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DER #10

Malathion: 13-week (Subchronic) Neurotoxicity Study in Sprague-Dawley Rats. Wil Research Laboratories. 1994. MRID 43269501. HED Doc No. (none).

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DATA EVALUATION REPORT

MRID No.: 432695-01
PC No.: 057701
Tox Chem No.: 535

Study Type: 82-7 Subchronic (13 Week) Neurotoxicity - Rat

Test Material: Malathion (96.4% purity from Lot 11029-01

Sponsor: Cheminova Agro A/S
Lemvig, Denmark

Testing Facility: WIL Research Laboratories, Inc.
Ashland, Ohio

Title of Report: A Subchronic (13-Week) Neurotoxicity Study of Malathion in Rats

Author: Ian C. Lamb, Ph.D.

Study No.: WIL 206006

Report Issued: June 9, 1994

Study Initiation Date: April 12, 1993

THIS IS A DRAFT REVIEW PENDING RESOLUTION BY THE HAZ-ID COMMITTEE OF THE POSSIBLE REQUIREMENT FOR ADDITIONAL SPECIFIC NEUROTOXICITY TESTING TO ASSESS FOR EFFECTS ON MAZE PERFORMANCE (LEARNING) AND EEG AND EMG RECORDINGS.

SEE PAGE 2 OF THIS DRAFT DER

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Executive Summary: Four groups of 25/sex Sprague-Dawley CRL:CD®BR strain rats were dosed as control, 50, 5000 or 20000 ppm malathion in their diets. These doses corresponded to 0, 4, 352 and 1486 mg/kg/day for males and 0, 4, 395 or 1575 mg/kg/day malathion for females. The rats were subjected to neurotoxicity assessments at pretest, weeks 3, 7 and 12 .

Neurotoxicity: For parameters of the FOB and motor activity, LEL exceeds 1486 mg/kg/day for males and 1575 mg/kg/day for females.

Systemic toxicity: Definite symptoms were noted in the high dose group only and included decreased body weight (males 9-20%) and females (9-13%) and food consumption and the presence of anogenital staining in both sexes and dried red material about the nose in females. **The LEL is 1486 (males), 1575 (females) mg/kg/day. The NOEL is 352 (males), 395 (females) mg/kg/day.**

ChE/AChE effects: Plasma ChE (males 12-20%, females 15-30%), RBC AChE (males 49-61% and females 49-53%) and brain (i.e. cortex 12-20% in females) was inhibited at 352 or 395 mg/kg/day. Higher levels of ChE/AChE inhibition were noted for the high dose group and male brain (i.e. mid brain 24%). **The LEL is 352 (males), 395 (females) mg/kg/day based on plasma ChE and RBC AChE, and 395 (females) mg/kg/day based on brain AChE. The NOEL is 4 mg/kg/day based on plasma ChE and RBC AChE in both sexes and brain AChE in females.**

Study Classification: **GUIDELINE.** This study satisfies the requirement for a series 82-7 subchronic neurotoxicity study. Published data, however, indicate possible evidence of neurotoxicity on parameters not assessed in the series 82-7 Guidelines. Additional specific neurotoxicity testing may be required to assess for effects on maze performance (learning) and EEG and EMG recordings.

Quality Assurance Statement: Provided

Good Laboratory Practice Statement: Provided

REVIEW

Experimental Constants

Test Chemical: Technical grade malathion, 0,0-dimethyl-S-[1,2-di(ethoxycarbonyl)-ethyl]-phosphorodithioate, was provided by Cheminova Agro A/S. The compound was designated as Lot No. 11029-01 and to be of 96.4% purity. The compound is a colorless to pale yellow liquid. The agent is described as stable when stored refrigerated, protected from light.

Test System: Sprague-Dawley Crl:CDBR strain rats were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. Rats were described as young adult, 41 days old at start of study weighing 159-218 grams (male), 145-190 grams (females). They were individually housed during the experiment, and received food (Purina certified rodent chow) and water ad libitum.

Basic Experimental Design:

The study consisted of four groups of 25 rats/sex that were dosed as either control, 50, 5000 or 20000 ppm of malathion. No rationale for selecting these dose levels was presented. There is no evidence that a dose range finding study was run.

