Irma

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

OPP ... AND
HEAL... ...IVISION
SCIE... ... REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES,
TOXIC SUBSTANCES

November 8, 2001

MEMORANDUM

Subject: EPA ID No.: 057801. Diazinon: Review of a single dose (MRID No.: 45184302, July 25, 2000) and a 28-day dosing (MRID No.: 45184301) studies with diazinon in human volunteers.

TXR # 0050237
PC Code: 057801
DP Barcode No.: D268244
Submission No.: S587363

From: John Doherty 11/15/01
ReRegistration Branch III
Health Effects Division 7509C

To: John Hebert
Special Review and ReRegistration Division 7509C

Through: Catherine Eiden 11/8/01
Branch Senior Scientist
ReRegistration Branch III
Health Effects Division 7509C

Action Requested and Response.

HED was asked to review single dose (MRID No.: 45184302, July 25, 2000) and repeated (28-day) dosing (MRID No.: 45184301) studies with diazinon in human volunteers. These studies were reviewed and copies of the DERs are attached.

These studies are further described and the Executive Summary is presented in the following table.

Study	Executive Summary
Acute single dose - human volunteers Covance Clinical Research, Study No.: NCP-8373 MRID No.: 45184302	In this study (MRID No.: 45184302) with human volunteers that was not classified for acceptability, a set of 41 males were dosed with capsules containing 0, 0.03, 0.12. 0.20, 0.21 or 0.30 mg/kg diazinon in epoxidized soybean oil as a single oral dose. There were 12 subjects receiving no diazinon (placebo control), subject that received the high dose of 0.3 mg/kg and there were 7 subjects in each of the other groups. The dose administration was made step wise over the course of the study with one subject receiving the next higher dose to assure that it would be tolerated before other subjects were dosed. Each subject was assessed for his physical well being and/or for reactions at pretest (3-4 assessments) and for 15 day after administration. Plasma cholinesterase (ChE) and red blood cell acetylcholinesterase (AChE) were assessed at screening, days -2 and -1, at zero hour (no treatment) and 1, 2, 4, 6, 8, 12, 24 and 48 hours and days 5, 8 and 15 after dosing. Postdosing values were compared with the predosing mean to determine the extent of inhibition. The dosed groups were also compared to the placebo group. The subjects were also assessed for clinical chemistry, hematology and urinalysis as well as pulse, blood pressure, heart rate and 12 point ERG. *Systemic toxicity.* There were no clinical reactions or changes in blood chemistry, hematology or urinalysis that were considered related to treatment. A single case of "back pain" described as mild and occurring on day 7 in the single subject dosed with 0.30 mg/kg that the study author considered possibly related to treatment was not considered a response to diazinon by the reviewers. **The LOAEL and NOAEL are > 0.21 mg/kg.** Note: There were an insufficient number of subjects dosed at 0.30 mg/kg to include this dose level in the evaluation of systemic effects. *ChE and AChE assessments.* The mean and standard deviation for plasma ChE and RBC AChE activities from all 41 subjects taken on day -2 were 4159 ± 816 (19.6%) and 8513 ± 1247 (14.7%), respectively, indicating that there was variation among the subjects. Based on comparison with each subjects predosing mean, plasma ChE was not inhibited at 0.03 mg/kg. At 0.12 mg/kg the groups mean indicated 40% inhibition after 8 hours and the group means for both the 0.20 and 0.21 mg/kg dose groups indicated approximately 59% inhibition. The single individual dosed at 0.30 mg/kg demonstrated 91% inhibition after 8 hours. The time to onset of inhibition was as early as 1 hour but it took about 4 to 8 hours to reach maximum inhibition. Recovery of activity began at about 24 hours but only some subjects fully recovered to their predosing level while others reached only about 71% of their predosing level. RBC AChE was not demonstrated to be inhibited even at the highest dose of 0.30 mg/kg/day. **The LOAEL is 0.12 mg/kg based on 40% inhibition of plasma ChE. The NOAEL is 0.03 mg/kg/day.**

Study	Executive Summary
28-day repeated dose - human volunteers. Novartis Corporation, Study No.: 972019, March 23, 1998. MRID No.: 45184301.	In this unclassified study (MRID No.: 45184301) four male managers at the Novartis Basel, Switzerland facility volunteered to be treated with oral doses of 0.03 mg/kg/day of diazinon (purity 99.5%) in gelatin capsules at breakfast time fo periods of 28-31 days. The subjects were assessed for physical responses, plasma cholinesterase (ChE) and red blood cell acetylcholinesterase (AChE), as well as clinical chemistry, hematology and urinalysis. ChE and AChE assessments were made on days -28,-27, -7, -1, 0 (6 hours after treatment), 1, 2, 3, 8, 10, 13, 14, 20 and after the last treatment on days 28, 29, 31 and on days 48 or 57 to assess for recovery. Not all assessments for all individuals were made each day. The mean ± standard deviations for pretreatment plasma ChE were in the range of 8476 ± 766 to 11262 ± 416 and for RBC AChE were 11097 ± 1845 to 12966 ± 1072 for the four subjects meaning that there was reasonably good precision in the assays. The only reported effect of treatment was an inhibition of plasma ChE. Plasma ChE started to show inhibition (22 to 42%) on day 8 in all o the four subjects and in three of the four subjects this inhibition increased over tim such that maximum inhibition was 47% to 55% on either day 20 or the subjects las day of treatment. Although the time to maximum inhibition differed, there was no indication that any one individual was either *especially* sensitive or insensitive to the plasma ChE inhibitory effects of diazinon. Posttreatment recovery was up to 86% to 92% of the predose mean by 17, 20 or 28 days after the last dose. RBC AChE values were generally *higher* than the pretest control values for each individual. One individual indicated a decrease of 10% in activity at day 20 but this was not considered related to diazinon because the values at the preceding and later intervals were in excess of or similar to the pretest mean. In conclusion, a daily dose of 0.03 mg/kg/day resulted in a maximum of 46-55% inhibition of plasma ChE that slowly recovers after cessation of treatment. RBC AChE was not affected at this dose level.

