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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 100-524. Evaluation of 21-Day Dermal Toxicity Study in Rabbits with Diazinon Technical

FROM: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/1/88
Section I, Toxicology Branch I (IRS)
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TO: George T. LaRocca, PM Team (15)
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THRU: Edwin R. Budd, Section Head
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Bdd 12/1/88 12/1/88

Record No.: 234061
MRID/Accession No.: 406608-07

Tox. Chem. No.: 342
Project No.: 9-0275

At the request of Special Review/Reregistration Division (Dona M. Williams), Toxicology Branch I/IRS has expedited an evaluation of the following study:

Diazinon Technical: 21-Day Dermal Toxicity Study in Rabbits; Ciba-Geigy Corporation; No. 842007; June 11, 1984.

The only finding of concern was an inhibition of acetylcholinesterase (AChE) activities in serum, erythrocytes (RBC) and brain of both sexes. NOELs were as follows:

Male rabbits: 1 mg/kg, based on inhibition of AChE activity in serum. The NOEL for RBC and for brain AChE activity was 5 mg/kg.

Female rabbits: Possibly < 1 mg/kg (LDT), based on significant inhibition of serum AChE activity. This is a tentative value, pending comments and additional statistical analyses from the TB/HED statistical team. The NOEL for RBC AChE activity was 5 mg/kg and for brain AChE activity was 1 mg/kg.

Classification of study: Core-Minimum

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Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/1/88
Section I, Tox. Branch I/IRS (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head
Section I, Tox. Branch I/IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 82-2. Repeated Dose Dermal Toxicity: 21-Day Study (Rabbit)

TOX. CHEM. NO.: 342

MRID NO.: 406608-07

TEST MATERIAL: Diazinon Technical; Purity: 97.1%; Brown liquid; Batch No.: FL-831737

STUDY NUMBER(S): 842007

SPONSOR: Ciba-Geigy Corporation, Agriculture Division, Greensboro, NC

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ

TITLE OF REPORT: Diazinon Technical: 21-Day Dermal Toxicity Study in Rabbits

AUTHOR(S): C. N. Tai and R. Katz

REPORT ISSUED: June 11, 1984

CONCLUSIONS:

Diazinon Technical was administered topically to New Zealand rabbits of both sexes at doses of 0, 1, 5 or 100 mg/kg (reduced to 50 mg/kg on day 8) for 5 days a week during a 3-week period. The only finding of concern was an inhibition of AChE activity in serum, RBC and brain. According to the testing laboratory, the NOEL was "at least 5 mg/kg" for both sexes, but Toxicology Branch I/IRS disagrees with this conclusion (see review for details).

NOEL: 1 mg/kg, males; based on inhibition of acetylcholinesterase (AChE) activity in serum. The NOEL for RBC and for brain AChE activity was 5 mg/kg.

Possibly < 1.0 mg/kg (LDT), females; based on significant inhibition of serum AChE activity; tentative value, pending comments from the TB/HED statistical team. The NOEL for RBC AChE activity was 5 mg/kg and for brain AChE activity was 1 mg/kg.

CLASSIFICATION: Core-Minimum

EXPERIMENTAL PROCEDURES

Treatment was started on January 23, 1984, and terminated on February 14, 1984.

Groups of 5 male and 5 female New Zealand rabbits were treated with Diazinon Technical (0, 1, 5 or 100/50 mg/kg) for 5 consecutive days/week, for 3 consecutive weeks. Because 4 males died in the high-dose group, the dose was reduced from 100 mg/kg to 50 mg/kg after 5 treatment days (7 study days). The test material was applied on intact skin as suspensions in 50% Polyethylene Glycol 300 (PEG 300), using 1 mL of a suspension/kg of body weight (based on weekly body weights). Suspensions of Diazinon Technical were prepared on the day of application. Immediately after application, the sites were occluded, a plastic collar placed around the neck of each rabbit, the occlusions removed 6 hours later, and the sites rinsed with water to remove excess test or control (PEG 300) substance. The rinsed application sites were wiped dry and the animals returned to their cages. At the initiation of treatment, males were approximately 10-11 weeks old and weighed 1.94-2.54 kg, whereas females were approximately 11-12 weeks old and weighed 1.81-2.31 kg.