For the control diet, a pre-mix was prepared by adding 300 ml acetone to 5 kg of feed (Purina Certified Rodent Chow) in a Hobart mixing bowl and mixing for 10 minutes. This pre-mix was then mixed for 15 minutes with enough feed to achieve a total batch size of 13 kg. For the test diets the appropriate amount of malathion was dissolved in 300 ml of acetone and processed into the feed as described above. The prepared feed was placed in labeled storage bags after the acetone was allowed to evaporate (approximately 24 hours). The test diets were prepared weekly and were stored at room temperature.

Analytical Chemistry:

The analytical report is in Appendix B, p. 1078 of the study report. Malathion levels in diet preparations were analyzed by gas chromatography with a flame ionization detector.

Stability: Samples stored for 14 days at room temperature were reportedly analyzed and the indicated recoveries were 91.3-94.1% of the target dose concentrations. The study report indicates that "no degradation or loss of the test material was apparent." (p. 1080)

Homogeneity: A test for sample homogeneity was made on pre-study dietary preparations. Replicate samples from the top, middle and bottom all yielded values considered adequately close to 100% of the nominal dose level in ppm, indicating adequacy of mixing.

Concentration: Dosing formulations were analyzed for malathion concentration during 10 of the weeks of dosing. the mean concentrations as reported were: 51.2 ppm (102%) (Group 2); 5117 ppm (102%) (Group 3) and 21042 ppm (105%) (Group 4). These data indicate that the desired dose levels were achieved. (p. 1080 of study report)

Statistics: The study report asserts that the following statistical tests were performed.

"All statistical tests for data other than the Functional Observational Battery (FOB) and Locomotor Activity were performed by Digital® MicroVAX® 3400 computer with appropriate programming. All analyses were two-tailed (except

as noted) for significance levels of 5% and 1%. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. Body weights, body weight changes, food consumption, cholinesterase determinations, brain weight data and brain dimensions were analyzed by a one-way analysis of variance (ANOVA). If significant differences were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. Histopathological findings in the treated groups were compared to the control group data by the one-tailed Kolmogorov-Smirnov test."

"Statistical analyses for cholinesterase values were conducted by Pharmaco LSR, Inc."

"All statistical tests for the FOB and Locomotor Activity data were performed using a personal computer installed with SAS/STAT statistical software. Each mean was presented with the standard deviation and the number of animals used to calculate the mean. Continuous FOB and Locomotor Activity data were analyzed using a two-way repeated measures ANOVA. If significant treatment or treatment-time interactions occurred, a one-way ANOVA was conducted at each time point. If significant treatment effects were observed at a time point, Dunnett's multiple T-test was conducted to determine significant treatment differences from the control group ($p<0.05$). FOB parameters yielding scalar (ordinal) or descriptive data were analyzed using the repeated measures SAS CATMOD procedure. If significant treatment or treatment-time interactions occurred, Fisher's Exact test or Dunnett's test were conducted to determine significant treatment differences from the control group. (These tests were performed by a Digital® MicroVAX®3400 computer with appropriate programming.)" (pp 24-25 of study report)

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Specific Methods and Results

1. Deaths and Clinical Signs All animals survived to scheduled euthanization.

TABLE 1: Selected Clinical Signs in Rats Dosed with Malathion for 90 Days
Total Occurrences/No. of Animals

Clinical Sign		Control	50 ppm	5000 ppm	20000 ppm
Dried Yellow Material anogenital area	M	0/0	2/1	1/1	97/12
	F	0/0	2/1	0/0	70/9
Dried yellow material urogenital area	M	0/0	0/0	0/0	41/5
	F	0/0	0/0	0/0	55/6
Dried orange material proximal tail	M	1/1	11/1	0/0	46/9
	F	0/0	0/1	2/1	38/8
Dried orange material anogenital area	M	0/0	0/0	0/0	49/9
	F	0/0	0/0	0/0	16/4
Dried orange material urogenital area	M	0/0	0/0	0/0	81/7
	F	0/0	0/0	0/0	9/3
Dried orange material entire length of tail	M	2/2	14/2	22/3	229/9
	F	0/0	0/0	37/3	147/8
Dried red material around nose	M	84/13	40/10	39/12	51/14
	F	21/6	28/6	12/6	91/5

Table 1 indicates that the high dose group rats had clinical signs related to urinary and bowel discharge irregularities as indicated by the presence of yellow and orange staining in the anogenital, urogenital areas and tail. Although the incidence of dried orange material over the entire length of the tail was increased in the 5000 ppm dose group only 3 males or females were affected. There were no similar increases in the more specific areas of the anogenital and urogenital areas in the 5000 ppm dose group for either sex. TB-I considers the 5000 ppm dose level a threshold dose rather than a definite LEL. The 20000 ppm dose level is considered a more definite LEL for clinical signs because 2 parameters (anogenital staining and red material around nose in females) were affected.

