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

004997

MAR 19 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Methyl Parathion Data Call-In: Rat/Rabbit Teratology;
Rat Chronic Feeding Study;
Accession Nos.: 259403,-04,-05,-12,-13 and -14;
Record No. 160444; EPA I.D. No. 4787-4;
Action Code No. 600; Caswell No. 372

TO: Edward Allen (PM #12)
and
Jay Ellenberger
Registration Division (TS-767)

FROM: Alan C. Katz, MS, DABT 3/12/86
Toxicology Branch
HED (TS-769C)

THROUGH: Clint Skinner, Ph.D, DABT 3-19-86
Head, Review Section III
and
Theodore M. Farber, Ph.D, DABT 3/19/86
Chief, Toxicology Branch

Action Requested:

Review captioned reports which were submitted in response to the data call-in of September, 1981.

Background:

This chemical is undergoing active Registration Standards Review. The following studies were reviewed:

1) Machemer, L.: Parathion-methyl, Evaluation for embryotoxic and teratogenic effects on rats following oral administration.(Unpublished Report No. 6825 prepared by Bayer AG Institute of Toxicology, Wuppertal, West Germany; submitted by Cheminova, Lemvig, Denmark; dated June 3, 1977). Accession No. 257512.

2) Renhof, M.: Parathion-methyl (Folidol M active ingredient), Study for embryotoxic effects on rabbits after oral administration. (Unpublished Report No. 12907, prepared by Bayer AG Institute of Toxicology, Wuppertal, West Germany; submitted by Cheminova, Lemvig, Denmark; dated September 4, 1984). Accession Nos. 259403 through 259405.

3) Bomhard, et al.: E 605-methyl chronic toxicological study on rats. (Unpublished study Nos. 9389 and 12559 prepared by Bayer AG Institute of Toxicology, Wuppertal, West Germany; submitted by Cheminova, Lemvig, Denmark; dated March 31, 1981). Accession Nos. 257513 and 257514.

Conclusions/Recommendations:

1) The rat teratology study is classified as Supplementary data and may be upgraded following submission of additional information, as specified in the attached DER. The reported results suggest that methyl parathion is embryo/fetotoxic at 1.0 mg/kg, but not at 0.3 mg/kg. No treatment-related fetal malformations were apparent. Maternal toxicity was not established, however.

2) No evidence of teratogenicity, embryotoxicity, fetotoxicity or maternal toxicity was reported in rabbits treated with methyl parathion by gavage at doses up to and including 3 mg/kg/day during days 6-18 of gestation. The data are classified as Supplementary. Additional information, as specified in the DER, is required in order to upgrade this classification.

3) The rat oncogenicity/chronic toxicity study is classified Core Supplementary. Deficiencies include: inadequate and incomplete reporting of histopathologic observations; lack of clinical observations, palpations for tissue masses or ophthalmic examinations; insufficient animals for clinical pathology laboratory determinations; and lack of a quality assurance statement. Findings included increased incidence of thyroid tumors and Leydig cell tumors in high dose (50 ppm) males, pituitary adenomas in mid dose (10 ppm) females, and adenocarcinomas of the uterus in mid and high dose females. Methyl parathion treatment caused inhibition of brain, plasma and RBC cholinesterase activity. However, there is no indication that nerve tissue was examined. The NOEL based on cholinesterase inhibition is 2 ppm. Body weights of high dose males and females were reduced. The NOEL for systemic toxicity is $\leq$ 50 ppm.

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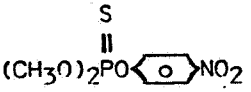
DATA EVALUATION REPORT

A. Compound:

Methyl Parathion; (O,O-dimethyl O-p-nitrophenyl phosphorothioate)



B. Study Report Citation:

Title: "Parathion-methyl: Study of Embryotoxic Effects on Rabbits After Oral Administration"

Testing Facility: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld

Report Number: 12907

Study Number: T9007877

Date: 9/4/84

Submitted to EPA by: A/S Cheminova, Lemvig, Denmark

Author: Dr. M. Renhof

C. Reviewed By: Alan C. Katz, M.S., D.A.B.T
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)
(Signature)
12/23/85
(Date)

D. Secondary Review By: Robert P. Zendzian, Ph.D.
Acting Head, Review Section IV
(Signature)
12/23/85
(Date)

E. Classification:

Supplementary data. This classification may be upgraded following submission of additional data (see "Discussion/Recommendations").

F. Conclusions:

No evidence of teratogenicity, embryotoxicity, fetotoxicity or maternal toxicity was reported in rabbits treated with methyl parathion by gavage at doses up to and including 3 mg/kg/day on gestational days 6-18. Additional data are required.

