4/18/1995

DER #9

Malathion: Acute Neurotoxicity Study in Sprague-Dawley Rats. Wil Research Laboratories. 1994. MRID 43146701. Hed Doc No. (none).

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

MEMORANDUM

SUBJECT: EPA ID# 057701 - Malathion: Review of a Series 81-8-SS Acute Neurotoxicity Study

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

Tox Chem No.: 535
MRID No: 431467-01
Submission No.: S464959
DP Barcode No.: D203034
PC No.: 057001

FROM: Brian Dementi, Ph.D., D.A.B.T.
Review Section III
Toxicology Branch-I
Health Effects Division (7509C)

Brian Dementi 4/18/95

and

John Doherty, Ph.D.
Review Section IV
Toxicology Branch-I
Health Effects Division (7509C)

TO: Linda Propst, PM Team 73
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Karl Baetcke, Ph.D.,
Chief, Toxicology Branch-I
Health Effects Division (7509C)

I. Conclusion

THIS IS A DRAFT REVIEW PENDING RESOLUTION BY THE HAZ-ID COMMITTEE OF THE REQUEST FOR HISTOPATHOLOGY SLIDES OF RETINAL TISSUE OF SELECTED RATS.

SEE PAGE 2 OF THIS DRAFT COVER MEMO

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I. Conclusion

The series 81-8-SS Acute Neurotoxicity Study (MRID No. 431467-01, WIL Labs # 206005, March 2, 1994) was reviewed and determined to be CORE MINIMUM. The LEL was set at 2000 mg/kg for both sexes. The NOEL was set at 1000 mg/kg.

The study is graded CORE MINIMUM as opposed to GUIDELINE because of certain inadequacies which are presented as follows: (1) erythrocyte and plasma cholinesterase data are inconclusive, due to inconsistent findings of inhibition, standard deviations being excessively large and poor dose response, all of which contribute to uncertainty in the assignment of LEL/NOEL for the blood cholinesterases, particularly plasma cholinesterase; (2) equivocal effect on motor activity at 1000 mg/kg; (3) equivocal presence of clinical signs such as salivation at all doses immediately

following dosing.

The Registrant is to be advised to submit the histopathology slides (or photomicrographs) of retinal tissue of both eyes of the male rat (#12011) exhibiting bilateral retinal rosette, plus those of another male rat selected at random from the study control group. Also to be submitted, for comparative purposes are slides (or photomicrographs) of retinal tissues of both eyes for control rat (# 14599) exhibiting unilateral retinal rosette (mild) in the WIL Labs Subchronic (13-Week) Neurotoxicity Study of Malathion (WIL # 206006).

II. Action Requested

Cheminova Agro A/S has submitted this series 81-8-SS (Acute Neurotoxicity Study in Rats) in response to the Data Call-In for malathion. Toxicology Branch I has reviewed the study and the following comments apply.

III. Comments

(1) The study was reviewed and determined to be CORE MINIMUM. The LEL was determined to be 2000 mg/kg and the NOEL 1000 mg/kg based on definite clinical signs and decreased motor activity.

(2) Additional comments on findings in other aspects of the study include: (a) one male animal of the 2000 mg/kg group was sacrificed in extremis (cholinergic signs) on the first day of the study; (b) clinical signs, such as salivation were reported the first day for all dose groups, but this effect is considered definitely related to the test material only at the high dose and equivocal at the lower doses. Animals of the high dose group also exhibited urogenital staining; (c) in the assessment of cholinesterase inhibition, the review concludes that the NOEL is 2000 mg/kg for brain cholinesterase as assayed in several regions. Plasma and erythrocyte cholinesterases were inhibited in males and females at 2000 mg/kg on Day 7, a finding which was sustained, in females only, at Day 15. Also, there was an equivocal inhibition of plasma cholinesterase for females at the mid and low dose levels as well - characterized by a poor dose response. Females appear to be more sensitive than males to malathion in terms of responsiveness to both of the blood borne cholinesterases. The lack of a dose response relationship and a clear NOEL for the enzymes constitutes an inherent weakness of the study; (d) under Locomotor Activity, there were numerical reductions in activity for males at all doses, but only the reduction at the high dose is here considered a definite effect. A decrease noted at the mid dose is considered equivocal; (e) under the topic of Neuromuscular Observations, among males of the high dose group there was recorded a reduction (statistically significant) in rotorod performance at Day 14 which is considered here as of only questionable biological significance.

