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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF PREVENTI ON, PESTICIDES AND TOXIC SUBSTANCES

TXR No. 0054632

June 28, 2007

Memorandum

SUBJECT: Malathion and Malaoxon: Review of Acute Dose Comparative Cholinesterase Study Protocol PC Code: 057701. Barcode: DP# 340518

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Action Requested:

HED was requested to review a study protocol and time-to-peak effect study for comparative cholinesterase activity for malathion and malaoxon, entitled "Request for Agency Concurrence on Selection of Dosage Levels and Times-to-Peak Effect for an Acute Comparative Cholinesterase Study with Malathion and Malaoxon" Dated June 4, 2007.

JUL 5 RECD, 2007

Registrant Proposal:

Based on the preliminary data presented from Study TCQ00021 (study not formally submitted yet), the Registrant believes the time-to-peak effect (TTPE) is 60 minutes for malathion and 80 minutes for malaoxon. In this study, post-natal day 11 pups were dosed with 150 mg/kg malathion and 10 mg/kg malaoxon and evaluated at 20, 40, 60, 80, 100, 120 and 150 minutes after dosing for malathion and at 20, 40, 60, 80, 100 and 120 minutes post dosing for malaoxon. The registrant has proposed the following dose levels for malathion: 0, 10, 25, 50 and 100 mg/kg and the following levels for malaoxon: 0, 0.5, 1, 3.5 and 7 mg/kg. The dosage levels were selected to bracket 10% inhibition for brain cholinesterase activity and 20% inhibition for RBC acetylcholinesterase (AChE) activity.

EPA Recommendations:

Dose Levels: The registrant proposed dose levels of 0, 10, 25, 50 and 100 mg/kg for malathion and 0, 0.5, 1, 3.5 and 7 mg/kg for malaoxon appear reasonable. The Agency applauds the use of four dose levels instead of the customary three dose levels. The Agency, however, suggests replacing the top dose levels of 100 and 7 mg/kg with 150 mg/kg malathion and 10 mg/kg malaoxon in the new study. These dose levels would be useful as a point of comparison between the two studies. Also, the current cholinesterase data in the treated groups appear to have very large standard deviations. Thus, the higher dose levels would ensure measurable inhibition levels, whereas inhibition at lower levels may not be statistically detectable. Thus, the higher doses with smaller variance would increase the confidence in any benchmark dose derived from these data in the future.

Sample size: Given the variation among the treated pups, the Agency suggests a power calculation be performed to support the final sample size used in the study to detect 10% brain AChE inhibition, and 20% red blood cell AChE inhibition.

Time to Peak Effect:

For malathion, the Agency notes the large variability in the data for both RBC and brain AChE activity and finds the appearance of a peak effect for brain inhibition in females prior to 80 minutes questionable since actual inhibition of AChE by a diethyl organophosphate would not be expected to reverse in the short time frame between 60 and 80 minutes. Thus, it is suggested to use 80 minutes as the TTPE to capture inhibition of both RBC and brain AChE. In addition, the Agency believes it does not seem appropriate to have a shorter TTPE for malathion (which requires activation to the oxon) as compared with malaoxon.

With respect to TTPE for malaoxon, the Agency believes that the available data support a peak time to inhibition of 80 minutes for both RBC and brain AChE.

Additional Comments on Study Protocol:

The available data from study TCQ00021 indicate very high standard deviations for some measurements, which suggest possible problems with dosing the pups. Thus, the protocol needs to provide more details relating to pup dosing. The Agency suggests the study use small syringes for the dosing, since the volume is very small and difficult to measure in a 1 ml syringe. The Agency uses a 500 ul gas-tight glass syringe for these small injections, and these are much more accurate.

The Standard Operating Procedure for the Cholineseterase assay should be provided to the Agency. It is also recommended that the techniques used to determine cholinesterase activity be re-evaluated so that less variance (smaller standard deviations) result from the measurements. The variation may be caused by several factors including dosing the pups, sample blood/brain collection and preparation. Would a narrower variance result if data were expressed as IU/mg of protein based on actual protein assay of the samples as diluted for assay?