G. Materials:

Test compound: Methyl parathion; Batch 230 206 015

Purity: 95.7%

Animals: Himalayan rabbits
Strain: CHBB:HM
Supplier: Thomae Co., Biberach a.d. Riss
Age: "Sexually Mature"; age not specified
Weight: Males- approx. 2.5 kg
Females- 2.1-2.9 kg

Diet: Ssniff K4 Rabbit Diet; Ssniff Specialfutter GmbH, Soest

H. Methods:

Mated females (15 per group) were administered methyl parathion in 0.5% aqueous Cremophor EL emulsion by gavage (5 ml/kg body weight) on Days 6 through 18 of gestation. The doses were 0, 0.3, 1.0 and 3.0 mg/kg. The control animals received the Cremophor EL emulsion.

Doses were selected based on the results of a rangefinding experiment. No details of the design or results of the rangefinder were given.

Room temperature was within a range of 20-24°C and relative humidity was approximately 45%. Lighting in the room was on a 12-hour on/off cycle. Food and water were provided ad libitum. Quarantine/acclimation conditions and duration were not specified. The method of identification of animals was not specified.

The study was terminated on Day 29, and the following observations or calculations were made: number of implantations; number of live and dead embryos; sex of all live fetuses; litter weights and mean fetus weight per litter; inspection of all fetuses for external malformations; examination of fetus heads for visceral malformations by the author's modification[1] of the Wilson technique[2] (the nature of this modification was not specified in the report); evisceration and examination of the abdominal and thoracic organs, followed by clearing of the fetuses with potassium hydroxide solution and evaluation of the skeletal system after staining with Alizarin Red S. Reference was made to "standardized" methods of examination[3,4] which were not described in the report itself, and copies of the reference articles were not provided with the report.

Data for weight gain, fetal and placental weights, and numbers of implantations, fetuses and resorptions were analyzed using the U test of Wilcoxon, Mann and Whitney. The Chi-square test with the Yates correction was used to analyze numbers of fetuses with bone alterations, as well as stunted fetuses and fetuses with other anomalies.

I. Results:

Two dams (1 control; 1 mid dose) died during the study. No treatment-related clinical signs were reported. Gross pathological findings

I. Results (cont'd):

at necropsy were not reported for any of the animals.

The following table, excerpted from the study report, summarizes the body weight gains of the pregnant females during the treatment period as well as over the course of the entire gestation period.

Dose in mg./kg.	Average weight gain in grams during treatment period	entire gestation period
0	-26.3	125.4
0.3	53.0	203.3
1.0	8.9	170.7
3.0	21.3	240.3

Data for individual weight gains were also included in the study report. However, no absolute body weight data were presented for the dams. Using the limited data presented in the study report, we have derived the following table, which demonstrates a wide range of values:

Dose (mg/kg)	Number of dams pregnant on day 29	Range of weight gain (loss) during treatment period	entire gestation period
0	12	(-265) - 105	(-230) - 360
0.3	15	(-30) - 175	(-40) - 430
1.0	14	(-200) - 300	(-40) - 490
3.0	15	(-130) - 130	20 - 460

There was no evidence of a treatment-related effect on maternal body weights.

Implantation, resorption and litter data may be summarized as follows:

Dose (mg/kg)	Mean No. of Implantations	Mean No. of Fetuses Male	Female	Total	Dead and Resorbed Embryos
0	6.4	2.3	2.0	4.3	2.1
0.3	6.3	2.5	3.5	6.1	0.3
1.0	6.6	2.8	3.1	5.9	0.7
3.0	7.7	3.5	3.5	6.9	0.7

I. Results (cont'd):

The high rate of post-implantation losses in the control group is primarily attributable to the loss of 2 complete litters (represented by a total of 15 implants) in that group. However, the incidence of litters with 1 or more post-implantation losses also appears somewhat higher than expected in the control group. The following calculations, although not included in the study report, are presented here to augment this evaluation:

Dose (mg/kg)	% Viable fetuses†	% Dams with 1 or more post-implantation losses
0	67	79
0.3	97	27
1.0	89	43
3.0	90	40

†Number of fetuses divided by number of implantations.

Historical control data with respect to post-implantation losses were not reported. There is no evidence of a treatment-related effect on post-implantation loss.

No treatment-related fetal malformations were apparent. Arthrogryposis was the most common malformation observed, and its incidence in treated groups was no greater than in the controls. Two mid dose fetuses showed multiple malformations which are not considered treatment-related, based on the nature and low incidence of malformations in the high dose group. Mean fetal and placental weights in the methyl parathion-treated groups were comparable to control values.

