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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 057801. Diazinon: Review of a series 82-7 subchronic neurotoxicity study and a special 28 day feeding study to verify the NOEL and LOEL and assess the time course for inhibition of plasma ChE and RBC and brain AChE.

TOX CHEM No.: 342
PC No.: 057801
Barcode No.: D214452
Submission No.: S485581

FROM: John Doherty, Ph.D., D.A.B.T. John Doherty 12/11/95
Section IV, Toxicology Branch I
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THROUGH: Marion Copley, DVM Marion Copley 1/19/96
Section Head
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I. CONCLUSION

The series 82-7 subchronic neurotoxicity screen study with diazinon (MRID No.: 43543902) was reviewed and determined to be ACCEPTABLE. The accompanying special 28-day study to reassess the NOEL and LOEL and the time course for plasma CHE and RBC and regional brain AChE (MRID No.: 43543901) was reviewed and determined to be SUPPLEMENTARY. The studies taken together satisfy the requirement for a series 82-7 subchronic neurotoxicity study.

These data establish the LOEL for inhibition of plasma CHE and RBC AChE at 30 ppm (0.18 mg/kg/day) and the NOEL at 0.3 ppm (0.018 mg/kg/day). The LOEL and NOEL for inhibition of brain AChE are 300 ppm (18 mg/kg/day) and 30 ppm (0.18 mg/kg/day). The

LOEL and NOEL for systemic effects are 3000 ppm (180 mg/kg/day) and 300 ppm (18 mg/kg/day).

II. Background and Action Requested

The Ciba-Geigy Corporation has submitted the required series 82-7 subchronic neurotoxicity study (MRID No.: 43543902) with rats and an accompanying special 28-day study (MRID No.: 43543901) to assess the time course for inhibition of plasma ChE and RBC and regional brain AChE. These studies were reviewed and the DERs are attached. They are identified in part IV below.

III. Toxicology Branch Comments

1. The studies when taken together satisfy the requirement for a series 82-7 subchronic neurotoxicity study. The special 28-day study was primarily designed to assess the time course for inhibition of plasma ChE and RBC and brain AChE as well as to assess for regional differences in brain AChE inhibition. The rational for selection of the NOEL and LOEL for plasma ChE and RBC and brain AChE and the time course and regional brain inhibition are discussed separately below.

2. **Assignment of NOEL and LOELs.**

a. Plasma ChE and RBC AChE. Both studies produced similar results which demonstrated that the NOEL and LOEL are 0.3 and 30 ppm for both plasma ChE and RBC AChE for both sexes. These values in ppm correspond to 0.018 and 1.8 mg/kg/day based on the 90 day study. The corresponding values for the 28 day study were 0.02 and 2.3. The values for the 28 day study are slightly larger because the younger rats eat more (consume more diazinon). As the rats get older, they eat less per kg of body weight. Thus the mean food consumption is lower for the longer experiment.

Only occasionally were there some statistical differences indicating possible inhibition of these enzymes at 0.3 ppm. This apparent inhibition was not consistent and there were also occasions where the readings for 0.3 ppm were actually higher than the control.

Both studies verified that females were more susceptible than males for plasma ChE inhibition. Males and females, however, were considered similar in their susceptibility to inhibition for RBC AChE.

b. Brain AChE. The two studies differed in that inhibition in the combined "cerebral cortex/hippocampus" was shown to be

statistically significant (25%, $p < 0.05$) at 90 days in the 30 ppm female group. Statistically significant inhibition of the separated cerebrum and hippocampus was not attained at 30 ppm in either males or females in the special 28-day study. Thus, TB-I considered that the NOEL and LOEL for inhibition of brain AChE should be 300 and 3000 ppm.

2. Time course for inhibition.

The special 28-day study assessed plasma ChE and RBC and regional brain AChE at weeks pretest, 1 and 4. In general, maximum inhibition was noted at week 2 and there were no major or remarkable differences between inhibition at the 2 for the regions of the brain assessed. Plasma ChE and RBC AChE showed in some cases for the 300 ppm but less so for the 3000 ppm a lesser inhibition at week four. It was also noted that week 4 control groups had high standard deviations to possibly result in a misleading interpretation. For example, a false indication of recovery of inhibition. Lesser degrees of inhibition were not noted in the 90 day studies when the assessments were made at weeks 4, 8 and 13. Overall, TB-I does not consider that these two studies provide any meaningful data to suggest that ChE/AChE adapts over the 90 days of dosing in these studies.

3. Regional brain AChE inhibition.

Females were more inhibited than males in both studies. Among the females at 300 ppm (the LOEL) there was 62-72% inhibition for all regions tested. This difference is probably not within the resolving power of the assay to demonstrate a marked difference in susceptibility of a specific region.

In males there was some indication that the cerebellum was more susceptible than the other regions because this region was the only region statistically significantly inhibited at each time interval tested (weeks 1, 2 and 4) but inhibition was only about 15%. There was no indication that the cerebellum in females was any more affected than the other regions in this sex at 300 ppm in this study. Thus, TB-I concludes that there are no marked regional brain differences demonstrated in this study.

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
Special Study. 28 day feeding to assess time course of ChE/AChE. Ciba-Geigy Study No.: F-00186 November 7, 1994	D*Z*N Diazinon MG87%	43543901	In a special study (MRID #43543901) designed to assess the time course for and regional brain inhibition of ChE/AChE, four groups of 15/sex Sprague-Dawley Crl $CD^{R}BR$ strain rats were dosed as control, 0.3, 30, 300 or 3000 ppm of diazinon (D*Z *N MG87%) corresponding to approximately 0.02, 2.4, 23 and 213 mg/kg/day in their diets and 5/sex/group were sacrificed at weeks 1, 2 and 4 and plasma ChE and RBC and brain AChE assessments were made. The central nervous system was dissected into the cerebellum, hippocampus, cerebrum, striatum and thoracic spinal cord in order to assess for potential differences in regional sensitivity. Systemic effects including cumulative body weight gain decreases of 26% in males and 39% in females and decreased food consumption (21% in males and 27% in females) and muscle fasciculations (3 males and 8 females at day 8) were noted in the 3000 ppm dose group only. At 30 ppm plasma ChE (males 59% and females 81%) and RBC AChE (39-58% males and 38-59% females) were inhibited. At 300 ppm brain AChE (62-72% at week 4 in females for all regions, only the cerebellum ~22% in males) was inhibited. Maximum inhibition of blood and brain ChE/AChE was attained at week 2 and essentially remained at that level by week 4 without evidence of adaptation. There were no marked or consistent differences in sensitivity in brain regions to AChE inhibition (refer to DER for discussion). **The LOEL for plasma ChE and RBC AChE is 30 ppm and the NOEL is 0.3 ppm. The LOEL for brain AChE is 300 ppm and the NOEL is 30 ppm.**	SUPPLEMENTARY

