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

007246

JUN 14 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ofternol 2 (Isofenphos) Insecticide - Review of 21-Day Dermal Toxicity Study in Rabbits

Tox Chem No. 447AB
HED Project No. 9-0592

FROM: Yiannakis M. Ioannou, Ph.D., Section Head
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THRU: Marcia van Gemert, PH.D., Acting Chief
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

MRID Nos: 409171-01

Registrant : Mobay Croporation, Stilwell, KS

Action Requested:

Review a 21-day dermal toxicity study in rabbits

Conclusions and Recommendations

Male and female rabbits (5/sex/dose level) were exposed to dermal applications of Oftanol 2 at dose levels of 0, 10, and 40 mg/kg/day for 6 hours a day, 5 days per week for 3 weeks. The only compound related effect reported was the inhibition of cholinesterase activity in plasma, erythrocyte and brain. Based on these effects, the LEL was 40 mg/kg/day (both sexes) and the NOEL 10 mg/kg/day.

The study was however classified as Core-Supplementary based on the following deficiencies (see also DER):

1. The purity of the test article was not reported.

2. The stability of the test article at room temperature for at least 7 days was not reported.

3. The dorsal area of each animal treated with offanol 2 was not reported

The study can be upgraded to Core-Minimum classification when these deficiencies are resolved.

FORMATION

NATIONAL SECURITY INFORMATION (EO 12065)

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EPA No.: 68D80056
DYNAMAC No.: 175-C
TASK No.: 1-75C
June 6, 1989

DATA EVALUATION RECORD

OFTANOL 2 INSECTICIDE

Subchronic Dermal Toxicity Study in Rabbits

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Prenta for

Date: June 6, 1989

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DYNAMAC No.: 175-C
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DATA EVALUATION RECORD

OFTANOL 2 INSECTICIDE

Subchronic Dermal Toxicity Study in Rabbits

REVIEWED BY:

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Date: 6/9/89

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DATA EVALUATION RECORD

GUIDELINE §82-2

STUDY TYPE: Subchronic dermal toxicity study in rabbits.

MRID NUMBER: 409171-01.

TEST MATERIAL: Oftanol 2 insecticide; 1-methylethyl 2-[[ethoxy[(1-methylethyl)-amino]phosphinothioyl]oxy]benzoate.

SYNONYM(S): Isofenphos.

STUDY NUMBER(S): 91333.

SPONSOR: Mobay Corporation, Stillwell, KS.

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA.

TITLE OF REPORT: Oftanol 2 Insecticide 21-Day Toxicity Study in Rabbits.

AUTHOR(S): David E. Bailey.

REPORT ISSUED: September 11, 1986.

CONCLUSIONS:

When male and female albino rabbits were exposed to daily dermal applications of oftanol 2 insecticide at levels of 0, 2.5, 10, or 40 mg/kg/day for 6 hours per day, 5 days per week for 3 weeks, there were no overt signs of toxicity or dose-related effects on body weight, food consumption, dermal irritation, hematology, or gross or microscopic pathology. There were no dose-related effects on the clinical chemistry parameters of dosed animals with the exception of erythrocyte, plasma, and brain cholinesterase activities, which were decreased in high-dose females when compared to control animals. A dose-related decrease was found in the erythrocyte cholinesterase activity of dosed males when compared to control animals. Increased body weight gain and food consumption were noted in low-dose and high-dose males but was not considered significant. Based on inhibition of cholinesterase activity in female rabbits, the Lowest-Observed-Effect Level (LOEL) is 40 mg/kg/day, and the No-Observed-Effect-Level (NOEL) is 10 mg/kg/day.

Classification: Core Supplementary.

A. MATERIALS:

1. Test Compound: Oftanol 2 insecticide; description: White viscous liquid; batch No.: 4030246; purity 100% (assumed).

2. Test Animals: Species: Rabbit; strain: HRA:(NZW)SPF; age: Not given; weight: 2.0-2.4 kg; source: Hazleton Research Products, Inc., Denver, PA.

B. STUDY DESIGN:

1. Animal Assignment: After 14 days of acclimation, animals were assigned to the following test groups using a computer-generated weight randomization program:

Test group	Dose (mg/kg/day)	Main study (3 weeks) Males	Main study (3 weeks) Females
1 Control	0	5	5
2 Low (LDT)	2.5	5	5
3 Mid (MDT)	10	5	5
4 High (HDT)	40	5	5

2. Dose Preparation: The liquid test material was used as received from the sponsor; the study author reported that the active ingredient was assumed to be 100%. The appropriate amount of test material was weighed and combined with the appropriate amount of vehicle (distilled water) to produce solutions for each specified dose level. Solution was easily attained by stirring. Dosing solutions were prepared weekly and stored at room temperature. Dose volumes were adjusted weekly to allow for animal weight changes.

