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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

014453

Subject: EPA Id No.: 057801. Diazinon: Review of a the analysis of blood for diazinon and the urine for the metabolites G-27550 and DETP following a single oral dose of diazinon to adult male volunteers.

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

From: John Doherty 1/24/01
ReRegistration Branch III
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Conclusions

ReRegistration Branch III (RRBIII) has reviewed submissions reporting the analysis of the blood for diazinon and the urine for the metabolite G-27550 (MRID No.: 45184304) and the urine for the metabolite DETP (MRID No.: 45184303) following administration of a single oral doses of diazinon to human volunteers. These studies indicate that analysis of the blood for diazinon would not be a meaningful assessment for the exposure to diazinon since even the highest dose administered (0.3 mg/kg) demonstrated only trace amounts of diazinon (maximum 5.9 ppb) whereas the limit of detection was 1.3 ppb.

Urinary analysis of the metabolite G-27550 indicated only about 10% of diazinon administered was recovered as this metabolite. There was much variation in the time of peak excretion and the mean recovery had large standard deviations. Overall, it was concluded that urinary analysis of G-27550 can

be described as a *semi-quantitative* assessment of exposure to diazinon.

The report (MRID No.: 45184304) on urinary DETP was considered incomplete and the registrant needs to provide additional calculations that express the ppb data in terms of diazinon equivalents. Please refer to the comments below and the DER for other issues that the registrant needs to address and study deficiencies.

Background

The Novartis Company (currently know as Synergen) has submitted a study with human volunteers in three parts. The first part (MRID No.: 45184302) concerns details of the administration of diazinon to the human volunteers and assessment of plasma cholinesterase (ChE) and RBC acetylcholinesterase (AChE) periodically before and after treatment. This part of the study describes the selection of the volunteers, conditions for their ethical treatment, dosing with diazinon and the times and conditions for collection of blood and urine. This part of the study will be reviewed separately since there are currently no guidelines for evaluating studies assessing cholinesterase with human subjects.

The remaining two parts of the study concern the analysis of blood for diazinon and the urine for the metabolite G-27550 (6-methyl-2-(1-methylethyl)-4(1H)-pyrimidinone, MRID No.: 45184303) and the analysis of the urine for the metabolite DETP (diethylthiophosphate, MRID No.: 45184304). The metabolites G-27550 and DETP are the hydrolysis products of the parent diazinon. The data on the analysis of the urine for G-27550 and DETP were evaluated for their insight into assessing potential exposure to diazinon. A copy of the DER is attached.

Specific Comments

1. **Acceptability of the study.**

This study was not classified for acceptability since there are no current guidelines for the classification of studies using human subjects. Several study deficiencies were noted that will have to be addressed by the registrant to resolve certain scientific issues for this study. These are as follows.

Study Deficiencies.

A. <u>Deficiencies considered critical to acceptability of the study.</u>

1. There was no analytical report that verified the concentration of the diazinon in the epoxidized soybean stock sample. The registrant needs to provide verification of the purity of the diazinon used in this study as well as verification that the stock sample was 8% diazinon.

2 There was no table or other data indicating the exact amount in grams of diazinon actually given to each individual. Such a table will have to be presented to validate that the individual subjects received

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their prescribed dose. This table should be structured so as to present the identity of the subject (in code so as not to unfairly reveal personal data), exact amount in grams of diazinon, the weight of the subject and the date that the test material was administered.

B. Other Comments Which the Registrant Needs to Address

i. The analysis of the data for the urinary analysis of DETP is considered incomplete and additional calculations converting the ppb data into recovery data in terms of µgram equivalents of diazinon should be provided by the sponsor.

ii. The registrant needs to provide a comparative analysis of both the DETP and G-27550 urinary data and explain why there is no correlation between the two when a correlation relative to the proportion of each metabolite to the other should exist and the same pattern with respect to time of maximum excretion would also be expected to exist.

In particular, the analysis for the metabolites in the urine that result from the hydrolysis of diazinon indicated only 10.7 ± 4.7% mean recovery based on the pyrimidinone metabolite G-27550 for all subjects dosed with diazinon. An explanation that accounts for the balance or approximately 90% of the administered dose needs to be provided by the registrant. For example, are there data which show that the metabolite G-27550 is further metabolized and all of these subsequent metabolites are excreted.

2. **Analysis of the blood for diazinon.**

The limit of detection for diazinon in the blood was 1.3 ppb which is considered a low level. However, only the highest dose of diazinon administered had detectable levels of diazinon and this was only 5.9 ppb or slightly higher than the limit of detection and this was at 4 hours after dosing. Although there were occasional findings of apparent diazinon for other dose levels. they were not considered by the reviewers to be a definite demonstration of the presence of diazinon in the blood.

Failure to demonstrate its presence in the blood following oral administration of diazinon, suggests that diazinon is rapidly metabolized and associates with the cholinesterase (inhibits) quickly. Data in Part 1 of this study (refer to MRID No..: 45184302) indicate that there is significant inhibition at dose levels of 0.12, 0.20 and 0.21 mg/kg even though there was no consistent indication of diazinon in the blood. Since diazinon was not found in the blood (except for the highest dose administered), analysis of the blood would not be a meaningful assessment for exposure to diazinon in either controlled experiments assessing for exposure or in clinical situations where attempts to identify the cause of poisoning in a patient.