C

82-7. Subchronic neurotoxicity - rats Ciba-Geigy Study No.: F-00176, August 26, 1994	D*Z*N Diazinon MG87%	43543902	In a subchronic neurotoxicity study (MRID No.: 43549302) 5 groups of 15/sex Sprague-Dawley Crl CD^R BR strain rats were dosed as controls, 0.3, 30, 300 or 3000 ppm corresponding to approximately 0.018, 1.8, 18 and 180 mg/kg/day of D*Z*N diazinon MG87% for 90 days with periodic assessments for clinical signs and FOB, motor activity and blood ChE/AChE. Regional brain AChE activity and neurohistopathology were assessed at termination. Principal clinical signs included (muscle fasciculations, 8/15 females; hyper-responsiveness and tremors, decrease in grip strength: 15-20% in males and 14-39% in females); body weight and gain and food consumption decrease in both sexes were noted at 3000 ppm only. **The LOEL for systemic and neurotoxicity effects is 3000 ppm (180 mg/kg/day) based on weight gain decrease and signs of nervous system perturbation. NOEL is 300 ppm (18 mg/kg/day).** At 30 ppm, plasma ChE (79%-86% in females, 37%-45% in males) and RBC AChE at 300 ppm (53-60% in females and 37%-75% in males) and brain AChE cerebral cortex/hippo-campus only (25%) were inhibited. Other regional brain AChE sources were inhibited at 300 ppm (55% - 75% in females) but only at 3000 ppm in males 62% - 73%). Conclusions regarding inhibition of brain AChE are deferred to an accompanying study (MRID No. 43543901) which was especially designed to assess regional brain AChE inhibition. **The LOEL for plasma ChE and RBC AChE inhibition is 30 ppm (0.18 mg/kg/day) and the NOEL is 0.3 ppm (0.018 mg/kg/day).**	ACCEPTABLE

1Reviewed by: John Doherty, Ph.D., D.A.B.T. 1/18/95
Section IV, Toxicology Branch I (7509C)
Secondary Reviewer: Linnea Hansen, Ph.D. 12/8/95
Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT 011873

STUDY TYPE: Special 28-day feeding ChE/AChE-rats

MRID NO.: 43543901 TOX. CHEM. NO.: 342
PC No.: 057801

TEST MATERIAL: D*Z*N Diazinon MG87%

STUDY NUMBER: F-00186

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Ciba Geigy Environmental Health Center

TITLE OF REPORT: "Cholinesterase Inhibition in 28 Day Feeding Study in Rats"

AUTHOR: Jane C.F. Chang

REPORT ISSUED: 11/7/94
In life phase: January 11, 1994 to February 10, 1994.

EXECUTIVE SUMMARY:

In a special study (MRID #43543901) designed to assess the time course for and regional brain inhibition of ChE/AChE, four groups of 15/sex Sprague-Dawley Crl CD^{R}BR strain rats were dosed as control, 0.3, 30, 300 or 3000 ppm of diazinon (D*Z*N MG87%) corresponding to approximately 0.02, 2.4, 23 and 213 mg/kg/day in their diets and 5/sex/group were sacrificed at weeks 1, 2 and 4 and plasma ChE and RBC and brain AChE assessments were made. The central nervous system was dissected into the cerebellum, hippocampus, cerebrum, striatum and thoracic spinal cord in order to assess for potential differences in regional sensitivity.

Systemic effects including cumulative body weight gain decreases of 26% in males and 39% in females and decreased food consumption (21% in males and 27% in females) and muscle fasciculation (3 males and 8 females at day 8) were noted in the 3000 ppm dose group only. At 30 ppm plasma ChE (males 59% and females 81%) and RBC ACHE (39-58% males and 38-59% females) were inhibited. At 300 ppm brain AChE (62-72% at week 4 in females for all regions, only the cerebellum ~22% in males) was inhibited. Maximum inhibition of blood and brain ChE/AChE was attained at week 2 and essentially remained at that level by week 4 without evidence of adaptation. There were no marked or consistent differences in sensitivity in brain regions to AChE inhibition (refer to DER for discussion). **The LOEL for plasma ChE and RBC AChE is 30 ppm and the NOEL is 0.3 ppm. The LOEL**

for brain AChE is 300 ppm and the NOEL is 30 ppm.

Classification: SUPPLEMENTARY. The study was designed to complement a series 82-7 subchronic neurotoxicity study and provides data that establish the time course of plasma ChE and RBC and brain AChE. It is noted, however, that the data from these two studies must be considered against the entire data base for assigning the NOEL and LOEL for plasma ChE and RBC and brain AChE.

Review

Experimental Constants:

Test Chemical:

Chemical:	D*Z*N^R Diazinon MG87% technical grade diazinon.
Lot # :	FL-880045, EHC Code No.: 0173-05
Supplier:	Ciba-Geigy Corporation
Purity:	88.0%
Description:	Amber liquid

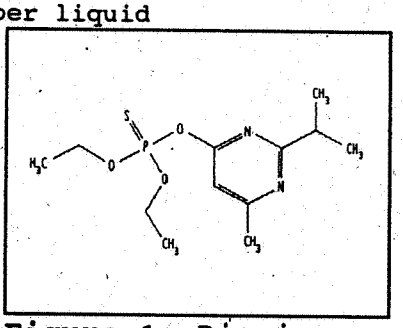

Figure 1 Diazinon

Analytical Chemistry: Data were presented (Table 9.1 of the study report) that indicated that analysis of two preparations of test diets showed concentration levels within 1% to 10.3% of the target dose level with homogeneity standard deviations of 12.1% or less. The lowest test dose of 0.03 ppm had the widest variation (-6.0% to 10.3% for two assays). This is acceptable variation such that the study is not considered compromised. Data on the stability of diazinon in feed (Appendix 10.2.3, page 74) indicated that samples of 0.3 and 3000 ppm showed < 3% degradation when stored at 25 degrees C for 44 days. Sample in the feed jar did not decompose after 16 days.

