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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. Diazinon Technical: 21-Day Dermal Toxicity Study in Rabbits - Statistical Evaluation of Acetylcholinesterase Activities in Erythrocytes, Serum and Brain in Order to Finalize NOELs

Tox. Chem. No.: 342

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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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In December, 1988, at the request of Special Review/Reregistration Division, 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. MRID/Accession No.: 406608-07

The only finding of concern was an inhibition of acetylcholinesterase (AChe) activities in serum, erythrocytes (RBC) and brain of both sexes. NOELs were reported in the expedited review 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 was 1 mg/kg.

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Based on the statistical analyses and comments from Bernice Fisher, Biostatistician, TB/HED statistical team (Attachment I), the NOELs for the inhibition of AChE activities in serum, RBC and brain of the male rabbits remain unchanged (that is, they are the same as those reported in the expedited review). The same applies to the NOELs for the inhibition of AChE activities in RBC and brain of the female rabbits. However, the NOEL for the inhibition of AChE activity in serum of the female rabbits is smaller than 1 mg/kg (LTD). At the 1 mg/kg dose level, the inhibition of AChE activity in the female rabbits was 30.7%, relative to the control value. This inhibition was statistically significant ($p < 0.05$) in the "trend test for slopes in time" (used by Ciba-Geigy, the testing facility) and not statistically significant in the Dunnett's test (used by Bernice Fisher, TB/HED statistical team). However, TB (IRS)/HED regards this inhibition as biologically significant.

According to Bernice Fisher (Attachment I, p. 8), "The results of the SAS calculations of the Standard Error of the Mean indicated that there was unusually large variability in the control group of rabbits in both sexes, especially in the serum ACHE levels. This large variability would thus reduce the reliability of the statistical conclusions of the trend analysis as well as the pairwise comparisons with controls."

In summary, the classification of this study (Core-Minimum) remains unchanged, but a NOEL for the inhibition of AChE activity in serum of the female rabbits was not determined (it is < 1.0 mg/kg, LDT).



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Subject: Diazinon Technical - 21 Day Dermal Study, Rabbits - Statistical Evaluation of Selected Biochemistry Data, AChE-RBC&Serum and Brain cholinesterase (u/l)

caswell no. 342

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Bernice Fisher 1/19/89

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Summary

In females, there was a significant decreasing linear dose trend in the levels of cholinesterase (u/l) in RBC, serum and brain with dermal doses of 1, 5, and 100 (reduced to 50 after 7 days) of diazinon. The pairwise comparison of female control and the 100/50 mg/kg dose level resulted in a significant difference in the RBC,serum and brain. Also in females, the pairwise comparison of of control and the 5mg/kg dose level resulted in a significant difference in the serum and brain at week 3 of the study.

In male rabbits, the dose related trends in the levels of cholinesterase (u/l) in serum were significantly decreased. No other significant findings were observed in males.

Attachment I

Background

The pharmaceutical division of Ciba-Geigy Corporation conducted a 21-day dermal toxicity study of diazinon technical in rabbits (laboratory study no. 842007) and issued it on June 11, 1984.

Diazinon technical was administered topically to both sexes of rabbits in doses of 0, 1, 5, or 100(reduced to 50 on day 8) mg/kg during 5 days a week for a three week period. Each group included 5 animals of each sex.

Dr. K. Locke requested a statistical evaluation of RBC, serum and brain cholinesterase outcome for the three week study with incremental doses of diazinon.

Analysis

Due to the high mortality (4 out of 5 male rabbits died) of males in the highest (100to50 mg/kg) dose group, the statistical evaluation of males included only three dose levels (0,1, and 5 mg/kg) of diazinon.

A SAS program (REG- regression analysis) was formulated to analyse trends in differences from week 0 to week 3 in cholinesterase in RBC and serum with dose increments of diazinon and also to test the significance of trends in data on week 3 for RBC, serum, and brain cholinesterase. The SAS program was also used to compute means and standard error of means of each of these groups, by each sex separately.

The statistical analysis of pairwise comparisons of controls and each dose level for the same 3 parameters ( RBC, serum, and brain cholinesterase) and the same time periods ( week 0 to week 3 for RBC and serum and week 3 only for RBC, serum, and brain) used Dunnett's test to evaluate these differences. The evaluation were based on data for each sex separately.

The following tables presents the mean, standard error of the mean and the statistical results based upon the use of the SAS program and Dunnett's test.

Table 1. Diazinon, Rabbits - RBC,ACHE Levels (u/l)- Week 3 minus Week 0 data, and SAS (REG & Means) & Dunnett's Test Results

| | Dose (mg/kg) | | | |
|---|---|---|---|---|
| Males | 0 | 1 | 5 | 100/50(a) |
| mean | 500.00 | 640.00 | 300.00 | na |
| $SE_m$ | 232.38 | 172.05 | 158.11 | na |
| Female | | | | |
| mean | 520.00** | 560.00 | 600.00 | -280.00* |
| $SE_m$ | 139.28 | 174.93 | 94.87 | 80.00 |

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a dose level reduced to 50 mg/kg on day 8

$SE_m$ Standard Error of Mean

na not applicable

Note: Significance of Trend denoted at Control.
Significance of pairwise comarison with control denoted at Dose level.