Dose (mg/kg)	Mean wt. (g) Fetus	Placenta
0	41.4	4.8
0.3	41.6	4.8
1.0	41.1	4.6
3.0	40.7	4.4

J. Discussion/Recommendations:

The results of this study suggest that oral administration of methyl parathion at doses up to and including 3.0 mg/kg/day during gestation (days 6-18) did not cause teratogenicity, embryo/fetotoxicity or maternal toxicity in rabbits. No evidence was presented to indicate that maternal toxicity would be expected at or near the highest dosage administered. The registrant should explain the rationale for the selection of doses in this

J. Discussion/Recommendations (cont'd):

study. Submission of a brief summary of the results of the rangefinding experiment used for selection of doses for this study may be sufficient. As noted in the 1982 Subdivision F Guidelines, "Unless limited by the physical/chemical nature or biological properties of the substance, the highest dosage level should include some overt maternal toxicity..."

While reporting the group mean body weight gain data in grams during the treatment period offers useful information, it does not provide a complete picture. Individual and group mean absolute body weight data must be presented for review.

Since dead fetuses were apparently counted with resorbed embryos, the combined data do not provide a sensitive means of analysis; these data should be separated in a manner which would allow evaluation of possible treatment-related differences with respect to the approximate time of death in utero.

In order to complete its evaluation, the Agency requires, in addition to the data cited above: a copy of the protocol and a description of any deviations from the protocol; a copy of each of the References (in English) #1, 3 and 4; individual clinical observations and necropsy findings for the dams; historical control data with respect to post-implantation losses in rabbits of the same strain; a description of conditions of storage of the test material and dosing mixtures; and analytical results with respect to homogeneity, concentration and stability of the test material in the dosing mixtures.

K. References:

1. Machemer, L. and Stenger, E. Zur Beurteilung der Foeten im teratologischen Experiment. Modifikation dr "Wilson-Technik". Arzneim. Forsch. (Drug Res.) 21, 144-145, 1971.

2. Wilson, J. In: Teratology, Principles and Techniques. Eds.: J.Wilson and J. Warkany, The University of Chicago Press, Chicago and London, 1965, 262-277.

3. Lorke, D. Zur Methodik der Untersuchungen embryotoxischer und teratogener Wirkungen an der Ratte. Arch. exp. Path. u. Pharmak. 246, 147-151, 1963.

4. Lorke, D. Embryotoxische Wirkungen an der Ratte. Arch. exp. Path. u. Pharmak. 250, 360-382, 1965.

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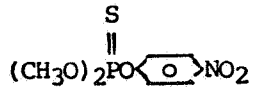
DATA EVALUATION REPORT

A. Compound:

Methyl Parathion; (O,O-dimethyl O-p-nitrophenyl phosphorothioate)



B. Study Report Citation:

Title: "Parathion-methyl: Evaluation for Embryotoxic and Teratogenic Effects on Rats Following Oral Administration"

Testing Facility: Bayer AG, Institut Fur Toxikologie, Wuppertal-Elberfeld

Report Number: 6825

Date: 6/3/77

Submitted to EPA by: A/S Cheminova, Lemvig, Denmark

Author: Dr. L. Machemer

C. Reviewed By: Alan C. Katz, M.S., D.A.B.T
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

(Signature)

12/10/85
(Date)

D. Secondary Review By: Robert P. Zendzian, Ph.D.
Acting Head, Review Section IV

(Signature)

12/16/85
(Date)

E. Classification:

Supplementary data. This classification may be upgraded following submission of additional data (see "Discussion/Recommendations").

F. Conclusions:

Results of this study suggest that methyl parathion is embryotoxic or fetotoxic at 1.0 mg/kg, but not at 0.3 mg/kg. Maternal toxicity was not established. Additional data are required.

G. Materials:

Test compound: Methyl parathion; BAYER 11405; Parathion M 100 technical; Prod. No. 2830

Purity: 94.4%

Animals: Wistar SPF
Supplier: F. Winkelmann, Borchen
Age: Females: 2 1/2 - 3 1/2 months (192-239 g)
Males: 3 - 5 months (300-400 g)

Diet: Altromin R feed

H. Methods:

Pregnant females (20-24 per group) were administered methyl parathion in 1.0% aqueous Cremophor EL emulsion by gavage (10 ml/kg body weight) on Days 6 through 15 of gestation. The doses were 0, 0.1, 0.3 and 1.0 mg/kg. The control animals received the Cremophor EL emulsion.