3. Preparation of Animal Skin: The dosing solution was applied to the test animals at the rate of 0.5 mL/kg body weight. The entire torso of each animal was shaved prior to study and at days 1, 5, 9, 12, 16, and 19. The dosing solution was applied (to an unspecified area of the dorsal aspect) under a nonabsorbent rubber damming; cloth wrapping was applied over the damming and secured for a period of 6 hours, after which time the test site was wiped with a moistened towel. Rabbits were treated 5 days/week for 3 weeks. Control animals were treated with the vehicle in volumes equivalent to test animals.

4. Food and Water Consumption: Animals received food (Purina Certified High Fiber Rabbit Chow #5325) and water *ad libitum*.

5. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, food consumption, hematology, clinical chemistry data, and organ weights were initially analyzed using Levene's test for homogeneity of variances. Homogenous data were analyzed using a one-way analysis of variance. Heterogenous data were analyzed by a series of transformations to homogeneity or analysis of variance of the rank-transformed data. Significant data were analyzed by Dunnett's T-test. The Terpstra-Jonckheere nonparametric trend test was used on both homogenous and heterogenous data.

5. Quality Assurance: A quality assurance statement was signed and dated February 20, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality and daily for signs of toxicity.

   Results: One control male was found dead on Day 14 of quarantine. Prior to dosing on Day 1, two control females, one low-dose female, and one high-dose female were removed from the study due to anorexia. These animals were all replaced with extra animals from the initial selection pool. One control female was sacrificed moribund on Day 3 (soft feces, anorexia, prostrate). All other animals survived to terminal sacrifice. Random clinical observations included urine stains, anorexia, thinness, and soft feces. None of these observations were considered compound related by the study author. No dermal irritation was found in dosed males or females.

2. Body Weight: Individual body weights were recorded prior to study initiation and weekly during the study.

   Results: Mean body weights of dosed females were slightly but not significantly decreased when compared to concurrent controls; mean body weights of dosed males were similar to controls (Table 1). Body weight gains of low-dose and high-dose males (0.27 and 0.30 kg, respectively) were reported to be significantly higher ($p < 0.05$) than concurrent controls (0.11 kg); body weight gains of dosed females were slightly decreased when compared to concurrent controls but were within the range of individual variation.

3. Food Consumption and Compound Intake: Consumption was determined weekly. Compound intake was not measured.

   Results: Food consumption was similar in dosed and control females (Table 2). Food consumption at week 3 and total food consumption from weeks 1 to 3 were significantly ($p < 0.05$) increased in dosed males when compared to concurrent controls; however, this increase is of no toxicological importance since food consumption of control males appears to have been slightly decreased during the study period.

TABLE 1. Representative Results of Mean Body Weight (± S.D.) and Mean Body Weight Gain (± S.D.) of Rabbits Treated Dermally with Oftanol for 3 Weeks[a]

Dose Group (mg/kg/day)	Mean Body Weight (kg) at Week 0	1	2	3	Mean Body Weight Gain (kg) 0-3 Weeks
Males					
0	2.25 ± 0.10	2.07 ± 0.17	2.25 ± 0.19	2.36 ± 0.15	0.11 ± 0.11
2.5	2.20 ± 0.07	2.25 ± 0.05	2.37 ± 0.09	2.47 ± 0.08	0.27 ± 0.09*
10	2.28 ± 0.08	2.16 ± 0.21	2.36 ± 0.11	2.48 ± 0.10	0.20 ± 0.09
40	2.25 ± 0.06	2.27 ± 0.15	2.44 ± 0.13	2.56 ± 0.11	0.31 ± 0.09*
Females					
0	2.24 ± 0.08	2.21 ± 0.18	2.45 ± 0.14	2.55 ± 0.07	0.29 ± 0.10[b]
2.5	2.20 ± 0.07	2.13 ± 0.15	2.29 ± 0.04	2.39 ± 0.04	0.19 ± 0.08
10	2.19 ± 0.14	1.96 ± 0.18	2.28 ± 0.14	2.38 ± 0.18	0.19 ± 0.07
40	2.25 ± 0.06	2.18 ± 0.23	2.31 ± 0.30	2.39 ± 0.28	0.14 ± 0.28

[a]Based on five animals/sex/dose with the exception of four females in the control group.

[b]Calculation corrected for animal No. E40220 which was sacrificed moribund on day 3.

*Significantly different from the control value ($p < 0.05$).

