

UNDATED

DER.#12

Malathion: 21-Day Dermal Toxicity Study in Rabbits. MB Research Laboratories. 1988. MRID 41054201. HED Doc No. 008714, 009385.

ADRENAL WIGHT Down at all
Dose (No dose response) in MALS
STAT. Sig. NO CONC
for this effect

DATA EVALUATION RECORD

Study Type: 21-Day Dermal Toxicity Study, Rabbits
OPP Guideline 82-2

P.C. Code: 057701

Tox. Chemical No.: 535

Test Material (purity): Malathion; (94% a.i.)

Synonyms O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate; O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate; diethyl mercaptosuccinate, S-ester with O,O-dimethyl phosphorodithioate; Cythion; AC6,601.

Citation: Oscar M. Moreno, 1988. 21-Day Dermal Toxicity Study with AC6,601 in Rabbits. MB Research Laboratories, Inc., Spinnerstown, PA. Study No. MB 88-9191. September 25, 1988. Unpublished.
MRID 41054201.

Sponsor American Cyanamid Company, Princeton, NJ.

Executive Summary:

In a 21-day dermal toxicity study in rabbits, groups of 6 male and 6 female New Zealand rabbits were treated dermally with undiluted technical grade malathion (94% purity) at dose levels of 0, 50, 300 or 1000 mg/kg/day for 6 hours/day, 5 days/week for 3 weeks.

At 1000 mg/kg/day, one male rabbit died on day 17 of acute mucoid gastroenteritis. Its death may have been treatment-related. With the exception of dose-related decreased cholinesterase activity in both males and females at 1000 and 300 mg/kg/day, no treatment-related toxic effects (other than one possible mortality in the 1000 mg/kg/day group) were observed in the study. No clinical signs of cholinesterase inhibition were noted. No treatment-related changes in body weights, food consumption, hematology, clinical chemistries, gross necropsies, organ weights or histopathology were observed. Dermal reactions at the application site were not observed. For males, the NOEL and LOEL respectively for cholinesterase inhibition were considered to be the following: for plasma inhibition, 50 mg/kg/day and 300 mg/kg/day (-13%); for RBC inhibition, 50 mg/kg/day and 300 mg/kg/day (-18%); for brain (cerebrum) inhibition, 300 mg/kg/day and 1000 mg/kg/day (-65%); and for brain (cerebellum) inhibition, 300 mg/kg/day and 1000 mg/kg/day (-41%). For females, the comparable NOELs and LOELs were the following: for plasma inhibition, 50 mg/kg/day and 300 mg/kg/day (-17%); for RBC inhibition, 50 mg/kg/day and 300 mg/kg/day

(-26%); for brain (cerebrum) inhibition, 50 mg/kg/day and 300 mg/kg/day (-19%); and for brain (cerebellum) inhibition, 300 mg/kg/day and 1000 mg/kg/day (-49%). For overall inhibition of cholinesterase activity in this study, for both males and females, the NOEL was 50 mg/kg/day and the LOEL was 300 mg/kg/day based on inhibition of both plasma and RBC cholinesterase activity. The systemic NOEL was 300 mg/kg/day and the LOEL was 1000 mg/kg/day based on possible mortality (1 male).

This study is **ACCEPTABLE** and **SATISFIES** guideline 82-2 for a 21-day dermal toxicity study in rabbits.

TB997:MALATH09.087



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

535

FILE

008714

OCT 18 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 21-Day Dermal Toxicity Study with Malathion in Rabbits

TOX Chem No.: 535
HED Project No.: 9-1413
MRID No.: 41054201

FROM: Brian Dementi, Ph.D., D.A.B.T.
Review Section III
Toxicology Branch-I
Health Effects Division (H7509C)

Brian Dementi 9/26/91

TO: William H. Miller, PM# 16
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Henry Spencer, Ph.D., Acting Section Head
Review Section III
Toxicology Branch-I
Health Effects Division (H7509C)

for 9/20/91

You will find appended the Data Evaluation Report (DER) for the malathion 21-day dermal toxicity study in rabbits. This study is rated Core-Supplementary. The study can be upgraded upon receipt of the cholinesterase protocol followed in the study.