Primary Reviewer: John Doherty 11/2/01
ReRegistration Branch III, HED 7509C
Secondary Reviewer: Jess Rowland 10/30/01
Science Information Management Branch, HED 7509C

TXR # 0050237

Date Evaluation Record

STUDY TYPE: Special study in human volunteers following a single oral dose

OPP Guideline No.: N/A

OPPTS No.: N/A

DP Barcode: D268244

Submission No.: S587363

PC Code: 057801

Test Material (purity): Diazinon

Citation: Boyeson, M.G., 2000, A Randomized, Double Blind, Ascending, Acute, Oral Dose Study of Diazinon to Determine the No Effect Level (NOEL) for Plasma and RBC Cholinesterase Activity in Normal, Healthy Volunteers. Covance Clinical Research Unit Inc., Madison, WI, Study No.: NCP-8373, July 25, 2000. MRID No.: 45184302 (3 volumes).

Sponsor: Novartis Crop Protection, Inc. Greensboro, N.C.

In Life Phase of Study: First volunteers started on July 28, 1998 and the last group of volunteers were released from the study on October 22, 1998.

Executive Summary:

In this study (MRID No.: 45184302) with human volunteers that was not classified for acceptability, a set of 41 males were dosed with capsules containing 0, 0.03, 0.12. 0.20, 0.21 or 0.30 mg/kg diazinon in epoxidized soybean oil as a single oral dose. There were 12 subjects receiving no diazinon (placebo control), 1 subject that received the high dose of 0.3 mg/kg and there were 7 subjects in each of the other groups. The dose administration was made step wise over the course of the study with one subject receiving the next higher dose to assure that it would be tolerated before other subjects were dosed. Each subject was assessed for his physical well being and/or for reactions at pretest (3-4 assessments) and for 15 days after administration. Plasma cholinesterase (ChE) and red blood cell acetylcholinesterase (AChE) were assessed at screening, days -2 and -1, at zero hour (no treatment) and 1, 2, 4, 6, 8, 12, 24 and 48 hours and days 5, 8 and 15 after dosing. Postdosing values were compared with the predosing mean to determine the extent of inhibition. The dosed groups were also compared to the placebo group. The subjects were also assessed for clinical chemistry, hematology and urinalysis as well as

pulse, blood pressure, heart rate and 12 point ERG.

Systemic toxicity. There were no clinical reactions or changes in blood chemistry, hematology or urinalysis that were considered related to treatment. A single case of "back pain" described as mild and occurring on day 7 in the single subject dosed with 0.30 mg/kg that the study author considered possibly related to treatment was not considered a response to diazinon by the reviewers. **The LOAEL and NOAEL are > 0.21 mg/kg.** Note: There were an insufficient number of subjects dosed at 0.30 mg/kg to include this dose level in the evaluation of systemic effects.

ChE and AChE assessments. The mean and standard deviation for plasma ChE and RBC AChE activities from all 41 subjects taken on day -2 were 4159 ± 816 (19.6%) and 8513 ± 1247 (14.7%) indicating that there was variation among the subjects. Based on comparison with each subjects predosing mean, plasma ChE was not inhibited at 0.03 mg/kg. At 0.12 mg/kg the groups mean indicated 40% inhibition after 8 hours and the group means for both the 0.20 and 0.21 mg/kg dose groups indicated approximately 59% inhibition. The single individual dosed at 0.30 mg/kg demonstrated 91% inhibition after 8 hours. The time to onset of inhibition was as early as 1 hour but it took about 4 to 8 hours to reach maximum inhibition. Recovery of activity began at about 24 hours but only some subjects fully recovered to their predosing level while others reached only about 71% of their predosing level. RBC AChE was not demonstrated to be inhibited even at the highest dose of 0.30 mg/kg/day. **The LOAEL is 0.12 mg/kg based on 40% inhibition of plasma ChE. The NOAEL is 0.03 mg/kg/day.**

Classification: This study is not being classified since there are no current acceptance criteria for studies that assess for systemic toxicity or cholinesterase inhibition in studies conducted with human volunteers. The study otherwise is considered to contain meaningful and useful data.

Compliance: Signed and dated Good Laboratory (GLP) and Good Clinical Practice (GCP) Compliance (7/26/2000), Quality Assurance (7/26/2000) and *No* Data Confidentiality claim (7/31/2000) statements were provided. Since this study used human subjects, the Good Clinical Practices Statement was included. The procedures for the use of human volunteers as indicated in 21 CFR 312 titles 50, 54, 56, 312 and 314 which are consistent with the Declaration of Helsinki and the Common Rule were said to be followed.

Review

I. Materials and Experimental Constants

A. Test material

Chemical: diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)-phosphorothioate).
Purity: 8% diazinon in epoxidized soybean oil.
Lot: FL-981726 (batch 781/44)
Expiration Date: July 15, 1999
Storage: Room temperature (15-30° C) protected from sunlight.

B. Vehicle

5

Material: Epoxidized soybean oil
Purity: Not stated.
Lot: FL-981714 (batch A GML 152)
Storage: Room temperature
Note: Material was supplied by the sponsor and no details on the original manufacturer were provided.

C. Test Subjects

Male human volunteers were the test subjects for this study. The original protocol included females but no females were included in the actual experiment as per the instructions of the sponsor. A total of 41 subjects were dosed (refer to Table 1) with either the placebo or one of the several doses of diazinon using the graduated increase in the dose. Two volunteers were treated on the first day with one being a Placebo and the other the lowest test dose. When no reactions were noted, additional volunteers were treated with the same dose and one other volunteer was treated with the next higher dose until it was shown that at 0.30 mg/kg the inhibition of cholinesterase was excessive and it was determined that no other volunteers should be treated at this or higher levels. In this study the volunteers that were supposed to be treated with the 0.30 mg/kg dose level were reassigned to be treated with only 0.20 mg/kg in order to obtain statistical data for inhibition at that dose level.

