicity. Dermal irritation was graded daily according to the scoring system presented in the Appendix B.

Hematologic evaluations were performed on each animal prior to initiation of the study and at the end of the study. Blood samples were collected from medial artery of the ear and the following parameters were examined:

Hemoglobin (HGB)	Leukocyte differential count
Mean corpuscular HGB (MCH)	Mean corpuscular HGB conc.(MCHC)
Leukocyte count (WBC)	Reticulocyte count
Erythrocyte count (RBC)	Mean corpuscular volume (MCV)
Platelet count	Blood Clotting Measurements
Packed cell volume	

Clinical chemistry parameters were also examined with blood samples for hematology. The following parameters were measured:

glucose	blood urea nitrogen
creatinine	total bilirubin
alkaline phosphatase	alanine transaminase

An enzyme induction study was conducted by measuring the activity of hepatic p-nitroanisole-o-demethylase in males and females of the satellite groups. Methodology for this assay is presented in Appendix C.

At necropsy the following organ weights were measured:

liver	kidney
heart	thyroid
adrenals	ovaries or testes

For pathology, all animals were grossly examined, and the following tissue samples were collected for histopathology:

kidney	liver	gallbladder
heart	lung	spleen
thymus	lymph nodes	salivary gland
pancreas	stomach	duodenum
jejunum	ileum	colon
ovary or testes	uterus	adrenal
thyroid	prostate	skin
mammary gland	skeletal muscle	urinary bladder
bone; bone marrow	eye	esophagus
trachea	aorta	cerebrum and cerebellum
brain stem	pituitary	

For statistical analyses, Dunnett's test was applied for analysis of differences between treated and controls animals. Bartlett's test was used to evaluate the homogeneity of variances.

RESULTS:

Toxicity and mortality:

No compound related toxic signs were observed in treated animals, and all test animals survived to the end of the study. Eye examinations at the end of the study did not reveal ocular toxicity.

Body weight:

Mean body weights of all test animals at various times are presented in Table 1. The mean body weights of treated and control rabbits were comparable.

Food consumption:

Tables 2a and 2b show mean food consumptions of all test animals. There was no significant difference in food consumption between treated and control animals.

Dermal irritation:

Dermal irritation indices were calculated and graphed. The results are presented in Figure 1. Skin irritation was found in all treated animals, and it was characterized by slight erythema and edema which developed within 5 days of treatment and persisted to the end of the study. The skin irritation was dose-related. In high dose animals, moderate to severe erythema and moderate edema were observed; erythema and edema were followed by dehydrated, desquamated, cracked, and coriaceous skin within 9 days of treatment. In the reversibility phase of the study, animals which received high dose test article appeared to be normal within 4 to 7 days after the withdrawal of the treatment.

Hematology:

The mean values of various hematological parameters measured are presented in Tables 3a,and 3b. There were sporadic changes in certain parameters, but these changes were often slight and not dose-related.

Clinical chemistry:

The clinical chemistry data are presented in Tables 4a and 4b. In treated males, there was a decrease in the mean value of creatinine in mid and high dose animals, but the changes were

not dose related. There was also a decrease in total bilirubin in the two groups of high dose females, and it was statistically significant when compared to the controls. However, the decrease in total bilirubin was not considered to be clinically significant. No change in bilirubin was found in the high dose animals which were maintained for an additional 14 days after the termination of treatment.

Enzyme induction:

The enzyme induction study by measuring the activity hepatic p-nitroanisole-o-demethylase did not indicate any significant increase in treated animals relative to the controls.

Organ weight:

The summary organ weight data indicated statistically significant decrease in mean testes weight of treated males relative to the controls (Table 5). However, this change was not compound-related. The individual animal data indicated that this difference was due to one control rabbit which had an enlarged testis with a benign teratoma which led to much higher testicular weight 18.43 gm) relative to that of the other controls (mean: 4.85 gm). No other significant organ weight changes were found.