3. **Analysis of the urine for the metabolite G-27550**

The metabolite G-27550 is chemically a relatively unique metabolite of diazinon and thus the analysis of the urine for G-27550 might provide a useful index of exposure to diazinon. However, in this

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study only about 10% of the diazinon was recovered as metabolite G-27550 (expressed as diazinon equivalents). In addition, there was little consistency in the time when this metabolite was excreted in the urine and the mean data for each dose group had large standard deviations (please refer to the DER attached). Lastly, there was poor quantitative agreement between the dose levels of 0.20 and 0.21 mg/kg. Overall, the available data with this metabolite indicate that urinalysis for G-27550 would be a *semi-quantitative* means of assessing exposure to diazinon.

4. **Analysis of the urine for the metabolite DETP**

The study report for the analysis of DETP was considered incomplete and additional calculations and preparation of summary tables are necessary to more fully evaluate the significance of DETP in the urine following oral administration of diazinon. Preliminary calculations indicate that there is 6.7 times as much DETP as G-27550 for one individual. The additional calculations are needed to verify the assumptions made in making this calculation and to determine if the group mean data also show a similar disparity in the presence of these two metabolites.

RRBIII recognizes that the metabolite DETP may come from other pesticides and possibly other sources and may have limitations for use in clinical situations where the urine would be analyzed in an attempt to determine the cause of poisoning. However, in closed experiments where the exposure to other chemicals is limited, the urinary analysis of DETP may have advantages over the metabolite G-27550.

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Diazinon/2000 Special Study: Excretion of G-27550 and DETP in humans/oral exposure.

Reviewer: John Doherty 1/24/01
ReRegistration Branch III, HED 7509C
Secondary Reviewer: Steve Knizner 1/24/01
ReRegistration Branch III, HED 7509C

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Study Type: Special Study - Human Pharmacokinetics - oral administration.

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

Test Material: Diazinon (stated as being 8% in epoxidized soybean oil - needs verification).

Citations:

Hughes, D.L. and C. Vaughn, 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. Part B. Analysis of DETP in the Urine. Covance Clinical Research Unit, Inc. Madison Wisconsin. July 25, 2000. MRID No.: 45184303. (0ne Volume). Reviewed in Appendix I.

Wong, A. J. and G. D. Anderson 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. Part C. Analysis of Diazinon in Blood and G-27550 in Urine. Developmental Resources / Clinical Support Department, Novartis Crop Protection, Greensboro, N.C. July 25, 2000. MRID No..: 45184304. (One Volume). Reviewed in Appendix I.

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

Executive Summary (prepared for the pharmacokinetic data only. A separate Executive Summary will be prepared for the cholinesterase inhibition data).

Blood and urine from the subjects (human males) dosed with placebo, or a single dose of 0.03, 0.12, 0.20, 0.21 or 0.30 mg/kg of diazinon was collected at selected intervals for up to 15 days. There were 12 subjects in the placebo group and 7 each in the groups dosed with diazinon except for the dose level of 0.30 which had only one subject. The blood (MRID No.: 45184304) was analyzed for diazinon and the urine was distributed to two different laboratories for analysis of the hydrolysis products of diazinon: DETP (diethylthiophosphate, MRID No.: 45184303) and G-27550 (6-methyl-2-(1-methylethyl)-4(1H)-pyrimidinone, MRID No.: 45184304). All analyses were by gas-chromatographic methods.

Blood. Diazinon was detected in blood only occasionally and the only time it was considered meaningful was for the single subject dosed with 0.30 mg/kg with a detected level of 5.9 ppb diazinon at 4 hours after dosing. Nearly all other assessments (but with some

exceptions) were indicated as being < 1.3 ppb or below the limit of detection.

Metabolite G-27550. Urinary analysis for G-27550 indicated that only a mean of 10.7±4.7% in terms of diazinon equivalents of the administered dose was recovered from all dosed subjects within 48 hours. There was 7.9±1.0, 11.3±2.7, 8.1±1.7, 13.4±5.3 and 25.3% total recovery at 48 hours of diazinon equivalents as G-27550 for the groups dosed with 0.03, 0.12, 0.20, 0.21 and 0.30 mg/kg, respectively. Individual subjects ranged in total recoveries of from 5.8% to 25.3% of diazinon equivalents. There was very little or no excretion after 48 hours. Most of the excretion occurred within 24 hours and mean recoveries in micrograms for the 6-12 hour interval were 25.1±5.7, 150.1±53.5, 161.6±65.5 and 646.5±409.6 and 1,817.2 (one subject) for the 0.03, 0.12, 0.20, 0.21 and 0.30 mg/kg/dose groups, respectively. The large standard deviations and the large difference between the groups dosed with 0.20 and 0.21 mg/kg indicate the variability of the excretion for this interval. For some subjects the maximum excretion was in the 0-6 hour interval for other subjects the maximum was in the 6-12 or 12-24 hour intervals.

Metabolite DETP. Urinary analysis generally indicated more of DETP than G-27550 on a ppb basis and there was poor correlation between the peak time for excretion of G-27550 and DETP. DETP appeared to have a more uniform pattern of excretion with most subjects excreting DETP in the first six hours postexposure. Calculations on the net recovery of DETP as diazinon equivalents were not provided. The differences in the quantity and time course patterns of G-27550 and DETP may relate to subsequent metabolism and mechanism of excretion differences between these two chemicals.

Conclusion. Overall, these data indicate that blood analysis for diazinon would not be a meaningful index of exposure to diazinon. They suggest that analysis of urine for G-27550 would be a *semi-quantitative* assessment of the exposure to diazinon. Additional calculations are needed to further evaluate DETP as an index of exposure to diazinon.