Reviewed by: Brian Dementi, Ph.D., D.A.B.T.
Section III, Tox. Branch I (7509C)
Secondary Reviewer: John Doherty, Ph.D.
Section IV, Tox. Branch I (7509C)

Brian Dementi 4/11/95

4/11/95

DATA EVALUATION REPORT

STUDY TYPE: 81-8SS Acute Neurotoxicity - Rat

Tox Chem No.	535
MRID No.	431467-01
PC No.	057001

TEST MATERIAL: Malathion (96.4% purity) from Lot # 11029-01

STUDY NUMBER: WIL 206005

SPONSOR: Cheminova Agro A/S
Lemvig, Denmark

TESTING FACILITY: WIL Research Laboratories, Inc.
Ashland, Ohio

TITLE OF REPORT: "An Acute Neurotoxicity Study of Malathion in Rats"

AUTHOR(S): Ian C. Lamb, Ph.D.

REPORT ISSUED: March 2, 1994

STUDY INITIATION DATE: April 12, 1993

EXECUTIVE SUMMARY: Malathion was evaluated for acute neurotoxicity and/or ChE/AChE inhibition in groups of 27/sex Sprague-Dawley Crl:CDBR strain rats at dosage levels of 0, 500, 1000 or 2000 mg/kg in corn oil. FOB, locomotor activity, histopathology and ChE/AChE assays were performed at pretest, peak effect (15 minutes post-dosing), day 7 and day 14.

NEUROTOXICITY: Definite effects were noted at 2000 mg/kg only. There was one death (male, moribund sacrifice, first day). This male rat showed tremors, labored respiration, stained fur (red and orange) and decreased defecation and urination. Only a few other rats showed clinical signs, but salivation

was excessive and body staining was evident in some high dose females. Motor activity was decreased (25%) at day 1 (peak effect time). The LEL is 2000 mg/kg. The NOEL is 1000 mg/kg.

CORE CLASSIFICATION: Minimum (erythrocyte and plasma cholinesterase data inconclusive - inconsistent findings of inhibition, standard deviations excessively large, poor dose response - rendering assignment of LEL/NOEL uncertain; motor activity LEL assignment equivocal as to 1000 or 2000 mg/kg level; clinical signs such as salivation evident at all doses immediately following dosing)

REVIEW: Quality Assurance Statement: Provided

GLP Statement: Provided

TEST ARTICLE: Technical grade malathion O,O-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 form the Charles River Breeding Laboratories, Inc., Portage, Michigan. Rats were described as young adult, 50 days old at start of study weighing 169-386 grams (male), 117-250 grams (females). They were individually housed during the experiment, and received food (Purina certified rodent chow) and water ad libitum.

EXPERIMENTAL DESIGN

There were 27 rats/sex/group included in the study. Each received by gavage a single dose of malathion in corn oil administered in a volume of 5 ml/kg at dosage levels of 0 (corn oil), 500, 1000 or 2000 mg/kg. Study assessment time points were pretest, 15 minutes (time of peak effect), 7 days and 14 days. All 27 animals/sex/group were observed twice daily for mortality and/or moribundity. Detailed clinical observations were recorded daily. Individual body weights were recorded pre-study and on days 0, 7, 14 and 15 (prior to euthanization). From the 27 animals/sex/group, 20/sex/group were designated as the "cholinesterase group", of which 5/sex/group were sacrificed pre-

7

study, 0 (15 minutes), 7 and 14 days and the remaining 7/sex/group, designated as the "neuropathology group", were sacrificed on day 15. The functional observational battery (FOB) (Appendix A, from p. 21 of study report) and locomotor activity tests (Appendix B, from p. 22 of study report) were performed on 12/rat/sex/group consisting of 7/sex/group from the "neuropathology group" plus 5/sex/group from the "cholinesterase group" at pre-test, 15 minutes and on days 7 and 14.

"After at least 15 days of observation, the 7 rats/sex/group in the "neuropathology group" were euthanized by carbon dioxide inhalation and then perfused in situ. The central and peripheral tissues were dissected and preserved. 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. Nerve tissues (Appendix C, from p. 25 of the study report) were prepared for a qualitative histopathological examination (embedded in paraffin or plastic, sectioned and then stained with hematoxylin and eosin) from five animals per sex in the control and 2000 mg/kg groups." (p. 24 of study report).