As disclosed in the DER, for the study at large, LEL = 300 mg/kg/day and NOEL = 50 mg/kg/day, findings which are based upon cholinesterase inhibition.

upgrade to Guideline attached (Dementi, 3/25/92)

Reviewed by: Brian Dementi, Ph.D., D.A.B.T. *Brian Dementi 9/26/91*
Review Section III, Toxicology Branch I (H7509C)
Secondary Reviewer: Henry Spencer, Ph.D., Acting Section Head *10/10/91*
Review Section III, Toxicology Branch I (H7509C)

008714

DATA EVALUATION REPORT

Study Type: 82-2 - 21-Day Dermal Toxicity Study with Malathion
in Rabbits

Project No.: 9-1413

TOX Chem No.: 535

MRID No.: 410542-01

Test Material: AC6,601 (technical liquid, purity 94%; impurities
were not listed)

Synonyms: Malathion; Phosphorodithioc Acid, S-[1,2-
bis(ethoxycarbonyl)ethyl]0,0-dimethyl ester

Study Number: MB 88-9191

Sponsor: American Cyanamid Company
Princeton, NJ

Testing Facility: MB Research Laboratories, Inc.
Spinnerstown, PA

Title of Report: 21-Day Dermal Toxicity study with AC6,601 in
Rabbits

Author: Oscar M. Moreno

Report Issued: September 25, 1988

Classification: Core-Supplementary (Registrant must provide
the protocol for cholinesterase activity used
in the study)

Conclusions: Clinical observations did not reveal any clear
evidence of compound-related effects. One male rabbit in the high-
dose group died on day 17 of the study. Death was attributed to
"acute mucoid gastroenteritis." Hence, for clinical
signs/mortality, LEL = 1000 mg/kg/day; NOEL = 300 mg/kg/day.

Body weight gain and food consumption were not altered at any dose; NOEL = 1000 mg/kg/day.

There were numerical declines in adrenal organ weight and organ to body weight ratios for male animals at all dose levels. These reportedly were not statistically significant.

There were no dose-related clinical chemistry findings.

NOEL = 1000 mg/kg/day.

Cholinesterases (erythrocyte, plasma and brain) were inhibited. For erythrocyte and plasma cholinesterase (both sexes), LEL = 300 mg/kg/day; NOEL = 50 mg/kg/day. Cerebrum, males, LEL = 1000 mg/kg/day; NOEL = 300 mg/kg/day; females, LEL = 300 mg/kg/day; NOEL = 50 mg/kg/day; cerebellum, both sexes, LEL = 1000 mg/kg/day; NOEL = 300 mg/kg/day. Overall, for cholinesterase inhibition, LEL = 300 mg/kg/day; NOEL = 50 mg/kg/day.

Histopathology did not indicate any effects at the highest dose tested. Lower dosed animals were not examined.

A. Materials:

1. Test Compound - In Appendix 2 to the final report, the sample of AC6,601 (Lot No. AC6015-136B) used in this

study was reported as assayed at 94% a.i. technical malathion (p. 50). The test article was stored at ambient room temperature and humidity. The appearance of the test material was that of a clear gold liquid.

Test Animals - Species: rabbit; Strain: New Zealand Albino; Age: Not indicated; Weight, 2.3 to 2.7 kg for animals of both sexes; Source: Ace Animals. The test animals were acclimated to the housing facility for at least 3 weeks prior to the initiation of the study. The animals were housed in suspended stainless steel cages. Fresh bedding was placed beneath the cages and changed twice weekly. Fresh Purina Rabbit Laboratory Chow and water were available ad libitum.