The animals were: 1) obtained from H.A.R.E. Rabbits for Research, Hewitt, NJ; 2) acclimated for 17 days; 3) assigned randomly to groups; 4) housed individually at 65 +_ 5° F, relative humidity of 50 +_ 20%, and light/dark light cycle of 12 hours; and 5) allowed unrestricted amounts of food (Certified Purina Rabbit Chow #5322) and water.

The following parameters were examined for all rabbits on the study:

1. Observation for toxic signs and mortality: once or twice daily.

2. Physical and ocular examination: before initiation of treatment and at the termination of the study.

3. Dermal examination and grading: once daily immediately prior to the application of the test substance (see Attachment I for the scoring system used).

4. Body weights and food consumption: weekly and at study termination.

5. Hematology and clinical chemistry: before initiation of treatment and at study termination. Non-fasted rabbits were bled from the auricular vessels. Serum was used for biochemistry; blood for hematology was collected with EDTA. The following tests were conducted on all animals during the predose period and

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at study termination:

Hematology

RBC Count	WBC Count
Hematocrit	Differential Platelet Count
Hemoglobin	Clotting Time
Red Cell Morphology	Reticulocyte Count

Biochemistry

Total Protein	Sodium
Albumin	Potassium
Globulin	Calcium
A/G Ratio	Chloride
Glucose	SGOT
BUN	SGPT
Total Bilirubin	GGT
Creatinine	Alkaline Phosphatase
Phosphorous (Inorganic)	Acetylcholinesterase (RBC, Serum, Brain)

Procedures used for the clinical laboratory tests were referenced. The following reference was cited from the determination of acetylcholinesterase activities:

> BMD Cholineasterase Reagent Set, Cat. No. 124117, Boehringer Mannheim Diagnostics, Inc., Houston, TX 77063

6. Necropsy: All animals, including the nonsurvivors, were necropsied and special attention was paid to treated and untreated skin. Selected cut surfaces of brain and spinal cord were also examined.

7. Organ weights: absolute and relative (organ/body weight ratios.

The following organs were weighed:

Adrenals	Liver
Brain, including brain stem*	Pituitary
Heart	Testes/Ovaries
Kidneys	

*Portions saved for acetylcholinesterase determinations were also weighed.

8. Histopathology: The following tissues were examined:

All gross lesions
All tissue masses

Brain, kidneys, and liver
Skin (treated and untreated areas)

STATISTICAL EVALUATIONS

Body weight, body weight gain, food consumption, hematology, clinical biochemistry and organ weight data were analyzed by the "Trend Test for Slopes in Time" (see Attachment II for details). Histopathology data were evaluated separately for each sex by Fisher's exact tests and for both sexes by computing "convolved probabilities."

RESULTS

Toxic Signs and Mortality

Four of 5 high-dose male rabbits died between study days 3 and 6. All other animals survived the scheduled experimental period. Toxic signs observed in the high-dose animals of both sexes included: anorexia, ataxia, fasciculations, tremors, diarrhea, hypoactivity, hypotonia, and salivation. These signs were generally observed during the first week of the test period. Except for one or two occurrences of anorexia, diarrhea, or soft feces, there were no gross signs of toxicity exhibited by low- or mid-dose animals.

Ocular Examinations

Ophthalmoscopic examinations performed on all animals during the third week of treatment revealed no abnormalities.

Dermal Reactions

Throughout the test period, all groups, including vehicle controls, exhibited very slight erythema (grade 1) at the treated sites. Well-defined erythema (grade 2) was occasionally observed in all treated groups of both sexes. Dry, flaky areas of skin were noted in all high-dose females and in one mid-dose male during the second week of treatment. In addition, red foci on the treated site were observed in the only surviving high-dose male. Edema was not observed in any animal during the course of this study.

Body Weight

There were no statistically significant differences in weekly mean body weights between the control and treated groups during the entire study. However, at the termination of the study, all treated animals gained more weight than the control animals. Comparing the initial and final body weights, the mean body weight gains for the control, low-dose, mid-dose and high-dose female groups were 9, 28, 24, and 19%, respectively. The

corresponding weight gains for the male groups were 17, 34, 24 and 13*%, respectively. (*Value for one surviving high-dose male rabbit).

Food Consumption

Overall, treated rabbits of both sexes at all dose levels tended to consume more food during the 3-week test period than did their respective controls. Compared with the controls, the mean percent increases in food consumption were: low-dose and mid-dose males and females, 6 to 16; high-dose females, 0.1; and high-dose male (single survivor), 23.

