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

010152

SEP 04 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Joint OPPT/OW/ORD Review of 1992 Aldicarb Human Study

TO: Addressees

FROM: William F. Satte, Ph.D., Chairman William F. Satte 9/4/92
ad hoc Joint OPPT/OW/ORD Review Group
Health Effects Division (H7509C)
Office of Pesticide Programs

Attached is our review of the new Acute Oral Toxicity Study in Humans of Aldicarb, our proposed reference dose (RfD) cover sheets for Aldicarb, and Aldicarb Sulfone for review by the Agency RfD Committee on September 22 and subsequent presentation to the SAP/SAB. At the request of OPPT/OW senior management, our group was formed to review the new acute human study of Aldicarb and to consider revisions to the RfDs on Aldicarb and Aldicarb sulfone in September. We held a series of 3 hour meetings over the course of the month of August. Ad hoc Joint Review Group Participants included: Amahl Mahfouz, Bruce Mintz, Jennifer Orme-Zavaleta, Ed Ohanian, Henry Kahn, and Helen Jacobs of OW, Suzanne McMaster and Dick Hill of OPPT, Karen Whitby, Kathleen Martin, Hugh Pettigrew of OPP, and Elaine Francis and Stephanie Padilla of ORD, and myself as chairman. Additional consultation was made with Chas Chapman of ORD on the pulmonary effects.

The primary review was drafted by me, and I take responsibility for the accuracy of the data evaluation report and the description of the conclusions of the group. Karen Whitby and Kathleen Martin of the Chemical Coordination Branch of OPP kept records of our deliberations from which, in part, conclusions, limitations, and weight of evidence sections were drawn. The participants varied in their level of attendance and participation, but there was considerable discussion on the key data and conclusions of the study review by members of OPPT, OW, and ORD. Additional statistical analyses were performed by Henry Kahn and Helen Jacobs of OW, and Hugh Pettigrew of OPP. Summaries of their analyses are attached as appendices to the Data Evaluation Report. The Results sections are divided into two general parts: first a description of what the study authors found and said about their findings; and second, a weight of evidence/conclusion section which summarizes the analyses and considerations of the review group.

1-76

010459

September 4, 1992
Ad hoc Joint Review Group

1992 Aldicarb Human Study Review

Table of Contents

010453

September 1, 1992
Ad hoc Joint Review Group

DATA EVALUATION REPORT[1]
COVER SHEET

STUDY TYPE: Acute Oral Toxicity Study in Humans

CAS No. 116-06-3 OPP TOX. CHEM NO: 011A

TEST MATERIAL: Aldicarb

SYNONYMS: Temik, 2-methyl-2-(methylthio) priopionaldehyde O-(methylcarbamoyl) oxime.

STUDY NUMBER: ICR Project No. 003237

SPONSOR: Rhone-Poulenc, Secteur Agro, 14-20 Rue Pierre Baizet 69009 Lyon, France

TESTING FACILITY: Inveresk Clinical Research Limited, Research Park, Riccarton, Edinburgh EH14 4AP Scotland

TITLE OF REPORT: A Safety and Tolerability Study of Aldicarb at Various Dose Levels in Healthy Male and Female Volunteers. Report No. 7786.

AUTHOR(S): P J Wyld, C E Watson, W S Nimmo, and N Watson

REPORT ISSUED: Study Completed 11 March and Issued 15 June 1992.

III. TEST MATERIALS AND SUBJECTS

Test compound White crystals. Purity: 99.0%;
The sample (Ref. No. 25DECQ89) was analyzed 7 January 1992 and certificate signed by J Thornton 8 January 1992.
"Supplies of the technical aldicarb were received at IRI on 7 January 1992 and stored in a freezer. A weighed amount of aldicarb was dissolved in deionised water. The solution was retained in a refrigerator for up to 2 weeks, providing pH < 7.0 before use."
The aldicarb was weighed on the day before dosing for each subject and dissolved in 150 ml of water. On the day of dosing, each solution "was made up to 200 ml with orange juice". Placebo was 200 ml of orange juice.

---

[1] Primary Reviewer: William F. Sette, Ph.D.
Toxicologist
Health Effects Division
Office of Pesticide Programs

3

C10453

010429

September 4, 1992
Ad hoc Joint Review Group

Human Studies Committee The protocol and lay summary were submitted to and approved by an independent Ethics Committee of the Inveresk facility and reported as conducted in accordance with the provisions of the declaration of Helsinki in 1964 as "concluded by the 29th Medical World Assembly in Tokyo (1975) the 35th Medical World Assembly in Venice (1983) and the 41st Medical World Assembly in Hong Kong (1989)". Approval was given on 19 December 1991. In addition, a quality assurance inspection was conducted on 27 January 1992 and a signed statement provided.

Test Subjects

Males and females 18-50 years old, 50-100 kg and within 15% of ideal body weight, with no clinically important abnormal physical findings or clinically relevant abnormalities in laboratory tests, ECG, blood pressure between 100-150/50-90 supine or standing, and between 50-90. Females were either post menopausal or surgically sterile. There were a variety of other exclusion criteria including a need for any medication during the five days before entry into the study, and any surgical or medical condition that might interfere with the absorption, distribution, metabolism, or excretion of the drug.

Subjects had been screened within 28 days of the beginning of the study including complete physical examination (e.g. height, weight, pulse, blood pressure, ECG (12 lead), blood for hematology and clinical chemistry, serum for HIV and hepatitis B antigens, and urine for drugs of abuse.

IV. STUDY DESIGN

The double blind, placebo controlled study included 38 men and 9 women, with 6 men and 5 women receiving both a dose and a placebo exposure, who were exposed to doses of 0, 10, 25, 50, 60, or 75 ug/kg of Aldicarb according to three study phases. The three phases were divided into a total of 8 sessions as described in report Table 1. Phase I consisted of two sessions, on January 9 and 17 in which 8 men received placebos, and groups of 4 men each received doses of 25, 50, or 75 ug/kg (Subject #20 got 60 ug/kg by mistake). On the basis of the results in these men, Phase II was conducted in three sessions on January 23, 27, and 30, where 8 men were given the placebo; 8 men were given 10 ug/kg; and 4 additional men were each given 25 or 50 ug/kg. Six men participated in two sessions, where each received a placebo at one session and a dose at the other (1 at 10, 3 at 25, and 2 at 50 ug/kg).
Phase III was conducted in 3 sessions on February 26, 27, and March 10. A total of 6 placebo exposures and four exposures each at 25 and 50 ug/kg were conducted. Five women participated in two sessions, where each received a placebo or a dose session (2 at 25 and 3 at 50 ug/kg). The number of subjects in each dose group is summarized below.

010459

September 4, 1992
Ad hoc Joint Review Group

	NUMBERS OF SUBJECTS IN DOSE GROUPS (UG/KG				
	0	10	25	50	75
MALES	16	8	8	8	4[2]
FEMALES	6		4	4	

The schedule of measures taken is given below.

SCHEDULE OF MEASURES TAKEN

	SCREEN	PRE-DOSE	HOURS AFTER DOSE						
			1	2	3	4	5	6	24
Pulse, BP	X	X	X	X	X	X	X	X	X
ECG	X								X
Hematology	X	X							X
Clinical chem	X	X							X
FEV_1, FVC		X	X	X	X	X	X	X	
Pupil size		X	X	X	X	X	X	X	
Saliva wt.		X	X	X	X	X	X	X	
Cholinesterase -16,-3H		X	X	X		X		X	21H
Blood in heparin		X	X	X		X			8H
Urine		X			0-8 H				9-24H
Signs/symptoms		X	X	X	X	X	X	X	X

Procedure

Subjects entered the clinic between 2-4 pm on the day before dosing. They were briefly examined and interviewed, and were trained on the pulmonary function device. They received food at 6 and 9 pm, but it was withheld after 11 pm. Beginning at around 8:30 am the next morning, subjects were given the 200 ml solution of orange juice with a breakfast of cereal, milk, toast, butter, marmalade, and tea. They were instructed to sip the juice throughout the breakfast. The time of dosing was defined as the time of the first drink of orange juice. Except for walking to testing areas, subjects were kept seated or recumbent for 6 hours after the dose, and fasted from liquids until 11:30 am and food until 12:30 pm. Normal activity except for strenuous exercise were permitted thereafter. No alcohol or other drugs were permitted on the study day "or until the last blood sample had been withdrawn". Tobacco was permitted. Screens between subjects's beds were

[2] One subject (#20) in this group received 60 ug/kg by mistake.

010459

010459

[redacted]429

employed to minimize contact and conversations.

Statistical Analysis

Statistical Analysis Software (SAS) was used to perform all statistical analyses.
All tests were two tailed and a 5% significance level used.
Vital signs, pulmonary function tests, saliva weight, and pupil diameter were evaluated by an analysis of covariance, with mean values for 1-6 hours post dose, and 1 hour values, with predose values as a covariate. If significant effects were seen at the 5% level, further analyses for linear trend and pairwise comparisons were made.

Cholinesterase data were analyzed using a repeated measures analysis of variance, comparing the mean of three pre-dose measures with the post dose time points (1, 2, 4, 6, 8, and 21 hours). "If a significant treatment effect was indicated at the 5% level and assuming the absence of a treatment*time interaction, the variation between treatment groups was subdivided into a number of contrasts so as to test for the presence of a dose related linear trend and also, of secondary interest, to make pairwise comparisons between each of the groups receiving Aldicarb and placebo."

Additional statistical analyses of blood pressure, cholinesterase, and salivation data were performed by Kahn and Jacobs of the Office of Water (blood pressure, ChE, salivation) and by Pettigrew of the Office of Pesticide Programs (salivation, pulmonary measures) and are described in detail in Appendix 1 and/or the text.

V. Methods and Results

The results sections contain a presentation of the data and the study authors's conclusions. EPA analyses and conclusions are presented separately, where different, under weight of evidence and conclusion sections.

A. Blood Pressure (Appendix 8)

Measurements were made of pulse(discussed in B.), systolic, and diastolic blood pressure with subjects both standing and supine according to the schedule given above.

Results

A decrease in mean supine systolic and diastolic blood pressure was noted in high dose men. The mean supine systolic and diastolic pressure (+/- S.D.), respectively for this group at the pre-dose interval and one hour after dosing were: Systolic 126.5+/-3; 110.3+/-10.1; Diastolic 77.5+/-3.4; 60.0+/-8.2 mm. But this effect was not statistically significant by the analysis of covariance.

010459

September 4, 1992
Ad hoc Joint Review Group

There seems to be little consistent effect in the females on blood pressure.

Weight of Evidence (WOE) Considerations/Conclusions

Pharmacologically, the effects of cholinesterase inhibitors on blood pressure is complex and both increases and decreases might be expected. The onset and duration of the changes seen fall within the first 6 hours after exposure. After discussion of the possible effects described above, further statistical analyses of the supine diastolic blood pressure data were conducted. A repeated measures analysis of variance showed a significant subject*time interaction but no significant overall dose effects or time*dose interactions.

For females, all three groups showed a decrease in this measure across the six hours after exposure with the high dose group showing the largest change, when examining the means over time for all dose groups (Appendix 1, Figure 11). For the 25 ug/kg a significant effect in a weighted analysis of the relative change at one hour in comparison to controls was seen; while Figure 11 shows the size of the decrease at 2 hours for the 50 ug/kg group was at least as large, this difference was not statistically significant.

For males, there was also a general decrease in this measure across time for all dose and control groups, with the largest decreases at the highest doses. Dunnett's tests, however, revealed a significant increase in this measure across all time intervals for men at 50 ug/kg. The unweighted analysis of the relative change in this measure at one hour vs time zero was significantly decreased for the 75 ug/kg group, but the weighted analysis was not significant.

In summary, there were a general decrease in the supine diastolic pressure seen in males and females which was greater in the higher dose groups and during the first two hours after exposure (Figures 11 and 12) and statistically significant for some, but not other, analyses at 25 ug/kg (females at one hour,), 50 ug/kg (an overall increase for males), or 75 ug/kg (males at one hour).

B. ECG Recordings and Pulse Measures (Appendix 8 and 9)

Sinus bradycardia was noted 24 hours after the dose in two men, subjects 22 and 26, following a placebo and a 10 ug/kg dose, respectively. While this effect is pharmacologically consistent with an anti-cholinesterase effect, it is not exposure related for subject 22. For subject 26, a decrease in supine pulse was also seen in this man with the greatest decrease 4 hours after the dose: 68 at time zero, 64,56,52,50,62,66, for 1-6 hours after the dose, and 76 24 hours after the dose.

In summary, subject 26 showed some decrease in pulse with the

010459

greatest decrease at 5 hours after the dose, and sinus bradycardia 24 hours after the dose, although supine pulse was increased at this time. No other changes in ECG were noted.

WOE/ Conclusions

Both pulse decreases and bradycardia are consistent with the pharmacology of anticholinesterases, and the pulse decrease is within the expected duration of effect. But for the exposed subject, the pulse and ECG measures are inconsistent with one another.

Further statistical analyses of all of the supine pulse data in males and females were performed. Dunnett's tests indicated a significant increase in supine pulse in males at 75 ug/kg and a significant decrease in supine pulse in females at 50 ug/kg, when comparing means of 1-6 hours of exposure with time zero. A repeated measures analysis of variance showed a significant within subject time effect, but no time*dose effect and no overall dose effect.

In summary, these effects do not support a conclusion of a consistent treatment related effect.

C. Urine Collection and Analysis (Appendices 10, 11)

Urine was collected predose, 0-8 and 9-24 hours, with volume and urinalyses results recorded and 20 ml aliquots frozen. A number of deviations for several subjects were noted on collection of urine volumes or for some urinalyses.

1. Urine volume. Although no statistics were performed, there was no apparent treatment effect on urine volume. For instance, the mean urine volume for the placebo men 0-8 hrs was 563+/-153 ml and for the men given 75 ug/kg, 581+/-184 ml. For women, mean placebo volume was 517 +/- 190 ml and for 50 ug/kg women, 410+/- 343 (N=3).

2. Urinalysis. No treatment related effects on urinalysis parameters were seen.

D. Hematology (Appendix 12) and Clinical Blood Chemistry (Appendix 13)

Hematology and clinical chemistry analyses were performed on blood samples taken at screening, predose, and 24 hours after the dose.

The following hematological parameters were measured: hemoglobin, total RBC count, hematocrit, mean cell volume (MCV), mean cell Hb, mean cell Hb concentration, WBC, Differential WBC analyses, and Platelet count.

There were no effects related to treatment expected or seen.

Clinical chemistry measures included BUN, glucose, AST, ALT, LDH, Na+, K+, Cl-, total protein, albumin, albumin/globulin ratio, Cholesterol, Alkaline Phosphatase, Ca+2, Creatinine, Triglycerides, Phosphate, Bilirubin, GGT, and Cholinesterase.

There were no effects related to treatment seen.

010459

[redacted]

010459

September 4, 1992
Ad hoc Joint Review Group

F. Adverse Events (Appendix 14)

Method

"Adverse events (any unwanted event) were allocated to a body system according to a recognised coding system (Costart) list of body systems. The adverse events are reported as recorded in the case report forms. Individual events have not been coded."
All signs were recorded, their intensity and severity noted, and fully described. Events were coded blindly as unrelated if neither time nor pharmacologically related, possibly related if time or pharmacologically related but not both, and definitely related if both. In general, time related was described as within 1-2 hours of dosing with recovery by 8 hours after dosing.

Symptoms of nausea, vomiting, sweating, diarrhoea, and abdominal cramps were recorded by enquiry. Involuntary movements were assessed by enquiry and observation. Slurred speech was evaluated by repetition of "Around the rugged rock, the ragged rascal ran". These signs and symptoms were recorded as present or absent "at each timepoint". No neurological examination, e.g.,the Romberg test for assessment of balance, or neurobehavioral tests were conducted. Subjects 1-8 had no pre-dose clinical signs assessed. We evaluated the adverse effects in terms of the criteria in Appendix 14, i.e., consistency with known effects of aldicarb or other anticholinesterases, as well as time of onset and duration, statistical significance and dose response relations, control incidence, objective/subjective nature of the signs, other signs or effects seen in the same subjects, and levels of cholinesterase inhibition.

Results

1. Sweating.

One man given 60 ug/kg showed profuse sweating, and this was regarded by the report authors as a definite sign of exposure. Another man in this group given 75 ug/kg showed palmar sweating immediately following the dose for 21 hours and at 24 hours and 10 minutes post dose for 1 hour and 5 minutes. This was considered to have no relation to treatment.

Sweating of palms and feet in one man given 50 ug/kg commencing within one hour of exposure, sweating of palms and forehead in one man given 25 ug/kg commencing within 2 hours of exposure, as well as sweating of palms in one man given placebo within one hour of exposure were all regarded as possibly related to treatment, but "not considered as possibly due to a systemic anticholinergic agent"(Sic).

September 4, 1992
Ad hoc Joint Review Group

Weight of Evidence Considerations

It is recognized and acknowledged that most if not all of these signs and symptoms are non-specific and may arise as a function of stress or other uncontrolled factors.

Both generalized and localized sweating was reported. In terms of the known effects of anticholinesterase agents, palmar sweating and other sites are considered an effector response from alpha adrenergic stimulation, while generalized sweating is a cholinergic effector response (Goodman and Gilman,p. 72). Of course, both sympathetic and parasympathetic ganglia are cholinergic. Thus, "low doses of anti-ChE agents cause, in general, augmentation of their secretory responses to nerve stimulation, and higher doses produce an increase in the resting rate of secretion"(ibid.,p.117). Thus, generalized sweating may result from stimulation of purely cholinergic pathways, while localized sweating may result from enhanced responsiveness of a cholinergic/adrenergic pathway. Palmar sweating is also a classic sign of fear or apprehension, which is also a symptom reported for cholinesterase inhibitors. Alternatively, this symptom may also be a non-specific response to stress.

Eliminating the 75 ug/kg man whose time of onset was too soon to be considered an effect of treatment yields an incidence of this response with increasing dose as follows: 1/16(0%); 0/8 (12.5%); 1/8 (12.5%); 1/8 (12.5%); 1/4(25%). There is some increased incidence as a function of exposure. However, no significant trend or comparison was found by Fisher's exact Test for this effect.

Examination of the summary table (Table 1) shows that except for subject 12, all subjects showed a time of onset consistent with the expected effect of Aldicarb. The duration of response for the subjects given 25-60 ug/kg also was consistent with expected effects, i.e., 1.5 to 6 hours after the dose, roughly.