The selection of doses for this study was based on the results of a rangefinding experiment in non-pregnant rats, using doses of 1, 3 and 7.5 mg/kg/day. Five rats per group were treated. All of the rats given 7.5 mg/kg/day died within 3 days. Two animals in the 3 mg/kg group died within 5 days, and dosing was discontinued for this group. No adverse clinical signs or reduced weight gain were reported in any of the rats given 1 mg/kg/day during the 10-day experimental period.

Except during mating, the animals were individually housed in Type II Makrolon cages. Room temperature was within a range of 20-23°C and relative humidity was approximately 60 %. Lighting in the room was on a 12-hour on/off cycle. Food and water were provided ad libitum. Quarantine/acclimation conditions and duration were not specified.

Untreated virgin rats were mated overnight, with 1 male and 2 females per cage. Mating was confirmed by observation of sperm in vaginal smears the morning after mating. The methods of identification and randomization of animals was not specified.

The study was terminated on Day 20. Fetuses were removed by caesarian section and subjected to examination for external anomalies; measurement of litter weight and calculation of average fetus weight per litter; determination of sex; and examination for visceral and skeletal malformations. About 30% of the animals were examined for visceral malformations by the author's modification[1] of the Wilson technique[2]. The nature of this modification was not specified in the report. The remaining fetuses were eviscerated and the abdominal and thoracic organs were examined; they were then cleared with potassium hydroxide solution and the skeletal system was evaluated after staining with Alizarin Red S. The report does not mention the method used to designate which of these examination techniques would be applied to each fetus. Reference was made to "standardized" methods of examination[3,4] which were not described in the report itself, and copies of the reference articles were not provided with the report.

H. Methods (cont'd):

Data for weight gain, fetal and placental weights, and numbers of implantations, fetuses and resorptions were analyzed using the U test of Wilcoxon, Mann and Whitney. The Chi-square test with the Yates correction was used to analyze numbers of fetuses with bone alterations, as well as stunted fetuses and fetuses with other anomalies.

I. Results:

None of the treated or control rats died during the study. No treatment-related clinical signs were reported.

The following table, excerpted from the study report, summarizes the body weight gains of the pregnant females during the treatment period as well as over the course of the entire gestation period.

Dose in mg./kg.	Average weight gain in grams during treatment period	entire gestation period
0	21.3	74.2
0.1	18.8	72.3
0.3	22.2	74.9
1.0	12.2*	63.1

*significantly different from control at $p < 0.01$

According to the investigator for the study, the above data indicate that "treatment with the 1 mg./kg./day dose had a toxic effect on the dams." Data for individual weight gains were included in a separate table in the study report. However, no absolute body weight data were presented for the dams. Using the limited data presented in the study report, we have derived the following table, which demonstrates a wide range of values:

Dose (mg/kg)	Range of weight gain (loss) during treatment period	entire gestation period
0	6 - 27	47 - 91
0.1	(-43)- 31	(-24)- 99
0.3	15 - 28	53 - 95
1.0	(-23)- 28	13 - 97

There were a total of 20 pregnant rats in each of the 4 groups. There was no apparent case in which an entire litter may have been lost.

I. Results (cont'd): 004997

Implantation, resorption and litter data may be summarized as follows:

Dose (mg/kg)	Mean No. of Implantations	Mean No. of Fetuses Male	Female	Total	Dead and Resorbed Embryos
0	10.4	3.9	5.1	9.0	1.4
0.1	11.3	3.9	6.1	10.0	1.2
0.3	10.2	4.4	4.1	8.5	1.7
1.0	9.3	3.7	3.9	7.5	1.7

The slightly lower number of implantations in the high dose group is not attributable to methyl parathion administration, since treatment did not begin until day 6. Although the number of fetuses in the high dose group was slightly reduced, it does not appear to represent a significant difference when viewed in the context of the non-treatment-related reduction in the number of implantations. The following calculations, although not included in the study report, are presented here to augment this evaluation:

Dose (mg/kg)	% Viable fetuses†	% Dams with 1 or more dead/resorbed embryos
0	87	75
0.1	88	45
0.3	83	60
1.0	81	55

†Number of fetuses divided by numoer of implantations.

No treatment-related fetal malformations were apparent. However, reduced mean fetal weights and an increased incidence of stunted fetuses (weighing less than 3 grams) were reported to be related to treatment at the high dose level. The incidence of stunted fetuses was also increased in the low dose group; however, the likelihood of a relationship to treatment was discounted on the basis that "the difference between the 0.1 mg./kg. group and the control resulted mainly from light fetuses of 2 dams." Fetal and placental weight data may be summarized as follows:

Dose (mg/kg)	Mean wt. (g) Fetus	Placenta	Mean number of stunted fetuses/litter	No. of litters with 1 or more stunted fetuses
0	3.40	0.62	0.50	5
0.1	3.27	0.57	1.65**	7
0.3	3.39	0.64	0.95	7
1.0	3.25*	0.62	1.35**	11

* significantly different from control at $p<0.05$
** significantly different from control at $p<0.01$

J. Discussion/Recommendations:

The data presented in the study report were not sufficient to conclude that maternal toxicity was demonstrated. While reporting the group mean body weight gain data in grams during the treatment period offers useful information, it does not provide a complete picture. Individual and group mean absolute body weight data must be presented for review.