Test System:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD^R(SD)BR
Source:	Charles River, Raleigh, NC
Age:	At study initiation: approximately 7 weeks.
Weight:	Males: 134-175; females: 118-147.
Housing:	Individually
Feed:	Purina Certified Rodent Chow^R #5002

Acclimation period: 21 days for males and 23 days for females.

Basic Experimental Design: Table 1 below illustrates the experimental design.

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Table 1. Experimental design for the special 28 day ChE/AChE study.

Group	Dose Level (ppm)	Rats/sex Sacrificed Day 7	Day 14	Day 28	Achieved Dosage mg/kg/day Males	Females
Control	0	5	5	5	--	--
Low	0.3	5	5	5	0.02	0.02
Mid-1	30	5	5	5	2.3	2.4
Mid-2	300	5	5	5	22.5	23.1
High	3000	5	5	5	213	210

Achieved dosage data are from Tables 9.11 and 9.12 on pages 51 and 52 of the study report.

Statistics: The study report asserts that the following statistical tests were performed. The probability of a type I error was set at 0.05 and statistical significance at the 0.01 level was also assessed.

Statistical Test	Parameter Investigated
Bartlett's test for homogeneity followed by one-way ANOVA and two-way Dunnett's "t" test.	Body weight, body weight gain and food consumption data.
ANOVA and two-way Dunnett's test	ChE and AChE activity data.

Results

1. Survival. All animals survived to scheduled termination.

2. Clinical signs. Two daily assessments for mortality and moribundity were made and comprehensive physical exams were made weekly. Only animals in the 3000 ppm dose group were reportedly affected.

In males:
"muscle fasciculation forefoot" (3 affected first noted at day 8)
In females:
"muscle fasciculation forefoot" (6 affected first noted on day 8)
"muscle fasciculation general" (8 affected first noted on day 8)
"diarrhea" (3 affected first noted on day 8)

It is noted that these symptoms are consistent with the symptoms reported in the 90 day study (MRID No.: 43543902).

3. Body weight and gain and food consumption. Weekly body weight data indicated no statistical differences in the weight of males but terminal (week 4) body weight for the high dose group was decreased 10.5%. High dose female body weight was significantly decreased ($p < 0.01$, 10%) at week 1 and decreased 11% at week 4 but not significantly.

Cumulative body weight body weight gain was decreased

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($p < 0.01$) at each weekly interval for the high dose male group with there being a 26% decrease after four weeks. Female cumulative body weight gain was decreased at each interval and there was actually a slight (-0.57 gm vs + 19.13 gms for controls) weight loss in the first week and at week four there was a 39% ($p < 0.01$) decrease in cumulative weight gain. Table 1 below compares the 90 day study and this study with respect to body weight gain.

Table 2. Body weight gain comparison at weeks 1 and 4 for the 90-day and 28-day studies with diazinon at 3000 ppm.

	Males		Females	
Interval	28-day	90-day	28-day	90-day
Week 1	54%	45%	(-0.57 gms)	(-5.49 gms)
Week 4	26%	25%	39%	49%

Data are in % decrease in body weight gain relative to the control. 28-day study is from Tables 9.7 and 9.8. Refer to accompanying DER for the 90 day study.

Food consumption was decreased significantly ($p < 0.01$) only at week one for males (21%) and females (27%).

The body weight gain and food consumption data compare favorably between the 28-day and 90-day studies.

4. ChE/AChE assessments. The assay methods used for blood and CNS ChE and AChE are appended. Assessments were made at pretest (serum and RBC) and at weeks 1, 2, and 4 for serum, RBC and brain. Blood samples were obtained from the orbital plexus of non-fasted rats under isoflurane anesthesia.

Brain samples were taken after blood collection. The animals were anesthetized with sodium pentobarbital and the whole brain removed and dissected into cerebellum, cerebral cortex, striatum and hippocampus using a modified procedure of Glowinski and Iverson. The thoracic spinal cord was also sampled for analysis.

Tables 9.13 (males) and 9.14 (females) photocopied form the study report illustrate the plasma ChE and RBC and brain AChE data from this study for all intervals assessed. The three types of cholinesterase are discussed separately as follows.

A. Plasma ChE. The NOEL and LOEL are considered to be 0.3 and 30 ppm. At 30 ppm there is 59% to 51% inhibition in males and 81% inhibition in females. At the highest dose level there is nearly total inhibition in both males (86%-96%) and females (96%-98%). There was no evidence of adaptation during the 4 week dosing period as would be indicated by lower inhibition at week 4.

Females were more sensitive than males as indicated by

the 30 (81%) and 300 (93-95%) ppm dose groups being more inhibited than males at 30 (51-59%) and 300 (76% - 88%).

The low dose group males reached statistical significance ($p < 0.05$) at week 1 but there was only 14% inhibition and this group did not sustain statistical significance at the succeeding weeks. Furthermore, this decrease is not considered related to treatment because females show greater sensitivity than males and females did not show statistically significant inhibition at 0.3 ppm although the females were 10% lower at this dose level.

B. RBC AChE. The NOEL and LOEL for inhibition of RBC ACHE was considered to be 0.3 and 30 ppm for both sexes. At 30 ppm there is 39% to 58% inhibition in males and 38%-59% inhibition in females. At higher levels there is again nearly total inhibition. There was some indication of adaptation especially noticeable in the male 300 ppm dose group which had 11%, 17% and 36% and the 3000 ppm dose group had 6%, 5% and 26% activity for weeks 1,2 and 4 respectively. Females, however, did not show a similar apparent effect.