Classification: This study is not being classified since there are no current guidelines for the classification of studies using human subjects. Several study deficiencies were noted that will have to be addressed by the registrant to resolve certain scientific issues for this study. Please refer to the list of study deficiencies on page 10 of this DER.

Detailed Considerations

A. *Treatment of the subjects.*

Note: The information in this section (Part A) was obtained in MRID No.: 45184302.

I. Test material

Chemical: diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)-phosphorothioate).
Purity: 8% diazinon in epoxidized soybean oil.
Source: Page 25 of the study report indicates that the test material was provided by the manufacturer (Novartis Crop Protection, Inc.)
There was no analytical report that verified the purity of the diazinon sample.
Lot: FL-981726 (batch 781/44)
Expiration Date: July 15, 1999

Storage: Room temperature (15-30° C) protected from sunlight.

II. Vehicle

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 of the vehicle were provided.

C. Test Subjects

Male human volunteers were the test subjects for this study. They were from 18 to 48 years of age and varied in weight from 64 to 99.5 kg and there were 10 individuals described as Black and the other 31 were described as Caucasian. 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 a system for graduated increase in the dose over the course of approximately 55 days. Two volunteers were treated on the first *day of dosing* with one being a Placebo and the other the lowest scheduled test dose. When no reactions were noted, additional volunteers were treated with the same dose the next *day of dosing* (not the next calendar day) 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.

Day of Dosing (actual date in parenthesis)							
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.

In the above table, the *day of dosing* refers to the day each group was dosed and is not the next day following the previous *day of dosing*. Table 2 of the study report (Volume 1) indicates that the *days of dosing* were 8/5, 9/9, 9/16, 9/23, 9/30, and 10/7 (all in 1998) meaning that there were intervals 35, or 7 days between the *day of dosing*. It should be noted that each subject was kept on the study for 17 days including the two days prior to dosing with two exceptions. One withdrew and another was retained for an additional week.

There was no table or other data presentation that indicated the exact amount of diazinon administered to each subject. Thus, the dose levels administered are not verified. This is considered a study deficiency.

B. Blood Plasma Analysis for Diazinon.

Two 10 mL blood samples were taken by venipuncture at check in day (-2) and at 0 hour (prior to dosing) and at 1, 2, 4, 6, 8, 12, 24 and 48 hours and on days 5, 8 and 15 for determination of plasma diazinon concentrations (refer to page 36 of MRID No.: 45184302 for description). The blood samples were sent to the Novartis Developmental Resources /Chemical Support Department in Greensboro, North Carolina for analysis of diazinon.

The blood samples were analyzed for diazinon using the "Draft" Analytical method 522-98 which was said to be validated separately under GLP standards. A description of this gas

chromatographic method with mass selective detection was provided in the report (refer to Appendix II of the report). The method was described as having a lower limit of detection of 1.3 ppb of diazinon based on the lowest fortification level used in the recovery studies. Data were presented that demonstrated that recoveries were 103.1 ± 15.3, 103.7 ± 14.1, 104.0 ± 11.1 and 107.2 ± 9.4 for samples at the 1.3, 5, 10 and 100 ppb fortification levels.

Table 5 (attached, photocopied from the study report) indicates that for almost all of the samples assessed the individual recoveries were < 1.3 ppb. The only times where appreciable diazinon (reviewer's discretion) was detected in the plasma were for the single volunteer (subject # 29) dosed with 0.3 mg/kg and the level was only 5.9 and 1.4 ppb at 4 hours and at 6 hours respectively. All other assessment times for this subject were < 1.3 ppb. Subject #32 who received 0.20 mg/kg had readings of 1.9 and 3.0 ppb at 1 and 4 hours but was < 1.3 ppb at all other times including the 2 hour assessment. Although these may be real, it is considered unlikely that the blood level of diazinon would be higher at 4 hours than at one hour for this individual and the result is questionable and not supported by other individuals. There were several other incidences of detection that were >1.3 but < 3 ppb and these occurred in the first several hours following treatment (with at least one exception).

Conclusion. The analysis of the blood plasma for diazinon indicates that generally the level of diazinon was below the limit of detection of 1.3 ppb. Occasionally assessments that were > 1.3 ppb did not exceed 5.9 ppb were noted. Thus, blood analysis for diazinon would not be a meaningful index of exposure to diazinon since there is substantial inhibition of plasma ChE at dose levels that do not have detectable levels of diazinon in the blood. Note. The review analysis for the inhibition of blood cholinesterase is being presented in a separate DER.

C. **Urinary Analysis for Diazinon Metabolites.**

Urine was collected during the time intervals on day -2 (prior to dosing), and at 0 to 6, 6 to 12, 12 to 24 and 24 to 48 hours post dose and for a 24 hour period on days 4, 7 and 14. The urine was separated into two portions and one portion was sent to the Novartis Developmental Resources/Chemical Support Department in Greensboro, N.C. for analysis of the metabolite G-27550 and the remainder was sent to the Covance Laboratories in Madison, Wisconsin for analysis of DETP. It was not specifically stated if the urine was equally distributed to each laboratory.