The Agency notes that the registrant deviated from the study protocol provided to the Agency (dated 1/17/07) when estimating percent cholinesterase inhibition values for both malathion and malaoxon. This was discovered and prompted the request for the actual study data (email 6/19/07). This then allowed the Agency to consider the actual cholinesterase activity levels as shown in the graphs provided to determine peak time to effect.

Malathion:

Sex	Group	Time (Minutes)	RBC Mean ChE ChE: U/G +/- S.D (n) /(Range)	% Decrease (b)	Brain Mean ChE ChE: U/G +/- S.D (n)/ (Range)	% Decrease (b)
Male	I	Control	60 min: 2.024± 0.202 (8) (1.77-2.4) 80 min: 2.396± 0.193 (8) (1.994-2.6)	N/A	60 min: 7.083± 0.423 (8) 80 min: 6.946± 0.685 (8)	N/A
	II	20	1.535±0.599 (7)/ (0.873-2.6)	24.2% (60)	5.051±2.67 (8)/ (1.2-8.5)	28.7% (60)
	III	40	1.725±1.016(8) / (0.569-4.0; note Huge range ©)	28% (80)	4.852±1.499(8) / (0.7-6)	30.1% (80)
	IV	60	1.254±0.457(7)/ (0.659-1.8)	38% (60)	4.973±2.021(8)/ (1.7-6.7)	29.8% (60)
	V	80	1.358±0.523(8)/ (0.786-2.2)	43.3% (80)	4.416±1.859(8)/ (1.9-6.8)	36.4% (80)
	VI	100	1.297±0.386(8)/ (0.9-2)	35.9% (60)	5.025±1.299(8)/ (2.9-6.6)	29.1% (60)
	VII	120	1.127±0.263(8)/ (0.73-1.5)	44.3% (60)	4.719±1.337(8)/ (2.5-6.2)	33.4% (60)
	VIII	150	1.455±0.190(8)/ (1.18-1.7)	39.3(80)	5.555±0.677(8)/ (4.4-6.2)	20% (80)
Female	I	Control	60 min: 1.868± 0.239 (7) (1.4-2) 80 min: 2.076±- 0.163 (8) (1.9-2.3)	N/A	60 min: 7.259± 0.524 (8) 80 min: 6.978± 0.492 (8)	N/A
	II	20	1.171±0.521(8): (0.7-2.1)	37.3% (60)	3.401±2.632 (8)/ (1-7.2; note Huge range) (a)	53.1% (60) (a)
	III	40	1.371±0.644(8) (0.71-2.6)	34% (80)	3.815±1.858(8) / (1.5-6.2)	45.3% (80)
	IV	60	0.867±0.283(7) (0.54-1.2)	53.6% (60)	3.23±1.467(8)/ (1.5-5.7)	55.5% (60)
	V	80	1.441±0.466 (8) (0.87-1.8)	30.6% (80)	4.562±1.605(8)/ (2.5-6.4)	34.6% (80)
	VI	100	1.196±0.321(8) (0.55-1.5)	36% (60)	5.076±1.381(8)/ (3-6.8)	30.1% (60)
	VII	120	1.281±0.281(8) (0.85-1.6)	31.4% (60)	4.394±1.560(8)/ (1.6-6.3)	39.5% (60)
	VIII	150	1.179±0.167(8) (1.0-1.37)	43.2% (80)	5.221±1.251(8)/ (3.2-6.9)	25.2% (80)

(a) 4/8 animals had brain ChE < 1.5 (average of 82% brain ChEI); 2 high values of 7.2 and 7.1 brain ChE influence the group average result.