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TABLE 2. Results of Mean Food Consumption (± S.D.) of Rabbits Treated Dermally with Oftanol for 3 Weeks

Dose Group (mg/kg/day)	Mean Food Consumption (g) at Week 1	2	3	Total Food Consumption 1-3 Weeks
		Males		
0	862 ± 415.1	1254 ± 240.1	987 ± 316.4	3103 ± 469.4
2.5	1254 ± 208.3	1358 ± 84.8	1249 ± 97.7*	3861 ± 340.6*
10	1180 ± 183.1	1356 ± 115.3	1248 ± 48.4*	3784 ± 285.0*
40	1201 ± 291.4	1373 ± 222.7	1311 ± 49.1*	3885 ± 423.7*
		Females		
0	1099 ± 555.5	1530 ± 108.7	1242 ± 72.5	3871 ± 579.5
2.5	1074 ± 282.3	1260 ± 166.5	1139 ± 89.4	3473 ± 231.8
10	635 ± 490.7	1368 ± 252.5	1231 ± 151.1	3234 ± 419.9
40	1167 ± 469.5	1369 ± 173.9	1208 ± 32.0	3744 ± 637.8

*Significantly different from control value ($p < 0.05$).

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from all animals for plasma and erythrocyte cholinesterase prior to study initiation and at weeks 1, 2, and 3. Blood was collected at terminal sacrifice for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)+
X Hemoglobin (HGB)+
X Leukocyte count (WBC)+
X Erythrocyte count (RBC)+
X Platelet count+
Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
Mean corpuscular HGB (MCH)
Mean corpuscular HGB concentration (MCHC)
Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)

Results: All clinical hematological parameters were similar for control and dosed males and females.

b. Clinical Chemistry

Electrolytes
X Calcium+
X Chloride+
Magnesium+
X Phosphorus+
X Potassium+
X Sodium+

Enzymes
Alkaline phosphatase (ALP)
X Cholinesterase (RBC, plasma, brain)
Creatinine phosphokinase
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)+
X Serum aspartate aminotransferase (SGOT)+
Gamma glutamyltransferase (GGT)

Other
X Albumin+
Albumin/globulin ratio
X Blood creatinine+
X Blood urea nitrogen+
Cholesterol
X Globulins
X Glucose+
X Total bilirubin+
Direct bilirubin
X Total protein+
Triglycerides

+Recommended by Subdivision F (October 1982) Guidelines.

Results: All clinical chemistry parameters, with the exception of cholinesterase activities, were similar in control and treated male and female rabbits. Representative cholinesterase activities are presented in Table 3. Erythrocyte cholinesterase showed a time- and dose-related decrease in activity in high-dose males and females. At week 2, these activities in males and females were 81 and 73%, respectively, of the controls. At week 3, these activities were [illegible] and 78%, respectively, of the control activities. Plasma cholinesterase showed a similar but less extensive dose-related decrease in activity when compared to the control values. For high-dose male and female animals at week 2 these activities were 88 and 76%, respectively, of the control values; at week 3, these activities were 82 and 78%, respectively of the control values. Brain cholinesterase activities at terminal sacrifice were slightly but not significantly reduced in high-dose males and significantly ($p < 0.05$) reduced (29%) in high-dose females.

Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	Aorta	Brain
Salivary glands	Heart	Peripheral nerve (sciatic nerve)
Esophagus	Bone marrow	Spinal cord (3 levels)
Stomach	Lymph nodes	Pituitary
Duodenum	Spleen	Eyes (optic nerve)
Jejunum	Thymus	
Ileum		
Cecum		
Colon		
Rectum		
XX Liver*		
XX Gallbladder		
Pancreas		

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*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. Representative Mean Cholinesterase Activity[a] of Rabbits Treated Dermally with Oftanol for 3 Weeks

Parameter/ Week	Dose Group (mg/kg/day) 0	2.5	10	40
Males				
Plasma				
1	100 ± 7.5	106 ± 4.2	98 ± 9.3	91 ± 4.5
2	100 ± 16.3	101 ± 6.6	91 ± 11.1	88 ± 11.0
3	100 ± 9.3	102 ± 12.0	88 ± 14.0	82 ± 28.1
Erythrocyte				
1	100 ± 11.3	97 ± 8.6	96 ± 9.4	85 ± 5.6
2	101 ± 13.4	98 ± 9.2	92 ± 8.1	81 ± 12.7
3	100 ± 23.1	89 ± 13.7	100 ± 15.9	77 ± 6.8
Brain				
1	100 ± 18.9	85 ± 21.6	110 ± 7.8	89 ± 11.1
Females				
Plasma				
1	100 ± 6.7	93 ± 4.7	89 ± 9.0	79 ± 8.7*
2	100 ± 4.8	96 ± 7.0	94 ± 11.4	76 ± 13.9*
3	100 ± 2.9	95 ± 13.1	100 ± 6.8	78 ± 10.9*
Erythrocyte				
1	100 ± 5.0	93 ± 10.8	99 ± 9.7	84 ± 7.1
2	100 ± 10.9	88 ± 16.3	91 ± 7.3	73 ± 4.8*
3	100 ± 3.8	101 ± 25.4	107 ± 24.0	78 ± 7.4*
Brain				
4	100 ± 9.6	101 ± 22.4	94 ± 10.2	71 ± 18.7*

[a]Cholinesterase activity reported as adjusted activity (%) = $\frac{\text{Individual Percent Activity at the Specified Interval}}{\text{Mean Percent Activity of Control Group at the Specified Interval}} \times 100.$

*Significantly different from control value ($p < 0.05$).