In the "cholinesterase group," plasma, erythrocyte and brain (regions: olfactory, cerebellum, hippocampus, cortex, brain stem, and midbrain) cholinesterase determinations were s cheduled to be evaluated on 5 animals/sex/group pretest, 15 minutes and days 7 and 15.

(NOTE: The dose levels chosen and peak effect time for this study were arrived at on the basis of a preliminary range-finding study which involved acute studies using dosages ranging 150 to 2000 mg/kg malathion. No remarkable findings were observed at doses up to 600 mg/kg. The time of peak effect for observing treatment related signs was approximately 15 minutes post-dosing).

STATISTICAL METHODS:

See the appended pp. 25-26 of the study report. HED statistician has examined the statistical approach and affirmed its validity and appropriateness for this acute neurotoxicity study as performed.

RESULTS:

All but one animal survived to scheduled euthanization. The one animal in question, a male in the 2000 mg/kg group was euthanized in extremis on the first day of study.

The study report advises that treatment-related clinical signs were observed in all dosed groups (p. 27 of study report). However, the evidence was more remarkable for the high dose group. Salivation occurring within 15 minutes of dosing was perhaps the most immediate and notable sign, as disclosed in Table 1A, pp 47-48, appended. Indeed, the study author advises that "The percentages of males and females in the 1000 and 2000 mg/kg groups with salivation were outside of the WIL FOB historical control data." (p. 29 of study report). Also observed were increased incidences of yellow material on the urogenital and anogenital areas among rats of both sexes in the high dose group. Such were observed primarily for one and/or two days post-dosing (Table 1, pp. 42-46, appended). The clinical picture is more remarkable (definite) at the high dose level even though certain clinical signs were observed at lower doses.

An inspection of body weight data as summarized in Table 2 (pp. 49-50, appended) of the study report indicates no remarkable differences between control and treated animals.

I. Functional Observational Battery (FOB)

Under the subtopic of Home Cage Observations, in terms of such parameters as posture, sleeping, convulsions, tremors, biting and eyelid closures as disclosed in Tables 4-11 (pp. 57-72) of the study report, there were no remarkable effects of dosing at any dose level or time point.

Under the subtopic of Handling Observations which includes such parameters as ease of removal from cage, ease of handling, lacrimation, chromodacryorrhea, salivation, piloerection, fur appearance, palpebral closure of eye lids, respiratory rate and character, deposits around nose, mouth and eyes, appearance of mucus membranes, eyes and skin, eye prominence and muscle tone - there were no remarkable findings except a small increased incidence of salivation and red deposits (nose, mouth) among males and females on day zero. There were no other effects noted at any time point or dose level as determined by inspection of tables 12-19 (pp. 74-120) of the study report.

Open Field Observations covers assessments for mobility, gait, convulsions (clonic, tonic), tremors, arousal, bizarre/stereotypic behavior, rearing, backing, grooming, urination and defecation. As disclosed in tables 20-27 (pp. 122-153) of the study report, there were no remarkable effects of malathion at any dose level or time point on the above named parameters.

Sensory Observations covers assessments of approach avoidance, touch response, startle response, tail pinch response,

olfactory orientation, eye blink response, forelimb extension, hindlimb extension and air righting reflex. As revealed in tables 28-35 (pp. 154-185), excepting a statistically significant effect in males of the low dose on startle response (see p. 178 of study report) there were no remarkable sensory observation findings. The noted finding occurred on the day 14 observation point. This is considered by this reviewer as a chance observation of no particular concern in view of the absence of such effect at high dose levels and at other time points.