C-1. **G-27550 (6-methyl-2-(1-methylethyl)-4(1H)-pyrimidinone).**

The urine samples were analyzed for the diazinon metabolite G-27550 by the "Draft" Analytical Method 633-98[5] which was described in the report. This method which was said to be validated under GLP, utilized gas chromatography and mass selective detection. The lower limit of detection was 1.0 ppb of metabolite G-27550. Table 2 of the study report indicated that recoveries of 97.1 ± 22.2, 93.5 ± 20.0, 93.2 ± 15.8, 86.0 (no standard deviation reported, only one sample analyzed) and 83.5 ± 0.7 percent were obtained for samples fortified with 1, 10, 100,

200 or 500 ppb metabolite G-27550. It is noted that samples of 1, 10 and 100 ppb all have large (15.8% to 22.2%) standard deviations indicating poor precision in this range.

Tables 6 and 7 (attached, photocopied from the study report) indicate the recovery of metabolite G-27550 from the urine of the subjects on this study. Table 6 illustrates the actual recovery in ppb and the amount in total µgrams. Table 7 illustrates the recovery in terms of the percent of diazinon *theoretically*[1] administered to the subjects and the percent G-27550 is in diazinon equivalents. Tables 6 and 7 show that by day 4 post treatment essentially no more metabolite G-27550 was excreted in the urine although there is an occasional indication of detection which may or may not be experimental artifact.

The mean recoveries in terms of diazinon equivalents for the 0-6, 6-12, 12-24 and 24-48 hour intervals were 2.6 ± 0.9%, 4.4 ± 4.3%, 2.6 ± 1.2% and 1.3 ± 0.7% respectively. Maximum excretion occurred for some subjects during either the 0 to 6 (i.e. subjects 19 and 41), 6 to 12 (most subjects) or 12-24 (i.e. subjects 9, 17, 18 and 40) hour collection intervals (see table). Thus, the excretion pattern with respect to time was variable among the many volunteers.

Study report Table 7 (appended) indicates that the recovery of G-27550 (expressed as diazinon equivalents) in the urine varied from a low of 5.8% (subject #33 who received a dose of 0.20 mg/kg) to a high of 25.3% (subject #29 who received the highest dose) and the mean recovery was 10.7 ± 4.7% for all dosed subjects. Table 7 also allows the calculation of the total recovery of G-27550 for each subject as well as group mean recovery. The relationship between the amount of diazinon administered (assuming that the nominal dose approximates the dose actually administered) and total recovery (0-48 hours) as metabolite G-27550 does not show a close relationship (compare the two columns labeled Achieved and Expected in Table 2).

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[1]The dose of diazinon in µgrams is called theoretical because it appears to be based on combining the subjects weight with his nominal dose. There is no evidence that this figure is based on an actual amount of diazinon weighed out and administered to the subject. Note: Since a nominal dose was factored with a absolute value (the weight of the subject) the term theoretical dose is considered appropriate.

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Table 2. Urinary metabolite G-27550 data following administration of diazinon.

Dose	mg diazinon*	Total G-27550 µgm	Percent	Multiple Achieved	Multiple Expected	Total Recovery***
0.03	2465±169	86±40	3.5%	(1)**	(1)**	7.9%
0.12	9135±1528	515±153	5.6%	5.9	4	11.3%
0.20	15729±1333	641±175	4.1%	7.5	6.7	8.1%
0.21	15852±215	1051±416	6.6%	12.2	7	13.4%
0.30	21,360	2698.9	12.6%	31.4	10	25.3%

Note subject #29 who is the only subject dosed with 0.30 mg/kg. *The dose in mg/subject that was *theoretically* administered to each subject. This is apparently derived by combining the weight of the subject with his nominal dose and not the actual weight of the diazinon administered. ** Assigned as . *** Total recovery as diazinon equivalents

The recovery is said not to have a close relationship because it would be a reasonable expectation, but not a definite correlation, that there would be an approximate linear relationship between the amount of diazinon administered (dose) with the amount of G-27550 in the urine. In particular, the dose of 0.20 mg/kg should have only slightly less than the dose of 0.21 mg/kg whereas the 0.21 mg/kg dose group has 64% more (1051/641 x 100 = 164% or 64% higher). The data from the 0.30 mg/kg dose level is less reliable since only one subject was dosed but shows over three times as much as predicted.

DETP (diethylthiophosphate).

DETP was assessed for in the urine by a gas chromatographic method that utilized flame photometric detection and this method was said to have been validated under GLP. The lower limit of detection was said to be 10 ng/mL (approximately 10 ppb). There was no table of recovery data presented although a reference was made to there being average recoveries of 70 to 120%.

There was no summary table for the results as was presented for the G-27550 metabolite. Table A1 was prepared by this reviewer to illustrate the findings of the analysis of DETP in the urine and in some cases to compare these data with the results of the analysis of metabolite G-27550. Since the metabolites G-27550 and DETP represent the hydrolysis products of diazinon, it would be expected that the peak time of excretion for each metabolite would be similar and that there would be a reasonable proportion for each metabolite at each time unless there were major differences in the means by which each is excreted via the kidney. Since in the several

comparisons (selected at random except for subjects #29 and # 30) made there was very poor correlation between the time for peak excretion of each metabolite and/or a proportional relationship at a given time interval.

It is noted that on a ppb basis there seems to be more of the metabolite DETP than metabolite G-27550. Calculations on the conversion of the data in ppb of DETP in the urine to total µgrams of DETP recovered were not provided by the sponsor as they were for the metabolite G-27550. Using the urinary volume for each subject as provided in Table 7 (it is assumed that this volume is the total volume for each subject) for the metabolite G-27550, some calculations on the recovery of DETP were made and an analysis for subject # 39 is as follows:

Table 3. Urinary DETP data and comparison with urinary G-27550 data. for subject # 39.