Pathology:

Gross pathology data did not show any compound-related findings other than the application site of the skin.

Histopathology data did not show any systemic toxicity, except the skin applicaton site. In 4/5 low dose males, 2/5 mid dose females, and 1/5 high dose females showed minimal subacute dermal inflammation, which was characterized by edema and infiltration of leukocytes in the dermis.

DISCUSSION

Groups of New Zealand white rabbits (5/sex/dose) were dermally applied a formulation of TREFLAN/COMMAND at concentrations of 5.0 10.0, and 20.0% for 6 hours/day and for 21 days. The applied volume was 1.0 mg/kg body weight. One group (5/sex) of control and a high dose group (5/sex) were maintained for additional 14 days after termination of treatment.

The results showed that a formulation of TREFLAN/COMMAND caused a dose-related increase in dermal irritation which was characterized by slight to severe erythema and slight to moderate edema. Systemic toxicity was not observed.

Command/trifluralin tox review

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Pages 11 through 21 are not included in this copy.

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___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

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Appendix A

Data excerpted from submission (EPA Accession No. 402796-01)

Protocol of Subchronic (21) Dermal Toxicity Study in Rabbits with TREFLAN/COMMAND PREPACK E.C.

Command/trifluralin tox review

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___ Identity of product impurities

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___ Description of product quality control procedures

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Reviewed by: Whang Phang, Ph.D. WPhang 5/4/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769C) M.vanGemert 5/19/88 006733

DATA EVALUATION REPORT

STUDY TYPE: Eye Irritation Study (Rabbits)

EPA ACCESSION No.: 402796-02 TOX. CHEM. No.: 463D

EPA ID No.: 1471-157/279-3074 RECORD No.: 205844/ 205842

CHEMICAL: TREFLAN/COMMAND PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 lbs of TREFLAN and 2.25 lbs of COMMAND® per gallon

TESTING FACILITY: Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, Indiana

CITATION: Negilski, D. S., Brown, G. E., and Markey, T.F. The acute ocular irritation of TREFLAN®/ COMMAND® PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 pounds of Treflan and 2.25 pounds of Command per gallon, in the New Zealand white rabbits. Study No. B-E-105-86; Lilly Research Laboratories. Sept 9, 1986. Submitted by Elanco Products Co. on July 29, 1987.

CONCLUSION:

The reported data indicated that the test article was an eye irritant causing moderate iritis, corneal dullness, slight to moderate conjunctivitis. The toxicity category for eye irritation for this article is II. The study is considered as Minimum.

METHODS AND MATERIALS: Groups of New Zealand white rabbits (3/sex) were obtained from Langshaw Farms, Michigan. These rabbits were 12 to 18 weeks of age and weighed approximately 2.77 and 3.10 kg for males and females, respectively.

The test article was an emulsifiable formulation containing 3 lbs of TREFLAN and 2.25 lbs of Command per gallon of formulation. TREFLAN or trifluralin is chemically identified as 2,6-dinitro-N,N-di-n-propyl-a,a,a-trifluoro-p-toluidine; Command, 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone.

Approximately 24 hours before dosing, the eyes of each rabbit was examined for any corneal lesions. Only animals with both eyes free of any ocular injury were selected for the study. One eye of each rabbit was applied 0.1 ml of test article into the conjunctival sac, and the eye lid was held closed for several seconds. The untreated eye of each rabbit served as the control.

The treated eyes were examined at 1 hour, 1, 2, 3, 7, 14, and 21 days. The effects were graded according to the scoring system presented in the Appendix. Sodium fluorescein dye was used to identify corneal lesions on days 1, 3, 7, and 14 or until a negative response was observed.

The treated rabbits were weighed at test initiation, 7, 14, and 21 days.

RESULTS:

The individual animal eye irritation data are presented in Table 1. All treated rabbits showed corneal dullness and opacity, slight to moderate iritis, moderate conjunctivitis after one hour of exposure to the test article. Conjunctivitis persisted for approximately 7 days in essentially all animals. Eye irritation was cleared in almost all rabbits by day 14 except one female rabbit which showed corneal dullness and vascularization for longer than 21 days.