2. Body Weight, and Food Consumption and Compound Intake - Assessed Weekly

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Males and females had reduced bodyweight in the high dose group only. Mean body weights for the high dose group males and females were reduced 9-20% and 9-13% respectively, for study weeks 1 through 13. The majority of these differences were statistically significant with respect to control values.

Food consumption was not altered at the low or mid dose levels of malathion. At the high dose level (20,000 ppm) food consumption was reduced for males during study weeks 0-1 and 6-7 and throughout the 13 week study period in the case of females.

According to the study report (p. 27), average amounts of test article consumed (mg/kg/day) were as follows:

Dose Level (ppm)	Males	Females	Ratio (F/M)
50	4	4	1.00
5000	352	395	1.12
20000	1486	1575	1.06

Conclusion (body weight): NOEL and LOEL = 5000 and 20,000 ppm, respectively, for both sexes.

3. Neurobehavioral Assessments

Functional Observational Battery The 5 rats/sex/group selected for the week 13 (91 days) cholinesterase determinations and the neuropathology animals (5/sex/group) were combined, yielding a total of 10 rats/sex/ group for purposes of conduction of the Functional Observational Battery (FOB) and Locomotor Activity assessment. The animals were assessed at prestudy and at weeks 3, 7 and 12 of treatment. The following parameters were reportedly investigated: (a) home cage observations; (b) handling observations; (c) open field observations (2 minute observation period); (d) sensory observations; (e) neuromuscular observations; and (f) physiological observations.

The report concluded that an examination of data as reported in Tables 7 through 46 of the study report confirms there were, with few exceptions, no remarkable effects of the test material evident at any dose level at any time point. The exceptions might be commented on as follows. One female in the high dose group has a gait alteration during the week 12 open field observation. We concur with the study director's opinion that this should not be considered evidence of

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neurotoxicity. Likewise, a statistically significant reduced grip strength in the low and high dose groups at the week 3 observation period is not considered evidence of neurotoxicity, particularly since these values were "higher than historical controls." (pp. 28-29 of the study report)

There was no evidence of the influence of test material on body temperature.

Motor Activity Assessments were made at the same intervals as for the FOB above. Locomotor activity was measured following the completion of the FOB, using the Digiscan Micro Animal System (Omnitech Electronics, Columbus, OH). This device employs a photobeam to record and quantitate an animal's motor activity. Data were collected in minute epochs, and the test session was 41 minutes in duration for each animal. Locomotor Activity was divided into two categories - total and ambulatory activity. Total motor activity is defined as a combination of fine motor skills (interruption of one or two adjacent photobeams) and ambulatory motor activity (interruption of three or more consecutive photobeams).

Table 2 indicates changes in activity for the study groups as expressed in terms of % of control activity, the data being calculated from data in Tables 51-54 of the study report (pp. 234-249) and discloses that for male animals there were statistically significant increases in total activity in the 50 ppm group at weeks 7 and 12 (137% and 141% of control, respectively, and at 7 weeks in the 5000 ppm group (141% of control). However, one would be hard pressed to attribute these increases to the test material, since the pretest activity for males in the 50 ppm group was 132% of control and at 5000 ppm, was 126% of control total activity. Also there is little evidence of a dose response and no effect was seen in females. With respect to ambulatory activity, there were statistically significant increases at week 12 in males at 50 and at 5000 ppm (138% and 132% of control, respectively), but again the pretest values were already somewhat elevated. In view of the lack of a dose response and the lack of response in females, the study does not demonstrate an effect of the test material on motor activity.

Conclusion (FOB and motor assessments): LEL > 20,000 ppm for both sexes.

4. Cholinesterase Assays

Plasma, erythrocyte and brain region cholinesterase determinations were performed on 5 rats/sex/group one week prior to the study initiation and during weeks 3, 7 and 13

(termination). Blood samples were taken from the inferior vena cava at the time of necropsy. Brain regions assayed included olfactory region, cerebellum, hippocampus, cerebral cortex, brain stem and midbrain. Cholinesterase assays were performed by a procedure using the Ellman reaction . For the more detailed descriptions as to the analytical procedures employed, See pp 22-23 of the study report (appended). Representative mean values ± s.d. (n=5) for RBC, plasma and brain cholinesterase activities, randomly selected (in this case, control animals at week 4) are reproduced as follows: other control groups had similar values and the S.D. for the groups were similar to those illustrated.