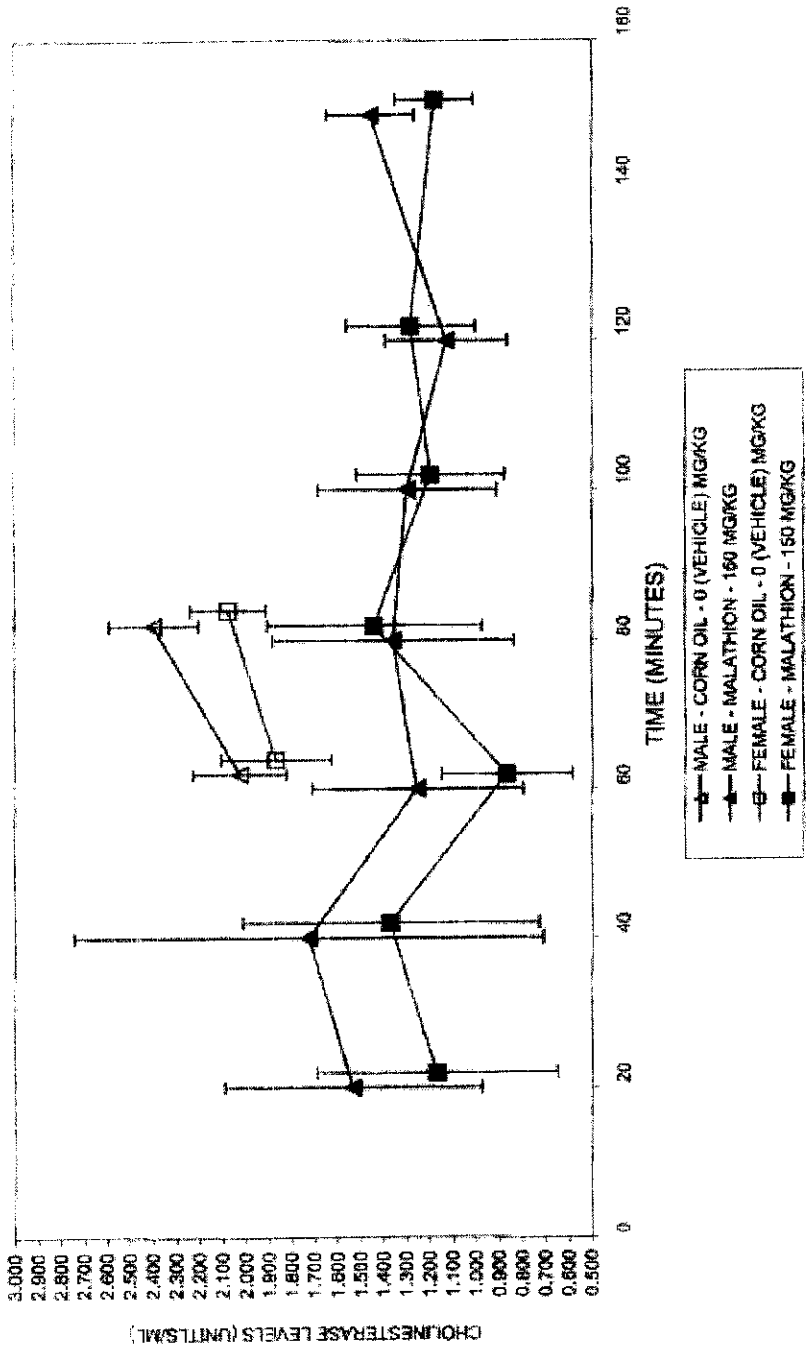
(b) Control value used by the registrant to estimate percent cholinesterase inhibition.

(c) Maximum value of 4 is 2X control values, but was included in the mean.