In summary, subject 20, given 60 ug/kg showed sweating most clearly related to aldicarb and was agreed to be a definite effect. Subjects with localized sweating at 50 and 25 ug/kg showed localized sweating consistent in time of onset and duration expected, while the placebo subject showed localized sweating consistent in onset but not duration of effect, and the subject given 75 ug/kg showed localized sweating inconsistent in both onset and duration with expected effects.

2. Lightheadedness

At 75 ug/kg, one man, subject 14, reported lightheadedness, commencing one hour and 5 mins post dose for 30 minutes. This

effect was not reported by any men in the placebo or lower dose groups. This was regarded as a possible effect of treatment, but was not explicitly discussed by the study authors.

Weight of Evidence Considerations/Conclusion

This was judged as an effect expected for Aldicarb and also with an onset and duration consistent with known effects.

3. Abdominal Cramps

One man, subject 34, given 25 ug/kg, reported abdominal cramping commencing about 20 hours after the dose. This man was described as drinking his dose all down at one time. Two men given placebo reported G.I. effects, one reporting intermittent flatulence starting 2 hours post dose for 9 hours, and another churning stomach for 30 minutes commencing 2 minutes after the dose. All three were classified as possibly related to treatment, but the cramps in the man given 25 ug/kg were discounted on the basis of the long time until onset of 20 hours.

WOE/ Conclusions

While abdominal cramps are considered a prominent feature of aldicarb exposure, only one male subject, 34, showed this effect. This may have been due in part to the administration of the material over a 10-15 minute period. However, for subject 34, the time of onset of these effects, 20 hours after the dose, were not considered as consistent with the effects of aldicarb and were concluded to be unrelated to exposure.

4. Headache

At 10 ug/kg, three men had headaches, two commencing 4-5 hours post-dose, and the third almost 8 hours post-dose. No headaches were seen in control men or in men given higher doses. This effect was not regarded by the study authors as being pharmacologically or temporally related to Aldicarb.

WOE/Conclusions

Headaches may be expected as a known effects of aldicarb exposure. They are evaluated in the summary table for each subject with respect to onset and duration. They occurred only at the lowest dose given in males and not females. However, we have no specific information on expected time of onset for headaches. These were considered to be possible effects in 2 of 3 men. No adverse events were seen in exposed females.

September 4, 1992
Ad hoc Joint Review Group

G. Pulmonary function tests (Appendix 15)

Pulmonary function measures, Forced Vital Capacity (FVC) and Forced Expiratory Volume 1 second, (FEV_1), were made using a Vitalograph Alpha spirometer, at least in triplicate at each timepoint and recorded values from the test giving the largest sum according to the American Thoracic Society criteria so that the recorded values for FVC and FEV_1 were from the test giving the largest sum of these 2 measures.

Results

There were statistically significant increases in FVC in men between 1-6 hours of dosing, with greatest changes at 10 ug/kg and 75 ug/kg. Both the trend and the overall F test were significant. Individual comparisons showed effects at 10 and 75 ug/kg, but not the intermediate doses. There was no statistically significant effect at one hour after the dose. The statistical report notes the 10 and 75 ug/kg groups showed increases in mean FVC also at 2 hours postdose, but no statistical analysis was presented. These effects were considered by the report's authors "contrary to the changes which would be expected from the pharmacological activity of Aldicarb".

No statistically significant effects were reported on FEV_1.

No effects on these pulmonary function were seen in females. In contrast to the males, data from females were remarkably stable during the hours after dosing.

Weight of Evidence / Conclusions

In poisoning cases with anticholinesterases, pulmonary congestion and even edema may result; thus one would expect impairment in pulmonary function. On the other hand, at low doses, an increase in FVC of FEV measures might be a result of cholinergic stimulation of the diaphragm. Thus, the observed effects could be seen as pharmacologically consistent with the known mechanism.

The time of onset and duration of effects on FVC in males was consistent with an anticipated effect of aldicarb exposure.

These effects were also statistically significant by the analysis of covariance for 1-6 hours, but not 1 hour, and possibly 2 hours after exposure. However, the pattern of findings of significant results at the lowest and highest dose are an inconsistent dose response pattern. In addition, there was considerable variability in the mean measures across time and different dose groups in men.

The method section notes that measurements were taken at least in triplicate (p.20), and concern was expressed that the number of

[redacted]

010459

010459

trials/ subject may have varied across treatment groups. Other comments included concern for the general insensitivity of spirometers, that there is a more recent recommendation by the American Thoracic Society for which measures to take, and that subjects given more trials could show a practice effect. On this last point, measures were not found to be larger for subjects involved in both placebo and exposure trials.

It was observed that the statistical significance of the between group comparisons may have been influenced by the sharp drop in FVC in males given placebos at 2 hours and more after exposure. Last, there was some concern about the erratic time course of the effects. As a result, we performed further statistical analyses to consider analyses of subjects with respect to their own pre-dose performances, as well as at different time intervals Appendix 2, Pettigrew). The one way analyses of variance at one hour showed no statistically significant effects and the repeated measures analysis of variance showed a significant dose x time interaction in the raw data, so main effects could not be tested. Thus we concluded that they could not support a conclusion of a treatment related effect. Based on all of the available information, then, we concluded that these effects could not be considered a toxicologically significant effect of treatment.

H. Pupillometry (Appendix 16)

Pupil diameters were measured by computer for subjects 1-21 and manually by pupillometer thereafter. Measurements were made predose and hours 1-6 post dose. After predose measurements for subjects 21-30, no further computer measurements were made. Manual measures were taken at 24 hours post dose and used as the baselines for statistics. Subjects 5 and 6 had their 4 hour pupillometry tests repeated due to unsatisfactory lighting on the first trials.

The report notes no dose related changes in pupil size in males or females.

WOE/Conclusions

No effects were seen in the raw data, summary statistics or figures which suggest any effect on pupil size for males or females.

I. Saliva (Appendix 17)

Salivation was measured by weight absorbed on dental plugs in five minute intervals according to an ICR standard operating procedure and measured pre-dose and hourly for the first six hours after exposure.

Results

010459

September 4, 1992
Ad hoc Joint Review Group

There was a general increase in mean saliva weight in the first hour after dosing for placebo and dose groups, which was attributed by the authors to the effects of breakfast. These increases were not statistically significant by the analysis of covariance. Neither in males were there statistically significant differences for the 1-6 hour post dose measures. For females, however, there was a marginally significant (p= 0.056) F test for increases at 1-6 hours post dose. Figure 17.2 (p 288) shows the dramatic increase for high dose females in mean saliva weights over time (See Appendix 1, Figure 15). In terms of numbers of subjects in each group showing a pattern of increase and peak one hour after exposure, 2/6 placebo subjects, 1/4 low dose subjects, and 2/4 high dose subjects showed this pattern.

WOE/Conclusions

Increased salivation is an expected effect of anticholinesterases, and the time of onset and duration were consistent with expectations of a treatment related effect. The female data at 50 ug/kg approached statistical significance by a two tailed F test performed by the study authors.

We conducted further statistical analyses and found a statistically significant increase by Dunnett's test for females given 50 ug/kg. A repeated measures analysis of variance showed no significant overall dose effect or dose*time interaction for either sex, but a significant subject*time interaction. There were also no significant differences when the relative change was evaluated between the control and dose groups of either sex.

There was no consistent pattern in saliva weight data in males, but there was a statistically significant decrease in saliva by Dunnett's test for males in the 10 and 50 ug/kg groups, and the 60 ug/kg man.

In summary, there is some evidence of an increase in saliva weight for females at 50 ug/kg, and supported by some statistical analyses.

J. Cholinesterase Determinations (Appendix 18)
Method

Blood samples for cholinesterase determinations were taken through indwelling cannulae or by venipuncture at -16 hrs, -3 hrs, pre-dose, and 1, 2, 4, 6, 8, and 21 hours post-dose. Additional samples were taken into (EDTA) tubes at predose and 1,2, 4, and 8 hours post dose. Samples were immediately centrifuged, plasma removed, and snap frozen in liquid nitrogen. Samples were retained at -20 degrees C for analysis of plasma. All analyses were

010459 [redacted] 010459

September 4, 1992
Ad hoc Joint Review Group

performed one day after dosing (except for Phase II, where Cholinesterase measurements were made as a batch at the end of Phase II.(p. 88) 23-30 January, p. 97.) Red blood cells (0.2ml) were mixed with 0.4 ml water, centrifuged at 10K rpm for 2 mins at 4 degrees C. "An aliquot of supernatant was then removed, snap frozen in liquid nitrogen and stored at -20 degrees C prior to assay." Samples were thawed in small batches (<11) and analysed within 15 minutes using a Hitachi 704 analyser and Boehringer Mannheim Kit-124 117 for acetylcholinesterase activity. Samples with activity above 120,000 I.U./l were diluted and re-assayed.

Results

In general, treatment of both males and females at all dose levels resulted in inhibition of both red blood cell and plasma cholinesterases, with the peak effects one hour after the dose, and the degree and duration of effect increasing with increasing dose. Effects on plasma lasted longer. In the repeated measures analysis of variance, statistically significant treatment*time interactions were seen for RBC and plasma cholinesterases in both sexes and a statistically significant dose related linear trend was found for both measures for both sexes.

Intensity of Effect

In males, the mean inhibition at one hour post dose in RBC AChE in comparison to time 0 was 3.8% at 10 ug/kg, 12% at 25 ug/kg, 29% at 50 ug/kg, and 38% at 75 ug/kg. In females, the mean inhibition at one hour post dose in RBC AChE was 20% at 25 ug/kg and 36% at 50 ug/kg.

For plasma ChEs, in males the mean inhibition at 10 ug/kg was 13%, at 25 ug/kg, 35%, at 50 ug/kg, 55%, and at 75 ug/kg, 70%. For plasma ChEs in females the mean inhibition at 25 ug/kg was 49%, and at 50 ug/kg, 67.6%.

Duration of Effect

At 10 ug/kg, there were statistically significant differences in males in RBC AChE at 1-4 hours(p=0.051 at 1 hr) and 21 hours after the dose, and in plasma ChE at 1-2 hours.

At 25 ug/kg, there were statistically significant differences in males in RBC AChE 1-4 hours and 8 hours after the dose, in females at 1-2 hours; in plasma ChE at 1-6 hours in males and 1-4 hours in females.

September 4, 1992
Ad hoc Joint Review Group

At 50 ug/kg, there were statistically significant differences in males in RBC AChE 1-6 hours after the dose, in females 1-4 hours; in plasma ChE at 1-21 hours after the dose in males and 1-4 hours after the dose in females.

At 75 ug/kg, there were statistically significant differences in males in RBC AChE 1-6 hours after the dose, and in plasma ChE 1-21 hours after the dose.

Sex Differences

There were marginally statistically significant differences in degree of RBC AChEI between males and females, with females showing greater inhibition, one hour after dosing at 25 ug/kg (p=0.058), and 50 ug/kg (p=0.049) and 21 hours after the dose at 50 ug/kg (p=0.51). Females showed significantly greater plasma ChE inhibition 1-4 hours after 25 ug/kg, but not 50 ug/kg. Placebo females showed significantly less effect than males in plasma ChEI 21 hours after the dose. In summary, effects were greater in females, but lasted somewhat longer in males.

Weight of Evidence Considerations/Conclusions

In general, the investigators seemed cognizant of the potential for "reversibility" of cholinesterase inhibited by carbamate pesticides: all samples were frozen and assayed as quickly as possible. There are, however, some problems with their stated method for determining red blood cell cholinesterase activity. The method for preparing the red blood cell cholinesterase appears to result in an ill-defined fraction which does not recover the majority of the cholinesterase activity, throwing a substantial amount of the activity away. The cholinesterase activity in the human red blood cell is anchored to the red blood cell membrane. The authors state that they hemolyzed (i.e., broke open) the red blood cell by diluting the packed erythyrocytes in water and then centrifuged this mixture for a short period of time and then took an aliquot of the supernatant. Some portion of the erythyrocyte membranes will pellet during this centrifugation procedure, and when the investigators take an aliquot of the supernatant, they are only sampling the portion of the cholinesterase which did not pellet during the centrifugation. It seems that most of the activity could be in the pellet after the spin. In order to validate this procedure, the investigators should present evidence that the pellet from this centrifugation does not contain cholinesterase activity. Moreover, this "washing" process will result in a substantial amount of decarbamylation of the inhibited cholinesterase, thereby underestimating the level of actual cholinesterase inhibition <u>in vivo</u>. In view of the above concerns, the data on RBC cholinesterase inhibition may have underestimated the amount and increased the variability in this

010459

measure, thus obscuring the extent of treament related effects and comparisons relying on this endpoint.

010459

measure, thus obscuring the extent of treament related effects and comparisons relying on this endpoint.

VI. Discussion, Study Limitations, and Conclusions

Summary

In general, the review group noted that this double blind study in healthy humans was acceptable for evaluating potential effects from acute oral exposures to Aldicarb, although certain limitations were noted. The study design consisted of administration first of 2 relatively high doses of Aldicarb and placebo doses to men. After a clear sign of aldicarb exposure (profuse sweating) along with considerable blood cholinesterase inhibition (65% plasma ChEI, 27% RBC ChEI) were noted in one male of four in the 0.075 mg/kg group (who was mistakenly given 0.060 mg/kg), further dosing at this level was not undertaken. The study ultimately incorporated five groups of subjects including placebo, 0.010, 0.025, 0.050, and 0.075 mg/kg aldicarb groups. Males (N=38) were included in each group, while females (N=9) only received the placebo, 0.025, or 0.050 mg/kg doses. Some subjects received both a placebo and a treatment dose in 2 testing sessions.

Subjects were given a light breakfast on the day of the study, including a drink of orange juice containing one of the doses of aldicarb or the placebo to be consumed over 15-30 minutes of the breakfast period. Subjects remained generally seated or recumbent for the first four hours after dosing. A number of biological parameters that are known to be affected by cholinesterase inhibitors were monitored before dosing, hourly for the first 6 hours after dosing, and at 24 hours after dosing. These measures included recording of signs and symptoms (e.g., sweating), measurements of pulse and blood pressure, evaluation of pulmonary functions (FEV-1 and FVC), saliva and urine output, pupil diameter measurements, and measurement of plasma and red blood cell cholinesterase activity.

All study subjects were evaluated with respect to the above consequences after dosing with aldicarb or placebo. Emphasis was placed on the first 6 hours after exposure, because it is known that the effects and cholinesterase inhibition caused by aldicarb, are acute and readily reversible. The major effects seen in the study and discussed as potentially treatment-related were effects on red blood cell and plasma cholinesterase inhibition, sweating, light-headedness, headaches, salivation, and supine diastolic blood pressure.

1. Cholinesterase measurements: Aldicarb treatment of both males and females at all dose levels resulted in statistically signifi-

cant inhibition of both red blood cell and plasma cholinesterases. Peak effects were noted at 1 hour after the dose, and the degree and duration of effect increased with increasing doses. Effects on plasma lasted longer. Effects were greater in females, but lasted somewhat longer in males. Enzyme effects were recovered 21 hours after dosing (except for low dose males and RBCs).

2. Sweating One male in the 75 ug/kg group who had mistakenly received 60 ug/kg, developed diffuse and profuse sweating that came on within 2 hours and abated within 6 hours of dosing. This clinical sign is the strongest indication of a cholinergic effect that occurred in t: study and was the reason the study directors discontinued dosing at the 75 ug/kg level. Two other treated males developed localized and mild sweating with onset within the first 2 hours of dosing which also abated within 6 hours of dosing. One man given placebo developed localized sweating beginning one hour after dosing and persisting for 11 hours. One man given 75 ug/kg developed localized sweating immediately after the dose for 21 hours. All four of these men showed sweating of the palms, either alone, or along with sweating of the soles or forehead.

3. Lightheadedness: One male given 75 ug/kg reported that he was lightheaded within one hour of dosing.

4. Headaches : Three men in the 10 ug/kg group reported headaches, two with onset within 6 hours of dosing, and one within 8 hours. One of the first two men had a second headache which began 10 hours after dosing.

5. Clinical effects in females: None of the females developed any clinical signs or symptoms consistent with cholinesterase inhibition or treatment.

6. Blood Pressure There was a general decrease in the supine diastolic pressure seen in males and females which was greater in the higher dose groups than in the lower dose groups and controls and during the first two hours after exposure (Appendix 1, Figures 11 and 12). The repeated measures analysis of variance did not show any effect of dose. There were significant decreases in this measure for males in the 75 ug/kg group at one hour, females in the 25 ug/kg group at one hour, but a significant increase in males in the 50 ug/kg group for 1-6 hours overall in comparison to controls. In contrast to these effects, there were no treatment related effects on either supine systolic or standing blood pressure in either sex.

7. Salivation: Repeated measures analysis of variance of saliva weights did not show any overall dose effect in either sex. For females, there was a statistically significant subject by time but not dose by time interaction; for males, neither was significant. Females group means show a consistent increase in saliva weight at the high dose of 50 ug/kg (Appendix 1, Figure 15). Dunnett's test for differences between means for 1-6 hours after exposure was statistically significantly increased in comparison to controls,

but for males this test showed a statistically significant decrease for 10 and 50 ug/kg groups and the 60 ug/kg man.

8. Pulse, respiratory parameters, pupil size, and urine volume: There were no treatment related changes in standing or supine pulse, pupil size, or urine volume in either males or females. As expected, there were no changes in hematology and clinical chemistry parameters. There were statistically significant increases in FVC in men at the 10 and 75 ug/kg dose, but these were not concluded to be treatment-related, based upon one way analyses of variance which were not statistically significant and upon the observation that the statistically significant findings were likely a result of a drop in control values during the session.

Discussion

The strongest indication of an adverse effect is the presence of diffuse and profuse sweating in a male given 60 ug/kg of aldicarb. This is a classical sign of cholinesterase inhibition. However, this effect was not seen in any other man given 75 ug/kg or any other subject.

The evaluation of localized sweating of the palms, soles, and forehead was considered more difficult. This effect was seen in two treated males, one at 25 ug/kg, and one at 50 ug/kg. No sweating was seen in women. Interestingly, in the Haines study (Union Carbide, 1971), all four males who received 100 ug/kg developed sweating within 2 hours of dosing, and two men were described as having sweating of the palms or forehead. Each of these men had other signs and symptoms and were clearly affected by aldicarb exposure. Men in that study given 25 or 50 ug/kg showed no sweating.