This study did not confirm a suggestion for inhibition of RBC AChE at 0.3 ppm in males as was indicated at week 13 in the 90 day study.

There was no marked difference between males and females with regard to RBC AChE inhibition.

C. Brain AChE. The NOEL and LOEL for inhibition of brain AChE was 30 and 300 ppm. AT 300 ppm, only the cerebellum (15%-22%, for each week) and striatum (15% week 2 only) of the males reached statistical significance. Note: The male striatal inhibition of 15% is not considered definitely an effect of the test material.

All five sections of the brain and the spinal cord reached statistical significance at 300 ppm for females and at 3000 ppm for both sexes. Thus, females are considered more susceptible than males. None of the comparisons at 30 ppm reached statistical significance and inspection of the data at 30 ppm indicated that in both sexes there were as many comparisons indicating slightly increased activity (about 10%) as there were indicating slightly decreased activity.

Each subsection of the brain for the 300 ppm female groups are discussed as follows: (the three percentages given are for the week 1, 2 and 4 respectively).

Striatum: 58%, 73% and 72% inhibited. No indication of adaptation.

Hippocampus: 51%, 72% and 71% inhibited. No indication of

adaptation.

Cerebral cortex: 47%, 62% and 72% inhibited. No indication of adaptation.

Cerebellum: 49%, 60% and 60% inhibited. No indication of adaptation.

Thoracic spinal Cord: 44%, 76% and 62% inhibited. Possible adaptation.

On this basis, TB-I does not consider that diazinon inhibits these subsections of the brain and central nervous system in females at markedly different levels. The cerebellum and spinal cord appear to be slightly less inhibited in females than the other three sections based only on the 60 to 62% inhibition at week 4 as compared to 72% for the other structures (this difference may not be within the resolving power of the assay). In the male, however, the cerebellum appears to be more susceptible than the other structures and was the only region considered actually inhibited at 300 ppm in the 28 day study. The 15-22% inhibition (statistically significant at each time interval in the 28 day study) is close to the 14% apparent inhibition (not statistically significant in the 90 day study) for the cerebellum.

The 90-day study indicated that at termination the "cerebral cortex/hippocampus" combined structure in females was inhibited 25% ($p < 0.05$) at 30 ppm. Progressively higher levels of inhibition were noted at 300 (75%) and 3000 (92%) dose groups. The males at 30 ppm in the 90 day study were actually 21% higher (not significant) for this combined structure. The special 28 day study did not assess a combined "cerebral cortex/hippocampus" structure but rather the cerebral cortex and hippocampus separately. Neither of these structures showed statistically significant inhibition at any time interval in the 28 day study. Figures 8.12 for cerebral cortex and 8.14 for hippocampus for females are attached to illustrate that there is no indication that inhibition in these structures is showing a progressive decline at 30 ppm and that the inhibition at 300 ppm remains constant after week 2.

Overall TB-I does not consider that this study shows marked biologically significant regional differences in the susceptibility to inhibition of brain AChE by diazinon although the cerebellum may at best tend to be more susceptible. In general, males are less sensitive than females.

Table 3 below compares the results of this study with the 90-day study.

Table 3. Comparison of plasma ChE and RBC and brain AChE data in the 28-day and 90-day studies with diazinon.

28-day study (MRID No.: 43543901	90-day study (MRID No.: 43539002	Conclusion
Plasma ChE NOEL and LOEL = 0.3 and 30 ppm for both sexes. At 30 ppm: Males inhibited 51%-59%. Females inhibited 81%.	NOEL and LOEL = 0.3 and 30 ppm. At 30 ppm: males inhibited 37%-45%; Females inhibited 79%-86%.	Very good agreement. The NOEL and LOEL are verified at 0.3 and 30 ppm. Females more sensitive than males.
RBC AChE NOEL and LOEL = 0.3 and 30 ppm for both sexes. At 30 ppm: Males inhibited 39%-58%. Females inhibited 38%-59%	NOEL and LOEL = 0.3 and 30 ppm. At 30 ppm: males inhibited 37%-74%; Females inhibited 53-61%.	Very good agreement. The NOEL and LOEL are verified at 0.3 and 30 ppm. Males and females about equally sensitive.
Brain AChE NOEL and LOEL = 30 and 300 ppm for both sexes. At 300 ppm the following (%) inhibition was noted for each section (males/females): cerebellum (15-22%/49-60%), cerebral cortex (10-14%, ns/47-72%), hippocampus (9-14%,ns/51-72%), striatum (5-15%/58-78%) and thoracic spinal cord (8-12%ns/54-76%).	NOEL and LOEL = 0.3 and 30 ppm for females only. At 30 ppm: the cerebral cortex/hippocampus was 25% inhibited. At 300 ppm: the cerebral cortex/hippocampus (75%), cerebellum (55%) and striatum (74%) were inhibited. Males were inhibited at 3000 ppm only (cerebellum 64% and cerebral cortex/hippocampus 77%). Regional sensitivity. Data for females imply cerebral cortex/hippocampus is more sensitive.	Poor agreement between studies with regard to possible effects in cerebral cortex/hippocampus noted in 90-day study. 28 day study does not verify inhibition in separated cerebral cortex and hippocampus at 30 ppm. Females established as more sensitive than males but no marked regional differences in the 5 structures established. Cerebellum may possibly be more sensitive. **NOEL and LOEL = 30 and 300 ppm for both sexes.**

CONCLUSION. This study by itself is SUPPLEMENTARY since it was designed to investigate primarily only ChE/AChE activity. This study is used to help the 90-day study (MRID No.: 43543902) establish NOEL and LOEL for plasma ChE and RBC and brain AChE and determine a time course for inhibition.

Quality Assurance Statement: Provided
Good Laboratory Practice Statement: Provided
Statement of No Data Confidentiality Claims: Provided.

Diazinon TOXR 11873

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Pages 13 through 19 are not included.