Table 1. Experimental Design and Diagram of the Ascending Dose Administration for the Study with Human Volunteers.

Test Groups and Date of Dosing and Termination							
Dose Level mg/kg	First (8/5/98)	Second (9/9/98)	Third (9/16/98)	Fourth (9/23/98)	Fifth (9/30/98)	Sixth (10/7/98)	Total
"Placebo"	1	2	1	2	3	3	12
0.03	1	6					7
0.12		1	4	2			7
0.20						7	7
0.21			1		6		7
0.30					1		1
Total	2	9	6	4	10	10	41/41
Termination	8/20/98	9/24/98	10/1/98	10/8/98*	10/15/98	10/22/98**	

*One subject (#21, a placebo) withdrew on 10/1/98 for personal reasons unrelated to treatment.
**subject (#40, 0.20 mg/kg/day) was listed as being retained on the study until 10/30/98.

Rationale for Dose Selection. The dose levels for this study were selected based on a tolerance study in which male volunteers from the management of the Novartis Company were dosed with 0.03 mg/kg/day for 28 to 31 days and no reactions or inhibition of cholinesterase were noted following the first dose. This study (MRID No.: 45184301) has been reviewed in a separate DER.

Dose Preparation and Administration Each individuals dose was said to be prepared by a certified pharmacist 24 hours in advance of administration (stored under refrigeration). There was no table indicating the exact amount in mg of test substance given to each individual. The pharmacist preparing the dosing samples was aware of each individual volunteer's dose and a second clinical staff member verified that each individual received his weighted dose. The dose was placed in a #1 or #0 gelatin capsule and administered to each individual with 250 mL of water. After a standardized breakfast, the volunteers were instructed to swallow the capsule whole (no biting or chewing. Verification of swallowing by mouth check was made by the study staff although the persons making the mouth check were not aware of the actual dosing material. Provisions were made to identify the dose level received by each individual in the case of a medical emergency.

Statistics:

The percent change from baseline was analyzed at the sample collection times using a one-way **analysis of variance (ANOVA)** is the SAS PROC MIXED program with plasma ChE or RBC AChE as the dependent variable and dose group as the independent variable. In addition, ANOVA was used to determine if there were significant differences between dose groups and placebo. If the ANOVA test was found not be significant at the 0.05 level, **Dunnett's test** was used to determine which treatment levels differed from the placebo group. The NOAEL for the study was determined to be the dose level at which there was no significant difference between the dose group and the placebo group. The **paired t-test** were used to determine whether significant percent change form baseline had occurred within a given dose group.

The statistical analysis results for the cholinesterase data were presented separately (Table 11 of the study report, pages 155-160).

II. Specific Methods and Results.

1. Reactions to Treatment and Disposition of the Volunteers.

All volunteers except one remained on the study until completion. A single volunteer (#21, assigned to the group that received the placebo) had some family problems (undisclosed) and dropped out of the study after day 8. There was no indication that this individual experienced any adverse effects or that his leaving the study would compromise the outcome. At worse it would only leave 11 individuals in the placebo group instead of 12 for the final assessment of ChE and other assessments.

The volunteers were instructed to report all "adverse events" from day -2 to study completion. An adverse event was defined as "any unintended change in the body structure (signs) or body function (symptoms), whether or not considered test material-related." . Although there were a total of 42 adverse events, there was only one adverse event that was considered *possibly* related to treatment by the study author The event that was considered possibly related to treatment was "back pain" that was described as mild and this occurred on day 7 and was in the single individual treated with 0.30 mg/kg. Since only one individual was treated at this dose level and there is no means to compare this event with others similarly treated, there is insufficient basis to conclude that this condition (which the reviewer gets occasionally) was definitely related to treatment. Since the event occurred on day 7, while the plasma ChE was recovering from maximal inhibition, it seems unlikely that the event was actually related to treatment.

The other events (listed in Table 4 of the study report) included some 22 different symptoms but there was no pattern showing that these were more frequent in the treated persons as the dose increased. For example, the total number of adverse events reported were 18/41 predose, 13/12 in the treated placebo group, 4/7 in the 0.03, 1/7 in the 0.12, 1/7 in the 0.20 and 4/7 in the 0.21 dose groups respectively. The placebo group was the only group that had symptoms described as moderate while all of the treated groups had symptoms described as mild.. With the exception of diarrhea (one incident in the placebo group), nausea (one incident each in the placebo and 0.03 dose groups), vomiting (one incident in the placebo group) and headache (one incident in the 0.03 mg/kg dose group) none of the other symptoms would be considered an obvious typical expected responses to a cholinesterase inhibitor.

There was also no effect on either diastolic or systolic blood pressure, heart rate, respiration (beats per minute), body temperature according to Table 10 of the study report.

Table 11 of the study report presents the results of a 12 lead ECO. There were no effects noted on ventricular rate, PR interval, ORS duration or the Qtc interval.

Conclusion (reactions and toxicity). It is concluded that there are no data presented to indicate that treatment with diazinon in this study resulted in reactions in the human volunteers. The fact that the single individual receiving the highest dose (0.30 mg/kg) experienced mild back pain on day 7 is noted but there is insufficient basis to conclude that this reaction was related to treatment.