Localized sweating is mediated by sympathetic neurons that are cholinergic and effector neurons that are adrenergic. This differs from generalized sweating which is mediated by cholinergic neurons only. Non-specific factors may also cause localized sweating, such as stress. This may be illustrated by localized sweating seen in one man given a placebo dose and one man given 75 ug/kg immediately after dosing.

The other clinical effects manifest in this study that were potentially referable to cholinesterase inhibition were the symptoms of lightheadedness and headaches. Both of these effects are common complaints of people exposed to organophosphates and carbamates. Only one male in the high dose group was lightheaded, but no simple tests of steadiness or balance, e.g., the Romberg test were performed. No females reported this symptom. Three men in the 10 ug/kg group reported headaches, two with time of onset

within 1-6 hours of dosing. Headaches were not seen at any higher dose which makes them more difficult to interpret as a treatment related effect. No headaches were seen in females or placebo subjects, or in men in the Haines study.

Interpretation of the blood pressure effects is also difficult. Cholinesterase inhibition may lead to increases or decreases in blood pressure. Observed changes were generally small in magnitude, limited to supine diastolic pressure, and statistically significant in some, but not other analyses. While there were general decreases in this measure and statistically significant results in females given 25 ug/kg at one hour, and in men given 75 ug/kg at one hour, they were not seen in 50 ug/kg women at one hour. Further, for men in the 50 ug/kg group, there was a statistically significant increase across 1-6 hours in comparison to controls. There were no significant effects noted on supine systolic or standing blood pressure in males or females. Likewise, no changes in blood pressure in the dose males in the Union Carbide study. These data limit the significance of these findings.

Cholinesterase inhibition would be expected to increase the flow of saliva. Consistent with this, females given 50 ug/kg showed higher output than controls, with marginal statistical significance. By contrast, in males three dose groups showed significant decreases in comparison to controls. In the Haines study, three men given 100 ug/kg showed salivation.

Study Limitations

A number of questions arose during the review of this study that made it more difficult to fully interpret the results. Some of these will be addressed further by the study authors in the near future. Others are simply limitations in the design and conduct of the study. These included the following:

1. The study included some, but not many other objective measurements of neurological and behavioral functions that might be affected by aldicarb.

2. It was not apparent what information the test subjects received before the study about the specific effects that might be expected from aldicarb exposure.

3. The list of symptoms that observers asked subjects about was not clear, or how the subjects were questioned about their symptoms, or the precise sequence of testing at each hour.

4. It was not stated whether observers confirmed the presence of

sweating reported by subjects.

5. Smokers were included, but not identified so that one could evaluate their responsiveness to treatment.

6. Consumption of aldicarb over a 15-30 minute period may produce a different spectrum of effects than a dose consumed as a bolus, as in the Haines study.

7. The small number of subjects, particularly in some dose groups, limited the power of the statistical analyses.

8. The method of measurement of RBC acetylcholinesterase may have underestimated the activity and increased the variability in the measurements, because cells may have been incompletely lysed and then pelleted by centrifugation, leaving only a portion of the membranes in the supernatant to be assayed.

Conclusion

This double-blind experimental study of the acute effects of aldicarb contributes further significant information about the potential toxicity of the chemical. However, the paucity of clearly and statistically significant chemically related effects other than inhibition of plasma and red blood cell cholinesterase activity, and the limitations noted make a definitive judgment of toxic and non-toxic doses difficult. Effects that were noted tended not to demonstrate statistical significance, dose response or dose effect relationships, or correspondence between males and females. Diffuse an' profuse sweating in one man given 60 ug/kg was the most clearcut sign of toxicity; the appearance of localized sweating in one man at 50 and one man at 25 ug/kg suggest some dose-related response, especially in light of the Haines study, where all males receiving 100 ug/kg showed sweating. Some other effects consistent with cholinesterase inhibition were noted in the range of 25-75 ug/kg. In conclusion, we consider the NOAEL for this study as 10 ug/kg and the LOAEL 25 ug/kg, based on the sweating seen in the men.

VII. REFERENCES

Taylor, P Anti-cholinesterase agents. In: Goodman and Gilman's The Pharmacological Basis of Experimental Therapeutics. 7th Edition. Macmillan: New York. 1985.

Table 1. Summary Table of Effects Reported for Males

Appendix 1. Analysis of Aldicarb Human Study Data. Memorandum, H.

010459

September 4, 1992
Ad hoc Joint Review Group

Kahn and H. Jacobs to W.Sette, 9/2/92.

010459

Appendix 2. Aldicarb: Statistical Analysis of Data from a Safety and Tolerability Study of Aldicarb at Various Dose Levels in Healthy Male and Female Volunteers-ICR Project No. 003237. Memorandum, H. Pettigrew to W. Sette, 9/2/92.

010459

010459

September 4, 1992
Ad hoc Joint Review Group

010459

010429

SUMMARY TABLE OF SYMPTOMS REPORTED FOR ALDICARB - GRAND ALLUVIUM STUDY

Group	Subject	Cholinesterase Change from 0 Plasma (%)	Inhibition to 2 hour Red Cell (%)	Signs and Symptoms[1]	Onset within 1-6 hour?	Duration within 1-6 hour?	Relationship to Aldicarb? Increase	Relationship to Aldicarb? Workgroup[2]
0	4	-0.1	20.3					
0	6	-4.8	-8.1					
0	7	-8.6	-10.4					
0	10	2.4	-2.8					
0	11	-6.8	0.4					
0	12	-13.8	4.7					
0	15	-8.5	-0.6					
0	18	-5.9	-0.2	Severe pains and fainted	Yes	No (11 HRS)	Possible	None
0	22	6.4	6.6					
0	25	0.4	0.8					
0	29	-1.5	-8.3					
0	30	-0.4	-3.8					
0	35	-2.4	-6.0					
0	36	-6.8	-8.4					
0	38	-2.5	-6.0					
0	40	-2.1	1.0					
0.010	24	-0.0	-0.4					
0.010	26	-2.5	1.8					
0.010	31	-14.0	0.4	Headache	Yes	Yes	None	Possible
"	"	"	"	Headache	Yes	Yes	None	Possible
"	"	"	"	Headache	Yes	No	None	None
0.010	32	-12.0	-3.4					
0.010	33	-10.0	-0.0	Headache	Yes	No	None	Possible
0.010	36	-15.0	-5.6					
0.010	41	-12.0	-4.1					
0.010	44	-7.0	-2.0	Headache	No	No	None	None
0.025	1	-37.4	-10.5					
0.025	3	-28.6	4.6					
0.025	9	-32.5	-12.0					
0.025	8	-37.7	-14.2					
0.025	25	-41.0	-2.6			~4HRS		
0.025	27	-46.0	-12.0	Severe pains	Yes	Yes	Possible	Possible
0.025	34	-48.1	-20.7	Abdominal Cramps	No	No	Possible	None
0.025	43	-36.2	-10.1					
0.050	2	-50.1						
0.050	5	-35.7	-26.0					
0.050	17	-36.3	-27.0					
0.050	19	-50.9	-28.4					
0.050	21	-37.0	-27.3					
0.050	26	-30.0	-20.9	Screaming pains and faint	Yes	Yes 4HR	Possible	Possible
0.050	37	-30.0	-20.0					
0.050	42	-26.0	-15.3					
0.066	28	-65.5	-27.1	Profuse sweating	Yes	Yes 1:23	Definite	Definite
0.075	13	-70.0	-40.2	Severe pains	No	No 21:4	None	None
0.075	14	-60.0	-41.2	Light headed	Yes	Yes :30	Possible	Definite
0.075	16	-74.7	-44.0					

[1] Symptoms which are possibly related to aldicarb exposure are listed. While localized sweating is listed there was considerable workgroup disagreement on whether it is consistent with the pharmacology of aldicarb.

[2] Definite: Symptoms that are pharmacologically, onset and duration related to aldicarb exposure.
Possible: Symptoms that maybe pharmacologically, onset or duration related to aldicarb exposure.
None: Symptoms that either are not pharmacologically or not onset and duration related to aldicarb exposure.

010459

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

010459

SEP 2 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Aldicarb: Statistical Analysis of Data From A Safety and Tolerability Study of Aldicarb at Various Dose Levels in Healthy Male and Female Volunteers- ICR Project No. 003237

TO: William F. Sette, Ph.D.
Peer Review Section (H7509C)

FROM: Hugh M. Pettigrew, Ph.D., Acting Section Head
Statistics Section (H7509C)

9/2/92

The purpose of this memorandum is to summarize the results of a preliminary analysis of data on Pulmonary Function Tests and Salivation from the recent human study. The analyses include repeated measures analysis of variance (ANOVA) applied to the Raw Data (unadjusted data) at times 0 through 6, the Difference Data, adjusting by subtracting the baseline (zero hour measurement) for each subject at each subsequent time point, and Percent Difference Data, reflecting the percent change from baseline calculated at each time point. In addition, 1-way analyses of variance were performed on the first-hour differences. The results of these analyses in general complement and confirm the results of the analyses carried out by INVERESK CLINICAL RESEARCH and submitted by the registrant.

The attached copies of computer printout are the result of applying a repeated measures analysis of variance program (BMDP2V- ANALYSIS OF VARIANCE AND COVARIANCE WITH REPEATED MEASURES) and a one-way analysis of variance program (BMDP7D-ONE- AND TWO-WAY ANALYSIS OF VARIANCE WITH DATA SCREENING)to the data. These results are summarized in the following pages. The complete printouts from the analyses are available in my office.

010459

010459

FVC- MALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: p=0.0344 Main effects cannot be tested.
Differences:	TimeXGroup Interaction: n.s. Time: p=0.0104 Group: p=0.0115
% Difference Data:	TimeXGroup Interaction: n.s. Time: p=0.0090 Group: p=0.0119
_-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s.(Bonferroni)

FVC- FEMALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: n.s. Time: p=0.0316 Group: n.s.
Differences:	TimeXGroup Interaction: n.s. Time: n.s. Group: n.s.
% Difference Data:	TimeXGroup Interaction: n.s. Time: n.s. Group: n.s.
1-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s. n.s.(Bonferroni)

010459

010459

SALIVA- MALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: n.s. Time: p=0.0056 Group: n.s.
Differences:	TimeXGroup Interaction: n.s. Time: n. s. Group: n.s.
% Difference Data:	TimeXGroup Interaction: n.s. Time: p=0.0454 Group: n.s.
1-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s.(Bonferroni)

SALIVA- FEMALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: n.s. Time: p=0.0153 Group: n.s.
Differences:	TimeXGroup Interaction: n.s. Time: n.s. Group: 0.0422
% Difference Data:	TimeXGroup Interaction: n.s. Time: n.s. Group: n.s.(p=0.0742)
1-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s. n.s.(Bonferroni)

010459

010459

FEV- MALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: n. s. Time: p=0.0395 Group: n.s.
Differences:	TimeXGroup Interaction: n.s. Time: p=0.0291 Group: n. s.
% Difference Data:	TimeXGroup Interaction: n.s. Time: p=0.0300 Group: n. s.
1-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s.(Bonferroni)

FEV- FEMALES

Repeated Measures ANOVA:

Raw Data:	TimeXGroup Interaction: n.s. Time: n. s. Group: n.s.
Differences:	TimeXGroup Interaction: n.s. Time: n.s. Group: n.s.
% Difference Data:	TimeXGroup Interaction: n.s. Time: n.s. Group: n.s.
1-way ANOVA:	Levene's Test: n.s. F-test: n.s. pairwise comparisons with control: n.s. n.s.(Bonferroni)

010459

010459

[redacted]

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

OFFICE OF WATER

SEP 2 1992

MEMORANDUM

SUBJECT: Analysis of Aldicarb Human Study Data

FROM: Henry D. Kahn, D.Sc. HDK
Chief, Statistics Section
Helen L. Jacobs, Statistician H.L.J.
Economic and Statistical Analysis Branch
Engineering and Analysis Division
Office of Science and Technology

TO: William Setto, Ph. D., Toxicologist
Science Analysis Branch
Health Effects Division (H7509C)
Office of Pesticide Programs

This memorandum provides summaries of our analysis of the data provided by Rhone-Poulenc from the study titled "A Safety and Tolerability Study of Aldicarb a Various Dose Levels in Healthy Male and Female Volunteers" (the "Study"). This study was conducted in early 1992 to assess the effects of aldicarb on healthy human volunteers.

We examined data on five response variables measured on all subjects over the duration of the study:

- red cell cholinesterase
- plasma cholinesterase
- supine pulse
- supine diastolic blood pressure
- saliva weight.

The following analyses were performed on the data for these five variables:

- Repeated measures analysis of variance
- Dunnett's test of dose effect compared to control using the lose mean estimated across time as the dependent variable
- Analyses of the relative difference between

measurements made at time = 0 (just prior to administration of dose) and time = 1 (1 hour after administration of dose); these analyses included univariate 1-way analysis of variance and Dunnett's test

Descriptions of the data and analyses are provided along with graphical figures that display the data in a manner that illustrates the analyses.

Overall Summary

Summaries of the results of each analysis on each variable are provided below. The results are mixed in that the results for plasma and red cell cholinesterase show consistent, statistically significant effects while the results for supine pulse, supine blood pressure and saliva weight display an inconsistent pattern of apparent differences and statistically significant and not significant results. In general, the results for cholinesterase indicate statistically significant effects over time, significant differences between control and dose groups and significant differences between relative change at one hour. These results are consistent with known effects of aldicarb on cholinesterase measures and are not surprising.

The results for supine pulse, supine diastolic blood pressure and saliva weight are mixed. In the repeated measures analysis there is no sigficant effect of dose for supine pulse, supine diastolic blood pressure or saliva weight for either sex. For supine pulse and supine blood pressure there are significant time effects but no dose-time effects for either sex; for saliva, the females have a significant time effect but no dose-time effect and males have neither time nor dose-time effects.

The effect of dose in comparison to control was evaluated using Dunnett's test with dose means calculated across all points in time at which measurements were made. For the supine pulse data, a two-tailed Dunnett's test showed the 0.075 male dose group had a significantly different mean pulse rate in comparison to control. No other dose group for either sex was significantly different. For the supine diastolic blood pressure data, the 0.050 male group mean was significantly different from control based on a two-tailed Dunnet's test. No other group for either sex was significantly different with respect to mean supine diastolic blood pressure. For the salivation data, based on Dunnett's test, the 0.050 female dose group mean was significantly greater than control mean and the means for the male 0.010, 0.050 and 0.060 dose groups were significantly less than the control mean. No other significant differences for salivation between dose group means and control means were found.

Relative change at one hour post dose in measures of supine

the dose group means with control. The one hour change was examined since the greatest change in cholinesterase levels usually occurs at one hour post dose. Pulse showed no significant differences among dose and control group means for either sex. For blood pressure, both the 0.075 males and the 0.025 females showed a significantly greater change at one hour in comparison to control. The relative change in salivation was not significantly different for any of the dose groups for either sex.

Of particular interest are the results for saliva weight in females (shown in Figure 15 below) and relative change at one hour for supine diastolic blood pressure for females (Figure 11). For the saliva data, the Dunnett's test shows a significant difference between the mean of the control group and the mean of the 0.050 group. The group means for each point in time, displayed in Figure 15, show the 0.050 group is clearly distinct and greater than the control and 0.025 group. The relative change at one hour in supine blood pressure for the females may be seen in Figure 11. On average the 0.025 and 0.050 groups both decrease at one hour. The 0.025 decrease is significantly different using analysis of variance weighted to account for heterogeneity of variance among the different dose groups. This decrease is probably real since all subjects in the 0.025 group decrease at one hour. The weighted analysis of variance is appropriate because of the differences in the within group variances. The mean blood pressure of the 0.050 group also decreases at one hour but the difference is not significant statistically. The decrease in the 0.050 group at one hour is due to the large decrease (80 to 65 mm Hg) in one subject. For the 0.050 group the blood pressure measurements at two hours post dose show a further decrease however statistical analysis of the two hour change was not conducted. The blood pressure measurements and change in plasma and red cell cholinesterase for each female subject are shown in the table below.

More complete description of the data, methodology and results and graphic figures follow.