Respiratory	Urogenital	Glandular
Trachea	XX Kidneys*	Adrenals
Lung	Urinary bladder	Lacrimal gland
	XX Testes	Mammary gland
	XX Epididymides	Thyroids
	Prostate	Parathyroids
	Seminal vesicle	Harderian glands
	Ovaries	
	Uterus	Other
		Bone (sternum and femur)
		Skeletal muscle
		X Skin (normal and treated)*
		X All gross lesions and masses

Histological examination was performed on control and high-dose animals.

Results:

a. Organ Weights: Mean absolute organ weights and organ-to-body weight ratios were similar in dosed and control males and females.

b. Gross Pathology: No treatment-related macroscopic findings were noted. No target organs were identified.

c. Microscopic Pathology:

1) Norneoplastic: Commonly occurring spontaneous lesions were seen in dosed and control animals; these included nonsuppurative pericholangitis, focal chronic-active hepatitis, and hepatocytic vacuolation. Minimal to slight diffuse subepithelial pleocellular infiltrates were observed in both treated and untreated skin (Table 4). No other dermal alterations were exhibited.

2) Neoplastic: No neoplastic changes were found in dosed animals.

*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Representative Nonneoplastic Findings in Rabbits Treated Dermally with Oftanol for 3 Weeks

	Dose Group (mg/kg/day)							
	Males				Females			
	0	2.5	10	40	0	2.5	10	40
Liver	(5)[a]	(0)	(0)	(5)	(5)	(0)	(0)	(5)
Nonsuppurative pericholangitis	2	0	0	2	3	0	0	3
Hepatocytic vacuolation	1	0	0	1	2	0	0	1
(Multi)focal chronic active hepatitis	0	0	0	1	0	0	0	1
Skin (untreated)	(5)	(0)	(0)	(5)	(5)	(0)	(0)	(5)
Subepithelial pleocellular infiltrate diffuse	2	0	0	2	2	0	0	1
Skin (treated)	(5)	(0)	(0)	(5)	(5)	(0)	(0)	(5)
Subepithelial pleocellular infiltrate diffuse	3	0	0	4	4	0	0	4
Subepithelial pleocellular infiltrate, focal	0	0	0	0	0	0	0	1
Kidney	(5)	(0)	(0)	(0)	(5)	(0)	(0)	(5)
Chronic nephropathy	0	0	0	0	0	0	0	1
Tubular dilation	1	0	0	0	0	0	0	0
Mineralization	0	0	0	0	1	0	0	0
Focal mononuclear infiltration	[illegible]	0	0	0	1	0	0	0

[a]Numbers in parentheses are the numbers of tissues examined histologically.

D. STUDY AUTHOR'S CONCLUSIONS:

Dermal administration of oftanol 2 at doses of 2.5, 10, and 40 mg/kg/day produced no dermal irritation. Significant dose-related cholinesterase inhibition (plasma, erythrocyte, and brain) was produced in females dosed with 40 mg/kg/day; erythrocyte cholinesterase was inhibited in males receiving the same dose. Based on cholinesterase inhibition, the LOEL is 40 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate, and the conduct of the study was acceptable; however, several deficiencies were noted. (1) The purity of the test chemical is assumed to be 100%, but there is no independent verification of this assumption. (2) No explanation is offered for the two early deaths of the control animals. Of 40 animals, one male died on the last day of quarantine, and one female died on day 3; four females had to be replaced due to anorexia prior to day 1. This represents 15% of the animals which were released from quarantine. (3) The stability of the test chemical in the dosage form (prepared weekly and stored at room temperature) is not documented. Each concentration of the prepared dosage form is not listed. (4) There is no justification provided for the choice of dosages. (5) The physical area (cm x cm) of application is not given and must be assumed as nonstandard between animals. This may affect the rate of percutaneous absorption of the test chemical.

Based on erythrocyte, plasma, and brain cholinesterase inhibition at 40 mg/kg, the LOEL for systemic toxicity is 40 mg/kg/day, and the NOEL is 10 mg/kg oftanol 2/day.