B. Study Design:

(The following is quoted or paraphrased from the study) - Animals were assigned as indicated to the following test groups:

<u>Test Group</u>	<u>AC6,601 Dose (mg/kg/day)</u>	<u>Number Rabbits/Group</u>	
		<u>Male</u>	<u>Female</u>
Control	0	6	5
Low (HDT)	50	6	6
Mid	300	6	6
High (HDT)	1000	6	6

"Prior to treatment, an area on the back of the animals, approximately 10-15% of total body surface, was clipped relatively free of hair. Clipping as done weekly thereafter as necessary. The test substance was used as received and applied by gentle inunction uniformly over the clipped area of skin. The treated sites were covered with a gauze patch secured with non-irritating tape. The trunk was then wrapped with an impervious plastic material and secured with non-irritating adhesive tape.

"The animals were exposed to the test substance for a six-hour period, five days a week for three weeks. At the end of each six-hour exposure, the tape, impervious material and gauze covering the exposed site were removed, the treated areas washed thoroughly with soap and water to remove extraneous material and dried with paper towels.

"Observations were made daily for signs of toxicity, mortality, food intake, general appearance and behavior, dermal effects and gross signs of pharmacotoxic effects and recorded for each individual animal. A mortality check was made each afternoon.

"Individual body weights and food consumption were taken prior to initial treatment and weekly thereafter.

"Hematology and clinical chemistries were performed on all animals prior to treatment initiation and at termination of the study.

"Hematology parameters were as follows: hematocrit, hemoglobin, erythrocyte count, leukocyte count (total and differential), platelet count and reticulocyte count.

"Clinical chemistry parameters were as follows: calcium, phosphorus, chloride, sodium, potassium, fasting glucose; serum alanine transferase, serum aspartate aminotransferase, gamma glutamyl transpepsidase, urea nitrogen, albumin, blood creatinine, bilirubin (total and direct) and total serum protein.

"Cholinesterase levels were determined in the serum and erythrocytes prior to treatment initiation and at terminal sacrifice, and in brain (cerebrum and cerebellum separately) at terminal sacrifice.

"A gross necropsy was performed on all animals. All surfaces, orifices, cavities, viscera and organs were examined. The weight of the liver, kidneys, gonads and adrenals were recorded and ratios to body weights determined.

"The following organs were taken and preserved in 10% neutral buffered formalin:

adrenals	skin (treated area)
kidneys	skin (mammary area)
liver	testes/epididymis
ovaries	gross lesions

"The cerebrum and cerebellum were taken and frozen for cholinesterase analyses.

"A histopathology examination was performed on the above tissues for the high-dose group (Group 4) and negative control group (Group 1).

"Statistical evaluations were made using analysis of variance techniques (ANOVA) on body weights, organ weights, organ/body weight ratios, food consumption, clinical chemistry, cerebrum and cerebellum cholinesterase and hematology parameters where appropriate. In instances of significant F values in the ANOVA, the data was further evaluated using Duncan's Multiple Range Test or Student's t-test. The limit of significance in both tests was 0.05. Both the ANOVA and Duncan's Multiple Range comparisons were used to analyze the differences between groups at a given period in the study.

"The statistical evaluation of treatment effects on serum and RBC cholinesterase values involved the use of pretest values as a covariate and was performed by the sponsor as follows:

"Pretreatment values for serum and RBC cholinesterase were correlated with the terminal serum and RBC values irrespective of the dose level. Hence, the pretreatment serum and RBC cholinesterase values were used as covariates to adjust the terminal serum and RBC mean values for the various dose groups. Analysis of covariance was used to obtain these adjusted means and their comparisons with the control using Student's t-test at the $p \leq 0.05$ level. No adjustment was needed to analyze the cerebrum and cerebellum means since values were measured only at study termination" (pp. 6 and 7). (Tox Branch statistician subsequently affirmed this to be an acceptable method for analysis of data.)