The material not included contains the following type of information:

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

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Reviewed by: John Doherty, Ph.D., D.A.B.T. John Doherty 1/18/96
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen, Ph.D. Linnea Hansen 12/11/95
Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 82-7. Subchronic Neurotoxicity Screen

MRID NO.: 43543902 TOX. CHEM. NO.: 342
PC No.: 057801

TEST MATERIAL: D*Z*N^R Diazinon MG87%. Technical grade diazinon.

STUDY NUMBER: F-00176

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Ciba-Geigy Environmental Health Center, Farmington, Conn.

TITLE OF REPORT: "90-Day Subchronic Neurotoxicity Study with D*Z*N^R Diazinon MG87% in Rats"

AUTHORS: John C. Pettersen and Robert L. Morrissey

REPORT ISSUED: August 26, 1994
[In life phase: March 22, 1993 to June 25, 1993].

EXECUTIVE SUMMARY:

In a subchronic neurotoxicity study (MRID No.: 43549302) 5 groups of 15/sex Sprague-Dawley Crl CD^R BR strain rats were dosed as controls, 0.3, 30, 300 or 3000 ppm corresponding to approximately 0.018, 1.8, 18 and 180 mg/kg/day of D*Z*N diazinon MG87% for 90 days with periodic assessments for clinical signs and FOB, motor activity and blood ChE/AChE. Regional brain AChE activity and neurohistopathology were assessed at termination.

Principal clinical signs included (muscle fasciculations, 8/15 females; hyper-responsiveness and tremors, decrease in grip strength: 15-20% in males and 14-39% in females); body weight and gain and food consumption decrease in both sexes were noted at 3000 ppm only. **The LOEL for systemic and neurotoxicity effects is 3000 ppm (180 mg/kg/day) based on weight gain decrease and signs of nervous system perturbation. NOEL is 300 ppm (18 mg/kg/day).**

At 30 ppm, plasma ChE (79%-86% in females, 37%-45% in males) and RBC AChE at 300 ppm (53-60% in females and 37%-75% in males) and brain AChE cerebral cortex/hippocampus only (25%) were inhibited. Other regional brain AChE sources were inhibited at 300 ppm (55% - 75% in females) but only at 3000 ppm in males 62% - 73%). Conclusions regarding inhibition of brain AChE are deferred to an accompanying study (MRID No. 43543901) which was especially designed to assess regional brain AChE inhibition.

The LOEL for plasma ChE and RBC AChE inhibition is 30 ppm and the NOEL is 0.3 ppm.

Classification: This study is classified as ACCEPTABLE and together with an accompanying study (MRID NO.: 43543901) satisfy the requirement for a series 82-7 subchronic neurotoxicity screen study with rats.

REVIEW

Experimental Constants:

Test Chemical:

Chemical:	D*Z*N^R Diazinon MG87% technical grade diazinon.
Lot # :	FL-880045, EHC Code No.: 0173-05
Supplier:	Ciba-Geigy Corporation
Purity:	88.0%
Description:	Amber liquid

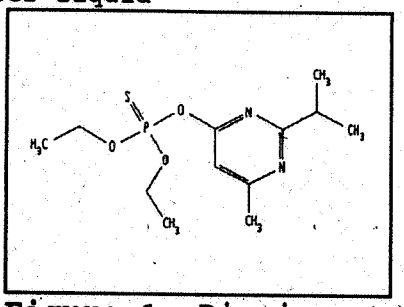

Figure 1 Diazinon

Analytical Chemistry: Test diets were prepared weekly and 8 of 13 preparations were assessed for diazinon. The mean concentration (with the mean relative standard deviation in %) in terms of D*Z*N Diazinon MG87% was determined to be 0.307 (4.2%), 30.40 (3.1%), 297.0 (3.4%) and 2999 (2.3%). Homogeneity was determined by using the relative standard deviation (standard deviation/average concentration) for the six diet samples analyzed per dose. The individual test diet analyses were not presented but summary tables were. Stability was stated as being assessed at room temperature and at 4 degrees C for the 0.3 and 3000 ppm diets some samples were assessed in open jars. No data were presented but statements attesting that diazinon was stable for 16 days in open jars at room temperature, for at least 35 days at 4 degrees C and 44 days at room temperature in a closed bucket.

Test System:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD^R(SD)BR
Source:	Charles River, Raleigh, NC
Age:	At study initiation: approximately 7 weeks.
Weight:	Males: 125-175; females: 113-148 (at assignment).
Housing:	Individually
Feed:	Purina Certified Rodent Chow^R #5002

Basic Experimental Design: Table 1 below illustrates the experimental design.

Table 1. Basic Experimental Design.

Group	Nominal (ppm)	Neurological Phase Rats/sex	ChE/AChE Phase Rats/sex	Achieved Dose Mg diazinon/kg/day Males	Females
Control	0	10	5	--	--
Low	0.3	10	5	0.017	0.019
Mid-1	30	10	5	1.7	1.9
Mid-2	300	10	5	17	19
High	3000	10	5	177	196

These dose levels were selected based on previously conducted 90 day (MIN882011, registrant's code) and two year (MIN882018 registrant's code) dietary toxicity studies. The high dose level was expected to produce about 50% inhibition of brain AChE and also produce non-fatal neurotoxicity (behavioral change, not specified). The lower doses except for 0.3 ppm were expected to produce significant plasma and RBC ChE/AChE inhibition.

The study design consisted of assessing for cage side observations on a daily basis and FOB and motor activity and ChE/AChE at pre-test (one week prior to exposure) and at weeks 4, 8 and 13.

Statistics: The study report asserts that the following statistical tests were performed. The criteria for statistical significance (type II error) was 0.05 except for Bartlett's test which was 0.001.

Statistical Test	Parameter Investigated
Bartlett's test for homogeneity for * parameters. One-way analysis of variance followed by Dunnett's t-test (two tailed) when significant differences were detected.	body weights and gains* clinical chemistry food consumption* feed efficiency* Figure-8 maze activity body temperature fore and hind limb grip strength hindlimb foot splay
Ranking of data followed with analysis of variance and Dunnett's test.	FOB Parameters: ease of removal, ease of handling, respiration, palpebral closure, piloerection, rearing counts, arousal, defecation, click response, righting reflex, hindlimb extensor strength. Histopathology findings.
Fisher's Exact test (two tailed) with Bonferroni's correction.	FOB parameters: abnormal gait, position of hindlimb when held by tail, approach response. Clinical observations.