Hematology

Decreases in the percentage of eosinophils (from 10 to 1) and basophils (from 6 to 0) were observed in the high-dose male (only survivor), when the pretreatment values were compared with those obtained at the termination of the study. In the females, the only statistically significant finding was a decrease in platelet counts (5.8%; $p < 0.05$) in the high-dose group, when the mean pretreatment values for this group were compared with those obtained at the termination of the study.

Considering that, before treatment, the percentage of eosinophils and basophils in the high-dose male group ranged from 0 to 10 and 1 to 9, respectively, the above changes do not appear to be treatment-related. A slight decrease in platelet count in the high-dose females also appears to be treatment-unrelated.

Clinical Biochemistry

Relative to controls, statistically significant ($p < 0.05$) increases in serum inorganic phosphorous (males) and serum glucose (females) were observed at the termination of the study in all treated groups. The percent increases in phosphorous in the low-dose, mid-dose and high-dose male groups were 12.7, 14.5 and 16.4 (one animal), respectively. The percent increases in glucose in the low-dose, mid-dose and high-dose female groups were 3.0, 6.2 and 7.8, respectively. These increases in the treated groups were apparently due to decreases in these parameters in the control groups. At the termination of the study, the pretreatment value for inorganic phosphorous was decreased by 19.1% (control males) and for glucose by 12.8% (control females). In the case of the Diazinon-treated groups, the predosing and terminal values for inorganic phosphorous (males) and glucose (females) were similar.

Relative to controls, other statistically significant ($p < 0.05$) findings included increases in serum albumin (8.8%), albumin/globulin ratio (38%) and sodium concentration (2%), all

in the high-dose females. However, in each instance the predosing control values were decreased at the termination of the study as follows: albumin (8.1%), albumin/globulin ratio (29%) and sodium (0.06%). The above increases, therefore, do not appear to be treatment-related.

Acetylcholinesterase Activities

Relative to controls, brain acetylcholinesterase (AChE) activity was inhibited in the mid-dose females and in the high-dose male (only survivor) and females. Serum and RBC AChE activities were inhibited in most of the treated groups, but the statistical significance of these inhibitions depended upon the type of evaluation used, i.e. trend tests vs. controls or trend tests for slopes in time using baseline (predosing) values. These data are summarized below.

Table I. Percent Inhibition of AChE Activities at the Termination of the Study, Relative to Control (Group 1) Values

Group	2	3	4
Diazinon (mg/kg)	1	5	100/50[a]
	Male Rabbits		
Brain	0	0	28.0[b]
RBC	0	0.8	38.9**[b]
Serum	4.1	22.6	63.6*[b]
	Female Rabbits		
Brain	0	18.1*	43.3**
RBC	7.6	8.3	31.8**
Serum	31.9*	35.4**	62.3**

*$p < 0.05$ **$p < 0.01$

[a]The initial dose of 100 mg/kg was reduced to 50 mg/kg on day 8.
[b]Values for one surviving rabbit.

Table II. Percent Change in AChE Activities at the Termination of the Study, Relative to Baseline (Predosing) Values

Group	1	2	3	4
Diazinon (mg/kg)	0	1	5	100/50[a]
		Male Rabbits		
RBC	+23.6	+29.9	+13.0	-20.0[b]
Serum	-1.3	+10.6	-21.4	-64.3**[b]
		Female Rabbits		
RBC	+24.5	+29.8	+33.0	-13.5**
Serum	-3.1	-15.6	-18.4	-56.5**

**p < 0.01

[a]The initial dose of 100 mg/kg was reduced to 50 mg/kg on day 8.
[b]Values for one surviving rabbit.

\+ = Increase in AChE activity
\- = Inhibition of AChE activity

Although not statistically significant (according to the testing laboratory), the decrease in serum AChE activity in male rabbits at 5 mg/kg is considered by Toxicology Branch I/IRS to most likely be related to the test material.

Necropsy

Treatment-unrelated red discoloration and dark diffuse lesions were observed in the stomach of the only surviving high-dose male. Nothing remarkable was observed in other animals on the study, including the nonsurvivors.

Organ Weights

Relative to controls, a statistically significant ($p < 0.05$) decrease was observed in the mean kidney/terminal body weight ratio in the high-dose females (0.82% in controls and 0.59% in the treated group). Other relative organ weights and all absolute organ weights were similar in the control and treated groups.