The test article treated eye of all rabbits showed positive response to sodium fluorescein dye instillation at 24 hours after treatment. Within 7 days, 4/6 rabbits showed negative reponse to dye instillation, and the remaining 2 rabbits also produced similar response by day 14.

All rabbits gained weight during the test, except one female which showed persistent eye irritation beyond 21 days.

DISCUSSION:

The reported data indicated that the test article was an eye irritant. The toxicity category for eye irritation for this article is II. The study is considered as Minimum.

TABLE I+

OCULAR IRRITATION SCORES* FOR RABBITS FOLLOWING
THE SINGLE OCULAR ADMINISTRATION OF
TREFLAN/COMMAND PREPACK E.C., AN EMULSIFIABLE CONCENTRATE FORMULATION (FN 5227) CONTAINING
THREE POUNDS OF TREFLAN AND 2.25 POUNDS OF COMMAND PER GALLON

STUDY B-E-105-86

RABBIT NUMBER	SEX	CORNEAL OPACITY							IRITIS							CONJUNCTIVAL HYPEREMIA							CONJUNCTIVAL CHEMOSIS						
			day							day								day							day					
		1hr	1	2	3	7	14	21	1hr	1	2	3	7	14	21	1hr	1	2	3	7	14	21	1hr	1	2	3	7	14	21
18747	M	1	1	1	1	<1	0		1	1	1	1	<1	0		2	2	2	2	1	0		3	2	2	2	1	0	
18748	M	1	1	1	1	<1	0		1	<1	<1	<1	0	0		2	2	2	2	1	0		3	3	3	2	1	0	
18749	M	<1	1	1	1	<1	0		<1	<1	<1	<1	<1	0		2	1	1	2	1	0		2	2	2	2	0	0	
18565	F	<1	1	1	2	2	<1	<1**	<1	1	1	2	1	0	0	2	2	2	2	2	1	0	3	3	3	3	3	1	0
18566	F	1	1	<1	<1	<1	0		1	<1	<1	0	0	0		2	2	2	1	1	0		3	2	2	1	0	0	
18567	F	1	1	1	1	0	0		1	<1	<1	<1	0	0		2	1	1	1	1	0		3	1	1	1	0	0	

*Based on the Consumer Product Safety Commission Scale for scoring ocular lesions (Appendix D.)
**Corneal vascularization persisting longer than 21 days.

+ DATA EXCERPTED From MRID Submission (EPA Accession No. 402796-02).

006733

Appendix

Data excerpted from submission (EPA Accession No. 400778-02)

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GRADES FOR OCULAR LESIONS

CORNEA

No ulceration or opacity.....0

Slight dulling of corneal luster.....<1

Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible.....(1)*

Easily discernible translucent areas, details of iris slightly obscured.....2

Nacreous areas, no details of iris visible, size of pupil barely discernible.....3

Complete corneal opacity, iris not discernible.....4

IRIS

Normal.....0

Slight circumcorneal injection.<1

Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive).....(1)*

No reaction to light, hemorrhage, gross destruction (any or all of these).....2

CONJUNCTIVAE

(Redness refers to palpebral and bulbar conjunctivae excluding cornea and iris.)

Vessels normal.....0

Some vessels definitely injected....1

Diffuse, crimson red, individual vessels not easily discernible.....(2)*

Diffuse beefy red.....3

CHEMOSIS

No swelling.....0

Any swelling above normal (includes nictitating membrane).....1

Obvious swelling with partial eversion of lids.....(2)*

Swelling with lids about half closed.....3

Swelling with lids more than half closed.....4

*Bracketed figures indicate lowest grades considered positive under the Federal Hazardous Substances Act Regulations (16 CFR 1500.42).