2. **Clinical Chemistry , Hematology and Urinalysis**

Inspection of Table 6 of the study report that presents the summary of results for clinical chemistry indicated that there were no changes in albumin, alkaline phosphatase, ALT, amylase, AST, BUN, calcium, chloride, cholesterol, CPK, creatinine, GGT, glucose, iron, LDH, lipase, phosphorous, potassium, sodium, total bilirubin, total CO2, total protein, triglycerides or uric acid. Assessments were made at screening, day -2, day 1, day 2 and day 15,

Inspection of Table 7 of the study report that presents the summary report for hematology indicated that there were no changes in basophils (% and absolute), eosinophils (% and absolute), hematocrit, hemoglobin, lymphocytes (% and abs), mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, monocytes (% and absolute), MPV, neutrophils (% and absolute), platelets, red blood cells, RDW and white blood cells.

Inspection of Table 8 of the study report that presents the summary report for urinalysis indicated that there were no effects on urinary pH, specific gravity, urobilinogen, urine creatinine (QT, RDN or timed)*,

3. **Assessment of Plasma ChE and RBC AChE.**

Two mL blood samples were collected via direct venipuncture (or indwelling catheter) into a tube containing EDTA-K2. The samples were assessed for both plasma ChE and RBC AChE by the method of Ellman (described in detail in Appendix F of the study report). The samples were collected and separated (plasma from RBCs) at the clinical unit and transferred to the analytical laboratory the same day for processing. There was no indication of the storage of the samples once removed from the subject or during transit to the analytical lab and there is no indication of the time lapse between the drawing of the blood and the actual analysis of ChE/AChE.

Plasma ChE The mean value at day -2 for 41 subjects for plasma ChE was 4159 ± 755 (18.1%) which included a minimum value of 2681 and a maximum value of 5958 µmole/L. The standard deviation of 18.1% is considered large but considering that there were 41 subjects, this would be expected from a diverse population of males of varying age and race.

Table 2 shows that there was inhibition relative to the placebo group that reached 40% for the dose group receiving 0.12 mg/kg at 8 hours. The dose groups receiving 0.20 and 0.21 mg/kg achieved a progressively higher mean percentages of plasma ChE inhibition of 51% to 60%. Statistical evaluation (Table 13, pages 155 to 160 of the study report) of these data indicated that all values for 0.12, 0.20 and 0.21 mg/kg at 4, 8 and 24 hours were statistically significantly decreased when compared to the predose baseline. Note: Figure l (attached) photocopied from the study report shows the mean plasma ChE activity at each time point for each dose group.

The high dose of 0.30 mg/kg was not included in the statistical analysis since only one individual was assessed at this dose level. There was only one individual dosed with 0.30 mg/kg and this individual's plasma ChE was 3517, 3516, 3408, 953 (↓72%), 288 (↓92%), 388 (↓89%), 453 (↓87%), 810 (↓77%), 1116 (↓68%), 1719 (↓51%), 2083 (↓41%) and 2496 (↓29%) for the 0, 1, 2, 4, 6, 8, 12, 24, 48 hour and 8th and 15th day assessments.

Recovery. Figure 1 from the study report (attached) plotting the mean group plasma ChE over the course of the 15 days of treatment and also including the predosing assessments is attached to illustrate the recovery over time. It is apparent from Figure 1 that full recovery to the predose mean was not always attained. This is especially evident for the single individual

receiving the dose of 0.30 mg/kg which recovers to only 71% of its predosing value. .

Table 2.. Plasma ChE and inhibition as a function of dose and time.

	Zero Hour	4 hours	8 hours	24 hours	15 days
Placebo (n=12)	4212±610	4336±631	4321±581	4317±576	4122±550
0.03 (n = 7)	4694±681 (–/↑11%)	4458±607 (↓5%/↑3%)	4390±613 (↓7%/↑2%)	4443±563 (↓5%/↑3%)	4189±633 (↓11%/↑2%)
0.12 (n = 7)	4405±1166 (–/↑5%)	3049±1292* (↓31%/↓30%)	2580±626* (↓41%/↓40%)	2872±667* (↓35%/33%)	3722±1019 (↓16%/↓10%)
0.20 (n = 7)	4210±916 (–/ =)	1942±575* (↓54%/↓55%)	1714±248* (↓59%/↓60%)	2073±202* (↓51%/52%)	3558±460 (↓15%/↓14%)
0.21 (n = 7)	4126±473 (–/↓2%)	1894±581* (↓54%/↓56%)	1789±615* (↓57%/↓59%)	2044±538* (↓51%/53%)	3334±501(a) (↓19%/↓19%)
0.30 (n = 1)	3517 (–/↓17%)	953 (↓72%/78%)	388 (↓89%/↓91%)	810 (↓77%/81%)	2496 (↓29%/39%)

Data are from Table 12 of the study report (page 147 to 154).

Data are in µmole/L. and are presented as the mean and the standard deviation.

The data in parenthesis are numerator indicating the percent inhibition when compared to the zero time for each dose group. The denominator indicates the percent inhibition relative to the placebo at each time interval. There was very good agreement between each means of assessment for inhibition.

The shaded area indicates the times and doses where there was significant inhibition (* $p < 0000$). Day 15 is not shaded for the 0.12, 0.20, 0.21 and 0.30 dose groups because there was appreciable recovery. Recovery was not to 100% of the predose means or equivalent to the controls. (a) this groups was statistically significantly less than the control ($p = 0.04$)

Was there any indication of any individuals being either hypersensitive or less sensitive to plasma ChE inhibition? The data table in Appendix EE that illustrated the percent plasma ChE inhibition change from predose baseline for each subject was perused to determine if there was any individual that was either hypersensitive or less sensitive to the plasma ChE inhibitory effects of diazinon. Table 3 presents an overview of this survey. Table 3 indicates that there are two subjects that may be less sensitive and two that mat be more sensitive since they had either the minimum or the maximum amount of ihibition for their group. . However, a *definite* conclusion that these individuals are actually that much different from the others in their group cannot be established by the data.

Table 3. Assessment for Individuals with Either Reduced or Hyper Sensitivity to Inhibition of Plasma ChE by Diazinon.