* $p<.05$ & ** $p<.01$

Table 2. Diazinon, Rabbits - RBC, ACHE Levels (u/l)- Week 3 data, and SAS (REG & Means) & Dunnett's Test Results

| | Dose (mg/kg) | | | |
|---|---|---|---|---|
| Males | 0 | 1 | 5 | 100/50(a) |
| mean | 2620.00 | 2780.00 | 2600.00 | na |
| $SE_m$ | 115.76 | 91.65 | 104.88 | na |
| Females | | | | |
| mean | 2640.00** | 2440.00 | 2420.00 | 1800.00* |
| $SE_m$ | 136.38 | 74.83 | 37.42 | 44.72 |

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a dose level reduced to 50 mg/kg on day 8

$SE_m$ Standard Error of Mean

na not applicable

Note: Significance of Trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p<.05$ & ** $p<.01$

Table 3. Diazinon, Rabbits - Serum,ACHE Levels (u/l)- Week 3 minus Week 0 data, and SAS (REG & Means) & Dunnett's Test Results

| | Dose (mg/kg) | | | |
|---|---|---|---|---|
| Males | 0 | 1 | 5 | 100/50(a) |
| mean | -8.60* | 60.80 | -140.00 | na |
| $SE_m$ | 65.93 | 44.75 | 26.83 | na |
| Females | | | | |
| mean | -25.20** | -90.00 | -114.60 | -384.40* |
| $SE_m$ | 54.01 | 33.38 | 47.79 | 58.64 |

a dose level reduced to 50 mg/kg on day 8

$SE_m$ Standard Error of Mean

na not applicable

Note: Significance of Trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p<.05$ & ** $p<.01$

Table 4. Diazinon, Rabbits - Serum, ACHE Levels (u/l)- Week 3 data, and SAS (REG & Means) & Dunnett's Test Results

| | Dose (mg/kg) | | | |
|---|---|---|---|---|
| Males | 0 | 1 | 5 | 100/50(a) |
| mean | 662.20 | 635.40 | 512.80 | na |
| $SE_m$ | 85.87 | 48.59 | 45.15 | na |
| Females | | | | |
| mean | 784.40** | 543.40 | 506.60* | 295.80* |
| $SE_m$ | 103.93 | 27.64 | 57.08 | 54.26 |

a dose level reduced to 50 mg/kg on day 8

$SE_m$ Standard Error of Mean

na not applicable

Note: Significance of Trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p<.05$ & ** $p<.01$

Table 5. Diazinon, Rabbits - Brain ACHE Levels (u/l)- Week 3 data, and SAS (REG & Means) & Dunnett's Test Results

| | Dose (mg/kg) | | | |
|---|---|---|---|---|
| Males | 0 | 1 | 5 | 100/50(a) |
| mean | 2874.00 | 3330.00 | 2986.00 | na |
| $SE_m$ | 171.31 | 204.62 | 172.96 | na |
| Females | | | | |
| mean | 3628.00** | 3788.00 | 2972.00* | 2056.00* |
| $SE_m$ | 178.25 | 228.11 | 120.64 | 154.42 |

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a dose level reduced to 50 mg/kg on day 8

$SE_m$ Standard Error of Mean

na not applicable

Note: Significance of Trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p<.05$ & ** $p<.01$

The outcome of this or any other statistical analysis of this study is to be considered not definitive for some of the following reasons.

The selection of the dose levels of 0, 1, 5, and 100 (then reduced to 50 on the 8 day) mg/kg was not justifiably spaced: i.e. 2 levels very close to zero and one level so toxic that is was reduced in both sexes after one week of a 3 week study. In addition 5 animals in a dose group was not sufficient to evaluate 3 or more factors that may show toxicity with diazinon.

The results of the SAS calculations of the Standard Error of the Mean indicated that there was unusually large variability in the control group of rabbits in both sexes, especially in the serum ACHE levels. This large variability would thus reduce the reliability of the statistical conclusions of the trend analysis as well as the pairwise comparisons with the controls.

With consideration of the above limitations that were found in the rabbit study, the following results were observed from the statistical evaluatons.

In males, cholinesterase levels among the 3 dose levels (0, 1, and 5 mg/kg) were not significantly changed in the RBC or the brain. Only in serum, the males had a significantly decreasing trend in ACHE levels in measurements of differences from week 0 to week 3, but also had unusually large variability in the control levels.

In female rabbits, the trends in the levels of cholinesterase in RBC, serum and the brain were all significantly decreasing with dose increments of diazinon. Also in all 3 sites there was a significant difference between the control and the highest (100 to 50) dose group. In addition, in the brain and in the serum, the cholinesterase levels in the control was also significantly different from the 5mg/kg dose level in week 3 of the study.

References:

Dunnett, C.W. (1955) A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J.Am. Stat. Assoc. 50, 1096-1121

Fleiss, J.L. (1986) The Design and Analysis of Clinical Experiments. pg.118