Reviewed by: Whang Phang, Ph.D. W Phang 5/4/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity and Primary Dermal Irritation (Rabbits)

EPA ACCESSION No.: 402796-02 TOX. CHEM. No.: 463D

EPA ID No.: 1471-157/279-3074 RECORD No.: 205844/ 205842

CHEMICAL: TREFLAN/COMMAND PREPACK E.C., an emulsifiable con-concentrate formulaiton (FN 5227) containing 3 lbs of TREFLAN and 2.25 lbs of COMMAND® per gallon

TESTING FACILITY: Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, Indiana

CITATION: Negilski, D. S., Brown, G. E., and Markey, T.F. The acute dermal toxicity and primary dermal irritation of TREFLAN®/ COMMAND® PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 pounds of Treflan and 2.25 pounds of Command per gallon, in the New Zealand white rabbits. Study No. B-D-103-86; Lilly Research Laboratories. Aug 21, 1986. Submitted by Elanco Products Co. on July 29, 1987.

CONCLUSION:

This study was intended to be used to fulfill the requirements for acute dermal toxicity and primary dermal irritation studies. For acute dermal toxicity, only one dose (5400 mg/kg) was tested. It produced no overt toxicity and was over the limit dose level. Under these conditions, the Agency's Guidelines for dermal toxicity study stated that a full study using a minimum of three dose levels might not be necessary. This study was considered as Minimum, and the toxicity category for the test article with respect to acute dermal toxicity was IV.

The data of the dermal irritation segment of the study indicated that the test article caused slight dermal irritation. For dermal irritation, the study was considered as Minimum, and the toxicity category for dermal irritation was III.

METHODS AND MATERIALS: Groups of New Zealand white rabbits (5/sex) were obtained from Langshaw Farms, Michigan. These rabbits were 12 to 18 weeks of age and weighed approximately 2.64 and 2.88 kg for males and females, respectively.

The test article was an emulsifiable formulation containing 3 lbs of TREFLAN and 2.25 lbs of Command per gallon of formulation.

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TREFLAN or trifluralin is chemically identified as 2,6-dinitro-N,N-di-n-propyl-a,a,a-trifluoro-p-toluidine; Command, 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone.

The back of each rabbit was shaved, and 5.0 ml/kg (5400 mg/kg) of the test article was applied onto the shaved area. However, the report failed to specified the size of the application site except to say that the application site was 10% of the body surface.

After treatment, the application site was cover with a non-occlusive dressing and an elastic sleeve for 24 hours after which time the treatment site was rinsed with warm water. A collar was placed on each animal for 48 hours.

The animals were observed for 14 days for signs of toxicity. Animals were weighed weekly, and the treated skin was graded for dermal irritation each day according to the scoring system presented in the Appendix.

RESULTS:

1). Acute dermal toxicity: All test animals survived to the end of the study (14 days), and no overt toxicity was observed at 5400 mg/kg. The report stated that gross pathology data did not indicate any compound related lesions, but no gross pathology data were presented in the report to substantiate this conclusion.

2). Dermal irritation: Table 1 presents the individual animal data on dermal irritation. Initially, all treated male and female rabbits showed slight dermal irritation as indicated by slight erythema and edema. After the removal of the test agent, the erythema and edema cleared. At day 14 of the test, the treated skin of 4/5 males and 3/5 females had returned to normal. At the end of the study most of the animals either had gained weight or retained the original weight.

DISCUSSION:

This study was intented to be used to fulfill the requirements for acute dermal toxicity and primary dermal irritation studies. For acute dermal toxicity, only one dose (5400 mg/kg) was tested. It produced no overt toxicity and was over the limit dose level (2000 mg/kg). Under these conditions, the Agency's Guidelines for acute dermal toxicity study stated that a full study using a minimum of three dose levels might not be necessary.

The data of the dermal irritation segment of the study indicated that the test article caused slight dermal irritation.