Dose group	Mean Range(a)	Time to max (a)	Comments on the Least Sensitive Individual (b)	Comments on Most Sensitive Individual (c)
Placebo (n=12)	(– to 10%)	N/A	N/A	#24 showed 10% *apparent* decrease at 1 hour only.
0.03 (n=7)	8% ± 4% 2% to 13%	6 to 12 h	N/A	#1 showed 13% *apparent* inhibition at 12 hours and then only 5% and 4% at 24 and 48 h but back again to 12% at day 5. Conclusion: Not increased sensitivity.
0.12 "	42% ± 9% 32% to 58%	4 to 12 h	#19 @ 32% max also appeared 100% recovered @ day 15. Of the 6 other subjects there was 37, 37, 39, 42, 52 and 58% max.	**#12** @58% and achieved max in only 4 hours also recovered to only 80% @ day 15. **#10** with 52% max follows similar pattern. **Increased sensitivity possible in two subjects.**
0.20 "	59% ± 12% 44 to 79%	4 to 6 h	**#35** @ 44% max is same as mean for lower dose completely recovers @ day 15. **Reduced sensitivity possible in one subject.**	#39 @ 79% and achieved max in only 4 hours also recovered to only 72% @ day 15. Next lower value in this group is only 65%. But three subjects in the 0.21 mg/kg/day dose group have 71, 72and 75% max. No specific hypersensitivity noted.
0.21 "	62% ± 11% 47 to 75%	4 to 6 h	**#23** @47% max but near max in only 1 hour. Recovers to 85% @ day 15. **Reduced sensitivity possible in one subject.**	**#27** @ 75% but two others #22 and 28 also have 71% and 72% max so no individual with hypersensitivity.
0.30 "	92%	6 h	N/A	N/A

N/A - not applicable.

(a) Data are from Appendix EE starting on page 844 of the study report. The mean for maximal inhibition was calculated form the highest percent inhibition noted within 24 hours (values after that time were lower because of recovery) for each individual. The range is the maximum inhibition noted within 24 hours. The time to ma is the time it took for the individuals to reach the maximum inhibition within 24 hours.

(b) The individual subject with the lowest maximum inhibition was compared with the other subjects in his group and in the next lower and higher dose group and a generalization made with regard to this subject being less sensitive.

RBC AChE. The mean value at day -2 for 41 subjects for RBC AChE was 8513 ± 1247 (14.65%) this included a *minimum* value of 6143 and a maximum value of 12549 µmole/L. The standard deviation of 14.65% is considered large but considering that there were 41 subjects, this would be expected from a diverse population of males of varying age and race.

There was only one individual dosed with 0.30 mg/kg and this individual's RBC AChE was 9145, 8552, 8517, 8796, 8691, 7976, 9255, 9756, 8709, 8587, 8988 and 7924 IU/mL for the 0, 1, 2, 4, 6, 8, 12, 24, 48 hour and 8th and 15th day assessments. These data indicate that there was no inhibition at the high dose level of 0.30 mg/kg. The lowest value relative to the 0 time (non-treatment) was at day 15 (↓13%). This would not be consistent for inhibition caused by an organophosphate which should have been noted at approximately 4 to 8 hours where plasma ChE was maximally or near maximally inhibited. Since RBC AChE would stay inhibited, there would not have been increases in activity at the 8th and 12th hour assessments.

Additional data demonstrating that there is no appreciable inhibition of RBC AChE in this study are shown in Table 4 which compares the results of the placebo and 0.20 and 0.21 mg/kg dose groups at several time intervals. Note: Figure 2 (attached) photocopied from the study report shows the mean RBC AChE activity at each time point for each dose group.

Table 4.. RBC AChE and inhibition as a function of dose and time (higher doses only).

	Zero Hour	4 hours	8 hours	24 hours	15 days
Placebo (n=12)	8427±1039	8490±978	8436±884	8539±854	8482±929
0.20 (n = 7	8709±612 (–/↑3%)	9016±608 (↑4%/↑6%)	8677±707 (=/↑3%)	8744±843 (=/↑2%)	8612±815 (↓1%/↑3%)
0.21 (n = 7)	≡ 8776±763 (–/↑4%)	7884±482 (↓10%/↓7%)	8186±743 (↓7%/↓3%)	8841±401 (↑1%/↑4%)	7896±526 (↓10%/↓7%)

Data are from Table 12 of the study report (pages 147 to 154)..
Data are in µmole/L. and are presented as the mean and the standard deviation.
The data in parenthesis are for the numerator the percent inhibition relative to the zero hour (non-treated) for each group and for the denominator the percent inhibition relative to the placebo control at each time interval. None of the differences are considered related to treatment.

Table 4 shows that although there are some apparent decreases in RBC AChE there is no pattern that would support a conclusion that there is inhibition caused by diazinon based on the mean group data. In particular, the dose of 0.21 mg/kg might suggest that there was inhibition at either the 4th or the 8th hour but at the 24th hour both comparisons show an apparent increase in activity. Table 14 (pages 158 to 160) of the study report) did not indicate either the 0.12 or 0.20 mg/kg dose groups were statistically significantly different from the placebo group at any time period after treatment. The 0.21 mg/kg dose group was statistically significant lower at the 4th hour ($p = 0.0011$, ↓7%) , day 5 ($p = 0.049$, ↓8%), day 8 ($p = 0.024$, ↓7%) and day 15 ($p = 0.025$, ↓7%) assessment times. These percent decreases are considered small and the p values are also considered border line. Also, there was as much apparent decrease in the later days as there was at the first assessment (4 hours) and this does not follow a pattern of inhibition of an enzyme by

an organophosphate.