Since dead fetuses were apparently counted with resorbed embryos, the combined data do not provide a sensitive means of analysis; these data should be separated in a manner which would allow evaluation of possible treatment-related differences with respect to the approximate time of death in utero.

In order to complete its evaluation, the Agency requires, in addition to the data cited above: a copy of the protocol and a description of any deviations from the protocol; a copy of each of the References (in English) #1, 3 and 4; individual clinical observations and necropsy findings for the dams; a description of conditions of storage of the test material and dosing mixtures; and analytical results with respect to homogeneity, concentration and stability of the test material in the dosing mixtures.

K. References:

1. Machemer, L. and Stenger, E. Zur Beurteilung der Foeten im teratologischen Experiment. Modifikation dr "Wilson-Technik". Arzneim. Forsch. (Drug Res.) 21, 144-145, 1971.

2. Wilson, J. In: Teratology, Principles and Techniques. Eds.: J.Wilson and J. Warkany, The University of Chicago Press, Chicago and London, 1965, 262-277.

3. Lorke, D. Zur Methodik der Untersuchungen embryotoxischer und teratogener Wirkungen an der Ratte. Arch. exp. Path. u. Pharmak. 246, 147-151, 1963.

4. Lorke, D. Embryotoxische Wirkungen an der Ratte. Arch. exp. Path. u. Pharmak. 250, 360-382, 1965.

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EPA: 68-02-4225
DYNAMAC No. 1-68A-1,2
February 24, 1986

DATA EVALUATION RECORD

METHYL PARATHION

Chronic Feeding Study in Rats

STUDY IDENTIFICATION: Bomhard, E., Loeser, E., and Schilde, B. E 605-methyl chronic toxicological study on rats. (Unpublished study Nos. 9889 and 12559 prepared by Bayer AG Institute of Toxicology, Wuppertal, West Germany, for Cheminova, Lemvig, Denmark; dated March 31, 1981.) Accession Nos. 257513 and 257514.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-24-86

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1. CHEMICAL: Methyl parathion; E 605-methyl; O,O-dimethyl-O-(4-nitrophenyl)-monothiophosphate.

2. TEST MATERIAL: E 605-methyl from batch No. 2306/06099, contained 94.8 percent active ingredient.

3. STUDY/ACTION TYPE: Chronic feeding study in rats.

4. STUDY IDENTIFICATION: Bomhard, E., Loeser, E., and Schilde, B. E 605-methyl chronic toxicological study on rats. (Unpublished study Nos. 9889 and 12559 prepared by Bayer AG Institute of Toxicology, Wuppertal, West Germany, for Cheminova, Lemvig, Denmark; dated March 31, 1981.) Accession Nos. 257513 and 257514.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Feb 24, 1986

Robert J. Weir, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner for
Date: 2-24-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Carcinogenicity/Chronic Effects
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-24-86

Alan Katz, M.S., D.A.B.T.
EPA Reviewer

Signature: Alan C. Katz
Date: 3/10/86

Clint Skinner, Ph.D., D.A.B.T.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

The oncogenic potential of E 605-methyl could not be completely assessed from the data provided. Under the conditions of the study, when E 605-methyl when was fed to Wistar rats for 2 years there was an increase in the incidence of thyroid adenomas in male rats receiving 50 ppm ($p \leq 0.05$) and pituitary adenomas in females receiving 10 ppm ($p \leq 0.05$). There were also nonsignificant ($p > 0.05$) increases in Leydig cell tumors of the testis in males receiving 50 ppm and in adenocarcinomas of the uterus at 10 and 50 ppm. Body weights were significantly decreased when compared to controls in males and females receiving 50 ppm. Brain cholinesterase was significantly decreased in males and females receiving 50 ppm (50 and 63 percent, respectively, $p \leq 0.01$) and marginally decreased (22 percent, $p \leq 0.05$) in males receiving 10 ppm. Red cell and plasma cholinesterase were decreased in both sexes at 10 and 50 ppm. There were marginal effects on clinical chemistry and hematologic parameters in females receiving 50 ppm and their toxicologic importance is dcubtful. There was a slight decrease in total protein at all intervals and plasma urea at 3 and 12 but not 6 and 24 months. Mean values for Hct and Hb were decreased and reticulocytes increased in females receiving 50 ppm at 24 months due to severe anemia in one animal. Changes in organ weights were not remarkable and had no histological correlates. Therefore, the LOEL for systemic toxicity is 50 ppm and the NOEL is 10 ppm. The NOEL based on cholinesterase inhibition is 2 ppm.