Neuromuscular Observations include assessments of such parameters as hindlimb extensor strength, grip strength, rotorod performance and hindlimb footsplay. An inspection of tables 36-43 (pp. 187-201) discloses that with the exception of reduced rotorod performance among males of the high dose group on day 14, and the finding of one male rat in the high dose group on day 1 with impaired hindlimb resistance (reduced, some weakness apparent), there were no remarkable effects of the test material on any of the test parameters. With respect to rotorod performance, the following table serves to illustaete the indicated finding of statistically significant reduced performance (seconds):

Dose Level	Pretest	Day 0	Day 7	Day 14
Control	102.5±38.61	104.4±33.04	109.6±27.6	109.4±22.2
Low	59.9	88.7	68.8	79.5
Mid	73.7	71.2	85.6	80.2
High	60.7±46.41	74.8±44.6	81.3±37.2	45.4±21.97*

* $p < 0.01$ vs. control

On day 14 there was a statistically significant reduction in rotorod performance time in the high dose male group. The mean values ± S.D. on day 14 for the control and high dose groups were 109.4 ± 22.2 and 45.4 ± 21.97 seconds, respectively. In commenting on this finding it should be noted that the pretest male control mean value was high in comparison to the high dose group: 102.5 ± 38.61 vs. 60.7 ± 46.41, and remained numerically high at the zero and 7 day time points as well. Thus, undoubtedly, the magnitude of the difference between control and high dose mean values at day 14 is explained in part as inherent and unrelated to treatment.

However, on day 14, the rotorod performance in males of the high dose group, while decreased with respect to the same group's performance on pretest, day 0 and day 7, is considered an equivocal (questionable biological significance) effect of the test material, and not of such import as to identify an LEL.

Physiological Observations entail assessments of catalepsy, body temperature and body weight, tables 44-47 (pp. 202-209) of the study report. There was no evidence of an effect of the test material on any of these parameters.

II. Locomotor Activity

The subtopics of total activity and ambulatory activity findings are summarized in tables 48-51 (pp. 210-225) of the study report. Locomotor Activity measurements were performed automatically using the Digiscan 'Micro' Animal Activity System (Omnitech Electronics, Inc.). This instrument utilizes a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify each animal's motor activity. The testing of treatment groups was done according to replicate sequence. Each animal was tested separately. Total activity (counts) at day 1 for males were 1596, 1386, 1215 and 1174 for the control, low, mid and high dose groups, respectively, or approximately 25% reductions at mid and high doses. None of the mean values for dosed groups was statistically significantly different from the control as assessed by Dunnetts Test. Decreased activity among males at the 2000 mg/kg level is considered to be an effect of the test material, particularly since both total and ambulatory activities were decreased by 25% or more. However, Table 49 also indicates a 24% decrease in total count and 27% decrease in ambulatory count for the 1000 mg/kg dose group which were not statistically significant, largely due to the high (50%) standard deviation. This equivocal finding at the mid dose poses additional difficulty in establishing a clear NOEL for the study.

Relevant to the finding of decreased activity in males at day zero, data from table 49, pp. 214-215 of the study report, are appended to this review.

III. Cholinesterase Determinations

Brain cholinesterase assays (olfactory region, cerebellum, hippocampus, cerebra cortex, brainstem, midbrain) were performed at WIL Research Laboratories, Inc. Plasma and erythrocyte samples were collected and prepared frozen at WIL Research Labs and submitted to Pharmac. LSR, Inc., East Millstone, NJ for cholinesterase assays. Samples were analyzed using a method that utilized the Ellman reaction. (p. 24)

The following is the Study Director's assessment of the cholinesterase findings in the study:

> "Mean plasma and RBC cholinesterase values for the 2000 mg/kg animals were lower on day 7, (to the extent of)

24% and 40% and 38% and 39% for males and females, respectively. The mean red blood cell cholinesterase value was decreased ($p<0.05$) in the 2000 mg/kg group females on day 7. Similar decreases were not observed on days 0 or 15, and no relationship to treatment was evident. The mean plasma cholinesterase value in the 500 mg/kg group females was lower ($p<0.05$) than the control group value at the day 7 evaluation. On day 15, for males, plasma and RBC cholinesterase values were comparable to the control group. For females, on day 15, these values were still depressed at the 2000 mg/kg level - 33% and 24% for plasma and RBC cholinesterase, respectively." (p. 32 of study report).

An inspection of Table 52 (pp. 234-237 of the study report, appended), indicates a poor dose response relationship for plasma and erythrocyte inhibition for both sexes. Among females, plasma cholinesterase appears inhibited possibly at all dose levels on days 0, 7 and 15, and erythrocyte cholinesterase may be inhibited at both the high and mid dose levels on day 7, though there is no evidence of a dose response. Among males, inhibitions of both blood enzymes do not appear to extend below the high dose level. In essence females appear to be more sensitive to malathion in terms of responsiveness of the plasma and erythrocyte cholinesterase. The lack of dose response relationships and clear indications of a NOEL for the blood enzymes constitutes an inherent weakness of the study, which contributes to the study's classification as Core minimum.