Histopathology

Treatment-related minimal hyperkeratosis of skin at the application site (back) was observed in 3 males and 3 females

from the high-dose group. All other changes (subacute lymphocytic or granulomatous inflammation of liver, kidneys and/or brain, the only other tissues examined)were dose-unrelated and regarded as incidental. Liver and kidneys in 3 (out of 4) nonsurvivors in the high-dose male group could not be examined because of autolysis.

COMMENTS

The only finding of concern in this study was an inhibition of AChE activity. According to the testing laboratory, "terminal serum, RBC, and brain cholinesterase activities in treated groups of both sexes exhibited dose-related, depressed trends, relative to both their respective baseline and/or control group values. However, reductions in cholinesterase activities which met the criteria of being both statistically ($p \leq 0.05$) and biologically significant ($\geq$ 20% reduction) only occurred for brain (high-dose females) and serum (high-dose of both sexes------. The no-observable effect level for the dermal application of Diazinon Technical to rabbits was considered to be at least 5 mg/kg while the maximum tolerated dose level was 50 mg/kg."

Based on mortality data, a MTD was probably 50 mg/kg for the male rabbits, but somewhat greater than 50 mg/kg for the female rabbits (there was no mortality in any female group). However, our interpretation of the AChE inhibition data differs from that of Ciba-Geigy's (testing laboratory). Regarding the inhibition of brain AChE activity, Toxicology Branch I/IRS regards an inhibition of 18.1% that is statistically significant as also being biologically significant. Based on this criterion alone, the NOEL for the females would therefore not be 5 mg/kg but 1 mg/kg (LDT). NOELs based on RBC AChE activities are 5 mg/kg for both males and females.

NOELs based on serum AChE activities are somewhat equivocal. If AChE inhibitions observed in the treated rabbits (Groups 2, 3 and 4) at the termination of the study are related to those observed in the concurrent controls (Group 1), the NOEL for serum AChE activity for the males is 1 mg/kg, and there is no NOEL for the females (due to a significant inhibition of serum AChE activity in the low-dose group; see Table I in the review).

In summary, NOELs, based on the inhibition of AChE activities in RBC, serum and brain, are as follows:

Male rabbits: 1 mg/kg; based on inhibition of AChE activity in serum. The NOEL for RBC and for brain AChE activity was 5 mg/kg.

Female rabbits: Possibly < 1.0 mg/kg (LDT); based on significant inhibition of serum AChE activity. This is a tentative value, pending comments and additional statistical analyses from the TB/HED statistical team. The NOEL for RBC AChE activity was 5 mg/kg and for brain AChE activity was 1 mg/kg.

Classification of study: Core-Minimum

Because this 21-day dermal toxicity study had only 5 rabbits/ sex/ dose level and because there were considerable quantitative differences among groups in the mean predosing AChE activities in serum and RBC, especially in the females, we asked our TB/HED statistical team to comment on the statistical procedures used by the testing laboratory and to also perform additional statistical analyses on the data. Their comments and analyses, when available, will be submitted separately.

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Attachment I

APPENDIX III

Scoring of Skin Reactions

Erythema and Eschar Formation	(score)
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Maximum possible	4

Edema Formation	(score)
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible	4

$$\text{Mean Score} = \frac{\text{Sum of All Scores}}{(\text{No. Time Points}) \times (\text{No. Animals})}$$

Attachment II

DIAZINON TECHNICAL: 21-DAY DERMAL TOXICITY STUDY IN RABBITS (MIN 842007)

APPENDIX V

Statistical Methods

Nonpathology Data: All numerical data that are obtained in the course of study will be submitted to the Computer Math Section or Scientific Systems for storage and for generation of interim/or final reports on programs developed by the Statistics Section of CIBA-GEIGY. These programs routinely list individual animal data and provide summary tables, and when the design requirements are met, generate statistical analyses. These analyses are designed mainly to test each parameter for the possible trends existing between treatment groups that comprise different doses of the same compound and a zero dose control. If a significant trend is found, the test procedure is applied again to the remaining treatment groups, excluding the highest dose group, and so on, in order to examine the significance of comparisons of dose groups against control. When appropriate, covariate analysis may be performed. Trend tests for overall means and slopes in time are also performed to detect the possible differences among the treatment groups with respect to the average response and the rate of change of response.