Core Classifications: Core Supplementary for oncogenicity and chronic toxicity because of deficiencies in reporting the histopathologic findings. Other study deficiencies discussed in Section 14 of the DER were: lack of clinical observations, palpations for tissue masses or ophthalmic examinations; insufficient animals were used for clinical laboratory studies and cholinesterase activity determinations; all guideline required tissues (in particular nerve) and animals at interim sacrifice were not evaluated histopathologically; and no quality assurance was provided.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for complete details.)

Wistar TNO/W 74 rats from Winkelmann, Borchen were used in the study. At study initiation, rats were 5-6 weeks old and males weighed an average of 85 g and females weighed 80 g. Animals were caged individually in a temperature-controlled room with a 12-hour light/dark cycle, and they were offered food (Altromin 1321) and tap water ad libitum.

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[1] Only items appropriate to this DER have been included.

Groups of 50 males and 50 females were fed diets containing 2, 10, or 50 ppm E 605-methyl, and 100 animals/sex served as the controls. An additional 10 animals/sex/group were used for interim sacrifices of 5/sex/group at 6 and 12 months.

Animals were examined daily for toxic signs. Body weights were determined weekly for 26 weeks and every 2 weeks thereafter. Food consumption by dose group and sex was determined weekly.

Clinical laboratory studies were conducted on samples collected from 5 animals/sex/group at 1, 3, 6, and 12 months and from 10 animals/sex/group at study termination. The following hematologic parameters were measured: erythrocyte, leukocyte, platelet, and reticulocyte counts, hemoglobin (Hb) level, hematocrit (Hct) value, and differential blood count. Thromboplastin time was determined at the end of the study only.

Plasma levels of alkaline phosphatase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and glutamate dehydrogenase, creatinine, urea, glucose, cholesterol, bilirubin, and total protein were determined.

Plasma and erythrocyte cholinesterase were determined using the method of Ellman[2] on five rats/sex/group at 1, 2, 4, 8, 13, 26, 52, 78, and 105 weeks and brain cholinesterase was determined on five/sex/group at study termination.

Urine was collected over 16 hours, and glucose, blood, pH, ketone bodies, bilirubin, urobilinogen, and sediment were determined semi-quantitatively; protein was quantitated.

Five males and five females/group (extra animals) were sacrificed at 6 and 12 months, and all of the surviving animals were sacrificed at 2 years. Animals that died or were sacrificed were dissected and examined grossly. The following organs were weighed for animals at interim sacrifices and at the terminal sacrifice: thyroid, heart, liver, lung, spleen, kidneys, adrenals, testes, and ovaries. In addition, the thymus was weighed in animals sacrificed at 6 and 12 months. Approximately 30 tissues for all animals were fixed in formalin for histopathologic evaluation. Tissues from animals at interim sacrifices were not examined histologically.

Means, standard deviations, and 95 and 99 percent confidence limits were determined, and group comparisons for significance were made with the Mann, Whitney, and Wilcoxon method. Mortality data were analyzed by the Fisher exact test.

B. Protocol: A protocol was not provided.

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[2]Ellman, G. L. Biochem. Pharmacol. 7 (1961): 88-95.

12. REPORTED RESULTS:

Dietary Analysis: No data on analyses of dietary levels, stability, or homogeneity of the test compound in the diets were presented.

Clinical Observations: Males and females receiving 50 ppm showed symptoms of cholinesterase inhibition (tremors) in the first 2 study weeks and during weeks 19-21. Three males and six females receiving 50 ppm died in the first 2 weeks. Cholinergic symptoms occurred sporadically in high-dose females after week 81. Since individual animal observations or summary tabulations were not presented, the incidence of these signs could not be determined. Mortality data at selected intervals are presented in Table 1. Mortality was significantly increased in high-dose females in the first 2 weeks of the study.

Body Weights and Food Consumption: Mean body weights were similar to controls in males and females receiving 2 or 10 ppm E 605-methyl but were significantly decreased ($p \leq 0.05$) in both sexes receiving 50 ppm throughout the study. Table 2 summarizes data at selected intervals during the study; decreases were 9 to 12 percent in males and 12 to 17 percent in females. Food consumption was similar in control and dosed groups, except that it was increased in the high-dose females (20 g/rat/day) when compared to controls (17 g/rat/day). The average compound intake in mg/kg/day was 0.090, 0.458, and 2.610 for males receiving 2, 10, and 50 ppm, respectively, and 0.144, 0.713, and 4.971 for females receiving the same doses.