Interval	Urine Volume	ppb DETP	µg DETP	µg G-27550*	Ratio**
0-6	650	4490	2,918.5	178.8	16.3
6-12	710	755	536.1	157.6	3.4
12-24	400	664	265.6	144.8	1.8
24-48	770	224	172.5	83.9	2.1
		Total	3892.7	565.1	6.9

*Data for G-27550 are from Table 6 of MRID No.: 45184304.
** Ratio of urinary DETP to urinary G-27550.

The ratio of DETP to G-27550 for total recovery is 6.9/1 verifying that much more DETP is excreted than the metabolite G-27550 for this subject #39. The mean data for DETP recovery needs to be calculated by the registrant in order to complete this analysis to determine more clearly the relationship between excretion of DETP and G-27550.

Table 1A also shows that metabolite DETP was nearly completely excreted before the 24-48 hour interval. Maximum excretion appeared to be in either the 0-6 hour interval for most subjects or the 6-12 hour collection interval for a few others. Never in the 12-24 hour interval as it was for the G-27550 metabolite for some subjects.

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Conclusions - Analysis of the Urine for Diazinon Metabolites.

Correlation of the Urinary Excretion of DETP and G-27550. In general, the results of the analysis of the metabolites G-27550 and DETP are not as consistent with one another as would be expected. Since G-27550 and DETP are the hydrolysis products of diazinon when diazinon is hydrolyzed the result should be equimolar amounts of each metabolite and there should have been a correlation between their content in the urine and the maximum time for excretion would be expected to be similar. There appears to be more of the DETP metabolite based on both tabulated ppb data and calculation of the quantity based on urine volume for one subject. Major differences in the extent of their subsequent metabolism once formed and the mechanisms for their excretion may explain these discrepancies. It is possible that each metabolite, but especially G-27550, may be further metabolized to other metabolites (conjugates etc.). If this is the case, the registrant should have explained these phenomena and attempted to account for the discrepancies in the results of the analysis of each metabolite.

Total Recovery. The total recovery for the G-27550 metabolite for up to 48 hours postdosing was only about 10.7 ± 4.7% of the administered dose (mean for all subjects receiving diazinon). Individual subjects ranged from a low of about 5% to a high of about 25%. Since there was no more excretion following the first 48 hours up to 15 days postdosing, it means that about 90% of the administered dose of diazinon was unaccounted for when metabolite G-27550 is used to assess for initial exposure to diazinon. There was also considerable variability in the time of peak excretion of G-27550. Thus, analysis for G-27550 may not be representative of the total or most appropriate assessment of excretion of diazinon administered even though it is considered to be a unique diazinon metabolite. Although G-27550 does have unique chemical properties that indicate that it would come from diazinon, its presence in the urine would have to be back extrapolated by a factor of 0.1 (or 10 times) to arrive at an approximation of diazinon originally administered. Another factor adding to the uncertainty of G-27750 as an index of exposure to diazinon is the large standard deviations for recovery.

Assessment of the metabolite DETP may be a better index of the exposure to diazinon based on preliminary calculations which indicate much more (i.e. 6.9 times as much, see Table 3 above) of this metabolite and a more uniform excretion pattern was noted. In particular, most subjects excreted maximum amounts of DETP in the first six hours postexposure. However, extensive additional calculations of the data would be required to make a more meaningful assessment of this issue. It must also be considered that DETP may not be the most appropriate metabolite for actual or practical field trials not having controlled exposure to diazinon only, since DETP may come from other sources besides diazinon. However, in closed systems such as a biomonitoring study, exposure to other potential sources of DETP can be regulated. ***The resolution of the most appropriate metabolite for assessing for diazinon exposure could not be derived from this study alone. This study does, however, raise concerns for the selection of G-27550 as the most appropriate metabolite for assessing exposure to diazinon.***

Classification

This study is not being classified since there are no current guideline for the classification of studies using human subjects. Several study deficiencies were noted that will have to be addressed by the registrant to resolve certain scientific issues for this study.

Study Deficiencies.

A. Deficiencies considered critical to acceptability of the study.

-There was no analytical report that verified the concentration of the diazinon in the epoxidized soybean stock sample. The registrant needs to provide verification of the purity of the diazinon used in this study as well as verification that the stock sample was 8% diazinon.

-There was no table or other data indicating the exact amount in grams of diazinon actually given to each individual. Such a table will have to be presented to validate that the individual subjects received their prescribed dose. This comment is considered critical to the acceptability of the study on technical grounds.

Other Comments

i. The analysis of the data for the urinary analysis of DETP is considered incomplete and additional calculations converting the ppb data into recovery data in terms of µgram equivalents of diazinon should be provided by the sponsor.

ii. The registrant needs to provide a comparative analysis of both the DETP and G-27550 urinary data and explain why there is no correlation between the two when a correlation relative to the proportion of each metabolite to the other should exist and the same pattern with respect to time of maximum excretion would also be expected to exist.

In particular, the analysis for the metabolites in the urine that result from the hydrolysis of diazinon indicated only 10.7 ± 4.7% mean recovery based on the pyrimidinone metabolite G-27550 for all subjects dosed with diazinon. An explanation that accounts for the balance or approximately 90% of the administered dose needs to be provided by the registrant. For example, are there data which show that the metabolite G-27550 is further metabolized and all of these subsequent metabolites are excreted.

iii. No females were included. The absence of females in this study is probably justified since it is difficult to include females in such studies because of possible effects on fetuses. Thus, the data generated reflect the excretion pattern of diazinon metabolites G-27550 and DETP in males not females. This may be acceptable unless review of the animal or any other data indicate sex differences in the pharmacokinetics of diazinon that would impact the utilization of the data from this study for risk assessment purposes for unique situations involving females. No reply from the registrant is requested or expected for this comment.