(J)

TABLE 1+

DERMAL RESPONSE SCORES AND BODY WEIGHT DATA FOR RABBITS TREATED TOPICALLY WITH A SINGLE DOSE OF TREFLAN/COMMAND PREPACK E.C., AN EMULSIFIABLE CONCENTRATE FORMULATION (FN 5227) CONTAINING THREE POUNDS OF TREFLAN AND 2.25 POUNDS OF COMMAND PER GALLON

STUDY B-D-103-86

Animal Number:	18732	18733	18734	18735	18736	18591	18553	18554	18557	18556
Sex:	M	M	M	M	M	F	F	F	F	F
Initial Wt. (kg):	2.56	2.52	2.60	2.88	2.62	2.97	2.68	3.15	2.78	2.84
7-Day Wt. (kg):	2.60	2.52	2.68	2.88	2.61	3.04	2.53	3.05	2.85	2.89
14-Day Wt. (kg):	2.70	2.52	2.79	2.99	2.80	3.10	2.68	3.28	2.93	2.97

DERMAL RESPONSE*
(erythema, edema, and desquamation)

Days after Treatment										
1	B2E1	B2E1	B2E2	B2E2	B2E1	B2E1	B2E2	B2E2	B2E2	B2E2
2	B3E2	B3E2	B3E3	B3E2	B2E2	B3E2	B2E2	B2E2	B3E2	B2E2
3	B2E1	B2E2	B3E2	B3E2	B2E2	B2E2	B2E2	B2E2	B2E2	B2E2
4	B1E1	B2E2	B2E2	B2E2	B2E1	B2E2	B2E2	B2E2	B2E2	B2E2
5	B1E1	B2E1F1	B2E2	B2E2	B2E1	B2E2F1	B1E1	B2E2	B2E2	B2E2
6	B1E1	B2E1F1	B2E2	B2E2	B2E1	B2E2F1	B1E1	B2E2	B2E2	B2E2
7	B1E1	B1E1F1	B2E2	B2E2	B2E1F1	B2E2F1	B1	B2E2	B2E2	B2E2
8	B1F1	B1F1	B1E1	B1E1	B1E1F1	B1E1F1	B1F1	B1	B1	B1E1
9	B1F1	B1F1	B1	B1	B1	B1	B1F1	B1	B1F1	B1
10	B1F1	B1F1	B1	B1	B1	B1	B1F1	B1	B1F1	B1
11	B1F1	B1	N	N	B1F1	N	B1F1	B1	B1	B1
12	F1	B1	N	N	F1	N	B1F1	N	B1	B1
13	N	B1	N	N	F1	N	F1	N	N	B1
14	N	N	N	N	F1	N	F1	N	N	N^1

*Based on the scoring system described in Appendix C. "B" represents erythema, "E" represents edema, and "F" represents desquamation.

"N" represents normal skin.

N^1 = represents normal skin except for epidermal dehydration.

+ DATA EXCEPTED FROM SUBMISSION (EPA ACCESSION NO. 402796-02)

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Appendix

Data excerpted from submission (EPA Accession No. 402796-02)

OBSERVATIONAL TERMS
DERMAL IRRITATION AND SYSTEMIC TOXICITY

Observations	Code
1. Erythema and Eschar Formation	
Very slight erythema (barely perceptible)	B1
Slight erythema (well-defined)	B2
Moderate erythema	B3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	B4
2. Edema Formation	
Very slight edema (barely perceptible)	E1
Slight edema (edges of area well defined by definite raising)	E2
Moderate edema (raised approximately 1 mm)	E3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	E4
3. Desquamation	
Lamella--3 mm or less in width (scales)	F1
Lamella--3 to 10 mm in diameter (flakes)	F2
Lamella--greater than 10 mm in diameter (sheets)	F3

Section III, Tox. Branch (TS-769C)
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Section III, Tox. Branch (TS-769C) M. van Gemert 5/19/88 6733

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation (Rats)