C. Results

1. Clinical Observation

One male rabbit (C4547) in the 50 mg/kg group was sacrificed on day 15 due to compromised health status (few feces, yellow nasal discharge, emaciation, lethargy, and ataxia). Necropsy revealed lung abnormalities. Another male animal (C4768) in the 1000 mg/kg group died on day 17 (signs included diarrhea, lethargy, unkempt appearance, soiling of the anogenital area, and evidence of a red rectal discharge). Death was attributed to acute mucoid gastroenteritis. The study author concluded that the health problem did not appear to be treatment-related

in the case of the former rabbit's (C4547) condition but that an effect of the test material in the latter case cannot be ruled out even though this is a common abnormality in New Zealand Albino rabbits (p. 8).

Clinical observations did not reveal any clear evidence of a compound-related effect. An inspection of Table 1 (pp. 11 to 18) reveals that certain animals in all groups, controls included, exhibited certain clinical signs of ill health such as diarrhea, few feces, yellow nasal discharge, soiled anogenital area, etc. However, the number of animals so affected was not dose-related or disproportionately expressed in dosed groups. In the control group there were two males and two females which displayed generally consistent health effects of the type described above during the course of study. Likewise, there were three males (one sacrificed) and three females so affected in the low dose group; one or two males and one female in the mid-dose group were affected and two males (one died) and one female exhibited the effects in the high-dose group. This reviewer does not consider that the clinical findings represent any clear evidence of an effect of AC6,601. The one death in the high dose group may have resulted from dosing. With respect to acute mucoid gastroenteritis as the cause of death in the animal in question, the study author conceded that "although this is a common abnormality in New Zealand Albino rabbits, a treatment

related effect can not be ruled out." (p. 8). NOEL = 300 mg/kg; LEL = 1000 mg/kg.

2. Body Weight - Body weight gain for the duration of the experiment (i.e., from pretest to termination) was similar for all groups including the control groups (Table 2, pp. 20 to 23). While at week 1 there were statistically significant differences in body weight for the low- and mid-dose groups with respect to the control group (Table 3, p. 24), such findings were not noted for the high-dose group or for any dose group beginning at week 1. This reviewer is of the opinion that body weight was not meaningfully affected at any dosage level by the test material. Hence, for this parameter, NOEL = 1000 mg/kg for rabbits of both sexes.

3. Food Consumption - An inspection of food consumption data did not, in the opinion of this reviewer, disclose any apparent effects of dosing with AC6,601 upon food consumption at any dosage level (Table 8, pp. 39 to 42).

4. Organ Weight and Organ/Body Weight Ratios - Review of Tables 4 (p. 27) and 5 (p. 31) did not disclose any effect of dosing upon absolute organ weights or organ/body weight ratios for the liver, kidneys or gonads. With respect to adrenals, there were no effects among females; however, for males both parameters, organ weight and the ratio, were less in all dose

groups with respect to the controls. The effect on absolute adrenal weights were not statistically significant. However, the organ/body weight ratios were significantly altered at all dosage levels. These mean values are tabulated as follows:

<u>Adrenals (Male)</u>	<u>Dosage Group</u>			
	<u>Control</u>	<u>Mid</u>	<u>Low</u>	<u>High</u>
Absolute Organ Weight, g	0.42 (0.36)*	0.29	0.32	0.28
Organ/Body Weight g/kg	.146 (.127)*	.098	.096	.095

* Mean value excluding rabbit #C4554.

These data suggest an effect of the test material; however, the absence of a dose response renders such a conclusion equivocal. The study author acknowledges this finding but expresses the view that "the difference appears to be primarily due to an abnormally high weight for the adrenals in one animal (C4554). The biological significance of the difference is questionable since the absolute adrenal weight from group 1 males is not statistically different from treated group males; the absolute and relative adrenal weights for control females are not different from treated females; there is no dose response relationship and no gross or histopathological changes clearly attributable to treatment were noted at sacrifice" (p. 8).