Table A1. Analysis of metabolite DETP in the urine for all subjects and comparison with metabolite G-27550 for randomly selected subjects.

Subj.	0-6 hr	6-12 hr	12-24 hr	24-48 hr	Comments
0.03 mg/kg Dose Group (Note: Data are in ng DETP/mL urine or ppb. Data in second row, when present, are for G-27550.)					
1	316	395	104	11	
3	958	795	88.4	18.4	
4	399	175	94.3	<	
5	**936** *42*	310 *43*	95.4 ***80***	18.8 *9.4*	Poor correlation between peak times for DETP and G-27550.
7	388	197	43.5	<	
8	90.7	260	25	16.4	
9	363	208	38.2	12.6	
0.12 mg/kg Dose group					
10	3200	1965	197	38.9	One of most sensitive and has highest for group for DETP.
12	965	439	153	35.3	One of most sensitive but lowest for group for DETP
15	2050	328	94.5	<	
16	843	314	21.6	<	
17	1470	1670	804	197	
18	680	97.4	256	26.6	
19	271	521	111	35.2	
Ave.	1354± 995 762 738		234± 262	22± 17	Mean has very large standard deviation. Thus, much variability. Most excretion in first 6 hours in 5 of 7 subjects.
0.21 mg/kg Dose Group					
13	4670	1680	316	<	
22	2190	7160	263	53.2	One of the least sensitive but has average DETP.
23	2070	824	220	30.1	
25	3080	1030	323	67.5	

Diazinon/2000 Special Study: Excretion of G-27550 and DETP in humans/oral exposure.

Subj.	0-6 hr	6-12 hr	12-24 hr	24-48 hr	Comments
27	7240	1610	330	61.8	
28	1770	1310	78.3	77	
30	**322** *127*	289 ***169***	161 *144*	46.6 *62*	Very low DETP but no unusual sensitivity. Seems low in both DETP and G-27550.
					Most excretion in first 6 hours in 6 of 7 subjects.
0.20 mg/kg Dose Group					
32	**2810** *117*	2130 *508*	963 **687**	61 *125*	Poor correlation between peak times for DETP and G-27550.
33	**3370** *130*	1170 ***230***	144 *ns*	47.3 *33*	Poor correlation between peak times for DETP and G-27550.
35	4070	933	390	74.7	One of the least sensitive but with average DETP.
37	1840	782	276	76.1	
39	4490	755	664	224	
40	2170	397	173	19.1	
41	2280 *639*	4090 *698*	502 *533*	81.8 *84*	Poor correlation between peak times for DETP and G-27550.
Mean	3004± 1008	1465± 1279	445± 293	83± 66	Mean has very large standard deviation. Thus, much variability. But most excretion in the first 6 hours in 6 of the 7 subjects . . .
0.30 mg/kg Dose Group					
29	**3590** *403*	3860 *858*	479 ***878***	74.4 *281*	Poor correlation between peak times for DETP and G-27550.

Data are from 51 to 63 of MRID No.: 45184303 for DETP metabolite and From Table 6 of MRID No.: 45184303 for the G-27550 metabolite. Data for G-27550 when presented are in the second row within the row for each subject.

TABLE 5. SUMMARY OF DIAZINON LEVELS FOUND IN BLOOD PLASMA SAMPLES AT SPECIFIED TIME INTERVALS

Diazinon Dose (mg/kg)	Volunteer #	Amount Diazinon (ppb) Found in Blood Plasma at Specified Interval												
		Day -2	Hour 0	Hour 1	Hour 2	Hour 4	Hour 6	Hour 8	Hour 12	Hour 24	Hour 48	Day 5	Day 8	Day 15
0	2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0	11	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	14	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	20	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	21	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	na	<1.3	na
0	24	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	26	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	31	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	34	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	36	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0	38	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0.03	1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0.03	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0.03	4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0.03	5	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0.03	7	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
0.03	8	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
0.03	9	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3