Hematology: Mean hematologic values at 3, 6, and 12 months were based on five animals/sex/group and those at 24 months on 10 animals/sex/group. According to the report there were "temporary significant variations in the reticulocyte count, hemoglobin level and hematocrit at 50 ppm which may have been the consequence of the treatment." Hb and Hct were slightly but significantly decreased ($p \leq 0.05$) when compared to controls at 24 months (Table 3). Data for reticulocytes in control and high-dose animals at 6, 12, and 24 months are presented in Table 3. There were no consistent increases in reticulocyte count over time.

Urinalysis: There were no definitive changes in urinary parameters to indicate an adverse effect from dosing. Urinary protein was increased when compared to controls in females receiving 50 ppm at all intervals, but the differences were only significant ($p \leq 0.05$) at 1 and 6 months.

Clinical Chemistry: The authors assessed that there were no toxicologically important changes in clinical chemistry parameters related to dosing and that all values were within the normal range. There was a significant increase in serum alkaline phosphatase (SAP) in high-dose females at 3 and 6 months, but there were no corresponding effects at 12 or 24 months (Table 4). SAP was sporadically decreased in males receiving 2 ppm but there were no dose-related

TABLE 1. Mortality and Percent Survival in Rats Fed E 605-Methyl for 2 Years

Dietary Level (ppm)	No. Animals Initially	Mortality (and Percent Survival) at Week			
		26	52	78	105
		MALES			
0	100	0(100)	1 (99)	5 (95)	21 (79)
2	50	0(100)	1 (98)	2 (96)	9 (82)
10	50	0(100)	1 (98)	3 (94)	9 (82)
50	50	3 (94)	3 (94)	4 (92)	5 (90)
		FEMALES			
0	100	1 (99)	1 (99)	9 (91)	28 (72)
2	50	1 (98)	3 (94)	5 (90)	11 (78)
10	50	0(100)	0(100)	5 (90)	15 (70)
50	50	8 (84)*	9 (82)*	11 (78)	21 (58)

*Significantly different from control value ($p \leq 0.05$).

TABLE 2. Mean Body Weights at Selected Intervals in Rats Fed E 605-Methyl for 2 Years

Week	Mean Body Weights (g ± SD)			
	Males/Dietary Level (ppm)		Females/Dietary Level (ppm)	
	0	50	0	50
0	83± 5	84± 7	80± 7	80± 7
13	334±23	300±30	188±16	161±11
26	383±29	330±34	213±17	180±14
53	424±32	374±36	240±25	198±21
79	429±34	389±36	256±31	221±28
104	414±40	379±49*	259±33	226±30*

*Significantly different from control value ($p \leq 0.05$); analysis by our reviewers (only data for 104 weeks).

TABLE 3. Selected Hematologic Data in Rats Fed E 605-Methyl for 2 Years

Sex/Dose (ppm)	Hb (g/dL) at 24 Months[a]	Hct (%) at 24 Months[a]	Reticulocytes (%) at Month 6[b]	12[b]	24[a]
Males					
0	16.1±0.8	48±2	0.9±0.2	2.0±0.2	2.5±0.4
2	15.2±1.1*	45±4	1.1±0.5	2.1±0.1	3.0±0.6
10	15.8±1.0	47±2	1.1±0.2	2.2±0.2	2.4±0.5
50	15.5±0.5*	46±2*	1.7±0.2*	2.2±0.2	2.5±0.3
Females					
0	15.8±0.7	47±2	1.4±0.4	2.2±0.2	2.5±0.6
2	15.6±0.7	46±3	1.6±0.3	2.2±0.1	2.5±0.2
10	15.5±0.5	45±2	1.4±0.2	2.1±0.1	2.6±0.4
50	14.5±1.8*	43±5*	2.2±0.6*	2.0±0.2	3.8±3.0*[c]

*Significantly different from control value ($p \leq 0.05$).

[a] N = 10.

[b] N = 5.

[c] One animal had a value of 12.3 percent and severe anemia (30 percent Hct; Hb = 9 g/dL). If this value is omitted as an outlier, the mean is 2.86±0.41 and not significantly different from the control value. The mean values for Hb and Hct omitting the animal in question are 15.0±0.77 and 44.4±2.65, respectively.