Was there any indication of any individuals being either hypersensitive or less sensitive to RBC ChE inhibition? During the first 24 hours postdosing the maximum apparent decrease in RBC AChE for an individual in the placebo group relative to the predose baseline was 23% for subject #26 and this occurred at 8 hours postdosing. Most of the other 11 subjects in the placebo group had activity in excess of the baseline but three subjects had 4, 5, or 6% decreases. In the 0.03 mg/kg dose group, maximum decease in activity was 7% and was not sustained after one hour. For the 0.20 mg/kg dose group, 4 of 7 subjects displayed a decrease and maximum decrease was only 6%. For the 0.21 mg/kg dose group, all but one subject displayed some decrease and the maximum decrease was 18% for subject #23 at 4 hours. However, this subject's RBC AChE was +10% at the next assessment time of 6 hours. Thus, there was no individual identified as having a hypersensitivity to inhibition of RBC AChE in this study.

Conclusion (ChE data). This study demonstrates a NOAEL and a LOAEL of 0.03 and 0.12 mg/kg for inhibition of plasma ChE following a single oral dose of diazinon. There was no definite inhibition of RBC AChE noted at any dose level. There were some indications but not definite conclusions that among this population of subjects that there were two subjects that showed reduced susceptibility and two subjects that showed increased sensitivity to plasma ChE by diazinon.

4. **Discussion/Study Conclusion**

This study is considered to have valuable information with regard to the sensitivity of adult males to a single oral dose of diazinon. It establishes that a dose level as low as 0.12 mg/kg results in consistent inhibition of *plasma* ChE with a mean of about 40% in a group of 7 volunteers. The inhibition can occur as early as one hour in some individuals but takes 4-6 or 8 hours to reach maximum inhibition. Recovery was slow and may be dose dependent, since the highest dose reached only about 71% of its predose activity after 15 days. Inspection of the individual response data did not indicate any individuals that were definitely hypersensitive or insensitive to the inhibition of plasma ChE by diazinon although there were two individuals each that may have been less sensitive or more sensitive than the norm for their dose groups.

RBC AChE was not considered to be inhibited at any dose level in this study and there was no indication that a single individual within any of the dosed groups was especially sensitive.

There were no clinical reactions to treatment noted and there were no effects on clinical chemistry, hematology, urinalysis, blood pressure, heart rate or noted by a 12 lead ECG.

This study is not being classified since there is no current policy for classifying studies conducted to assess for the systemic effects or inhibition of cholinesterase in humans.

5. **Study Deficiencies/comments:**

-There was no table or other data indicating the exact amount of diazinon actually given to each individual.

-No females were included. In animal studies, females tend to be more sensitive than males showing inhibition at lower doses and more inhibition at the same dose for many studies.

-One volunteer withdrew from the study after day 8. Thus, there was no assessment of ChE on day 15. Since this individual received the placebo, there is no reason to suspect that this individual's departure would compromise the study in any way.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

Figure 1. Mean Plasma Cholinesterase Activity Values (µmole/L) by Dose Level and Time.

Figure shows the recovery over time for plasma ChE inhibition and that full recovery is not attained for all dose groups with there being approximately only 63% recovery for the individual dosed with 0.30 mg/kg.

Figure 2. Mean RBC Cholinesterase Activity Values (µmole/L) by Dose Level and Time.

Figure shows that there was no pattern to indicate inhibition of RBC AChE over time.

Document Received MRID Numbers 45184301
45184302

Page _____ is not included in this copy.

Pages 15 through 16 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert 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.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

√ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewer: John Doherty 10/23/01
ReRegistration Branch III
Secondary Reviewer: Jess Rowland 10/20/01
Science Information Management Branch, 7509C

TXR# 0050237

Data Evaluation Record

Study Type: Non-Guideline Special Study - human volunteers

DP Barcode: D268244 Submission No.: S583763
PC Code: 057801

Test Material: Diazinon (purity 99.5%, Batch No.: AMS 140/7)

Sponsor: Novartis Crop Protection

Citation: Bellstein, P. 1998, "Tolerance Study in Novartis Managers Upon Repeated Oral Administration of Diazinon. Novartis Crop Protection, Inc. Basel, Switzerland. Study No.: 972019, March 23, 1998, MRID No.: 45184301.

In-Life Phase: There is no indication of the actual dates when the subjects were treated and their blood samples assessed. However, It appears that the study was conducted in the late summer and fall of 1997.

Executive Summary:

In this unclassified study (MRID No.: 45184301) four male managers at the Novartis Basel, Switzerland facility volunteered to be treated with oral doses of 0.03 mg/kg/day of diazinon (purity 99.5%) in gelatin capsules at breakfast time for periods of 28-31 days. The subjects were assessed for physical responses, plasma cholinesterase (ChE) and red blood cell acetylcholinesterase (AChE), as well as clinical chemistry, hematology and urinalysis. ChE and AChE assessments were made on days -28.-27, -7, -1, 0 (6 hours after treatment), 1, 2, 3, 8, 10, 13, 14, 20 and after the last treatment on days 28, 29, 31 and on days 48 or 57 to assess for recovery. Not all assessments for all individuals were made each day.

The mean ± standard deviations for pretreatment plasma ChE were in the range of 8476 ± 766 to 11262 ± 416 and for RBC AChE were 11097 ± 1845 to 12966 ± 1072 for the four subjects meaning that there was reasonably good precision in the assays. The only reported effect of treatment was an inhibition of plasma ChE. Plasma ChE started to show inhibition (22 to 42%) on day 8 in all of the four subjects and in three of the four subjects this inhibition increased over time such that maximum inhibition was 47% to 55% on either day 20 or the subjects last day of treatment. Although the time to maximum inhibition differed, there was no indication that any one individual was either *especially* sensitive or insensitive to the plasma ChE inhibitory effects of diazinon. Posttreatment recovery was up to 86% to 92% of the predose mean

by 17, 20 or 28 days after the last dose. RBC AChE values were generally *higher* than the pretest control values for each individual. One individual indicated a decrease of 10% in activity at day 20 but this was not considered related to diazinon because the values at the preceding and later intervals were in excess of or similar to the pretest mean. In conclusion, a daily dose of 0.03 mg/kg/day resulted in a maximum of 46-55% inhibition of plasma ChE and slowly recovers after cessation of treatment. RBC AChE was not affected at this dose level.