PROTOCOL TQC000021: ORAL (GAVAGE) ACUTE DOSE TIME OF PEAK CHOLINESTERASE DEPRESSION STUDY OF MALATHION AND MALAOXON IN JUVENILE RATS

RBC CHOLINESTERASE LEVELS
JUVENILE PUPS - MALATHION

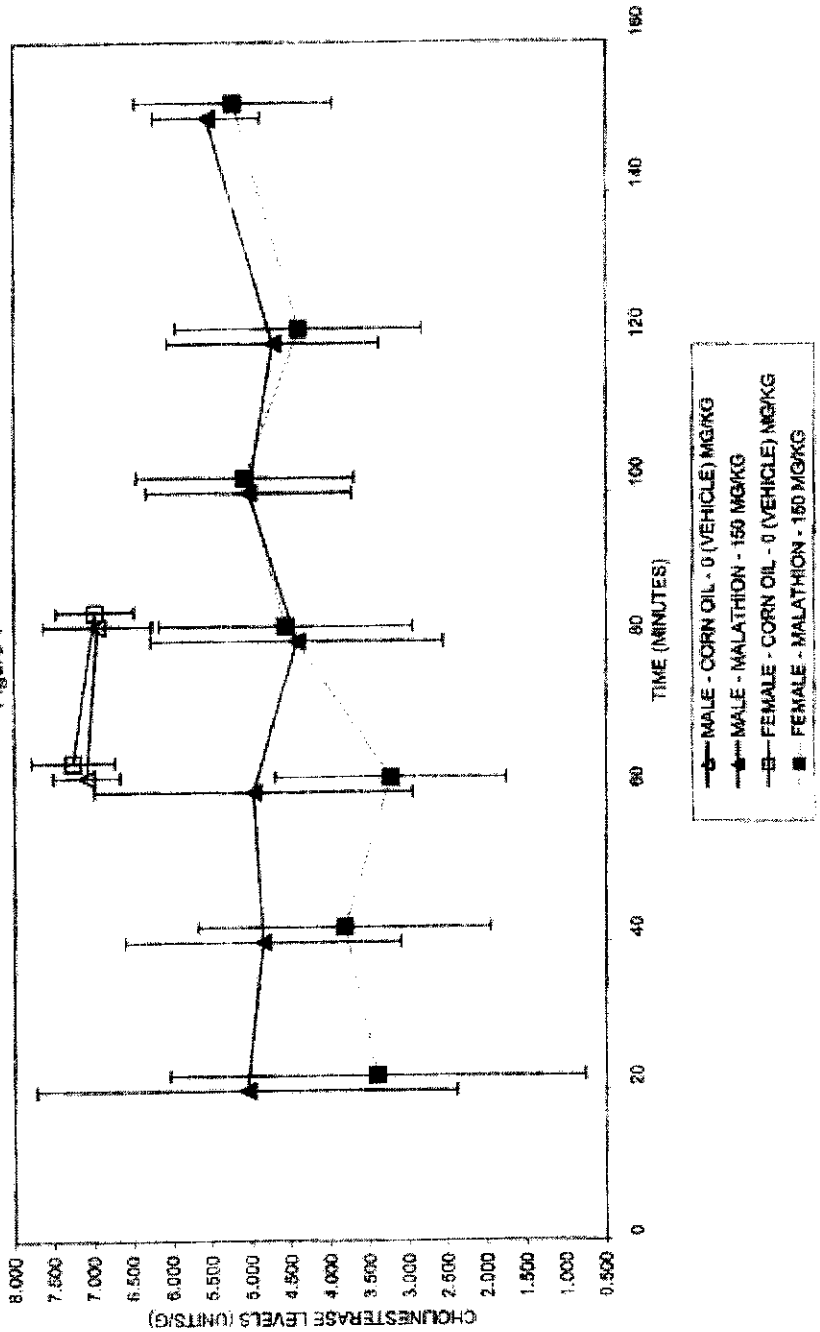
Figure 2



PROTOCOL T&C000021 ORAL (GAVAGE) ACUTE DOSE TIME OF PEAK CHOLINESTERASE DEPRESSION STUDY OF MALATHION AND MALAOXON IN JUVENILE RATS