	Male	Female
RbC(IU/ml)	0.88±0.08	0.99±0.08
Plasma(IU/ml)	0.358±0.041	0.933±0.379
Brain (hippocampus) (IU/g)	3.33±0.83	4.2±1.30

Table 3 presents cholinesterase inhibition data, calculated as per cent of the respective control values. Computations were derived from cholinesterase activity data as presented in Tables 55 and 55a (pp. 250-263 of the study report)

The study author claims that mean plasma cholinesterase was decreased in the 5000 ppm group males (18-20%) at the week 3 (statistically significant) and week 7 evaluations, and in the 20,000 ppm group, male and female mean plasma cholinesterase was statistically significantly decreased (50-54% and 55-76%, respectively) at the week, 3, 7, and 13 evaluations. Furthermore, mean plasma cholinesterase in the 50 ppm group males and females and the 5,000 ppm group females were unaffected by the test article administration. (p. 30 of study report). We concur in this assessment, with the possible exception of the 30% inhibition (not statistically significant) in the 5000 ppm female group at week 7. To the extent that the non-statistically significant 20% reduction in males of the 5000 ppm group is considered in the study author's opinion as an effect, the 30% reduction in females at the same dose level and time point is here considered an effect, particularly since inhibition was a remarkable 76% among females at the next high dose at week 7.

With regard to erythrocyte cholinesterase inhibition, we concur with the study report that inhibition of the enzyme was statistically significant among males and females, at all time points in the mid and high dose groups. Significant

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inhibition was not observed at 50 ppm for either sex at any time point.

As to the subject of brain region cholinesterase inhibition, the study author provides the following tabulation for the 20,000 ppm group (p. 31 of study report): (Note - NA actually means that activity was not altered with respect to the control)

		Percent Reduction					
Region	Week	Males			Females		
		3	7	13	3	7	13
Hippocampus		19	18	NA	44**	38**	47**
Olfactory Region		13	11	34*	31*	27*	50**
Midbrain		17	16	24**	18	35**	40**
Brainstem		17	11	18**	22	17	36**
Cerebellum		NA	NA	NA	20*	16	32**
Cerebral Cortex		11	26**	23	32**	40**	53**

NA = Not applicable
* = Statistically significant at $p<0.05$
** = Statistically significant at $p<0.01$

The study author claims that the majority of the differences from the control group, primarily in the females, were statistically significant. (p. 31 of study report) We concur that the majority of the differences were statistically significant among females, but not so among males. For all but the cortex, there was no statistically significant inhibition in any brain region after 3 or 7 weeks of study in the case of males. Furthermore, there was no inhibition observed in the cerebellum of males at any time point. By contrast, inhibition among females was much more pronounced than in males and occurred at earlier time points. Thus, brain cholinesterase inhibition among females was more pronounced than among males at the high dose level.

The study author goes on to say that brain region cholinesterase inhibition in the 50 and 5000 ppm group males and females were apparently unaffected by the test article. He considers a statistically significant 18% inhibition in the cortex at week 3 among females of the 5000 ppm group as transient in nature and not of neurotoxicological significance. (p. 31 of study report). To the contrary, we

are of the opinion that collectively the statistically significant finding in the cortex and, the following inhibition for females at the mid dose level, though not statistically significant, constitutes sufficient evidence to conclude an effect for females at this dose level: 13% inhibition at week 7 in the hippocampus; 29% inhibition at week 13 in the olfactory region; 16% inhibition at week 13 in the brain stem; and 20% and 12% inhibition at weeks 7 and 13, respectively, in the cortex. Note, for females inhibition was evident at all time points in the cortex at 5000 ppm dose level.

Conclusion

For plasma and erythrocyte cholinesterase inhibition, LEL = 5000 ppm, NOEL = 50 ppm for both sexes. For brain cholinesterase inhibition, LEL = 5000 ppm, NOEL = 50 ppm, for females.