EPA ACCESSION No.: 402796-02 TOX. CHEM. No.: 463D

EPA ID No.: 1471-157/279-3074 RECORD No.: 205844/ 205842

CHEMICAL: TREFLAN/COMMAND PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 lbs of TREFLAN and 2.25 lbs of COMMAND® per gallon

TESTING FACILITY: Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, Indiana

CITATION: Negilski, D. S., Brown, G. E., and Markey, T.F. The acute inhalation toxicity of TREFLAN®/ COMMAND® PREPACK E.C., an emulsifiable concentrate formulation (FN 5227) containing 3 pounds of Treflan and 2.25 pounds of Command per gallon, in the Fischer 344 rat. Study No. R-H-078-86; Lilly Research Laboratories. Aug 26, 1986. Submitted by Elanco Products Co. on July 29, 1987.

CONCLUSION:

Groups of Fischer 344 rats (10/sex/dose) were exposed by inhalation to the test article at analytical or gravimetric concentrations of 4.74 and 0.66 mg/L. In high dose animals, 5/10 males and 7/10 females died; no death occurred among the low dose rats.

The report is lacking data on clinical observations and gross pathology. Not enough doses were tested to allow accurate calculation of a LC_{50} value.

This study is considered as Supplementary. Attempts should be made to obtain greater percentage of the particles whose sizes are smaller than 1.5 micron when this study is repeated

METHODS AND MATERIALS: Groups of Fischer 344 rats (10/sex/dose) were obtained from Charles River Breeding Laboratories, Inc., Michigan. These rats were 8 to 9 weeks old and weighed approximately 150 gm for males and 130 gm for females.

The test article was an emulsifiable formulation containing 3 lbs of TREFLAN and 2.25 lbs of Command per gallon of formulation. TREFLAN or trifluralin is chemically identified as 2,6-dinitro-N,N-di-n-propyl-a,a,a-trifluoro-p-toluidine; Command, 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone.

The experimental procedures are summarized below, and the details are excerpted from the submitted report and presented in the Appendix.

Two groups of Fischer 344 rats (10/sex/dose) were exposed to aerosolized test chemical at nominal concentrations of 39.99 and 4.03 mg test article/L of air with "nose only" exposure. The total air flow through the nozzle was 15 L/min, and the delivery rates for low and high dose were approximately 32.3 and 3.6 ml/hr, respectively. The animals were exposed to the test article for 4 hours and remained in position for an additional 13 minutes as 15 L/min of air was flushed through the chamber.

The exposure concentration were determined on a nominal, total gravimetric or analytical, and activity basis. The analytical concentrations were determined from eight samples collected at the "respiratory level" of the animals. The distribution of particle sizes was also determined.

The treated animals were observed daily and weighed on days 0, 1, 3, 5, 7, and 14. Gross pathology was performed on animals which died or sacrifice.

RESULTS:

The exposure nominal concentrations of the test article were analyzed. The results are prested below:

Analytical Results of the Exposure Concentrations

Nominal Concentration (mg formu./L of air)	Gravimetric (Analytical) concentration (mg formu./L of air)	Activity oncentration (mg/L of air)
39.89 (high)	4.74 ± 0.262	Treflan = 2.23±0.095 Command = 1.64±0.070
4.04 (low)	0.66 ± 0.034	Treflan = 0.34±0.016 Command = 0.25±0.011

Particle sizes of the aerosolized test article were determined. Representative results are presented in Table 1. Majority of the the particles were greater than 1.5 micron.

In high dose males, the body weight dropped from treatment days 1 to 5; for high dose females the reduction of body continued to the end of the study (Table 2a). In low dose animal the treatment had no effect on the body weight (Table 2b).

The mortality data are presented in Table 3. In high dose animals, 5/10 males and 7/10 females died during the study. All the animals in the low dose group survived. The report stated that the LC_{50} was between 0.66 and 4.74 mg formulation/L for females and 4.74 mg formulation/L for males.