Results[1]

1. Survival. All rats survived to terminal sacrifice.

2. Clinical signs. There were few reactions to treatment reported and these were at the 3000 ppm dose group only. Among males, two rats were reported to have "hyper-responsiveness" and this was noted on day 29 and based on the individual animal data (Appendix 10.3.1) it lasted for about one week. Among females "muscle fasciculations" and "tremors" were reported. 8/15 females were reported to have at least some fasciculations and two of these 8 also had tremors. Thus 7 rats were not affected. The tremors were noted on days 8 to 22 only (Appendix 10.3.2). The muscle fasciculations involved the pinna, forefoot or hind leg and foot. They were noted as early as day 8 and in some but not all cases may have lasted until termination.

3. Body weight and food consumption. Body weight and feed consumption were assessed weekly. The study author asserts that only the high dose group was affected. Table 2 below illustrates body weight and cumulative body weight gain data at weeks 1, 4 and 13.

Table 2. Body weight and gain data for rats dosed with diazinon for 90 days.

	Males					Females				
Week	Control	0.3	30	300	3000	Control	0.3	30	300	3000
1 Bw[1]	149.1±12.2	149.7	147.9	149.4	148.9	131.6±10.1	130.9	129.8	130.3	130.4
Gain[2]	52.6±19.3	53.4	53.3	47.8	28.8**	23.8±9.6	18.2	19.7	18.7	-5.49**
4 Bw	425.1±31.7	403.9	404.1	406.0	379.1**	259.5±23.3	258.3	257.4	259.0	226.2**
Gain	167.1±26.1	154.9	154.0	156.6	125.0**	69.7±13.8	67.5	65.5	65.7	34.6**
13 Bw	607.8±61.3	551.0	570.4	564.7	538.8	314.7±37.7	307.4	313.0	332.6	286.32
Gain	349.3±54.8	305.5	322.4	320.3	280.6**	128.0±33.1	118.0	122.1	138.4	99.50

1. Bw = body weight in grams from table 9.5 (males) and 9.6 (females). 2. Gain = the cumulative body weight gain for the interval in grams. The standard deviation is presented for the controls only. The treated animals had similar standard deviations.
** $p < 0.01$, study report statistics.

Overall, the data demonstrate that there is an initial reduction in body weight/weight gain in the high dose group for both sexes. Male body weight was statistically lower on weeks 1-6 and females lower at weeks 1 to 9 and on week 11. Terminal body weight was 11% decreased for males and 9% decreased for females, neither was statistically significant. Cumulative body weight

[1][Note: The methods and procedures used by the Ciba-Geigy Laboratory as assessed by positive controls were reviewed elsewhere. Refer to Appendix I, attached.]

gain was 20% ($p < 0.01$) decreased for males and 22% (not significant) for females for the 13 week study. Statistical differences in weekly weight gain were at week one only (except for males at week 10). Generally the weekly weight gain was similar to the controls.

The terminal low dose (0.3 ppm) male group body weight was 9% less and the cumulative weight gain was 13% less than the control group but there was no progressively higher weight loss at 30 and 300 ppm dose levels. At week 10, the mean weekly body weight gain (15.96±17.80) was decreased (about 40%) for the 300 ppm male group relative to the control group (27.19±9.35). This was considered an incidental finding because the 3000 ppm dose group gain was increased (18.65±5.87 gms) and no other significant reductions were noted for the 300 ppm dose group.

For females, the 0.3 and 30 ppm dose groups were very similar to the controls at all intervals. The 300 ppm dose group was eventually 12% higher (not significant) at termination.

Food consumption was significantly decreased for males (up to 18% less) in the first two weeks of the study and for females (up to 34% at week 1 and about 15% later) also at weeks 1, 2 and 4 but was generally comparable with the control group after that time. Feed efficiency was reported to be decreased 30% for males and to a negative value for females in the first week but reportedly stabilized after that time. The decrease in food consumption in the early weeks corresponds to the decrease in body weight gain.

Conclusion (body weight and food consumption): NOEL and LOEL = 300 and 3000 ppm.

4. Ophthalmoscopic Examination. Ophthalmoscopic examinations were said to be made at pretest and at termination on all animals (except those for ChE assessment at termination. No treatment related effects were noted. Two incidents of "dry cornea" in the female 3000 ppm dose group were not considered related to treatment.

5. FOB assessments. Assessments were made at one week pre-test and at weeks 4, 8 and 13. The following parameters were reported to be investigated.

Home Cage Measurements	Manipulative Measurements
posture	ease of removal from cage
tremors	respiration character
convulsions	position of hindlimbs when held by tail
stereotypy	pupillary size
bizarre behavior	lacrimation
fecal color	staining
fecal composition	eye prominence
gait	palpebral closure
	piloerection

fur appearance
salivation
vocalization
ease of handling in hand

Open Field tests
- arousal level
- number of rears
- number of defecations
- pools of urine
- head position
- convulsions
- gait
- stereotypy
- bizarre behavior

Reflex tests
- approach response
- touch response
- click response
- pupil response
- eye blink response
- tail pinch response
- placing reflex
- righting reflex

Neuromuscular tests
- hindlimb extensor strength
- grip strength (fore and hind limb)
- hindlimb foot splay

Physiological measurements
- body tone
- body weight
- rectal temperature
- muscle tone

The study author asserts that effects were noted in the 3000 ppm dose group only. Table 3 below illustrates the findings for effects determined in the FOB assessments.

Table 3. Effects of diazinon observed at FOB assessments.