Changes at 1 and 2 Hours in Supine Diastolic Blood Pressure and 1 Hour in Cholinesterase Levels in Females

Group	Subject	Cholinesterase Inhibition Change from 0 to 1 hr. Plasma (%)	Cholinesterase Inhibition Change from 0 to 1 hr. Red Cell (%)	Supine Diastolic Blood Pressure mm Hg 0 hr.	mm Hg 1 hr.	mm Hg 2 hr.	Pressure Change 0-1 hr. (%)	Change 0-2 hr. (%)
0.000	47	-3.4	-2.5	70	70	80	0.0	14.3
0.000	50	-3.8	-4.6	64	60	66	-6.3	3.1
0.000	51	-4.1	-0.8	70	74	76	5.7	8.6
0.000	152	-6.2	3.7	70	70	70	0.0	0.0
0.000	155	-3.5	-5.2	90	90	80	0.0	-11.1
0.000	158	-6.6	-24.9	84	80	80	-4.8	-4.8
0.025	45	-55.7	-22.9	75	65	70	-13.3	-6.7
0.025	48	-99.9	-26.4	75	72	62	-4.0	-17.3
0.025	154	-43.5	-17.4	78	70	70	-10.3	-10.3
0.025	157	-41.3	-13.9	86	80	80	-7.0	4.7
0.050	44	-82.1	-48.9	80	68	70	-18.8	-12.5
0.050	49	-76.2	-45.0	55	58	56	5.5	1.8
0.050	153	-57.4	-23.9	80	80	70	0.0	-12.5
0.050	156	-55.5	-22.4	80	80	70	0.0	-12.5

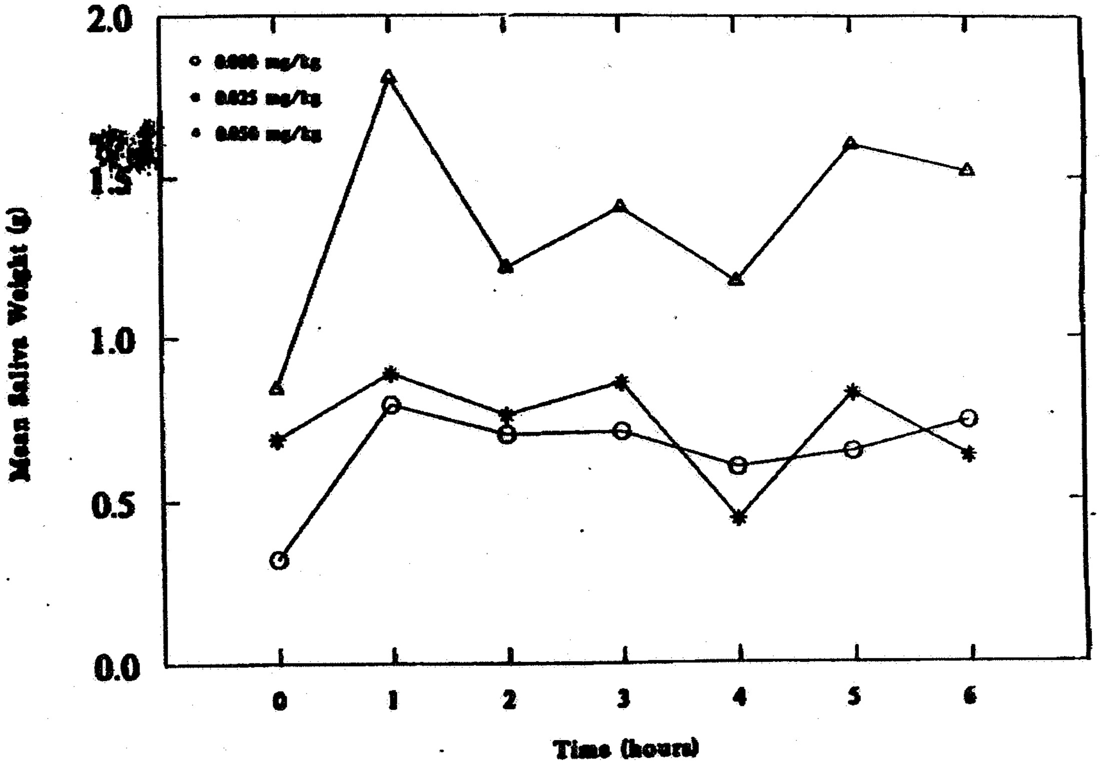


Figure 15. Mean Saliva Weight versus Time for Aldicarb Doses in Females

010459

33

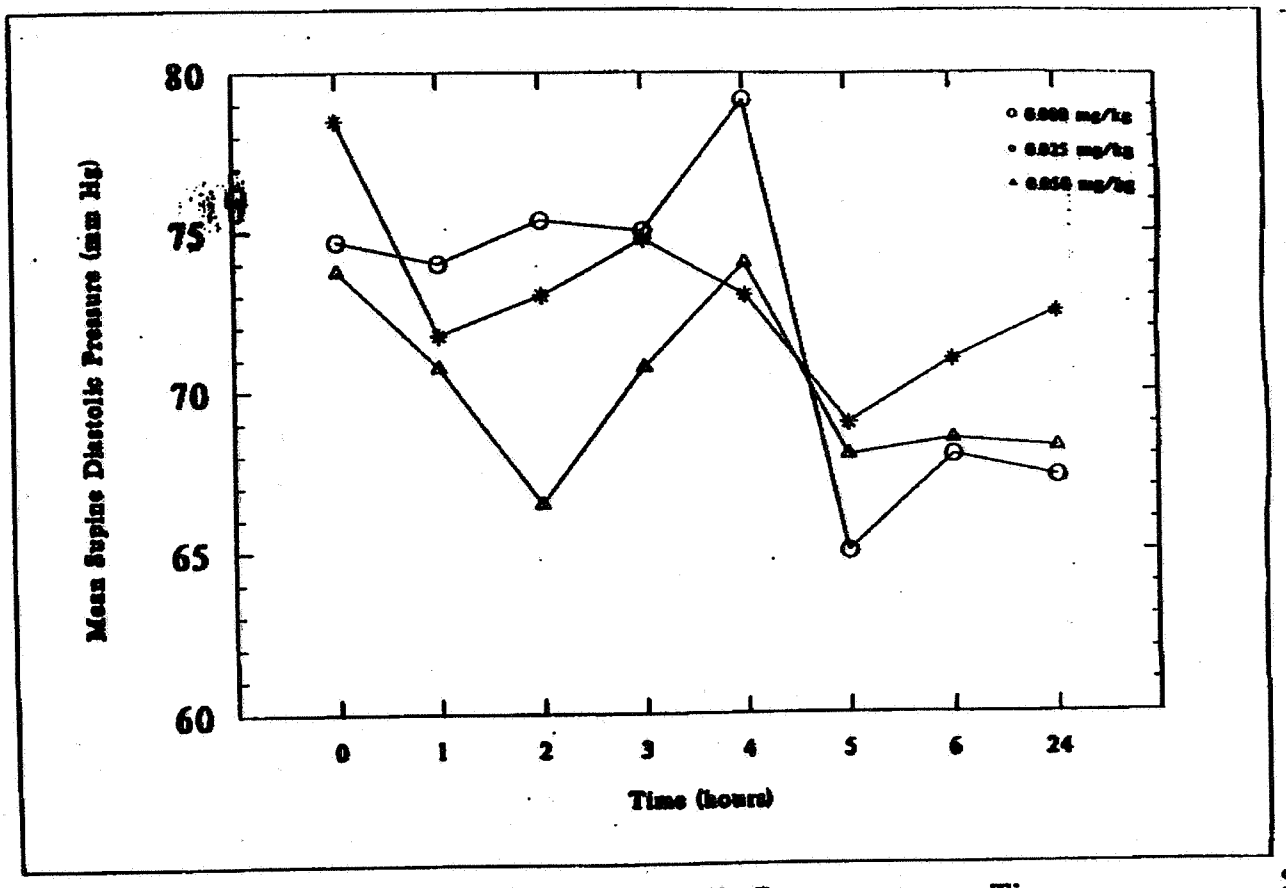


Figure 11. Mean Supine Diastolic Pressure versus Time for Aldicarb Doses in Females

34

010459

010459

SUMMARY OF DATA

The following is a summary of the data analyzed:

-- 5 response variables:

- red cell cholinesterase (IU/l)
- plasma cholinesterase (IU/l)
- supine pulse (beats per minute)
- supine diastolic blood pressure (mm Hg)
- saliva weight (grams)

-- Male (n=44 subjects), Female (n=14 subjects)

Time before/ after administration of dose (hours)	blood pressure	pulse	saliva	red cell cholinesterase	plasma cholinesterase
-16				✓	✓
-3				✓	✓
0	✓	✓	✓	✓	✓
1	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓
3	✓	✓	✓		
4	✓	✓	✓	✓	✓
5	✓	✓	✓		
6	✓	✓	✓	✓	✓
8				✓	✓
21				✓	✓
24	✓	✓			

Dose (mg/kg)	Sample Sizes Female	Male
0.000	6	16
0.010	0	8
0.025	4	8
0.050	4	8
0.060	0	1
0.075	0	3
	14	44

[redacted]

010459

6

DATA CONVENTIONS

-- Measurements taken at -16 (16 hours before administration of dose), -3 (3 hours before administration of dose), and 0 (administration of dose) averaged for red cell cholinesterase and plasma cholinesterase. Only single predose measurements were available for the remaining variables.

-- Subject 020, a male, was administered a dose of 0.060 mg/kg of Aldicarb instead of 0.075 mg/kg. Data from this subject was statistically analyzed at the 0.060 mg/kg dose level.

-- Repeated Measures Analyses were not adjusted for predose levels. That is, predose measurements entered the analyses at time zero. This allowed statistical tests of time effects within subject and across pretest groups for the doses.

-- Dunnett's comparisons of dose means to the mean of the control group were conducted on means estimated across time.

-- Relative Difference Testing:

- Relative difference measures were created for each of the five response variables as follows:

$$\frac{h_0 - h_1}{h_0}$$

where

h_1 is the response variable at time = 1 (1 hour after administration of dose), and

h_0 is the response variable at time = 0 (administration of dose)

- Relative differences can be either positive or negative. Typical transformations such as logarithmic not appropriate. Normality assumed.

- In a majority of relative differences for the response variables, for both sexes, between dose variances differ. Therefore, weighted analyses of the relative differences were conducted to correct for unequal variance. Weights were the inverse of the variance.

STATISTICAL METHODS

-- Repeated Measures Analysis:

For each dependent variable, analyses were run using the REPEATED option in PROC GLM of SAS. The model for each variable is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \pi_{i(k)} + \epsilon_{ijk}$$

where

μ	= the overall mean
α_i	= the effect of the i^{th} dose of Aldicarb
β_j	= the effect of the j^{th} time
$\alpha\beta_{ij}$	= dose*time interaction
$\pi_{i(k)}$	= the effect of the kth subject in the i^{th} dose group
ϵ_{ijk}	= random error.

Here, $\pi_{i(k)} \sim N(0,\sigma_\pi^2)$, $\epsilon_{ijk} \sim N(0,\sigma_\epsilon^2)$ and σ_π^2 is independent of σ_ϵ^2.

-- Dunnett's Test of dose effect as compared to the control.

Both one and two tailed Dunnett's tests were conducted on each dependent variable. These were run using PROC GLM with the DUNNETT option to the Means statement. Dose means are calculated across time.

-- Tests of Relative Differences at 1 Hour:

- Univariate one-way analyses of variance performed separately for 5 response variables - separately for males and females - weighted and unweighted (5x2x2=20 analyses); relative difference measures $(h_0 - h_1)/h_0$ used for

  supine diastolic blood pressure
  supine pulse
  saliva weight
  red cell cholinesterase
  plasma cholinesterase

MODEL: $(h_{0ij} - h_{1ij})/h_{0ij}$ for response variable $= \mu + \alpha_i + \epsilon_{ij}$

where μ = overall mean

α_i = effect of dose i (i = 0.000, 0.025, 0.050 for females)
(i = 0.000, 0.010, 0.025, 0.050, 0.060, and 0.070 for males)

ϵ_{ij} = random error $\epsilon_{ij} \sim N(0,\sigma^2)$ for unweighted analysis
$\epsilon_{ij} \sim N(0,\sigma_i^2)$ for weighted analysis

- Dunnett's test of individual dose level means vs. control (dose=0.000) at significance level α=0.05; t-test of means vs. control at α=0.05.

-- graphical presentations

- Relative differences vs. dose (separately for male and female - 10 figures): observations, means, and 95% confidence interval about means presented

  supine diastolic blood pressure
  supine pulse
  saliva weight
  red cell cholinesterase
  plasma cholinesterase

- Average response vs. time (averages for different doses denoted by different symbols); separately for male and female (10 figures)

  supine diastolic blood pressure (mm Hg)
  supine pulse (beats per minute)
  saliva weight (grams)
  red cell cholinesterase (IU/l)
  plasma cholinesterase (IU/l)

[redacted]

010[illegible]

010459

SUMMARY OF RESULTS

A synopsis of results is presented below for each dependent variable. These results do not include evaluations of assumptions and model diagnostics. These evaluations should be conducted.

Red Cell Cholinesterase:

- Repeated measures analyses, unadjusted for predose levels, indicate statistically significant within subject time and time*dose effects at the α=0.05 for both sexes. Overall test of between subject dose effects is statistically significant at α=0.05 for both sexes. Dose effect statistically significant 1 and 2 hours after dose for both sexes. Dose effects not statistically significant at other time periods.

- Dunnett's two tailed test of means indicates that mean levels of cholinesterase for the females dose group of 0.025 mg/kg and the male dose group of 0.01mg/ kg are not statistically different from the control. However, doses of 0.05 and higher have a statistically different effect on red cell cholinesterase levels at the α=0.05 level for both sexes. The exception to this is the male subject with a dose level of 0.06 mg/kg. Means of cholinesterase levels in females monotonically decrease as dose increases. Mean cholinesterase levels do not monotonically decrease for males.

  Dunnett's one tailed test that the dose mean is statistically lower than the mean of the control indicated at the α =0.05 level that the mean red cell cholinesterase for females is statistically lower than the control for both dose groups. One tailed Dunnett's test for males yielded the same results as the two tailed test.

- Statistical test of the relative change of cholinesterase levels at one hour for both sexes are the same as those indicated by the Dunnett's testing of response means with the exception that the 0.060 mg/kg dose group is statistically different from the control.

Plasma Cholinesterase:

- Repeated measures analyses, unadjusted for predose levels, indicate statistically significant within subject time and time*dose effects at the α=0.05 for both sexes. Overall dose effect is statistically significant for males (p=0.0321) and nonsignificant for females (p=0.0947). Dose effect statistically significant 1 and 2 hours after dose for both sexes. Four hours after dose, a statistically significant dose effect at α=0.05 is present in the males. For females, dose is marginally none significant (p=0.0543) at hour 4. Dose effects are not statistically significant at other time periods.

- Dunnett's two tailed test of means indicates that means levels for the 0.01 and 0.06 dose groups for males and 0.025 for females are not statistically different from the control. However, doses of 0.05 and higher have a statistically different effect on mean plasma cholinesterase levels at the α=0.05 level for both sexes. The exception to this is the male subject with a dose level of 0.06 mg/kg. Means of cholinesterase levels decrease monotonically (except for dose=0.06 mg/kg) as dose increases for both sexes. Dunnett's one tailed lower test of differences yield the same results as the two tailed tests.

- Statistical test of the relative change at one hour of plasma cholinesterase indicates statistically different levels from the control group for all dose groups for both sexes.

Supine Pulse:

- Repeated measures analyses, unadjusted for predose levels, indicate a statistically significant within subject time effect but no time*dose effects at the α=0.05 for both sexes. Overall dose effect is not statistically significant for either sex at the α=0.05 level for both sexes.

- Dunnett's two tailed test of means indicates a statistically significant difference, at α=0.05, between the mean of the control group and that of the 0.075 mg/kg dose group for males. No other statistically significant differences between dose group means and the mean of the control group were declared for either sex.

  When a one tailed lower Dunnett's test is applied, the mean pulse of females in the 0.05 mg/kg dose group is statistically lower than that of the females in the control group. No statistical differences are declared for the males.

- Statistical test of the relative change in supine pulse indicates no statistically different levels from the control group for all dose groups for both sexes.

Supine Blood Pressure:

- Repeated measures analyses, unadjusted for predose levels, indicate a statistically significant within subject time effect but no time*dose effects at the α=0.05 for both sexes. Overall dose effect is not statistically significant for either sex at the α=0.05 level for both sexes.

- Dunnett's two tailed test of means indicates a statistically significant difference, at α=0.05, between the mean of the control group and that of the 0.050 mg/kg dose group for males. No other

statistically significant differences between dose group means and the mean of the control group were declared for either sex. When a Dunnett's one tailed lower test is applied, no statistically significant differences are declared for either sex.

- Statistical test of the relative change in supine blood pressure at one hour indicates a statistical difference between the control mean and that of the male dose group=0.075 mg/kg for the unweighted analysis. In the weighted analysis, the mean of this dose is no longer statistically different from the control. However, in the weighted analysis, the mean relative differnce for the females in dose group =0.025 mg/kg is statistically different from the control. No other blood pressure means were statistically different from that of the control group for all dose groups for both sexes.

Salivation:

- Repeated measures analyses, unadjusted for predose levels, indicate a statistically significant within subject time effect but no time*dose effects at the α=0.05 for females. Neither time nor time*dose within male subjects were statistically significant. Overall dose effect is not statistically significant for either sex at the α=0.05 level for both sexes.

- Dunnett's two tailed test of means indicates a statistically significant difference, at α=0.05, between the mean of the control group and that of the 0.050 mg/kg dose group for females. For males, the means for grams of saliva from dose groups 0.01, 0.05 and 0.06 were statistically lower than that of the control. No other statistically significant differences between dose group means and the mean of the control group were declared for either sex.

- Statistical test of the relative change in salivation at one hour indicates no statistical differences between the mean of the control group verses those of the dose groups for either sex.

010459

010459

[redacted]

RESULTS OF RELATIVE CHANGE ANALYSES

	Female		Male	
	Overall	Doses Different from Control (α=0.05)	Overall	Doses Different from Control (α=0.05)
Unweighted Analysis				
Blood Pressure	NS	--	NS	0.075 (p=0.0500)
Pulse	NS	--	NS	--
Saliva Weight	NS	--	NS	--
Red Cell Cholinesterase	p=0.0060	0.050 (p=0.0018)	p=0.0001	0.025 (p=0.0001) 0.050 (p=0.0001) 0.060 (p=0.0002) 0.075 (p=0.0001)
Plasma Cholinesterase	p=0.0001	0.025 (p=0.0001) 0.050 (p=0.0001)	p=0.0001	0.010 (p=0.0003) 0.025 (p=0.0001) 0.050 (p=0.0001) 0.060 (p=0.0001) 0.075 (p=0.0001)
Weighted Analysis				
Blood Pressure	p=0.0424	0.025 (p=0.0140)	NS	--
Pulse	NS	--	NS	--
Saliva Weight	NS	--	NS	--
Red Cell Cholinesterase	p=0.0097	0.025 (p=0.0397) 0.050 (p=0.0035)	p=0.0001	Not Available
Plasma Cholinesterase	p=0.0001	0.025 (p=0.0001) 0.050 (p=0.0001)	p=0.0001	Not Available