For clinical observations, the author concluded that high dose animals showed weight loss, lethargy, ataxia, dyspnea, rales, tympanites, chromorhinorrhea, chromodacryorrhea and nasal exudate. Convulsion was observed in a male rat which died on the day of treatment. Low dose animals showed hypoactivity and dyspnea on the day of treatment. However, no clinical data were presented to substantiate these conclusions.

For gross pathology, the author concluded that predominant findings were gaseous distension of the stomach and intestine in high dose males and some females which died. The rats in the low dose animals which survived to the end of the study did not show any gross lesions. Again, the report failed to included the gross pathology data of individual animals.

DISCUSSION:

When two groups of Fischer 344 rats (10/sex/dose) were exposed by inhalation to the test article at analytical or gravimetric concentrations of 4.74 and 0.66 mg/L, 5/10 males and 7/10 females of the high dose died, while no death occurred among the low dose animals.

The study had many deficiencies which included data on clinical observations and gross pathology were not included in the report, and not enough doses were tested to allow accurate calculation of a LC_{50} value. In addition, attempts should be made to obtain greater percentage of particles whose size was smaller than 1.5 micron.

The study is classified as Supplementary.

TABLE 1*

ACTIVITY PARTICLE SIZE ANALYSIS WITH THE SIERRA IMPACTOR

R-H-078-86 036352 ACD-12261 GROUP I 06 AUG 86 036352 ACTIVITY

SAMPLE FLOW RATE = 3 LPM

SIZE (MICRON)	ACTIVITY ON STAGE (MG)	PERCENT	CUM % < SIZE	LOG (SIZE)	PROBIT
> 21.00	0.71	20.28	79.72	1.32	5.83
17.00	0.02	0.58	79.14	1.23	5.81
6.80	0.25	7.09	72.05	0.83	5.58
4.10	0.66	19.10	52.95	0.61	5.07
2.60	0.68	19.45	33.50	0.41	4.57
1.50	0.75	21.69	11.81	0.18	3.82
0.84	0.17	4.84	6.96	-0.08	3.52
0.54	0.20	5.63	1.33	-0.27	2.78
FILTER	0.05	1.33			
TOTAL=	3.48				

AMEAD = 5.32 G.S.D. = 3.30 R = 0.9727

PROBIT = 3.60 + 1.93 [LOG SIZE (MICRON)]

* DATA EXCERPTED FROM SUBMISSION (EPA ACCESSION NO. 402796-02)

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TABLE 2a **

INDIVIDUAL BODY WEIGHTS OF MALE AND FEMALE FISCHER 344/Crl RATS EXPOSED NOSE ONLY FOR FOUR HOURS TO A LIQUID DROPLET AEROSOL (4.74 MG/L) OF TREFLAN/COMMAND PREPACK E.C., AN EMULSIFIABLE CONCENTRATE FORMULATION (FN 5227) CONTAINING THREE POUNDS OF TREFLAN AND 2.25 POUNDS OF COMMAND PER GALLON.

STUDY R-H-078-86

ANIMAL NUMBER	SEX	BODY WEIGHT (G)*						BODY WEIGHT CHANGE, (G)
		PRE-EXPOSURE	DAY 1	DAY 3	DAY 5	DAY 7	DAY14	
1001	M	146	123	0	0	0	0	
1002	M	152	125	122	138	154	195	+43
1003	M	160	0	0	0	0	0	
1004	M	147	123	122	139	146	185	+38
1005	M	139	118	104	0	0	0	
1006	M	140	117	100	89	0	0	
1007	M	150	130	138	146	156	193	+43
1008	M	146	121	126	131	122	0	
1009	M	140	137	152	162	172	201	+61
1010	M	148	125	127	141	153	191	+43
Mean +/- S.D.	M	147+/- 6.4	124+/- 6.1	124+/-16.8	135+/-22.5	151+/-16.4	193+/- 5.8	+46
1051	F	128	108	0	0	0	0	
1052	F	131	112	102	91	0	0	
1053	F	130	111	0	0	0	0	
1054	F	138	118	103	102	0	0	
1055	F	133	112	113	115	121	145	+12
1056	F	139	117	122	133	128	93	-46
1057	F	133	113	102	117	122	96	-37
1058	F	138	118	117	130	120	0	
1059	F	127	110	107	114	103	0	
1060	F	138	117	105	118	119	0	
Mean +/- S.D.	F	134+/- 4.5	114+/- 3.6	109+/- 7.6	115+/-13.7	119+/- 8.4	111+/-29.2	-24

* Body weight of '0' represents an animal that has died.