Parameter	Week	Males Control	Males 300	Males 3000[1]	Females Control	Females 300	Females 3000
Forelimb grip strength[2]	-	693±76	723±102	778±153ns(+12%)	708±97	728±89	725±115
	4	1715±219	1565±215	1465±258ns(-15%)	1173±187	1220±201	718±209**(-39%)
	8	2078±348	2040±342	1770±270ns(-15%)	1333±172	1358	963±251**(-28%)
	13	2018±361	1888±479	1615±422ns(-20%)	1410±155	1383	1045±255**(-26%)
Hindlimb grip strength	4	1173±250	1080±115	979±178ns(-17%)	580±112	588	433±81*(-25%)
	8	1353±214	1420±121	1213±271ns(-10%)	673±154	663	533±67ns(-21%)
	13	1398±226	1450±196	1125±305ns(-20%)	825±153	805	713±123ns(-14%)
Hindlimb foot splay	4	No apparent effect			9.3±1,7	7.4±1.7	6.3±0.8**
	8				8.5±2.3	7.4±1.7	6.3±1.3ns
	13				7.5±2.4	6.0±1.3	5.8±1.2ns
Muscle fasciculations	4	No apparent effect			Noted in the high dose group only following the grip strength assessment. Six animals showed both front and hind limb effects and one animal showed hind limb only.		
	8						
	13						

Data are from Tables 9.19 for males (p. 124 and 125) and 9.20 for females (p. 179, 180 and 181).
[1] Data for the control and 300 and 3000 ppm dose groups are presented only since no effects were noted in the 0.3 or 30 ppm dose group.
[2] Grip strength are in grams.
* $p < 0.05$ and ** $p < 0.01$.

Other parameters showing isolated incidents in the high dose group and possibly related to treatment were hunched gait (one male, at all time intervals), slight tremors at week 4 (one

male). Increased dehydration in females (4 of 10 at week 4) and a decrease in rectal temperature (0.7 degrees C) at week 13 were also reported. Some other deviations were noted (i.e. approach response score of 2 in the low dose group) but were not considered treatment related because of a lack of a dose response.

CONCLUSION (FOB assessments). NOEL and LOEL = 300 and 3000 ppm. AT 3000 ppm there is a decrease in grip strength in both males and females and a decrease in hindlimb foot spay in females. These parameters may not actually be neurotoxicity but a reflection of the change in body weight.

6. Motor activity. Assessments were made at pretest, and at weeks 4, 8 and 13 after completion of the FOB assessments. Activity was assessed using a Figure 8 maze (San Diego Instruments) and computer assisted computation. Each maze contained 8 separately counted photobeams at standardized locations in the perimeter. The test sessions were one hour in duration with 5 minute epochs. White noise (70dB) and normal room lighting conditions were maintained during testing.

The study author asserts that motor activity was unaffected by treatment. Figures 8.10 for males and 8.14 for females photocopied from the study report supports that conclusion. Other time interval plots of motor activity were similar. The 300 ppm male group was consistently lower but inspection of the individual animal data indicated 1 rat (i.e. #58) with unusually low motor activity, thus lowering the mean for this group. For example, the pretest, week 4, week 8 and week 13 total session scores for this rat were 113, 82, 113 and 54 and the range for all the other animals in this group was 135-367, 110-398, 136-408 and 81-320 respectively. Thus, TB-I concurs that there were no effects on motor activity.

7. ChE/AChE assessments. Assessments were made by taking blood samples from the orbital plexus from five unfasted animals designated for ChE assessment under isoflurane anesthesia during weeks 4, 8 and 13. At week 13 the animals were anesthetized with pentobarbital and the whole brain was removed and separated into the cerebellum, cerebral cortex plus hippocampus and striatum (3 subsections). The specific method of assay for CHE/AChE was described in Appendix 10.10.6 , page 548.

Tables 9.25 and 9.26 photocopied from the study report illustrate the results of the ChE/AChE assays. The following discussion identifies the NOEL and LOEL for each source of ChE or AChE.

Plasma ChE: The NOEL and LOEL are 0.3 and 30 ppm for both sexes. At 30 ppm:

-Males were inhibited 37%-45% (all $p < 0.01$).
-Females were inhibited 79%-86% " ".

Females were more susceptible than males. There was no indication of time difference in the extent of inhibition for assays at weeks 4, 8 or 13. Inhibition at 300 and 3000 ppm was usually greater than 80%.

RBC AChE: The NOEL and LOEL are also 0.3 and 30 ppm for both sexes. At 30 ppm:

-Males were inhibited 37%-75% (all $p < 0.01$).
-Females were inhibited 53-60% " ".

At 0.3 ppm males were also inhibited 11% ($p < 0.05$) at week 13 only but this is not considered compound related (small magnitude and not consistent at other time intervals). Note also that at week 8, this groups was 11% increased over the control group.

Males and females are considered about equally affected.

Brain AChE. Females were apparently more sensitive than males.

-Females had a NOEL and LOEL of 0.3 and 30 ppm. At 30 ppm only the cerebral cortex/hippocampus was 25% ($p < 0.05$) inhibited. At -300 ppm, the cerebellum (55%, $p < 0.01$), cerebral cortex/ hippocampus (75%, $p < 0.01$) and striatum (74%, $p < 0.01$) were all inhibited.

The apparent effect of 25% inhibition at 30 ppm in the combined "cerebral cortex/hippocampus" was not verified in the special 28 day study (MRID No.: 43543901 (DER attached) when the separated cortex and hippocampus sections were assessed at weeks 1, 2 and 4.

-Males had a NOEL and LOEL of 300 and 3000 ppm. At 3000 ppm the cerebellum and cerebral cortex/hippocampus were inhibited by 64% and 77%. The data for the striatum showing a large increase at 300 ppm were considered compromised due to sampling technique.

Comments on the regional effects of AChE. These data suggest that the cerebral cortex/hippocampus region is more susceptible to inhibition in females than the other regions (i.e. LOEL of 30 ppm). TB-I has determined not to make conclusions regarding inhibition of brain AChE based on this study alone. The accompanying study (MRID No.: 43543801, accompanying DER) was designed to more thoroughly assess the regional susceptibility of brain AChE.

8. Neurohistopathology. At study termination, the first ten rats/sex/group were anesthetized with sodium pentobarbitone and sacrificed by whole body perfusion with 2.5% buffered glutaraldehyde and were necropsied. The following central and peripheral nerve tissues and muscle structures were prepared for histopathology (fixatives were 10% NBF or BG).