Pathology Data: All data from microscopically investigated animals will be recorded by the pathologists into the Pathology Data Base. The data will be tabulated and tables will be generated by the N032 pathology data system. If sample sizes are adequate, these data will be analyzed separately for each sex by Fisher's exact tests and for both sexes by computing convolved probabilities.

References:

1. Fisher, R. A. (1958). Statistical Methods for Research Workers. 13th Edition, Hafner Publishing Co., Inc., NY, (pp. 356).

2. Feller, W. (1950). An Introduction for Probability Theory and Its Applications, 3rd Edition, John Wiley and Sons, NY, (pp. 266).

3. Scheffe, H. (1959). Analysis of Variance. John Wiley and Sons, NY, (pp. 55-59).

4. Barlow, R. E.; Batholomew, D. J.; Brenner, J. M.; and Brunk, H. D. (1972). Statistical Inference Under Order Restrictions. John Wiley & Sons, NY, (pp. 183-188, 198-207, 214-215).

5. Snedecor, G.W., Cochram, W.G. (1968). Statistical Methods. Sixth Edition. The Iowa State University Press. Ames, IA, (pp. 377-379).

Explanation of the Summary of Statitical Analysis in the Computer Program Printout - Diazinon Tech: 21-Day Dermal Study in Rabbits (MIN 842007)

The statistical procedure in the computer program has been developed for studies to evaluate toxicity of a substance where treatment groups comprise a series of doses of the substance and a zero dose control. It is assumed that the effect of the substance, if any, will be to increase (or decrease) the mean response as the dose is increased. We consider, therefore, an analysis based on the assumption that the responses are monotonically ordered. The test for trend at each time point (except at pre-dose where an F test for the equality of group means is performed [1]) is a test based on a specific contrast (maximin contrast) and is applied to all treatment means using optimum choice of scores [2]. The scores are derived to produce the largest minimum power for monotone alternatives. It tests the following null hypothesis using the critical values of an ordinary t-test.

$H_0 : \mu_1 = \mu_2 = \ldots = \mu_k$ (μ_i = mean of the ith treatment group $i = 1, 2, \ldots, k$)

against the monotone alternatives

$H_1 : \mu_1 \geq \mu_2 \geq \ldots \geq \mu_k$ with at least one strict inequality,

or $\mu_1 \leq \mu_2 \leq \ldots \leq \mu_k$ with at least one strict inequality.

Upon rejection of the null hypothesis, a maximum contrast is applied again to all but the highest dose mean. If this second contrast is found to be significantly different from zero when tested by an ordinary t-test (with the same degree of freedom as in the previous test), then a maximin contrast is calculated again to all but the highest and the second highest dose means, and so on until a contrast is found to be not significantly different from zero when tested by an ordinary t-test (with the same degree of freedom as the previous tests) or if there are no more comparisons with control.

The result of applying the sequence of maximin contrasts in this prescribed manner results in the comparison of dose levels with a zero dose control and is indicated to the right hand side of the means table as:

**: Dose vs. Control, flagged at p <.01,
*: Dose vs. Control, flagged at p <.05,
NS: Not significant p >.05.

In addition, the "p-value" given directly below the means table indicates the significance of the test for trend among all treatment groups (the maximin contrast applied to all treatment groups).

Trend tests for overall means and slopes in time [3] are also performed to detect the possible differences among the treatment groups with respect to the average response and the rate of change of response.

The average response and slope are calculated for each animal from its baseline measurement and all measurements collected up to the last dosing period. Animals with any missing measurements are excluded from the analyses.

A significant trend test for overall means implies a significant increase (or decrease), in the average response as the dose is increased. Likewise, a significant trend test for slope in time implies a significant increase (or decrease) in the rate of change of response from time to time as the dose is increased.

Mean body weights and mean feed consumed were plotted over time for each group/sex.

REFERENCES: [1] Scheffe, H. (1959) Analysis of Variance. John Wiley and Sons, pp. 55-59.

[2] Barlow, R. E., Bartholomew, D. J., Bremner, J. M. and Brunk, H. D. (1972). Statistical Inference Under Order Restrictions. John Wiley and Sons, pp. 183-188, 214-215.

[3] Snedecor, G.W., Cochran, W.G. (1968). Statistical Methods. Sixth edition. The Iowa State University Press. Ames, Iowa. pp. 377-379.

Darlene M. Looney 4/17/84
Darlene M. Looney Date
Statistician I

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