** DATA EXCERPTED From Submission (EPA Accession No. 402796-02)

TABLE 26+

INDIVIDUAL BODY WEIGHTS OF MALE AND FEMALE FISCHER 344/Crl RATS EXPOSED NOSE ONLY FOR FOUR HOURS TO A LIQUID DROPLET AEROSOL (0.66 MG/L) OF TREFLAN/COMMAND PREPACK E.C., AN EMULSIFIABLE CONCENTRATE FORMULATION (FN 5227) CONTAINING THREE POUNDS OF TREFLAN AND 2.25 POUNDS OF COMMAND PER GALLON.

STUDY R-H-078-86

ANIMAL NUMBER	SEX	BODY WEIGHT (G)* PRE-EXPOSURE	DAY 1	DAY 3	DAY 5	DAY 7	DAY14	BODY WEIGHT CHANGE, (G)
2001	M	159	156	170	186	193	218	+59
2002	M	142	139	154	167	178	210	+68
2003	M	160	157	169	180	191	218	+58
2004	M	154	150	164	177	188	216	+62
2005	M	139	133	146	161	169	194	+55
2006	M	159	156	170	187	195	216	+57
2007	M	150	146	161	176	188	217	+67
2008	M	141	134	147	160	174	196	+55
2009	M	143	137	153	166	176	206	+63
2010	M	155	151	166	177	190	218	+63
Mean +/- S.D.	M	150+/- 8.3	146+/- 9.5	160+/- 9.3	174+/- 9.7	184+/- 9.1	211+/- 9.3	+61
2051	F	119	117	123	130	139	152	+33
2052	F	127	124	134	141	150	158	+31
2053	F	130	129	133	140	148	155	+25
2054	F	129	121	126	136	144	153	+24
2055	F	126	122	132	137	143	154	+28
2056	F	130	130	135	141	146	156	+26
2057	F	133	131	139	145	152	159	+26
2058	F	125	122	132	139	145	153	+28
2059	F	123	118	123	133	137	149	+26
2060	F	129	127	131	140	145	154	+25
Mean +/- S.D.	F	127+/- 4.0	124+/- 5.0	131+/- 5.2	138+/- 4.3	145+/- 4.6	154+/- 2.9	+27

* Body weight of '0' represents an animal that has died.

+ DATA Excerpted From Submission (EPA Accession No. 402-796-02)

TABLE 3

MORTALITY DATA FOR MALE AND FEMALE FISCHER 344/Crl RATS
EXPOSED NOSE ONLY FOR FOUR HOURS TO A LIQUID DROPLET AEROSOL OF TREFLAN/COMMAND PREPACK E.C.,
AN EMULSIFIABLE CONCENTRATE FORMULATION (FN 5227)
CONTAINING THREE POUNDS OF TREFLAN AND 2.25 POUNDS OF COMMAND PER GALLON

STUDY R-H-078-86

(DATA EXCERPTED From Submission, EPA Accession No. 402796-02)

GROUP NUMBER	TOTAL GRAVIMETRIC CONCENTRATION (mg/L)	SEX	OBSERVATION DAY														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	4.74	M	1			1		1	1						1		
		F			1	1			2				1		1	1	
2	0.66	M															
		F															

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Appendix

Data excerpted from submission (EPA Accession No. 402796-02)

850

Command/trifluralin tox review

Page _____ is not included in this copy.

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851