For purposes of comparison, the male control group mean values for adrenal weight and adrenal/body weight ratio were calculated for five control animals excluding animal #C4554. Excluding this animal, which had an adrenal weight notably higher than other animals of that group, did help to normalize the mean values with respect to those of the dosed groups. Independent analysis of variance computations by Tox Branch on the adrenal/body weight data confirmed the significance of an effect with rabbit #C4554 included among control animals ($p = 0.001$), but also revealed as significant ($p = 0.017$) the analysis when rabbit #C4554 was excluded from control data. Furthermore, dose groups were compared with the control group using Dunnett's test, and all pairwise comparisons were significant with or without inclusion of rabbit #C4554. Hence, a NOEL was not identified for effects on adrenal weight in male rabbits.

5. Dermal Reactions - The Guidelines require under "Observations of Animals" various clinical observations, including examination of skin and fur. While no effects on the skin were noted previously, a close examination for erythema and edema at the end of each week of exposure yield data which indicate that treated animals, as opposed to controls, exhibited mild dermal effects. Table 10 (p. 45) showing these results is duplicated below:

Mean Erythema and Edema Scores

<u>Group</u>	<u>Week 1</u>	<u>Week 2</u>	<u>Week 3</u>
Control	0	0	0.04
Low Dose	0.25	0.25	0.32
Mid Dose	0.33	0.75	0.88
High Dose	0.13	0.54	0.54

the study author concluded that "application of the test article caused slight irritation of rabbit skin" (p 8). This slight irritation from a practical standing is considered innocuous.

6. Laboratory Investigations

- (a) Hematology - Examination of Table 11 (p. 46) and the statistical treatment of hematology data (Table 13, p. 54) did not disclose any remarkable effects of dosing upon hematology parameters. Possible exceptions include statistically significant increases in basophils for mid- and high-dose groups and a possible dose-related increase in reactive lymphocytes predominately in females (p. 47). However, such values fall within normal ranges for these animals.
- (b) Clinical Chemistry - In the opinion of this reviewer, none of the clinical parameters examined was altered in a biologically meaningful way by any dosage level of malathion. It should be noted that

among males, SGOT was numerically reduced in the high-dose group and the study author notes a decline in sodium among females at the high-dose. In view of the lack of an effect on so many parameters for both males and females, this reviewer considers the effects noted on SGOT and sodium to be a consequence of random variation.

(c) Cholinesterase Data - Mean values for RBC and plasma cholinesterase are presented in Table 15 (p. 90) of the study. The same table also presents mean values for brain (cerebrum and cerebellum) cholinesterase. The following is a tabulation of percent of control activity for each dose group for the various enzymes.

		% of Control		
		<u>50</u> Low	<u>300</u> Mid Δ	<u>1000</u> High
Serum	(M)	88.5	87.2 - 1.3	42.9*
	(F)	100.5	82.9 - 17.6	52.5*
RBC	(M)	103.3	82.3 - 21.0	25.1*
	(F)	92.0	74.4* - 17.6	26.1*
Cerebrum	(M)	93.0	97.5 - 19.6	34.5*
	(F)	112.0	80.8	47.5*
Cerebellum	(M)	117.7	145.4*	59.2*
	(F)	117.7	112.0	50.8*

* Statistically significant at $p < 0.01$.