BRAIN CHOLINESTERASE LEVELS
JUVENILE PUPS - MALATHION

Figure 1

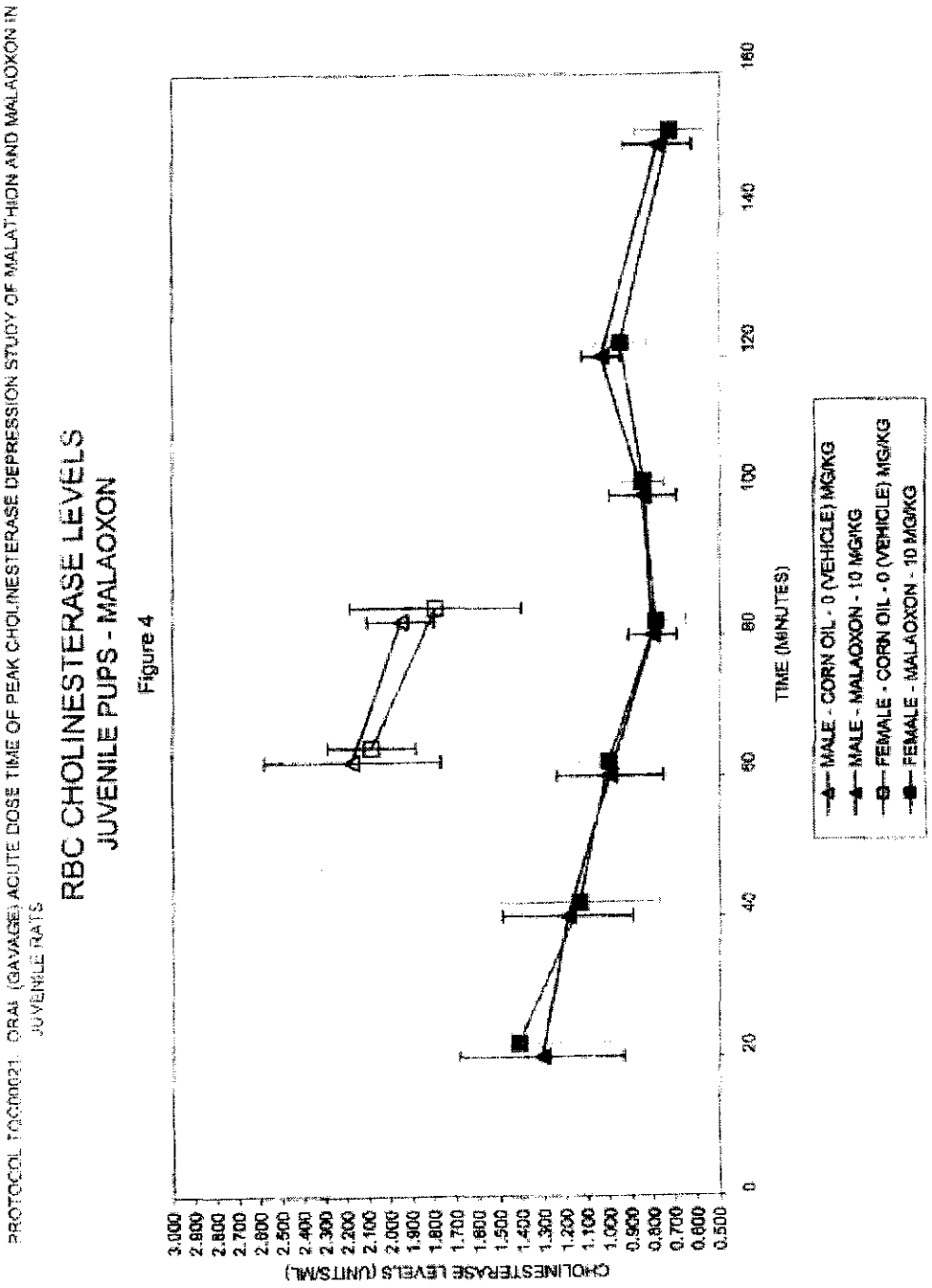


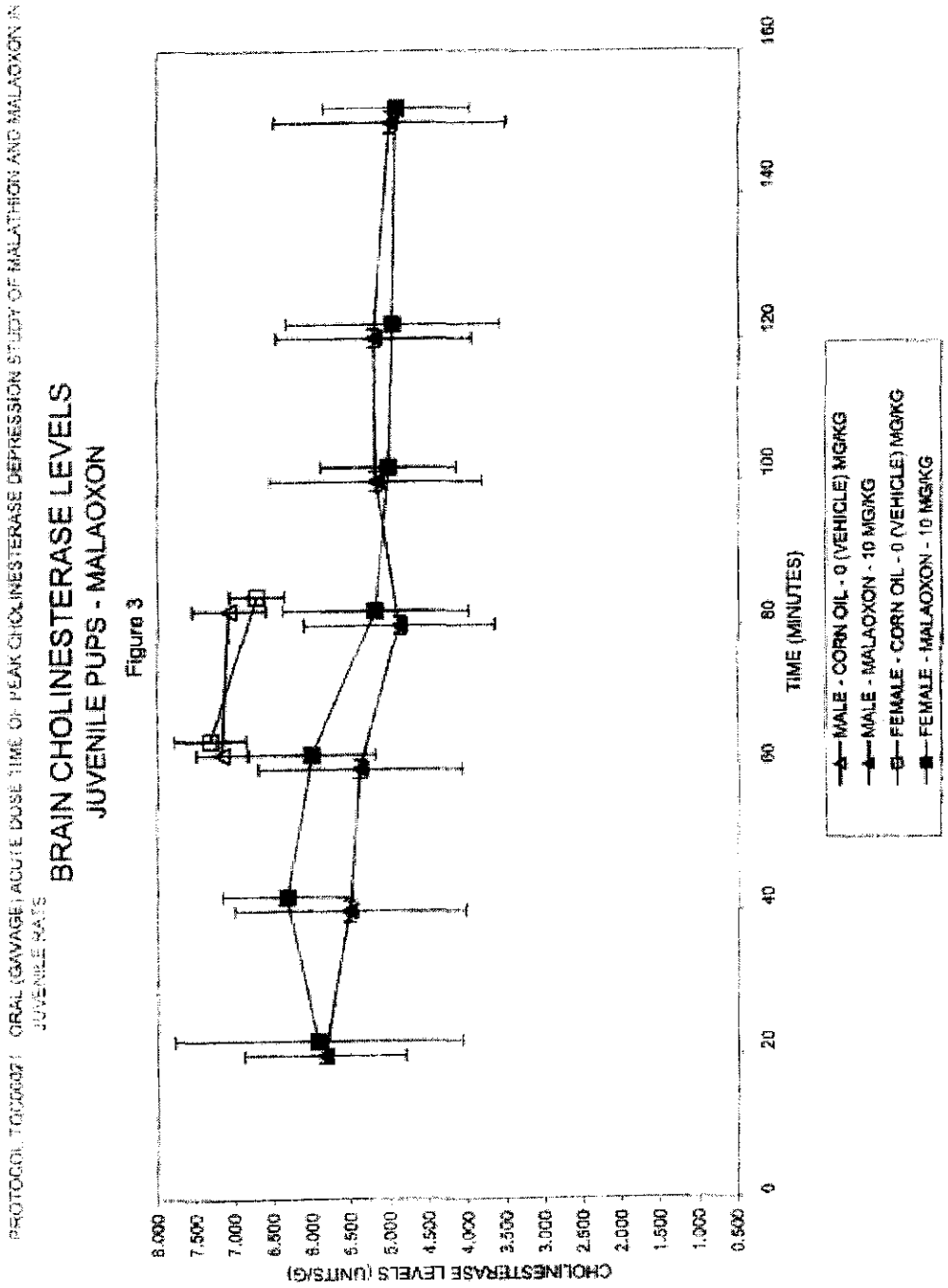
Malaoxon:

Sex	Group	Time (Minutes)	RBC Mean ChE ChE: U/G +/- S.D (n) /(Range)	% Decrease (e)	Brain Mean ChE ChE: U/G +/- S.D (n)/ (Range)	% Decrease (e)
Male	I	Control	40 min: 2.176± 0.405 (8) 60 min:1.955± 0.153 (8)	N/A	40 min: 7.166± 0.332 (8) 60 min: 7.079± 0.474 (8)	N/A
	II	10	1.311±0.376 (7)/ (0.7-1.79)	39.8% (40)	5.836±1.040 (8)/	18.6% (40)
	III	20	1.195±0.297(8) / (0.799-1.56)	38.9% (60)	5.512±1.483(8) /	22.1% (60)
	IV	40	1.0±0.243(8)/ (0.84-1.5)	54% (40)	5.386±1.313(8)/	24.8% (40)
	V	60	0.807±0.110(8)/ (0.6-0.94)	58.7% (60)	4.891±1.227(8)/(4.97 with duplicate) (2.97-6.7) (1 outlier of 2.97)	30.9% (60)
	VI	80	0.848±0.153(8)/ (0.6-0.96)	61% (40)	5.181±1.36(8)/ (2.1-6.2) (1 outlier of 2.1)	27.7% (40)
	VII	100	1.039±0.088(8)/ (0.89-1.1)	46.9% (60)	5.215±1.257(8)/ (3-6.6)	26.3% (60)
	VIII	120	0.786±0.154(8)/ (0.6-1.0) (a)	63.9(40)	5.002±1.485(8)/ (3-6.9) ©	30.2% (40)
Female	I	Control	40 min: 2.087± 0.202 (8) 60 min: 1.796±- 0.39 (8) (1.93 w/o outlier control value 0.851)		40 min: 7.313± 0.468 (8) 60 min: 6.718± 0.357 (8)	N/A
	II	10	1.416±0.47(8) (0.83-2.0)	32.2% (40)	5.927±1.852 (8)/ (1.82-7.7) (1 outlier of 1.82)	19% (40)
	III	20	1.138±0.365(8) (0.73-1.9)	36.6% (60)	6.323±0.836(8) / (4.9-7.1)	5.9% (60) (13% vs 40 min)
	IV	40	1.005±0.138(8) (0.81-1.1)	51.8% (40)	6.003±0.818(8)/ (4.9-7.0)	17.9% (40)
	V	60	0.793±0.137 (8)/ (0.63-1.0)	55.8% (60)	5.193±1.189(8)/ (3.1-6.8)	22.7% (60)
	VI	80	0.848±0.096(8)/ (0.73-1.0)	59.4% (40)	5.019±0.861(8)/ (3.6-5.7)	31.4% (40)
	VII	100	0.953±0.115(8) (0.89-1.1)	46.9% (60)	4.97±1.37(8)/ (3.4-6.7)	26% (60) (d))
	VIII	120	0.729±0.154(8)/ (0.5-0.97) (b)	65.1% (40)	4.921±0.936(8)/ (3.6-6.4)	32.7% (40)

(a) 3/8 animals had RBC ChE values <1.
(b) 2/8 animals had RBC ChE values at 0.5, and this group had the lowest range of RBC ChE activity.
(c) 3/8 animals had Brain ChE values in the 3 range.
(d) less of variation in brain ChE activity; 1 animal had tremors.
(e) Control value used by the registrant to estimate percent cholinesterase inhibition.

Note: error for time points on graph: Time points should be 10, 20, 40, 60, 80, 100 and 120 min.





Note: error for time points on graph: Time points should be 10, 20, 40, 60, 80, 100 *and 120 min.*

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Chemical: Malathion

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