010459

FIGURES

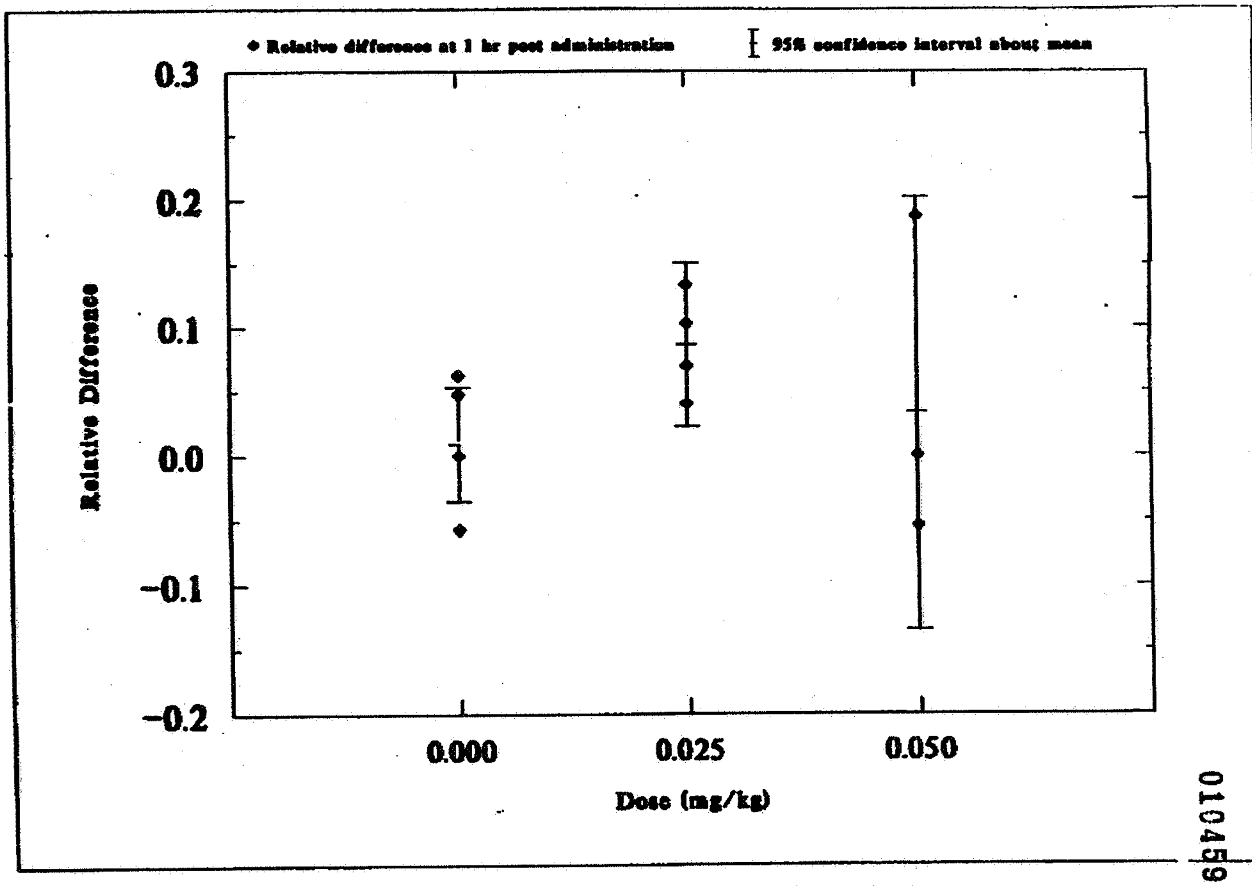


Figure 1. Relative Differences in Supine Diastolic Pressure versus Aldicarb Dose in Females

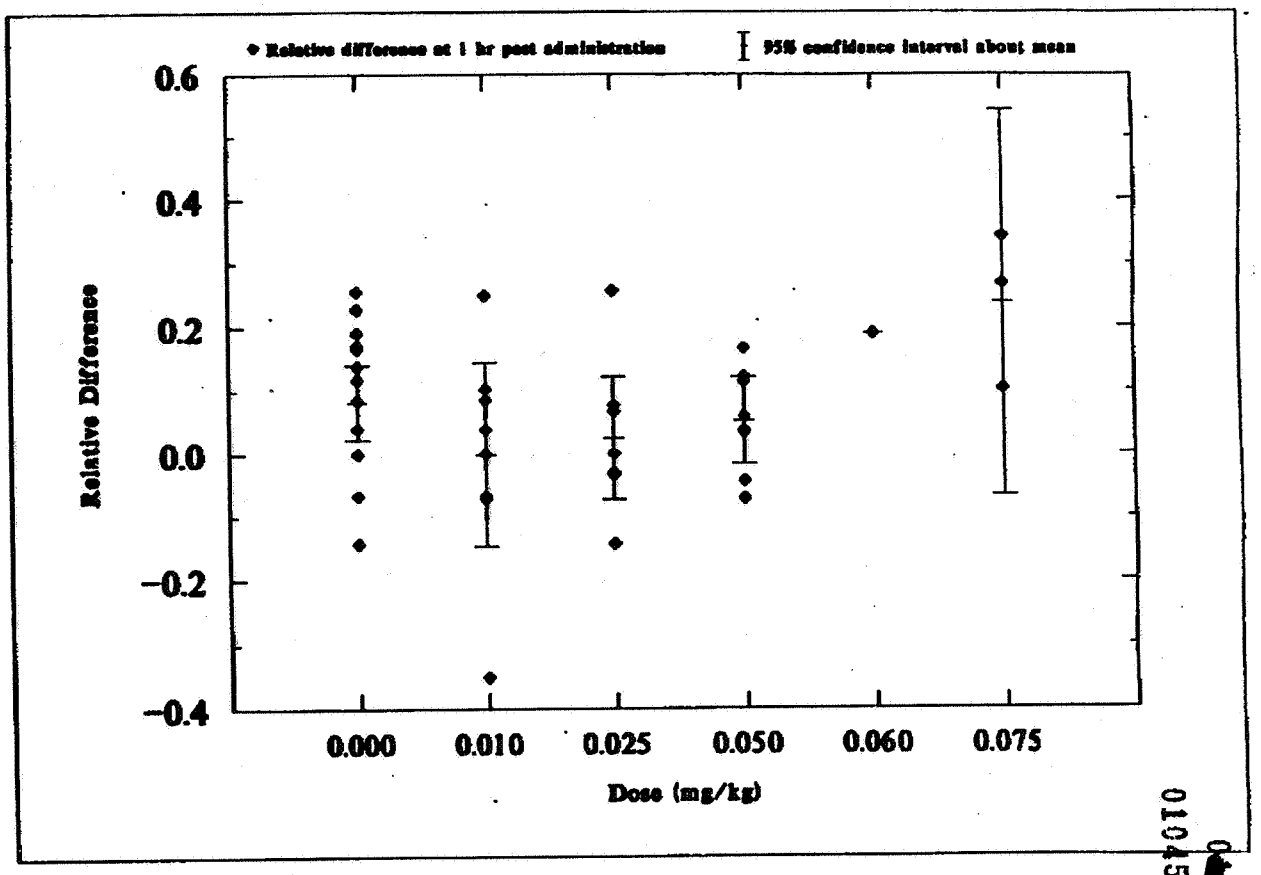


Figure 2. Relative Differences in Supine Diastolic Pressure versus Aldicarb Dose in Males