ABLE 6. SUMMARY OF G-27550 LEVELS FOUND IN URINE SAMPLES AT SPECIFIED TIME INTERVALS

		INTERVAL																								
		-2 Days			0-6 Hours			6-12 Hours			12-24 Hours			24-48 Hours			4 Days			7 Days			14 Days		
olunteer No.	DOSE (mg/kg)	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg	Vol (ml)	G-27550 ppb	µg
2	0	1220	<1.0		1010	<1.0		295	<1.0		470	<1.0		1017	<1.0		1780	<1.0		1505	<1.0		1570	<1.0	
6	0	1110	<1.0		870	<1.0		880	<1.0		634	<1.0		1525	<1.0		790	<1.0		970	<1.0		895	<1.0	
11	0	880	<1.0		470	<1.0		475	<1.0		415	<1.0		880	<1.0		1158	<1.0		1660	<1.0		1210	<1.0	
14	0	1580	<1.0		710	<1.0		630	<1.0		340	<1.0		2550	<1.0		1040	2.8	2.9	1120	<1.0		1110	<1.0	
20	0	1070	<1.0		580	<1.0		ns	ns		1160	<1.0		460	<1.0		750	<1.0		710	<1.0		1620	<1.0	
21	0	1090	<1.0		184	<1.0		ns	ns		470	<1.0		740	<1.0		ns	ns		655	<1.0		ns	ns	
24	0	1020	<1.0		1020	<1.0		2030	<1.0		1224	<1.0		1202	1.1	1.3	2285	<1.0		2380	<1.0		1970	<1.0	
26	0	1870	<1.0		740	<1.0		2058	<1.0		226	<1.0		440	<1.0		1705	<1.0		1480	<1.0		1840	<1.0	
31	0	1316	<1.0		866	<1.0		2132	<1.0		450	<1.0		1020	<1.0		1300	<1.0		910	<1.0		1360	3.1	4.2
34	0	1840	<1.0		530	<1.0		760	<1.0		440	<1.0		1160	<1.0		1760	<1.0		1720	<1.0		1745	<1.0	
36	0	4160	<1.0		1360	<1.0		820	<1.0		950	<1.0		3320	<1.0		3660	<1.0		4405	<1.0		2560	<1.0	
38	0	2030	<1.0		990	<1.0		440	<1.0		750	<1.0		2320	<1.0		3610	<1.0		1565	<1.0		1490	<1.0	
1	0.03	2060	<1.0		1380	25	34.5	455	46	20.9	383	64	24.5	1735	12	20.8	1540	2.2		2415	<1.0		1730	<1.0	
3	0.03	1610	<1.0		990	26	25.7	400	49	19.6	355	83	29.5	810	8.7	7.0	1260	<1.0		1470	<1.0		1140	<1.0	
4	0.03	1380	<1.0		825	28	23.1	545	48	26.2	430	93	40.0	1530	11	16.8	1230	<1.0		1930	<1.0		1714	<1.0	
5	0.03	1435	<1.0		675	42	28.4	405	43	17.4	275	80	22.0	860	9.4	8.1	1190	<1.0		980	<1.0		2135	<1.0	
7	0.03	2710	1.4	3.8	850	52	44.2	600	52	31.2	360	13	4.7	1360	11	15.0	3110	<1.0		1360	<1.0		2184	<1.0	
8	0.03	2610	<1.0		2050	15	30.8	1150	26	29.9	1370	18	24.7	1340	10	13.4	2845	<1.0		5130	<1.0		2250	<1.0	
9	0.03	2560	<1.0		550	57	31.4	970	31	30.1	435	108	47.0	1275	14	17.9	3276	<1.0		1590	<1.0		2575	<1.0	
10	0.12	1240	<1.0		560	108	60.5	515	299	154.0	400	359	143.6	1676	29	48.6	1700	<1.0		1340	<1.0		1650	<1.0	
12	0.12	1810	1.3	2.4	1335	102	136.2	780	277	216.1	610	214	130.5	2040	39	79.6	1430	1.6	2.3	1570	<1.0		2020	<1.0	
15	0.12	1460	1.1	1.6	810	243	196.8	750	146	109.5	810	107	86.7	1490	82	122.2	1600	<1.0		2700	<1.0		2506	<1.0	
16	0.12	2390	1.8	4.3	2760	79	218.0	2115	75	158.6	2120	47	99.6	5015	6.9	34.6	3860	1.2	4.6	2840	<1.0		3172	<1.0	
17	0.12	1990	<1.0		1000	132	132.0	1065	207	220.5	800	387	309.6	770	213	164.0	1360	<1.0		3710	<1.0		1590	<1.0	
18	0.12	1390	<1.0		670	95	63.7	350	306	107.1	330	624	205.9	540	40	21.6	1162	<1.0		1966	<1.0		1420	<1.0	
19	0.12	1350	1.5	2.0	680	207	140.8	440	193	84.9	1070	88	94.2	1860	36	67.0	1930	<1.0		1395	<1.0		1250	<1.0	
32	0.2	1690	<1.0		1480	117	173.2	480	508	243.8	324	687	222.6	1960	64	125.4	2770	<1.0		2280	<1.0		2170	<1.0	
33	0.2	1670	<1.0		1390	130	180.7	800	230	184.0	ns	ns		2790	33	92.1	3310	<1.0		1710	<1.0		2570	<1.0	
35	0.2	1630	<1.0		840	245	205.8	510	193	98.4	470	215	101.1	1070	81	86.7	1210	<1.0		825	<1.0		920	<1.0	
37	0.2	2370	<1.0		960	221	212.2	670	316	211.7	270	856	231.1	970	97	94.1	2820	<1.0		1945	<1.0		1670	<1.0	
39	0.2	830	<1.0		650	275	178.8	710	222	157.6	400	362	144.8	770	109	83.9	1430	<1.0		1240	<1.0		1920	<1.0	
40	0.2	2060	<1.0		1350	92	124.2	320	231	73.9	1620	179	290.0	3010	15	45.2	2850	<1.0		1785	<1.0		2120	<1.0	
41	0.2	2300	<1.0		650	639	415.4	280	698	195.4	430	533	229.2	1060	84	89.0	1310	<1.0		1025	<1.0		830	<1.0	
13	0.21	1870	<1.0		985	174	171.4	1125	149	167.6	870	325	282.8	2070	36	74.5	2410	<1.0		1770	<1.0		880	<1.0	
22	0.21	2905	<1.0		770	131	100.9	2017	699	1409.9	900	242	217.8	1172	82	96.1	1130	<1.0		1560	<1.0		2070	<1.0	
23	0.21	1334	<1.0		530	255	135.2	2023	223	451.1	260	402	104.5	272	26	7.1	1705	<1.0		1250	<1.0		1320	<1.0	
25	0.21	1232	1.0	1.2	730	196	143.1	2052	374	767.4	250	846	211.5	630	140	88.2	1350	1.5	2.0	1160	<1.0		1520	<1.0	
27	0.21	1560	<1.0		279	767	214.0	2104	246	517.6	430	417	179.3	1100	77	84.7	1050	<1.0		1340	<1.0		1220	<1.0	
28	0.21	610	1.2	0.7	950	290	275.5	2111	404	852.8	280	86	24.1	950	86	81.7	1030	1.1	1.1	830	<1.0		810	1.1	0.9
30	0.21	1965	<1.0		1320	127	167.6	2124	169	359.0	810	144	116.6	860	62	53.3	1475	<1.0		1690	<1.0		2320	<1.0	
29	0.3	840	<1.0		467	403	188.2	2118	858	1817.2	400	878	351.2	1218	281	342.3	1490	3.6	5.4	1090	<1.0		1070	2.3	2.5