Continuing with the Study Director's assessment: "On day 0, brain-region cholinesterase values for the 2000 mg/kg males were depressed by 12% in the olfactory region, when compared to the control group. By day 7, brain-region cholinesterase values were decreased 11% and 13% in the olfactory and brainstem regions, respectively, for the 2000 mg/kg males and decreased 17% in the brainstem region for the 1000 mg/kg males. Only the decrease in the 1000 mg/kg male brainstem region was statistically significant ($p<0.05$). No differences were observed at the day 14 evaluations. No such differences were observed in treated females." (p. 32 of the study report)

An independent inspection of the cholinesterase data in Table 52A (pp. 238-245 of the study report) on brain region cholinesterase inhibition does not disclose findings that would justify a conclusion that brain cholinesterase was inhibited in any brain region in either sex. In fact the essentially consistent findings across the several brain regions serves to reinforce the conclusion that 2000 mg/kg

is a NOEL for brain cholinesterase inhibition as assessed for both sexes.

Pathology

A. Non-perfused animals

As assessed on 5 rats/sex/group, mean values for brain and brain region weights, both absolute and relative to final body weight, in addition to brain region weights relative to brain weight are summarized in tables 54-65 (pp. 247-292) of the study report. An inspection of these data does not disclose any remarkable effects of dosing. There were a few statistically significant findings which could indicate an effect at the high dose level, but any such conclusion should be viewed as equivocal. The following is a comment on these particular statistically significant changes:

Among females of the high dose group on day 15 there was reduced mid-brain weight (79% of control) (p. 261 of study report) and a reduced mid-brain weight relative to brain weight (82% of control) (p. 293 of study report). However, for this same study group, mid-brain weight as expressed relative to final body weight was 88% of the control (not statistically significant) and no different from the other dose groups (p. 278 of study report) - which serves to support a conclusion that possible effects of the test material on mid-brain weight in females at the high dose as questionable at worse. Brain weight relative to body weight among females at the high dose level was increased 7.4% on day 15 (p. 277 of study report). As perhaps a corollary to this finding, brain stem and cerebral cortex weights relative to body weight were also increased by 19% and 15%, respectively (p. 278). Since there were no substantial alterations of absolute brain weight (p. 261) or brain stem or cerebral cortex (p. 262) weight, the findings observed on a body weight basis appear to be essentially reflections of a numerical reduction (10%) of bodyweight for the group (p. 277 of study report) and hence, not to be viewed as an adverse effect of dosing.

Among males of the high dose group on day 7 there was a 37% increase of olfactory bulb weight to body weight ratio (p. 271 of study report). As before in the case of females in the high dose group, this finding may be largely accounted for by the 13% reduction of body weight for this group (p. 271).

In summary the few statistically significant brain or brain region weight changes relative to body weight noted are considered to be unrelated to dosing.

B. Perfused Animals

Mean brain weights and brain dimensions for the 7 rats/sex/group assessed on day 15 for this study component are recorded in table 66 (pp. 295-296). An inspection of this table does not disclose any significant findings.

Microscopic examination (histopathology) findings for 5 rats/sex/group in this study component (groups 1 and 4) are summarized in table 67 (pp. 297-303). It is noteworthy that only the control and high dose group animals received microscopic examinations. As commented on by the Study Director in the text (p. 34 of study report) and confirmed by inspection of table 67, one high dose group male had axonal degeneration in the lumbar root and bilateral retinal "rosette". Digestion chambers were noted in the lumbar dorsal root fibers of another high dose group male and in the sciatic and tibial nerve of another high dose group male. Digestion chambers and axonal degeneration in the sciatic nerve were also observed in one control group male rat.

The histopathology slides (or photomicrographs) of retinal tissues of both eyes of the male rat (#12011) exhibiting bilateral retinal "rosette", plus those of a control rat in this study are being requested of the registrant for independent characterization. For comparative purposes, slides of retinal tissues of both eyes are also being requested for a control male rat (#14599) exhibiting unilateral retinal "rosette" (mild) in the WIL Labs Subchronic (13-week) Neurotoxicity Study of malathion (study number WIL206006).

14

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