Classification: This study is not being classified since there is no current system for the classification of studies with human volunteers treated with cholinesterase inhibitors.

Compliance: Signed and Dated Statements of No Data Confidentiality Claim (July 14, 2000), Good Laboratory Practice (August 26, 1999) were provided but there was no Quality Assurance Statement. There was also no statement that indicated which set of guidelines were followed for the ethical treatment of human subjects in this type of research.

Review

A. General Methods and Materials

1. Test material:

Chemical: Diazinon (IUPAC name: thiophosphoric acid O,O'diethyl-O"-(2-isopropyl-6-methyl-pyrimidin-4-yl)ester.

Batch number: AMS 140/7
Purity: 99.5%
Expiration Date: September 1997

2. Test Subjects:

Subjects: Four male volunteers described as managers of the Novartis Company with responsibility for human safety and as passing a screening examination. No information on their age, race or height, or other characteristics were provided. No information on their smoking, drinking, current medication or unusual dietary habits were indicated. A set of rejection criteria was provided and this included having low plasma and/or RBC cholinesterase levels. Table 1 below illustrates the plan for this study with regard to assessments for reactions to treatment.

Two batches of capsules (were prepared based on the individuals body weight. These were one batch of 2.47 mg diazinon and used for subjects # 1 who weighed 79.5 kg, subject #2 who weighed 83.5 kg and subject # 3 who weighed 84 kg.. Another of another batch of 2.85 mg diazinon was for subject #4 who weighed 95 kg. Thus, all subjects received their dose of approximately 0.03 mg/kg/day. An analytical report was provided to verify that the capsules contained 95 to 100% of their nominal content.

18

Table 1. Protocol for the 28-31 day subchronic study with human volunteers.

Assessment	Time(s)
4 male subjects (designated as Subjects #1, #2, #3 or #4) dosed with 0.03 mg/kg/day at day 0 to day 28, 29 or 31.	
Screening Examinations (extensive physical exam) *and preliminary tests.*	Day -28 subjects #1, 2 and 3. Day -27 subject 4. Baseline values (except serological) were obtained on days -27 (#1 and #2), -7 (#3 and #4) and on day -1 (all subjects). All subjects reassessed for fitness on day -1.
Treatment period and post treatment period plasma and RBC cholinesterase assessments for inhibition.	Day 0 (day of treatment) - 6 hours post-administration and on days 1, 2 or 3, 8, 10 (apparently only 3 subjects) and 13, 14 and 20 during treatment and on the last day of treatment on days 28, 29 or 31 and on recovery days 48 and 57.
Clinical assessments (hematology and clinical chemistry and urinalysis).	Days 8, day 13or 14, day 20, day 28, 29 or 31. Apparently no post treatment assessments were made.
Final examination (physical)-neurological status, sitting systolic/diastolic blood pressure, heart rate and rhythm and other parameters as in the screening exam.	After the last intake of the test compound: Day 28 for #3 and #4, day 29 for #2 and day 31 for #1.
Special urinalysis for optional kinetic and metabolic analysis (see page 16 of the study report).	From one subject (number not specified) on days -7, -4, 0 (fractioned into 0-6h, 6-12h and 12-24h), 1, 3, 7, 14 and 28.

Statistics

There was no section in the study report that described the statistical procedures used to evaluate the study.

B. Specific Methods and Results.

1. Reactions to treatment.

The report states that "treatment with diazinon was without adverse effects" since no abnormalities were reported by the subjects. The report did not state specifically how the routine physical assessments were made or characterize the system used for the evaluations during the treatment period.. Table 3 of the study report indicates that the cardiovascular. pulmonary

19

lymph nodes abdomen (liver and spleen) and "brief neurological status" all had "nad" or "no abnormality detected" indicating that examinations were made at pretest, during treatment and at the last day of treatment.

2. Assessment for cholinesterase (ChE)

Blood samples were taken and plasma and erythrocytes were prepared (apparently by centrifugation). Plasma ChE was assessed by the method of Ellman et al (1961 reference provided).

Erythrocyte acetylcholinesterase (RBC AChE) was prepared by centrifuging whole blood separating the reb blood cells and washing twice with phosphate buffered saline and hemolyzed with 1% Triton X-100. The activity was assessed at 37° C utilizing a modification of the method of Augustinsson (1978 reference) using acetylthiocholine iodine as the substrate.

Enzyme activity was expressed as U/l of plasma or U/l of erythrocytes where 1 Unit (U) = 1 µmol of substrate converted per minute. Mean ± standard deviation values for plasma ChE for the four subjects based on three pretest assessments were 8692±285 (3.3%), 8476 ± 766 (9.0%), 11262 ± 416 (3.7%) and 10461 ± 767 (7.3%). Judging by the fact that the standard deviations were all < 10% the assessments of the plasma ChE were considered to reflect good precision in the assays. Mean ± standard deviations for RBC AChE were 12475 ± 1433 (11.5%), 11097 ± 1845 (16.6%), 12966 ± 1072 (8.3%) and 12046 ± 1010 (8.4%). RBC AChE tends to usually have higher standard deviations when human blood is assessed and these results are considered typical of assessments made under the given conditions.

Treatment assessments were compared with the individual subjects pretreatment values to determine the extent of inhibition. Since only one assessment was made at each assessment time, no statistical standard deviation was determined.