5. Histopathology and Perfusion Studies

"At study termination, 5 animals/sex/group were euthanized by carbon dioxide inhalation and then perfused *in situ* with buffered sodium nitrite solution followed by 1.5% glutaraldehyde - 4.0% formaldehyde solution for neuropathological examination. The central and peripheral tissues were preserved from all animals and dissected from 5 animals/sex in the control and high dose groups (Groups 1 and 4, respectively). Brain weight (excluding olfactory bulbs) and brain dimensions (length and width) were recorded. Any observable gross changes, abnormal coloration or lesions of the brain and spinal cord were recorded. The central nervous system tissues were embedded in plastic, sectioned and stained using methodology deemed appropriate by the resident pathologist. The following nerve tissues were evaluated for a qualitative histopathological examination in the control and 20,000 ppm groups.

"Central Nervous System tissues"

Brain - forebrain, center of cerebrum, midbrain, cerebellum and pons, and the medulla oblongata
Spinal cord - at cervical swellings C_3 - C_8 and at lumbar swellings T_{13} - L_4
Gasserian ganglion/Trigeminal nerves
Lumbar dorsal root ganglion at T_{13} - L_4
Lumbar dorsal root fibers at T_{13} - L_4
Lumbar ventral root fibers at T_{13} - L_4
Cervical dorsal root ganglion at C_3 - C_8
Cervical dorsal root fibers at C_3 - C_8

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Cervical ventral root fibers at C_3 - C_8
Optic Nerves
Eyes

"Peripheral Nervous System tissues[b]

Sciatic nerve (mid- thigh region and at sciatic notch)
Sural nerve
Tibial nerve
Peroneal nerve
Forelimbs[c]
Tail[c]
a - embedded in paraffin
b - embedded in plastic
c - preserved but not examined" (pp. 23-24 of study report)

Conclusions

For non-perfused animals, the study report claims there were no adverse effects observed on absolute brian weight, brain region weights, brain and brain region weights relative to final body weights or brain region weights relative to brain weights at any dose level or time point. Mean relative (to final body weight) brain, hippocampus, cerebellum, cerebral cortex and brainstem weights in the 20,000 ppm group males and/or females were significantly increased when compared to the control group at all time points. This finding was attributed to the low mean body weights observed in the 20,000 ppm (HDT) group. (p. 32 of study report). An independent (TB-1) analysis of the relevant data as presented in Tables 56 through 67 of the study report enables our concurrence with these conclusions of the study author. There were no obvious effects of dosing on any of the parameters examined.

Among perfused animals, we concur with the study author's conclusions that neuromorphological (histopathological) examinations of the tissues of the nervous system under study did not disclose any remarkable adverse effects among the high dose group animals, the only dosed group examined. A noteworthy observation is that histopathology of the eyes disclosed an incidence of "retinal-focal dysplasia (rosette formation) mild" in the control group. None were reported for the high dose group. (Table 69 of study report). Also, there were no compound related effects upon absolute brain weights, or mean brain length or width measurement. (Table 68 of study report).

Comments

Brain region cholinesterase inhibition in this study was evidently more pronounced for females than for males (see percent reduction in enzyme activity for both sexes of the 20,000 ppm test

group, p. 8 of this review). However, before concluding that females are more susceptible than males, the question of test material intake must be considered. The study report shows that the mean test material intake was 1486 and 1575 mg/kg/day for males and females, respectively, based on food consumption. The intake ratio for females vs males was 1.06 (see p. 5 of this review). This finding alone might be used to explain to some degree, at least, the more remarkable extent of cholinesterase inhibition found for females, but a closer examination of dietary intake is indicated.

Independent TB-1 calculations of food consumption for the high dose group show the following:

	Mean Food Consumption, g/kg/day		
Weeks	Male	Female	Ratio (F/M)
0-3	106.0	102.3	0.965
0-7	87.9	88.3	1.005
0-13	74.3	78.8	1.060

We thus confirm the study's findings that female intake of malathion (based on dietary intake) was 6% in excess of that of males on the average over the 13-week study period. However, we also find that for the first three weeks of study, female intake was 97% that of males, and for the first 7 weeks was equivalent to that of males. Hence, compound intake differences on a per kg body weight basis, between males and females of the high dose group cannot explain the more remarkable cholinesterase inhibition observed in brain regions of females - at least not for the 3 week and 7 week time points and therefore not likely so for the entire 13-week period. This study indicates that females are more vulnerable than males to the cholinesterase inhibiting potential of malathion. Indeed in this study as discussed previously, the next lower dose, 5000 ppm, is considered an effect level for brain cholinesterase inhibition for females but not for males.