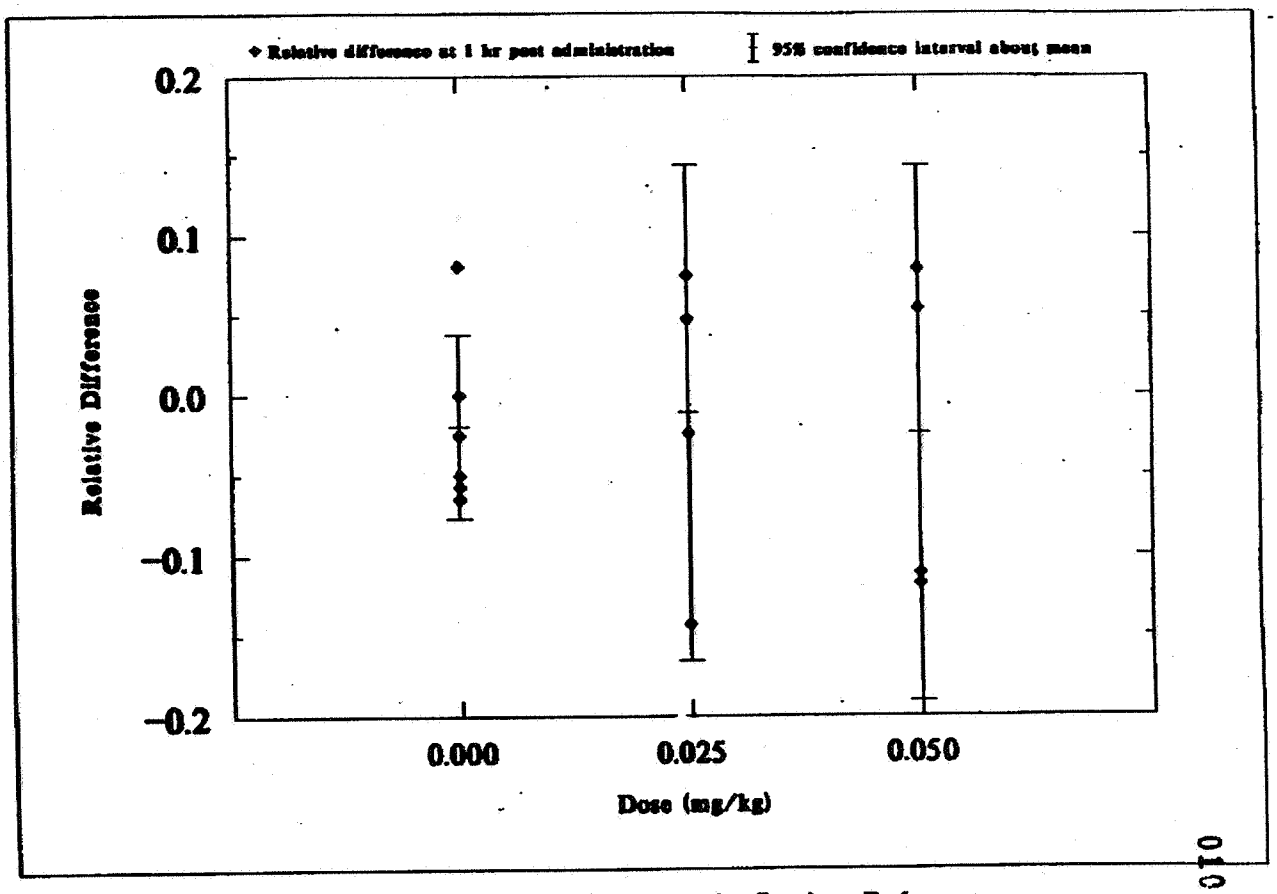


Figure 3. Relative Differences in Supine Pulse versus Aldicarb Dose in Females

46

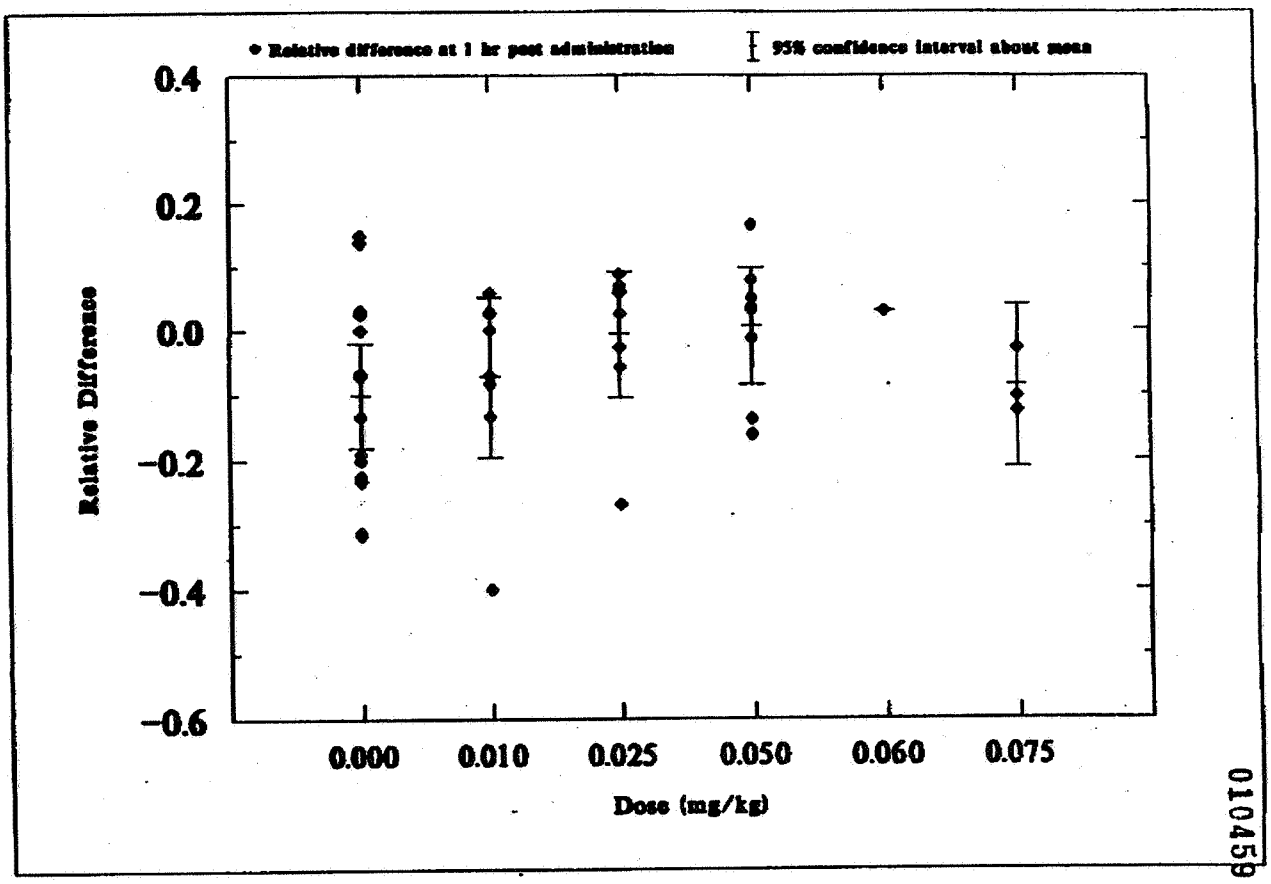


Figure 4. Relative Differences in Supine Pulse versus Aldicarb Dose in Males

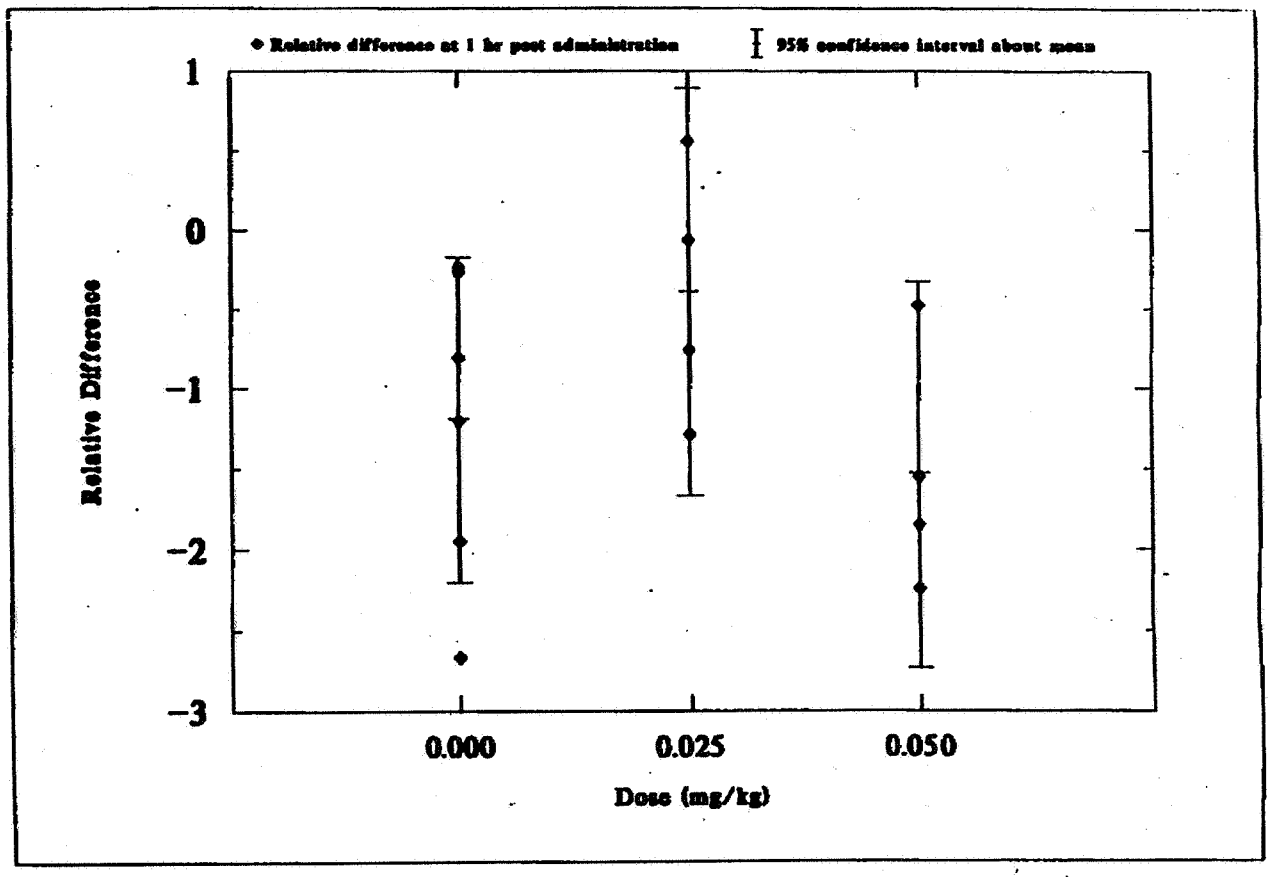


Figure 5. Relative Differences in Saliva Weight versus Aldicarb Dose in Females

010459

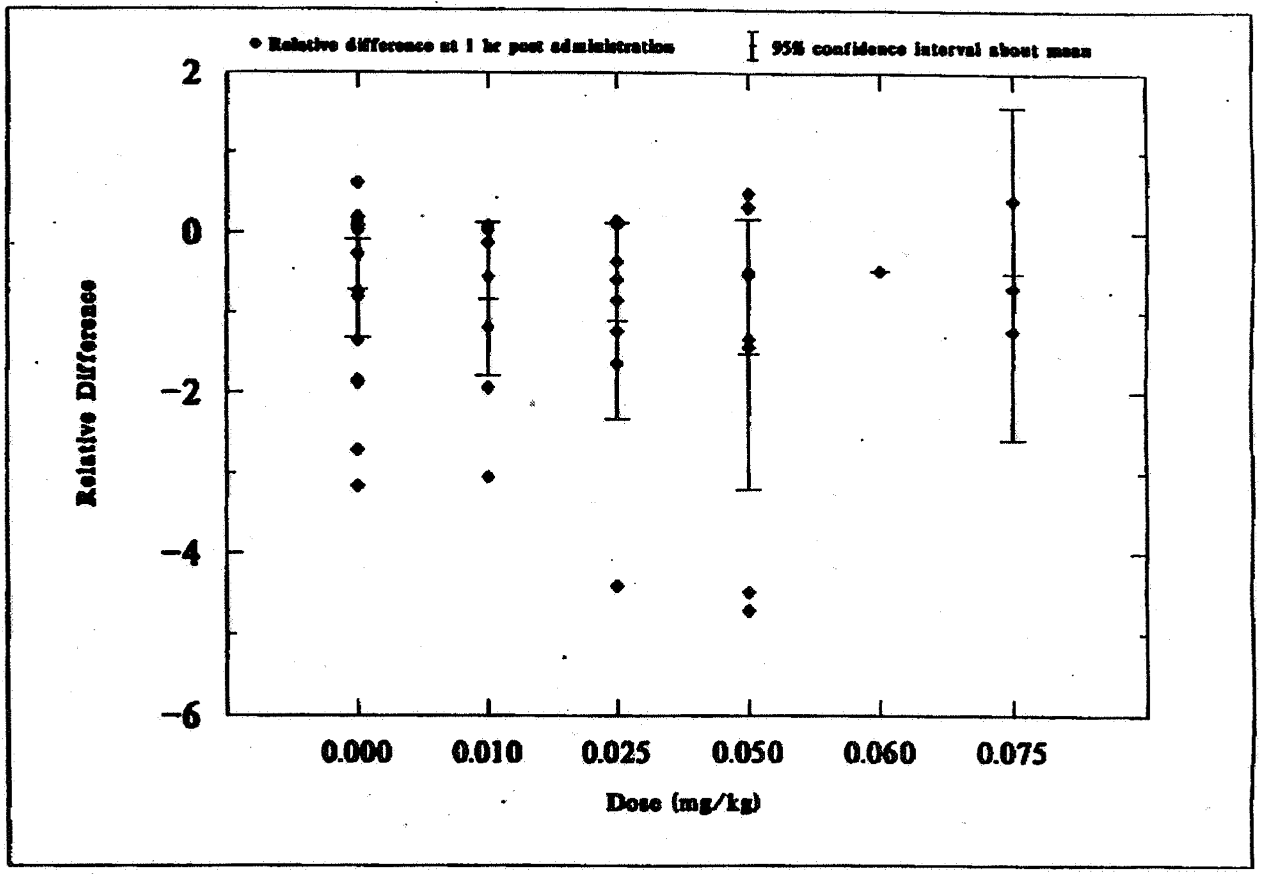


Figure 6. Relative Differences in Saliva Weight versus Aldicarb Dose in Males

010459

49

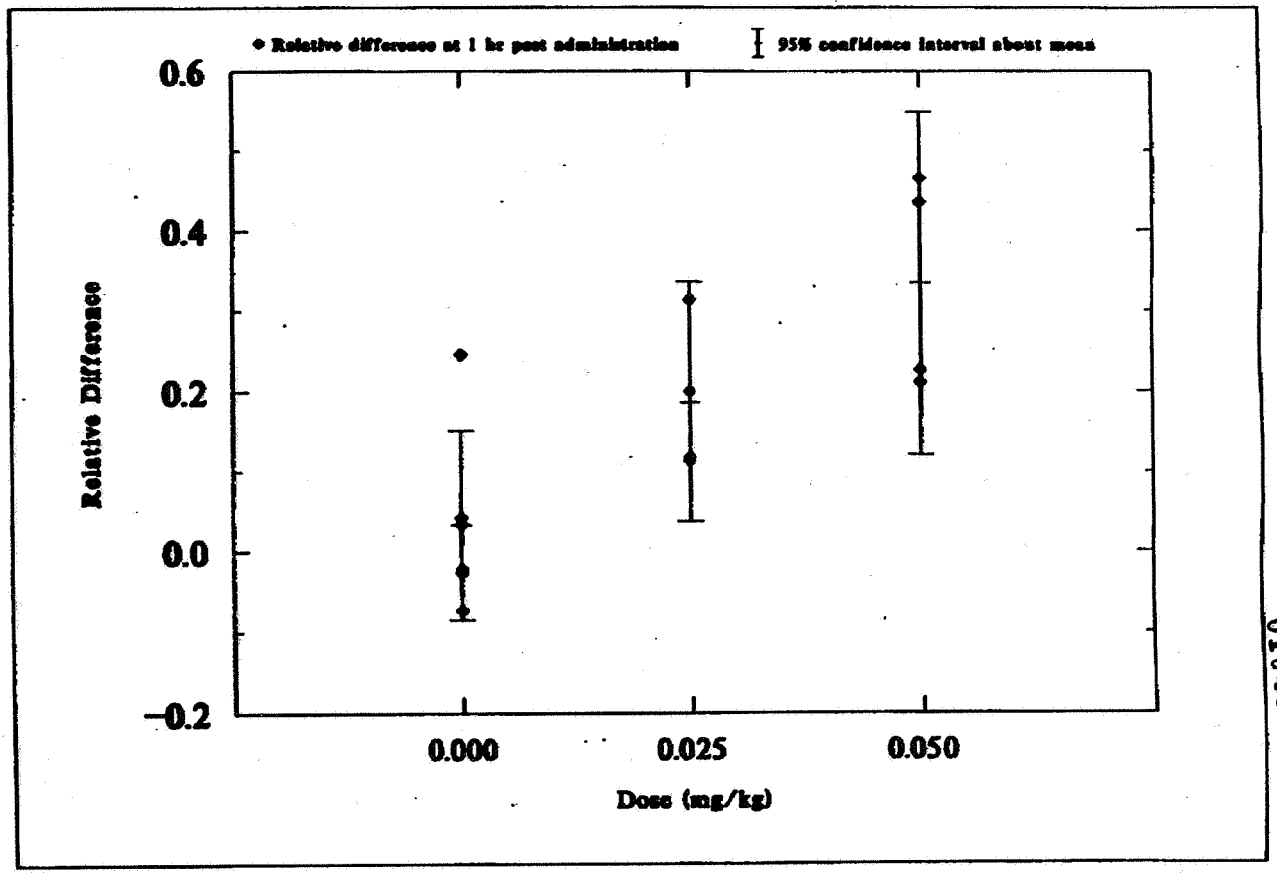
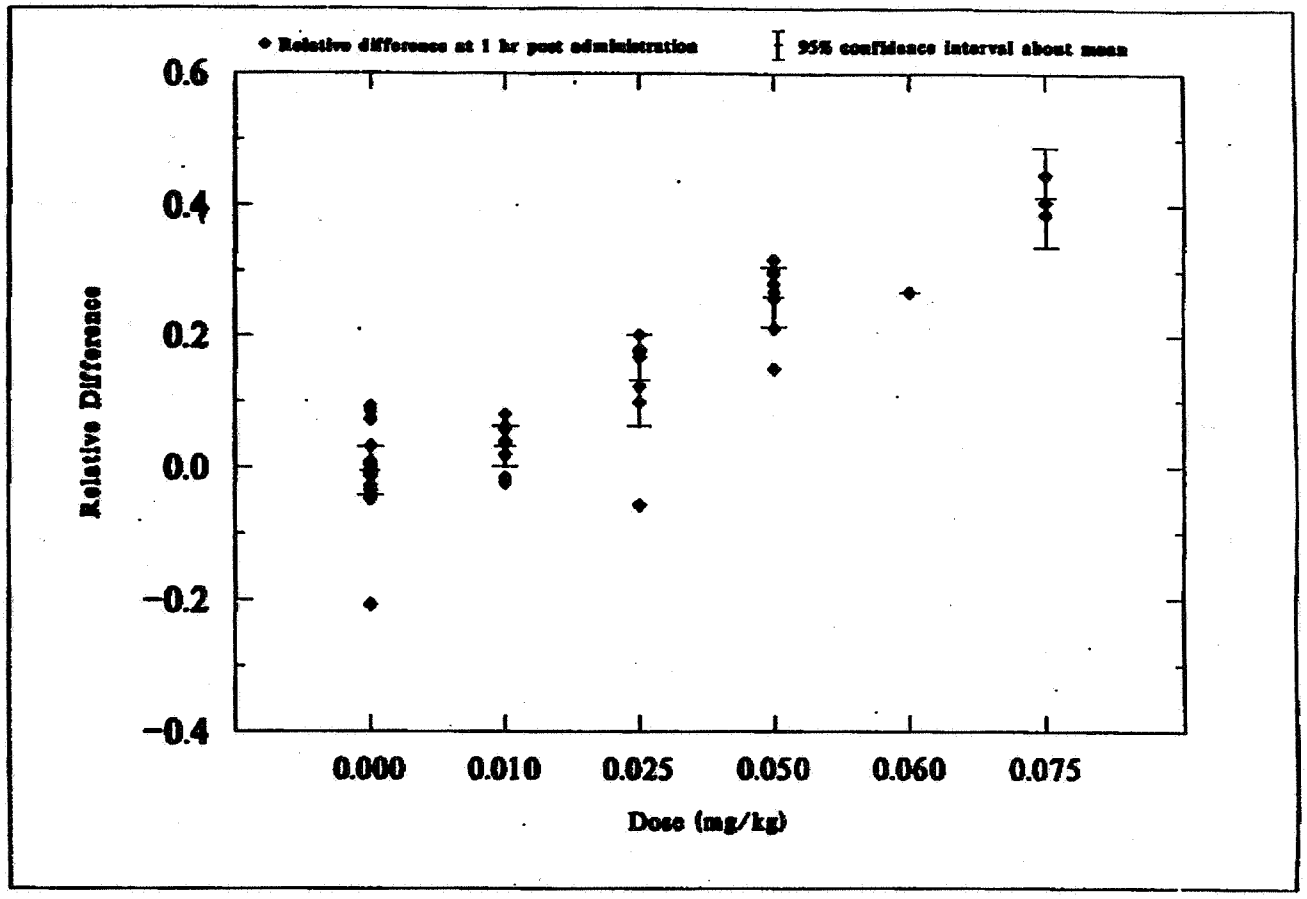


Figure 7. Relative Differences in Red Cell Cholinesterase versus Aldicarb Dose in Females



010459

Figure 8. Relative Differences in Red Cell Cholinesterase versus Aldicarb Dose in Males

010459

51

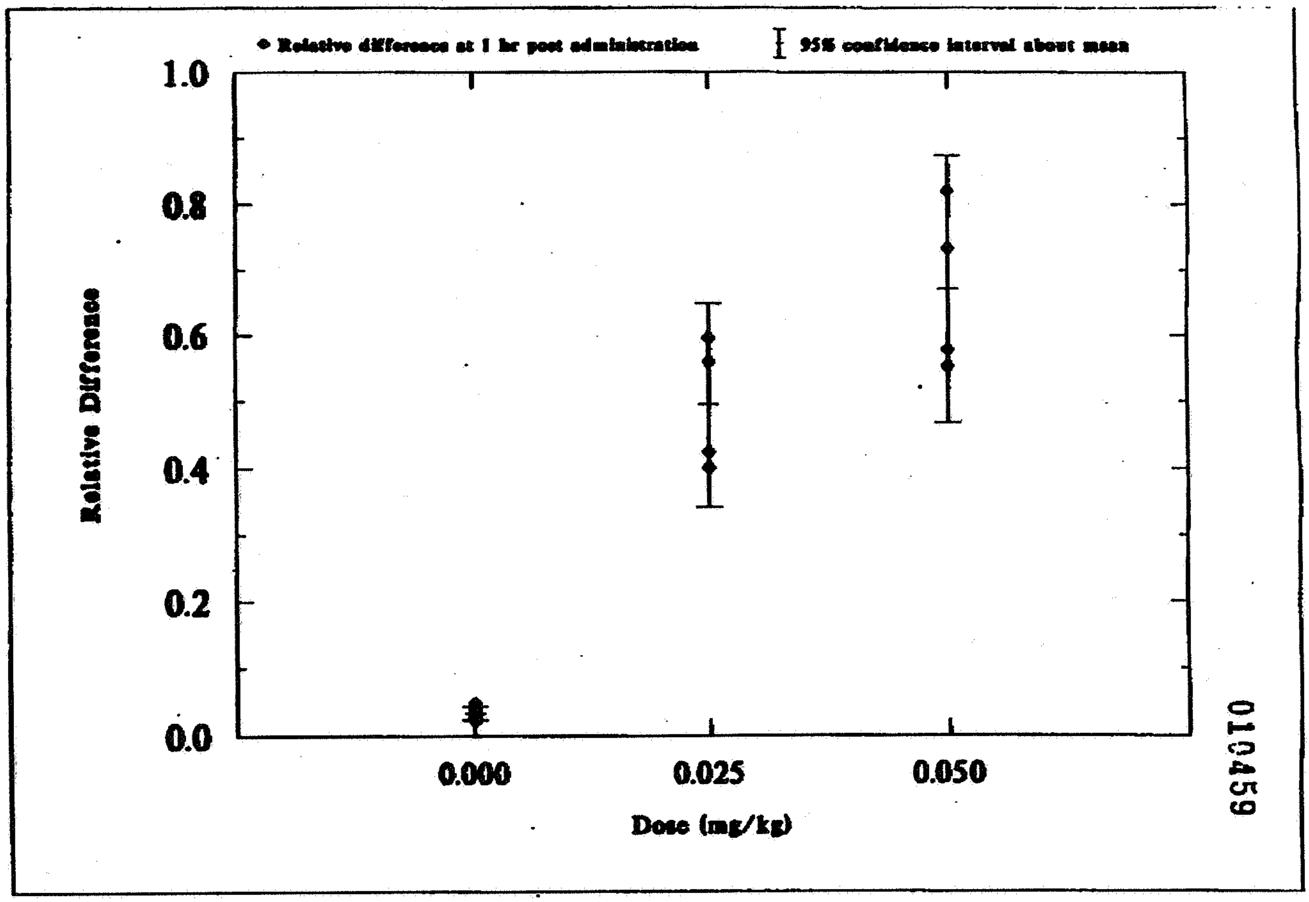


Figure 9. Relative Differences in Plasma Cholinesterase versus Aldicarb Dose in Females

010459

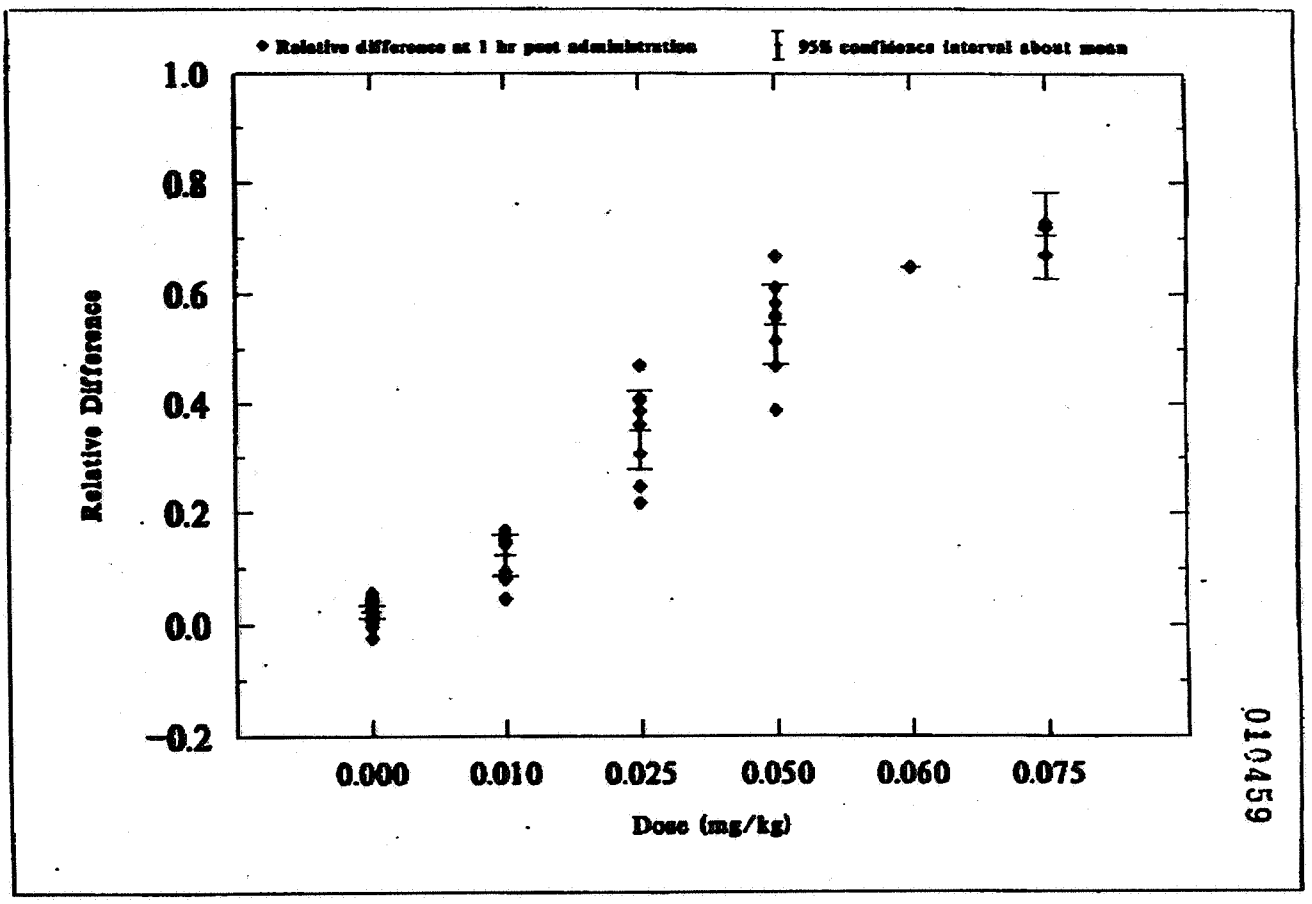


Figure 10. Relative Differences in Plasma Cholinesterase versus Aldicarb Dose in Males

(16153)