no sample

TABLE 7. PERCENT OF DOSE EXCRETED AS G-27550 IN URINE AT INTERVALS FROM 0-48 HOURS POS DOSING

				Interval (Hours)								
				0-6		6-12		12-24		24-48		
Subject No.	Dose (mg/kg)	Body Wt (kg)	Dose (µg)	G-27550 Found (µg)	% of Dose	G-27550 Found (µg)	% of Dose	G-27550 Found (µg)	% of Dose	G-27550 Found (µg)	% of Dose	Total %
1	0.03	89.0	2670	34.5	2.6%	20.9	1.6%	24.5	1.8%	20.8	1.6%	7.5%
3	0.03	76.5	2295	25.7	2.2%	19.6	1.7%	29.5	2.6%	7.0	0.6%	7.1%
4	0.03	80.0	2400	23.1	1.9%	26.2	2.2%	40.0	3.3%	16.8	1.4%	8.8%
5	0.03	73.7	2211	28.4	2.6%	17.4	1.6%	22.0	2.0%	8.1	0.7%	6.9%
7	0.03	83.6	2508	44.2	3.5%	31.2	2.5%	4.7	0.4%	15.0	1.2%	7.6%
8	0.03	85.7	2571	30.8	2.4%	29.9	2.3%	24.7	1.9%	13.4	1.0%	7.7%
9	0.03	86.6	2598	31.4	2.4%	30.1	2.3%	47.0	3.6%	17.9	1.4%	9.7%
10	0.12	78.2	9384	60.5	1.3%	154.0	3.3%	143.6	3.1%	48.6	1.0%	8.7%
12	0.12	73.6	8832	136.2	3.1%	216.1	4.9%	130.5	3.0%	79.6	1.8%	12.7%
15	0.12	68.7	8244	196.8	4.8%	109.5	2.7%	86.7	2.1%	122.2	3.0%	12.5%
16	0.12	98.6	11832	218.0	3.7%	158.6	2.7%	99.6	1.7%	34.6	0.6%	8.6%
17	0.12	86.0	10320	132.0	2.6%	220.5	4.3%	309.6	6.0%	164.0	3.2%	16.0%
18	0.12	62.2	7464	63.7	1.7%	107.1	2.9%	205.9	5.5%	21.6	0.6%	10.7%
19	0.12	65.6	7872	140.8	3.6%	84.9	2.2%	94.2	2.4%	67.0	1.7%	9.8%
32	0.2	88.7	17740	173.2	2.0%	243.8	2.7%	222.6	2.5%	125.4	1.4%	8.6%
33	0.2	78.7	15740	180.7	2.3%	184.0	2.3%	ns	ns	92.1	1.2%	5.8%
35	0.2	73.1	14620	205.8	2.8%	98.4	1.3%	101.1	1.4%	86.7	1.2%	6.7%
37	0.2	76.9	15380	212.2	2.8%	211.7	2.8%	231.1	3.0%	94.1	1.2%	9.7%
39	0.2	71.6	14320	178.8	2.5%	157.6	2.2%	144.8	2.0%	83.9	1.2%	7.9%
40	0.2	74.7	14940	124.2	1.7%	73.9	1.0%	290.0	3.9%	45.2	0.6%	7.1%
41	0.2	86.8	17360	415.4	4.8%	195.4	2.3%	229.2	2.6%	89.0	1.0%	10.7%
13	0.21	95.2	19992	171.4	1.7%	167.6	1.7%	282.8	2.8%	74.5	0.7%	7.0%
22	0.21	78.8	16548	100.9	1.2%	1409.9	17.0%	217.8	2.6%	96.1	1.2%	22.0%
23	0.21	69.9	14679	135.2	1.8%	451.1	6.1%	104.5	1.4%	7.1	0.1%	9.5%
25	0.21	71.3	14973	143.1	1.9%	767.4	10.2%	211.5	2.8%	88.2	1.2%	16.2%
27	0.21	80.1	16821	214.0	2.5%	517.6	6.2%	179.3	2.1%	84.7	1.0%	11.8%
28	0.21	66.4	13944	275.5	4.0%	852.8	12.2%	24.1	0.3%	81.7	1.2%	17.7%
30	0.21	66.7	14007	167.6	2.4%	359.0	5.1%	116.6	1.7%	53.3	0.8%	9.9%
29	0.3	71.2	21360	188.2	1.8%	1817.2	17.0%	351.2	3.3%	342.3	3.2%	26.3%
		Overall Average =			2.6%		4.4%		2.6%		1.3%	10.7%
		Overall Std Dev =			0.9%		4.3%		1.2%		0.7%	4.7%

ns - no sample

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