Brain (blocks 1-10, coronal blocks of about 3 mm thickness)
Each brain block was embedded in paraffin and cut at 8 microns. One section from each block was stained with hematoxylin and eosin. A second section from each block was stained with luxol fast blue.

Spinal cord (with ganglion): cervical, thoracic, lumbar and sacral

These sections were fixed within the spinal column, decalcified in formic acid and trimmed to present a cross section and oblique section that was said to included many nerve root and ganglia, embedded in paraffin and cut at 5 microns. One section from each spinal cord was stained with hematoxylin and eosin; a second section was stained with luxol fast blue. Additional cross sectional blocks from the cervical and lumbar regions were used to trim out at least one nerve root ganglion from each area. These were embedded in glycol methacrylate, sectioned at 2 microns and stained with cresyl fast violet. One gasserian ganglion from each animal was also embedded in glycol methacrylate, section at 2 microns and stained with cresyl fast violet.

Peripheral nerves:
left and right sciatic nerve, left and right fibular nerve, left and right tibial nerve, left and right lateral cutaneous sural nerve. The left samples were also embedded in glycol methacrylate, sectioned at 2 microns and stained with cresyl fast violet. The right samples were saved for special staining (if needed).

Skeletal muscle: (right thigh) and Eyes (with optic nerve) were prepared in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

Also some nonnervous system tissues showing gross lesions as selected by the pathologist.

Samples from the control and high dose groups were assessed microscopically. Of all the tissues examined from the high dose group, only the sacral spinal cord nerve root axons indicated a possible effect of minimal or mild degeneration in 2 of 10 females (refer to table attached). The low and mid (2) dose groups were then assessed and no degeneration was noted in these animals. The control group had one animal affected (refer to animal #118, page 583) which was described as focal.

One 3000 ppm female group rat (refer to page 588) was minimally affected (animal number 176) and also described as focal. Thus, this rat is essentially similar to the control. The other female rat (animal number 177) in the 3000 ppm dose group was mildly affected and described as multi focal. The spinal cord for this rat was also described as having poor fixation.

The study author dismissed the initial concern for a potential significant lesion in the sacral spinal cord nerve root. The justification included that the data were not statistically significant, there were no similar lesions in the low or mid dose groups and the severity was similar for the control and for one of the affected 3000 ppm animals. This leaves only one animal with a higher level of severity and this animal had poor fixation in the suspect nerve area. Overall TB-I concurs with the study author. There is insufficient basis to

conclude that this lesion was attributed to the test material. In addition this animal (# 177) did not show unusual adverse effects in the FOB or motor activity assessments.

Study Conclusion. This study is classified as ACCEPTABLE. The study established NOEL and LOELs for clinical signs and FOB parameters and plasma ChE and RBC AChE but there were no motor activity effects noted. Interpretation of brain AChE data were deferred for review in conjunction with the accompanying study (MRID No.: 4353901).

Study deficiencies include:

-No individual analysis data for the test diets.
-No data for the stability studies.

These deficiencies are not considered to compromise the integrity or interpretation of the study.

Quality Assurance Statement: Provided.
Good Laboratory Practice Statement: Provided.
Statement of Data Confidentiality: Provided No claim of confidentiality asserted.

Appendix I

Positive Controls for Neurotoxicity Studies

Ciba-Geigy Laboratory

Chemical	Study Identification	Treatment Information	Results and Comments
Triadimefon (acute)	F-00175 (with diazinon) January 20, 1994 MRID No.: 431322-04 HED Document No.: pending.	150 mg/kg in corn oil by gavage. Hsd Sprague-Dawley SD strain rats.	FOB: increased rearing and arousal, bizarre behavior. Motor activity: Starts only slightly higher than control but remains elevated.
Triadimefon (acute)	F-00166 (with profenofos) September 1, 1993 MRID No.: 429398-02 HED Document pending	100 mg/kg males and 150 mg/kg females in corn oil HSD Sprague Dawley strain rats.	increased rearing, arousal, tail pinch response, motor activity, compulsive sniffing, Straub tail and exophthalmos.
Propoxur (acute)	F-00166 (with profenofos) September 1, 1993 MRID No.: 429398-02 HED Document No.: pending	6 mg/kg ip in corn oil. Hsd Sprague Dawley strain rats.	FOB : diarrhea, lacrimation, salivation, miosis, impaired respiration, increased urination, soiled fur, stereotypy (compulsive licking and repeated opening and closing of the mouth), abnormal gait (ataxic, hunched, crouched, waddling, immobile or hindlimbs splayed or dragged) impaired rr, decreased forelimb and hindlimb grip strength, impaired extensor reflex, flattened posture, decreased arousal, tremors, increased ease of handling, muscle fasciculation, decreased rearing, impaired tail pinch response, dehydration, decreased body temperature and decreased motor activity.

Acrylamide (subchronic)	F-00167 (with profenofos) March 1, 1994 MRID No.: 432133-03 HED Document: Pending	16 mg/kg in distilled water by gavage 5 days/week for 90 days Hsd Sprague-Dawley strain rats.	
Trimethyltin chloride (subchronic)	F-00167 (with profenofos) March 1, 1994 MRID No.: 432133-03 HED Document: Pending	3 mg/kg in distilled water by gavage once a week for f weeks Hsd Sprague-Dawley strain rats	Deaths, convulsions, other minor singular incidents., sharply decreased body weight, abnormal gait, impaired rr, hindlimb extensor, forelimb grip strength, hypotonia of muscle, tremors, arousal ease of handling, body tone, dehydration, palpebral closure, piloerection, soiled fur, decreased motor activity. Pathology: hippocampus - necrosis gliosis, nerve cell loss, ganglion-generalized chromatolysis and some nerve degeneration, peripheral and spinal nerves had axonal degeneration and chromatolysis in the spinal cord.

Diazinon TOXR 11873

Page_____ is not included in this copy.

Pages 32 through 37 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

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✓ FIFRA registration data.

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