53

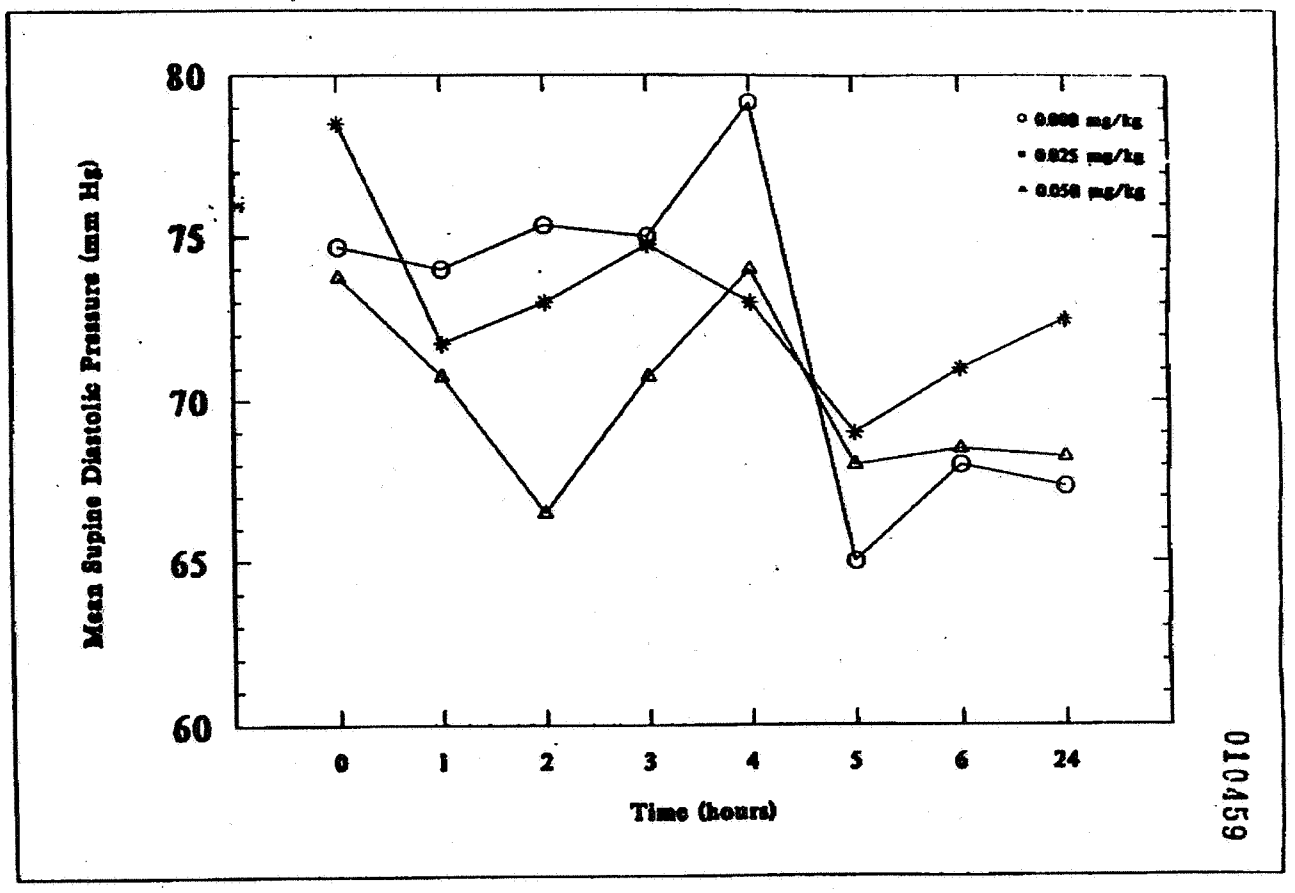


Figure 11. Mean Supine Diastolic Pressure versus Time for Aldicarb Doses in Females

54

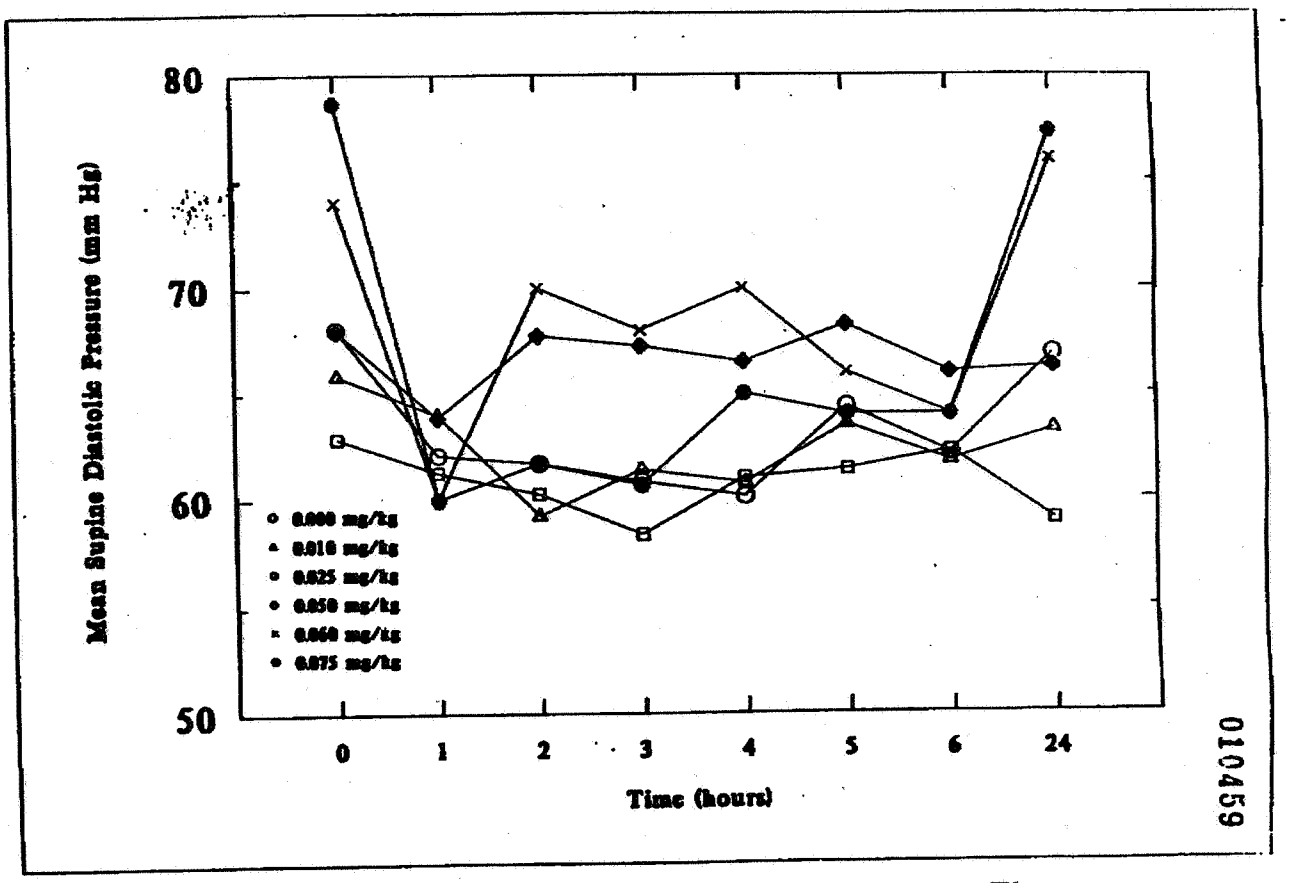


Figure 12. Mean Supine Diastolic Pressure versus Time for Aldicarb Doses in Males