Plasma ChE. Table 2 indicates that each of the four subjects demonstrated inhibition of plasma ChE by day 8 or after 8 administrations of 0.03 mg/kg/day of diazinon. The following generalizations can be made from inspection of table 2.

-*Time to onset of inhibition.* There were no assessments for two of the four subjects at day 3 (#1 and #2). Subject #4 indicated 89% activity at day 3 but his value was 93% activity at day 0 and 99% at day 2 so it cannot ve concluded that this individual actually had inhibition prior to day 8. Similarly subject #3 had activity ranging from 92% to 95% the first three days and this is considered to be too small a difference to represent actual inhibition. Thus, day 8 is considered the time that all four subjects demonstrate inhibition. There is 22 to 42% inhibition at this time for all subjects. The more exact time to onset of inhibition cannot be determined since there were no assessments between day 3 and day 8.

- *Maximum inhibition and individual sensitivity.* Maximum inhibition ranged from a low of 46% (subject #4 at day 28) and 47% (subject #3 at day 20) to a high of 54% (subject #1 at day 31) or 55% (subject #3 at day 20). At least one subject (#1) showed maximum inhibition at day 31 but the other three subjects appeared to reach maximum by day 20. This is considered a narrow range and thus, none of the four subjects involved is considered at this time to be *especially* sensitive or insensitive to the inhibitory effects of diazinon.

-*Recovery.* None of the four subjects recovered to100% of their predose measurement for plasma ChE even after 28 days following their last dose. Three of the subjects (#1, #3 and #4) had recovered to 88% to 92% of their activity in 17-20 days while the subject # 2 recovered to only 86% after 28 days.

Table2. Plasma ChE and RBC AChE activity as a percent of the predosing mean for each subject over the course of the treatment and recovery periods.

	Plasma ChE				RBC AChE			
	#1	#2	#3	#4	#1	#2	#3	#4
Pretest Mean (1)	8692 ±285	8476 ±766	11262 ± 416	10461 ± 767	12475 ± 1433	11097 ± 1845	12966 ± 1072	12046 ± 1010
Day 0	93%	87%	95%	93%	119%	119%	108%	115%
Day 1	94%	91%	93%	99%	118%	122%	104%	112%
Day 2	96%	98%	–	–	129%	137%	–	–
Day 3	–	–	92%	89%	–	–	99%	111%
Day 8	59%	78%	65%	58%	105%	107%	97%	98%
Day 10a	66%	77%	–	60%	119%	119%	–	103%
Day 10p	58%	72%	–	–	–	–	–	–
Day 13	52%	–	–	–	119%	–	–	–
Day 14	–	64%	49%	55%	–	127%	111%	113%
Day 20	54%	53%	45%	56%	110%	109%	90%	103%
Last Dosing Day								
Day 28	–	–	51%	54%	–	–	97%	120%
Day 29	–	60%	–	–	–	141%	–	–
Day 31	46%	–	–	–	127%	–	–	–
Post Treatment (Recovery)								
Day 48	88%	–	88%	92%	129%	–	99%	124%
Day 57	–	86%	–	–	–	117%	–	–
Maximum inhibition	54%	47%	55%	46%	N/A	N/A	N/A	N/A

(1) Data are in U/l of plasma or erythrocytes and are expressed as the mean ± the standard deviation for at least three predosing determinations. Data are from Table 1 (plasma ChE) and 2 (RBC AChE) of the study report. Shaded area is considered inhibited. Maximum inhibition is 100% - % remaining activity. - Not assessed at this interval. On day 10, AM and PM samples were taken.

RBC AChE. RBC AChE did not indicate that any of the individuals demonstrated inhibition. Although subject #3 had only 90% of his pretreatment activity at day 20 suggesting 10% inhibition. The previous asse4ssemnt for this individual indicated activity of 111% of the pretreatment mean, This same person's activity was 97% on day 28. Day 28 was the last day of treatment and if RBC AChE was in fact inhibited, it would have taken several days at least to recover to the pretreatment level. Overall, it is concluded that none of the four subjects demonstrated inhibition of RBC AChE.

3. Clinical Chemistry, Hematology and Urinalysis.

Assessments were reported for blood content of C-reactive protein, glucose, cholesterol, gamma-globulin, ALT, creatinine, uric acid and alkaline phosphatase. Hematology assessments included white and reb blood cell counts, hemoglobin, hematocrit, mean cell volume, mean corpuscular hematocrit, MCHC, platelets, differential blood counts. None of the clinical chemistry or hematology parameters demonstrated a change in response to diazinon treatment.

Urinalysis was limited to test strip analysis by Combur 9 test stipe) and only "normal" was entered for each assessment time. The exact parameters of the urine that are assessed for by the Combur 9 test stripe were not identified in the report. No data on the "special urinalysis for optional kinetic and metabolic analysis" were presented.

C. **Discussion and Conclusion.**

This study is not being classified at this time since there is currently no system for classification of studies with human subjects treated with cholinesterase inhibitors. This study is considered to have very useful data with regard to assessing for the potential for diazinon to inhibit plasma ChE and RBC AChE at a dose level of 0.03 mg/kg/day for dosing periods of 28 to 31 days. Only plasma ChE was noted to be inhibited. All subjects showed inhibition at day 8 and recovered several days after the last dose and maximum inhibition of 47 to 55% was noted at approximately the last day of dosing. meaning that all four subjects responded in approximately the same qualitative and quantitative manner.

D. **Study Deficiencies.**

-There was no specific description or results of the procedure(s) used to evaluate each subject on a daily basis to determine if they were experiencing any physical or mental reactions.

-A more exact time to onset of inhibition of plasma ChE cannot be determined from this study since there were no assessments made between day 3 and day 8 when activity was first considered to be sufficiently reduced to be considered inhibited.

-There is also insufficient data to more firmly establish if maximal inhibition in *all* subjects occurs within the 31 days of treatment since one subject continued to decline at day 31.