The study did not disclose any evidence of an effect of malathion on the nervous system except brain cholinesterase inhibition. Parameters evaluated included those of the FOB, motor activity, brian morphology and peripheral and central nervous system histopathology. The absence of an effect among these parameters in the face of ~~substantial~~ brain cholinesterase inhibition requires some comment. It may be that the rat possesses considerable compensatory capability (e.g. nervous system plasticity, cholinergic receptor down regulation, etc.) to mitigate the consequences of cholinesterase inhibition. Such phenomena, if

detected, might be considered by some to constitute an adverse effect.

Where malathion is concerned, there are published works reporting effects on behavior (Kurtz 1977) and learning, electroencephalography (EEG) and electromyography (EMG) (Desi et al. 1976) in the rat at doses reportedly extending below those that measurably inhibited brain AChE.

Kurtz (1977) evaluated the effects of malathion in the rat on conditioned avoidance behavior and erythrocyte, plasma and brain cholinesterase inhibition, at dosage levels ranging 25-150 mg/kg, as administered via single intraperitoneal injections. Avoidance performance was significantly impaired 1 hour post administration of 50 mg/kg, a dose which did not significantly inhibit blood or brain cholinesterase activity. At doses of 100 and 150 mg/kg the enzymes were inhibited in addition to the impairment of avoidance performance.

In a subchronic feeding study, Desi et al. (1976) evaluated learning rate (maze study) during 21 days of dosing, and EEG and EMG changes after 90 days of dosing. This latter publication is reviewed under a separate DER, appended.

References

Desi, et al (1976). Arch. Environ. Contam. Toxicol. 3, 410-425

Kurtz, P. (1977) Toxicol. Appl. Pharmacol. 42, 589-594

TABLE 2				
	Total Activity (% of Control)			
	Study Week			
	Pretest	3	7	12
50 ppm				
Males	132	91	137*	141*
Females	97	102	99	112
5000 ppm				
Males	126	113	141*	131
Females	117	101	100	95
20,000 ppm				
Males	127	93	129	127
Females	113	83	93	94
	Ambulatory Activity (% of Control)			
	Study Week			
	Pretest	3	7	12
50 ppm				
Males	129	92	134	138*
Females	99	102	102	117
5000 ppm				
Males	119	112	133	132*
Females	120	101	99	100
20000 ppm				
Males	123	86	123	120
Females	121	85	95	94

TABLE 3 - CHOLINESTERASE INHIBITION (% of Control)								
	Males				Females			
	Pretest	Week 3	Week 7	Week 13	Pretest	Week 3	Week 7	Week 13
Plasma								
50 ppm	95	89	100	91	93	126	75	149
5000 ppm	107	82*	80	88	91	99	70	85
20,000 ppm	100	47*	46*	50*	104	45*	24*	30*
RBC								
50 ppm	97	102	99	98	110	105	97	101
5000 ppm	105	49*	39*	51*	107	49*	47*	51*
20,000 ppm	120	47*	32*	37*	121	36*	33*	32*
Hippocampus								
50 ppm	98	96	97	89	96	75	100	114
5000 ppm	96	89	102	98	99	79	87	91
20,000 ppm	97	81	82	110	94	56*	62*	53*
Olfactory								
50 ppm	95	122	102	107	98	91	121	75
5000 ppm	102	117	95	105	101	92	101	71
20,000 ppm	104	87	89	66*	103	69*	73*	50*
Midbrain								
50 ppm	103	99	100	98	99	111	79	112
5000 ppm	102	97	88	93	95	102	87	100
20,000 ppm	97	83	84	76*	92	82	65*	60*

TABLE 3 - CHOLINESTERASE INHIBITION (% of Control)								
	Males				Females			
	Pretest	Week 3	Week 7	Week 13	Pretest	Week 3	Week 7	Week 13
Brainstem								
50 ppm	100	100	107	95	102	110	112	104
5000 ppm	98	111	92	94	102	95	97	84
20,000 ppm	99	83	89	82*	102	78	83	64*
Cerebellum								
50 ppm	98	89	104	92	102	106	92	105
5000 ppm	91*	87	96	89	99	100	97	100
20,000 ppm	94	95	97	87	100	80*	84	68*
Cortex								
50 ppm	99	101	106	101	101	94	85	98
5000 ppm	110	101	92	107	102	82*	80	88
20,000 ppm	109	89	74*	77	103	68*	60*	47*

* = significant difference from control at $p \leq 0.05$

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MRID # 43269501

Dec # 10

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