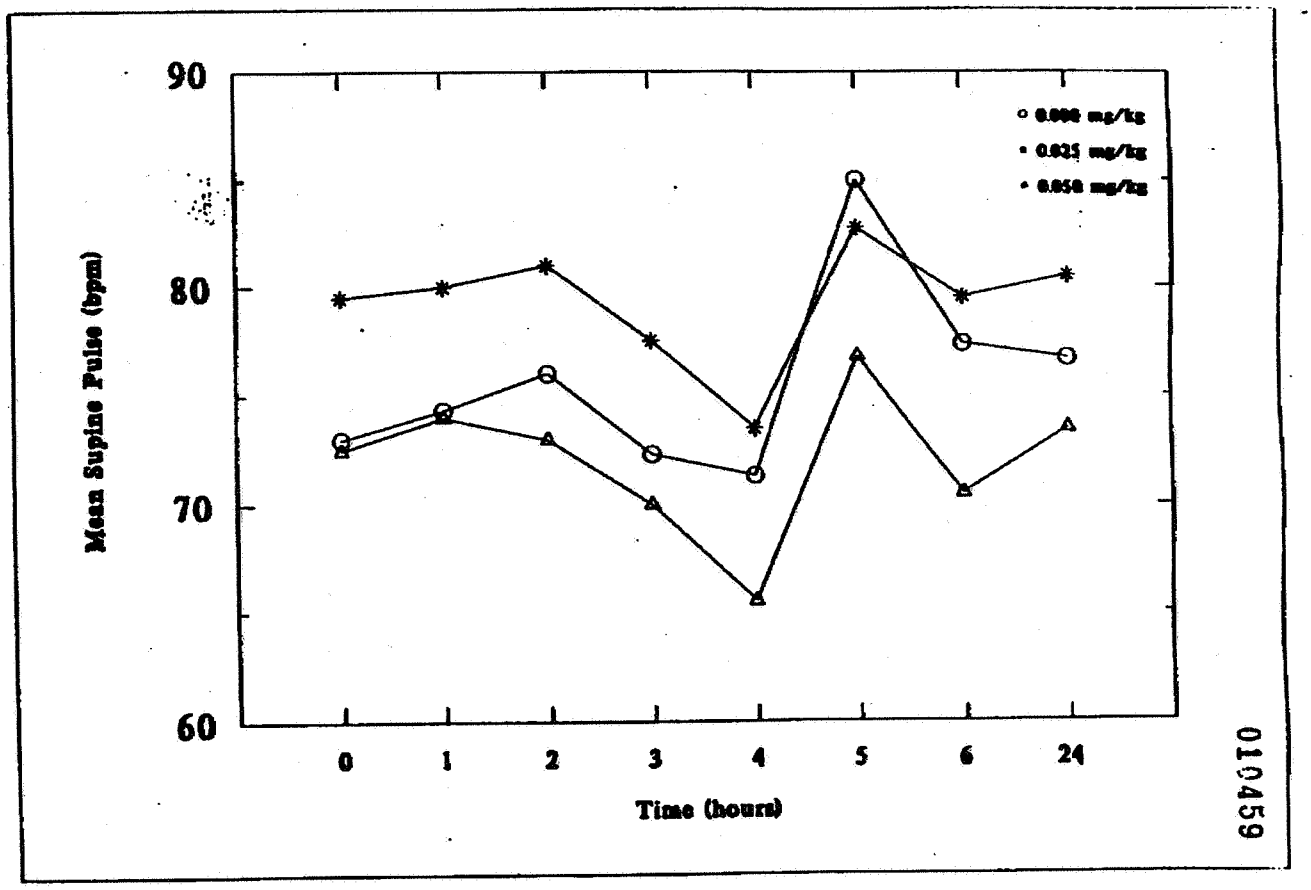


Figure 13. Mean Supine Pulse versus Time for Aldicarb Doses in Females

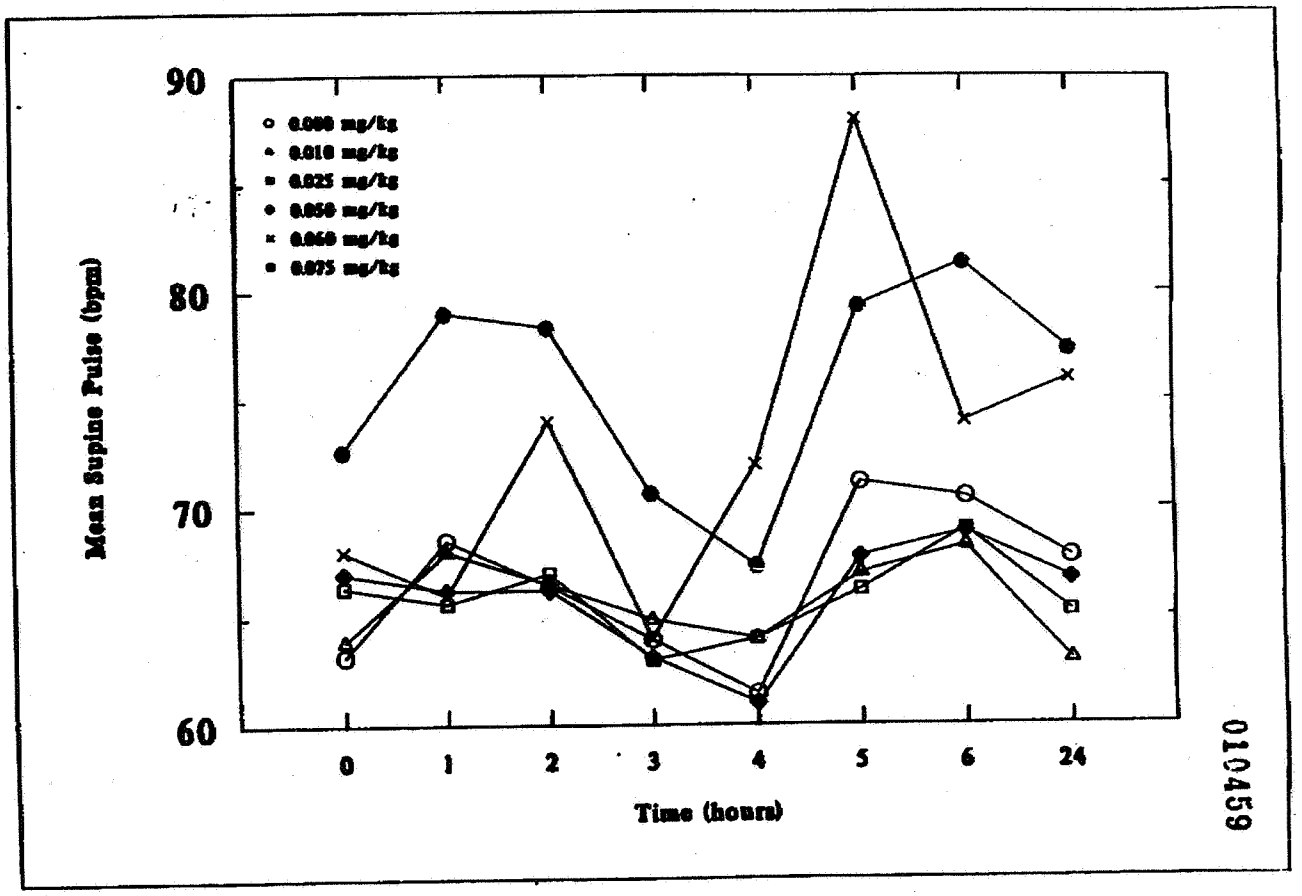


Figure 14. Mean Supine Pulse versus Time for Aldicarb Doses in Males

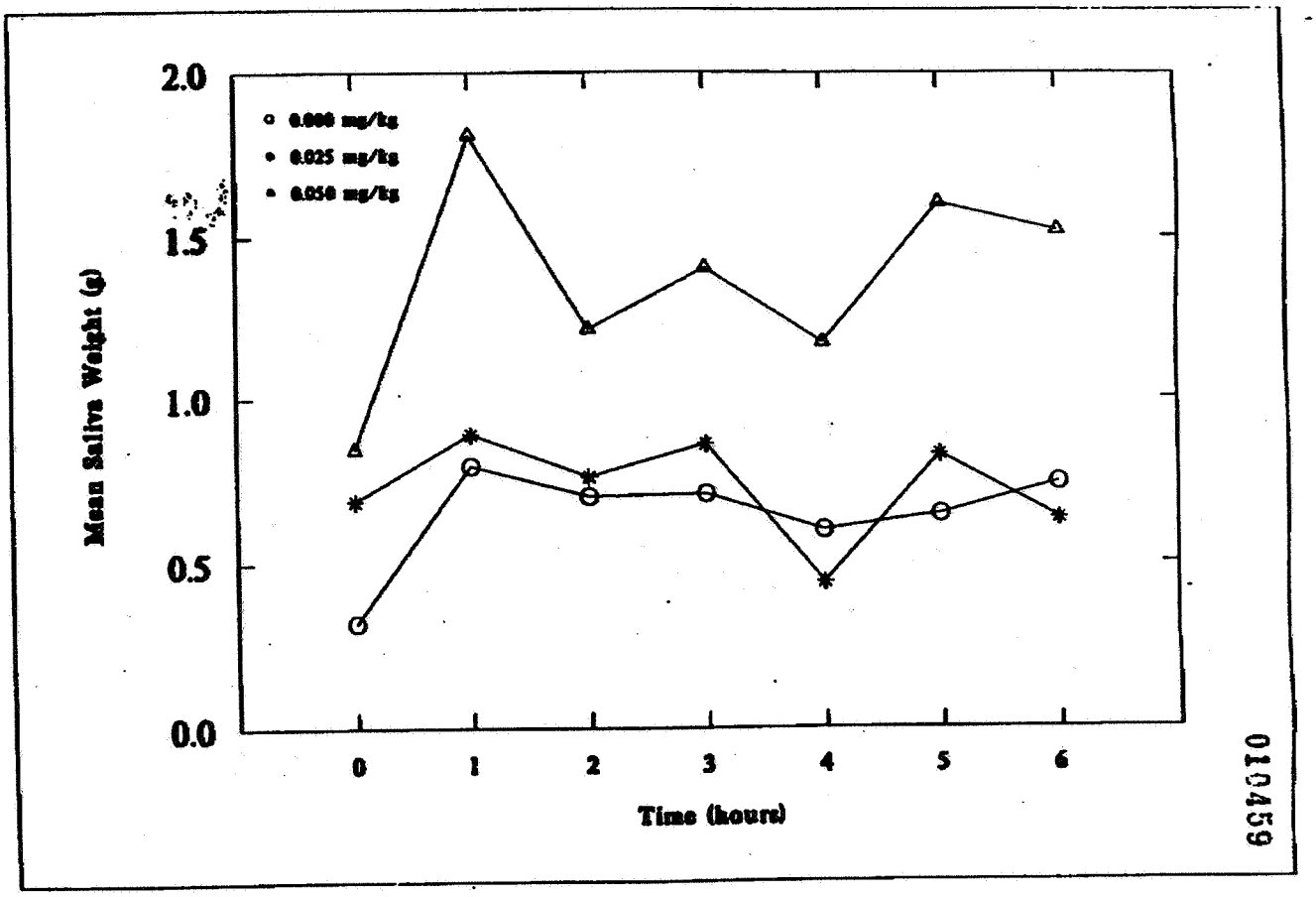


Figure 15. Mean Saliva Weight versus Time for Aldicarb Doses in Females

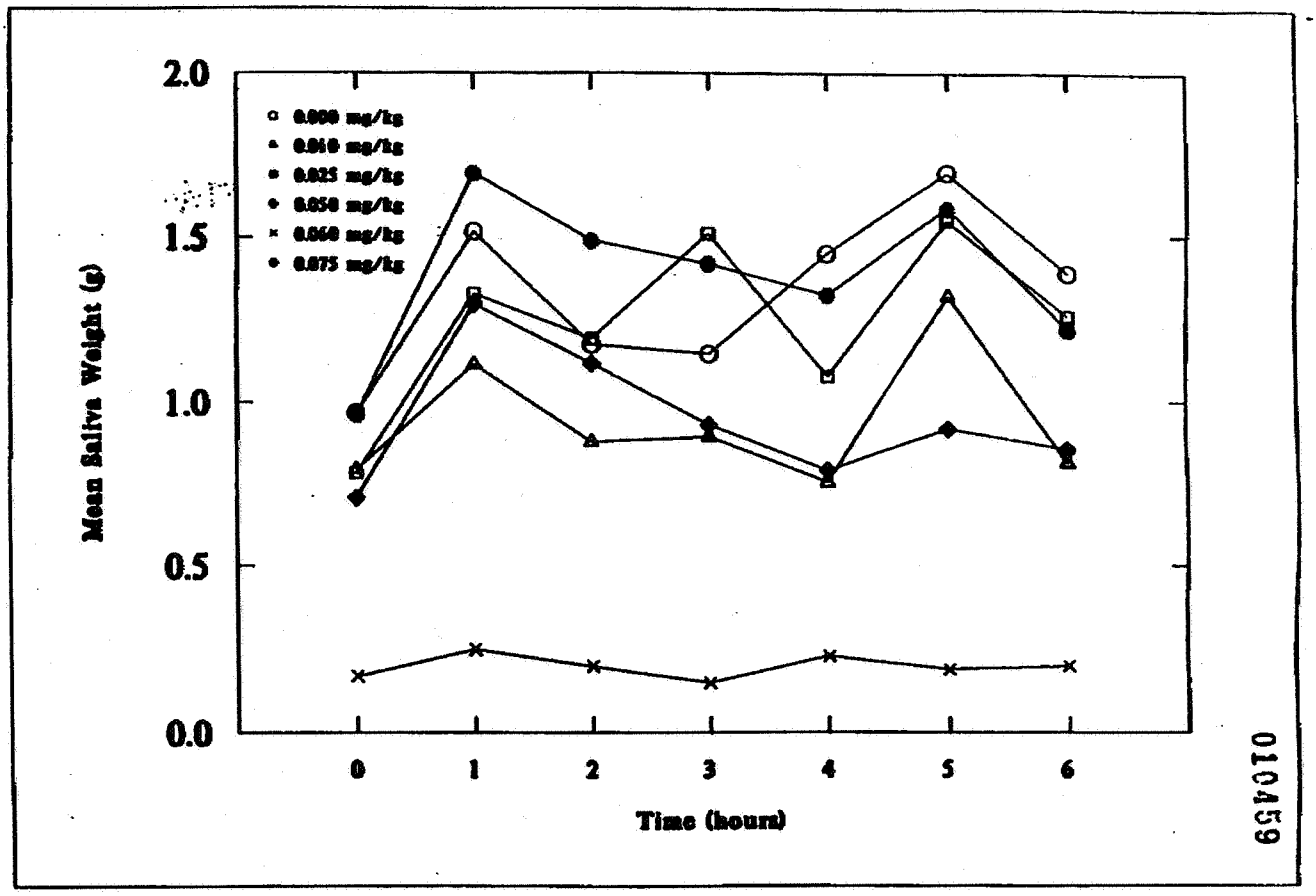


Figure 16. Mean Saliva Weight versus Time for Aldicarb Doses in Males

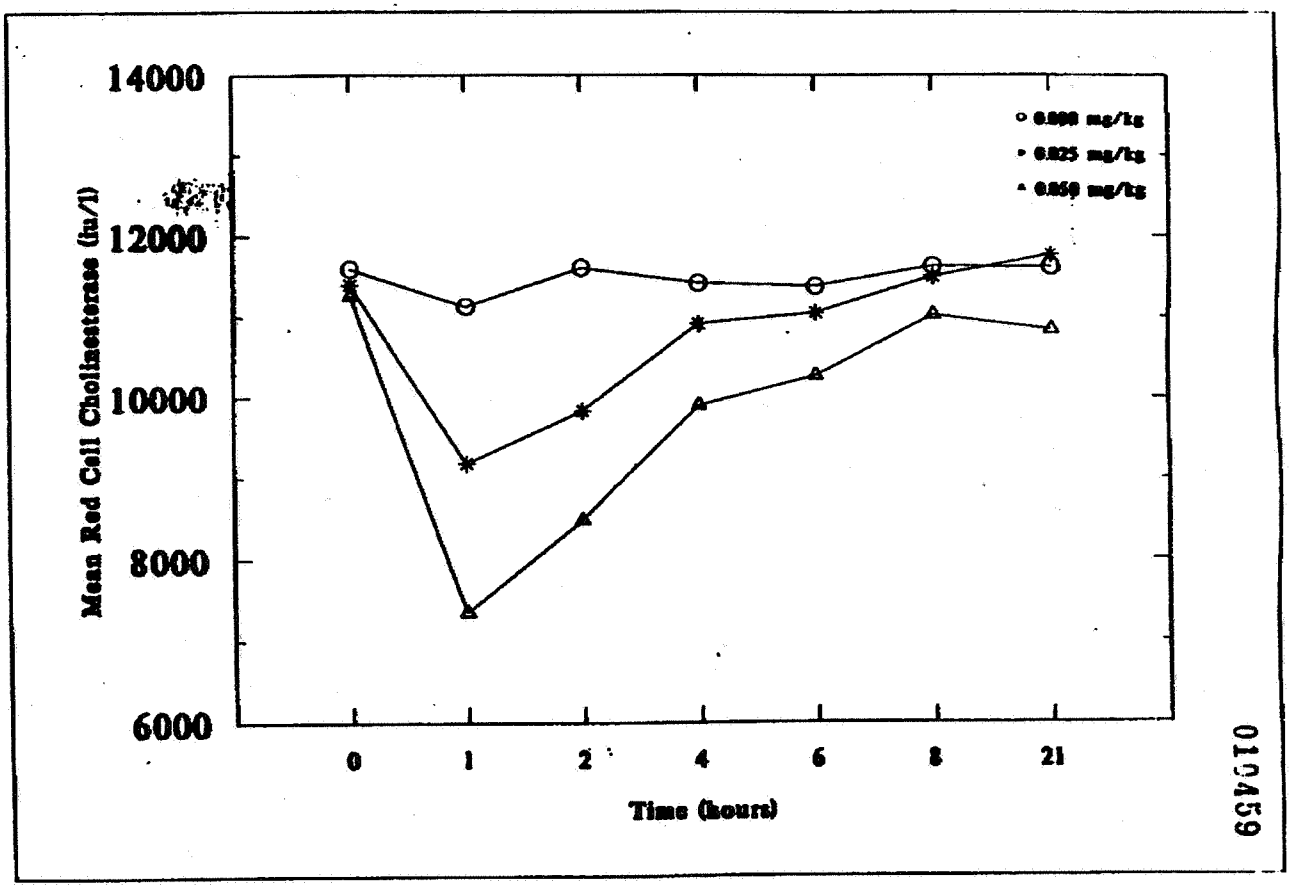


Figure 17. Mean Red Cell Cholinesterase versus Time for Aldicarb Doses in Females

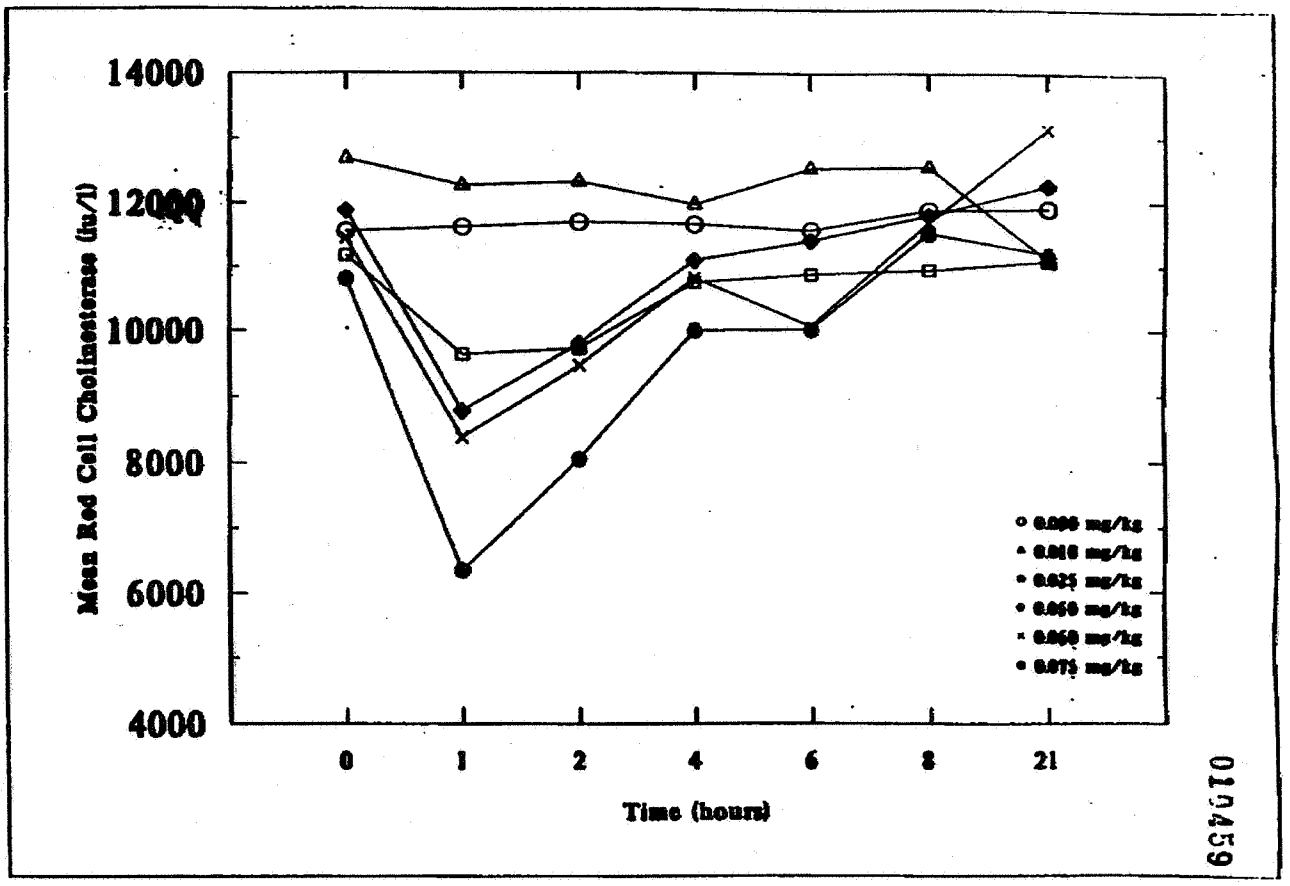


Figure 18. Mean Red Cell Cholinesterase versus Time for Aldicarb Doses in Males

010459

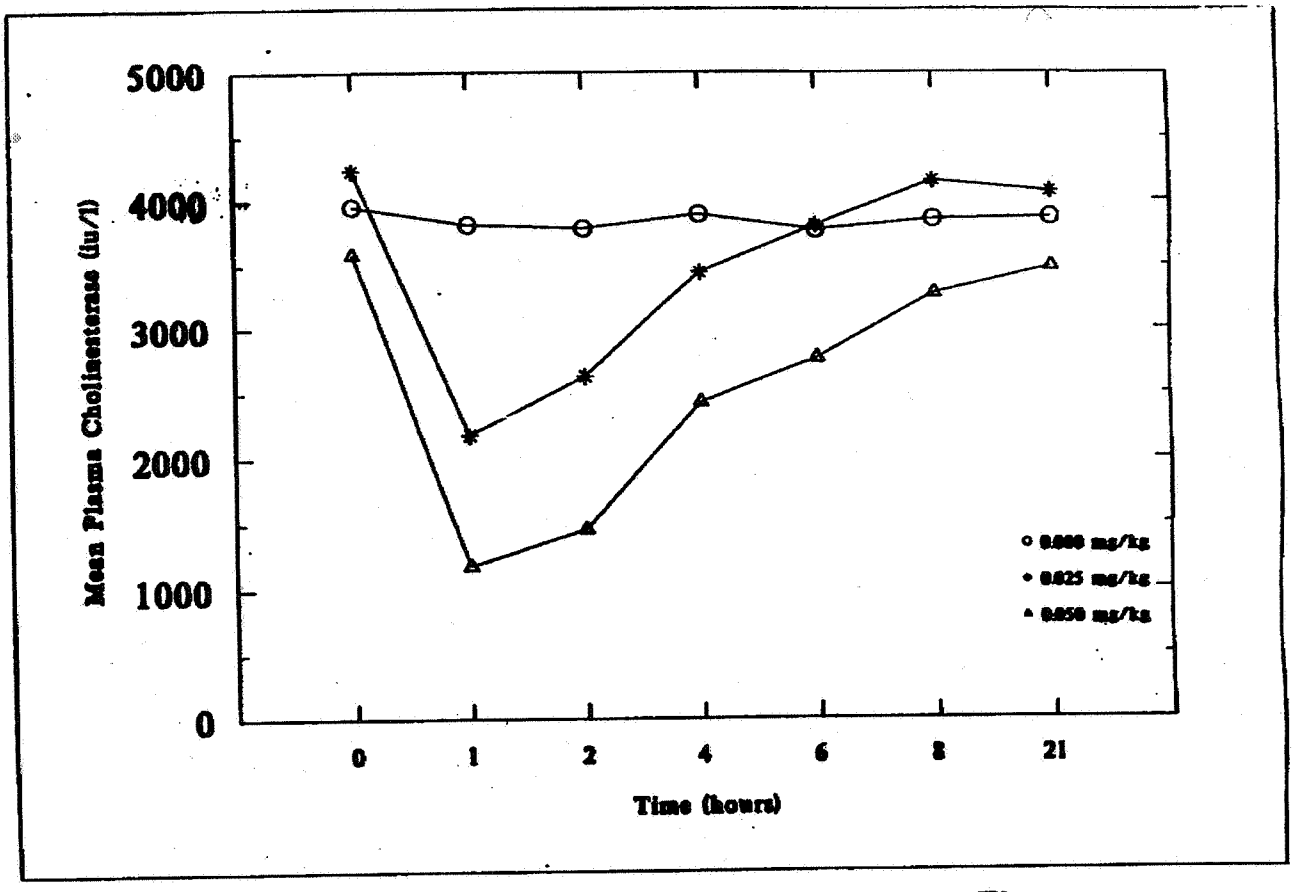


Figure 19. Mean Plasma Cholinesterase versus Time for Aldicarb Doses in Females

010459

010459

62

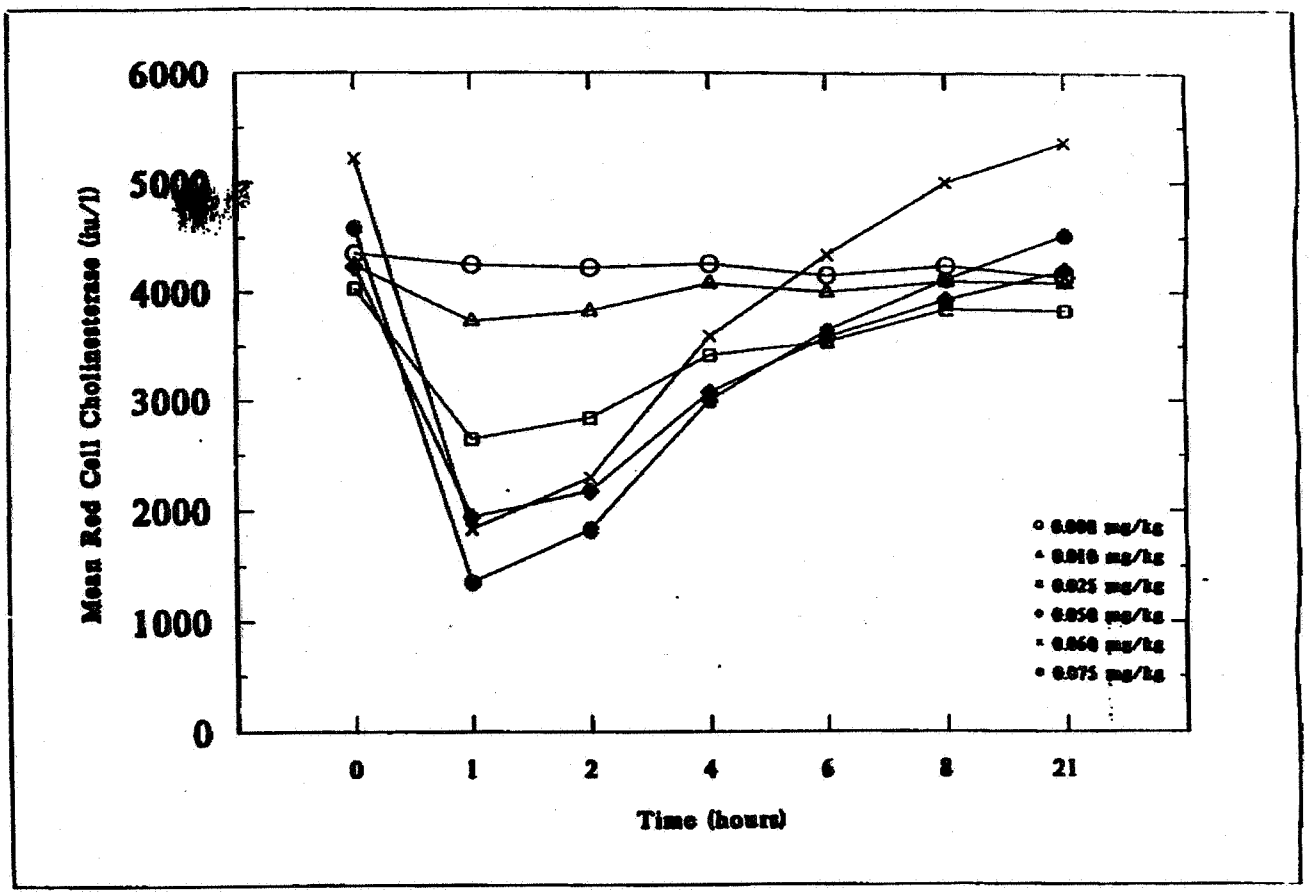


Figure 20. Mean Plasma Cholinesterase versus Time for Aldicarb Doses in Males