It is clear from these data that treatment with AC6,601 substantially inhibited all forms of cholinesterase assayed, ^{though methods were not submitted.} All high-dose inhibitions were statistically significant at $p \leq 0.01$. At the mid-dose, erythrocyte cholinesterase was also significantly ($p \leq 0.01$) inhibited in females. Erythrocyte cholinesterase for males was numerically inhibited at the mid-dose. Serum cholinesterase for males ^{NOT 50; 58.5 vs 57.2} and females was numerically inhibited to the extent of about 15 percent though neither was statistically significant. Cerebral cholinesterase for females was inhibited to the extent of about 19 percent, but was not reported as statistically significant. This reviewer is of the opinion that the mid-dose should be viewed as an effect level for serum and erythrocyte cholinesterase of both sexes. All of these mid-dose findings appear to be part of a dose response, with one of them (erythrocyte activity, female) being statistically significant. Likewise, this reviewer considers inhibition of cholinesterase of the cerebrum of females at the mid-dose to be an effect of dosing. Hence, for plasma and erythrocyte cholinesterase of both sexes, LEL = 300 mg/kg/day and NOEL = 50 mg/kg/day. For the cerebrum, male LEL = 1000 mg/kg/day, NOEL =

LEL is
NO
NOEL
INHIBITION
AT MID
Dose

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300 mg/kg/day, female LEL = 300 mg/kg/day, NOEL = 50 mg/kg/day. For cerebellum, both sexes, LEL = 1000 mg/kg/day and NOEL = 300 mg/kg/day.

Overall for cholinesterase inhibition, LEL = 300 mg/kg/day, NOEL = 50 mg/kg/day.

7. Gross Necropsy Observations

Necropsy finding as presented in table 16 (p. 95) did not disclose any dose-related effects.

8. Histopathology Observations

Results of the histopathologic examination of the tissues indicated above are presented in the pathology report (Appendix 2 to the study).

An examination of this report did not reveal any remarkable non-neoplastic or neoplastic dose-related findings.

There may have been some skin (non-neoplastic) effects in one or two female animals of the high-dose group (Appendix 2, p. 10). This reviewer does not consider this to provide sufficient evidence that the high dose will elicit such effect (acanthosis of epidermis, hyperemia of dermis, hyperkeratosis of epidermis and (inflam lympho sa/dermis)).



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

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MAR 25 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: CORE Upgrade of 21-Day Dermal Toxicity Study with
Malathion in Rabbits

Tox. Chem. No:535
HED Project No.: 2-0805
I.D. No.: 057701
Record No.: S 408703

FROM: Brian Dementi, Ph.D., D.A.B.T.
Review Section III
Toxicology Branch I
Health Effects Division (H7509C)

Brian Dementi 3/16/92

TO: Joanne Edwards
PM Team 74
Reregistration Branch
Special Review and Reregistration
Division (H7508C)

THRU: Henry Spencer, Ph.D.
Acting Section Head, Review Section III
Toxicology Branch I
Health Effects Division (H7509C)

Head 3/16/92

*K/B
3/19/92*

Action Requested

The study in question, a 21-day dermal toxicity study with malathion (MRID# 41054201), was reviewed in Toxicology Branch on September 26, 1991. The study was rated CORE supplementary pending submission of the protocols used for cholinesterase assays performed in the study. The Registrant has now submitted the requested cholinesterase procedures, and accordingly requests the study be upgraded.

CONCLUSION

Toxicology Branch recommends that the review of the 21-day dermal toxicity study of malathion be upgraded to CORE guideline.

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TOXICOLOGY BRANCH ONE-LINERS

Tox Chem No. 535

EPA

File Last Updated _____

Accession/
MRID

TOX Category
CORE Grade/
Doc. No.

Study/Lab/Study #/Date

Material

LD50, LC50, PIS, NOEL, LEL

Results:

21-DAY DERMAL TOXICITY
STUDY WITH AC-6601 IN
RABBITS/MRS RESEARCH LABS,
EM, SPANNERSTOWN, PA / STUDY
NO. M888-9191 / SEPT. 25, 1988
AC-6601
(MILLATHION)
4/05/92-01
STUDY UPGRADE FROM CORE
SUPPLEMENTARY TO CORE QUINOLINE
(REGISTRANT HAS SUBMITTED REQUESTS AS
FOR CHINESE/VIETNAMESE ADJUDGMENTS AS
REQUIRED IN THE ORIGINAL GUIDELINES
FOR THE UPGRADE)

M/H

CORE Quinoline