TABLE 4. Mean Serum Alkaline Phosphatase in Female Rats Fed E 605-Methyl

Dietary Level (ppm)	Alkaline Phosphatase Levels (U/mL) at Month			
	3[a]	6[a]	12[a]	24[b]
0	135±26	168±12	162±18	165±27
2	156±24	155±33	121±21*	142±37*
10	144±39	137±55	124±16*	147±40
50	196±34*	231±33**	189±45	161±41

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Mean ± SD of five animals/sex/group.

[b]Mean ± SD of 10 animals/sex/group.

TABLE 5. Mean Serum Protein in Female Rats Fed E 605-Methyl

Dietary Level (ppm)	Total Serum Protein (g/dL) at Month				
	1[a]	3[a]	6[a]	12[a]	24[b]
0	6.45±0.28	6.99±0.25	6.96±0.25	7.01±0.33	6.65±0.30
2	6.15±0.22	6.60±0.23	6.34±0.17**	6.59±0.33	6.79±0.45
10	6.05±0.08**	6.65±0.35	6.50±0.33	6.92±0.46	6.83±0.34
50	5.36±0.41**	6.17±0.23**	5.85±0.10**	6.24±0.51*	6.03±0.51**

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]Mean ± SD of five animals/sex/group.

[b]Mean ± SD of 10 animals/sex/group.

TABLE 6. Mean Serum Urea in Rats Fed E 605-Methyl

Dietary Level (ppm)	Serum Urea (mg/dL) at Month				
	1	3	6	12	24
Males					
0	40.1±5.4	55.7±8.7	54.2±9.9	42.2±6.8	45.8±6.4
50	38.5±7.1	51.1±2.9	53.9±9.4	49.6±6.9	54.7±8.3*
Females					
0	59.2±8.1	49.8±4.1	56.3±4.5	49.4±8.3	49.5±4.5
50	70.6±8.5	62.1±7.3*	62.1±6.2	64.2±9.0*	53.6±8.5

*Significantly different from control value ($p \leq 0.05$).

effects. Total protein was significantly lower than controls at all intervals of analysis for high-dose females (Table 5); there were no effects in dosed males. Creatinine levels were decreased at several intervals for 50-ppm females, but it was reported that all values were within the normal range; there were no effects in dosed males. Plasma urea was increased compared to controls in 50-ppm males at 24 months and in 50-ppm females at 3 and 12 months but not at 6 or 24 months (Table 6). The authors considered the value for 50-ppm males at 24 months within the normal range of spontaneous variability and of no toxicologic importance. The increases in mean serum total protein and urea in females receiving 50 ppm were considered of doubtful toxicologic importance since the "increased values for single animals were marginally within or outside" the normal range. There were random changes, both increases and decreases, in blood sugar in dosed groups, but there were no changes consistent with time or dose. There were no dose-related effects on other clinical chemistry parameters.

Cholinesterase: There was a statistically ($p \leq 0.01$) and biologically significant decrease in brain cholinesterase activity in both males and females receiving 50 ppm E 605-methyl for 2 years when compared to controls (Table 7). There was also a significant ($p \leq 0.05$) decrease in brain cholinesterase activity at 2 years in males but not in females receiving 10 ppm. Table 8 presents data for erythrocyte and plasma cholinesterase as percent of control value at intervals during the study. Plasma cholinesterase was more severely inhibited in high-dose groups than was erythrocyte activity and inhibition was greater in females than males.

Organ Weights: At the terminal sacrifice, there were significant decreases in the absolute weight of heart, lung, liver, spleen, and kidney in both males and females receiving 50 ppm when compared to controls. However, this was probably accounted for by a significant decrease in body weight since there were no significant decreases in organ-to-body weight ratios for any of these organs (Table 9). Mean weights of thyroid, testes and ovaries and their weight relative-to-body weight were similar in control and dosed rats. At the interim sacrifices at 6 and 12 months, organ weights were determined for five animals/sex/group. At 6 months, the mean weights of heart and kidney were decreased in males receiving 50 ppm, and heart weight in females receiving 50 ppm was decreased compared to control ($p \leq 0.05$). At 12 months, there were several sporadic significant changes in mean organ weights in dosed groups when compared to controls; there was an apparent dose-related decrease in heart weight in males and a decreased liver weight in high-dose males when compared to controls. Mean thymus weight in 50-ppm males (88±26 mg) was significantly lower ($p \leq 0.05$) than in controls (178±37 mg). The authors considered all changes in organ weights attributable to varying mean body weights. These were no statistical analyses of the data on organ-to-body weight ratios, however, data were presented for individual animals (see Reviewers' Discussion).

TABLE 7. Brain Cholinesterase in Rats Fed E 605-Methyl for 2 Years

Dietary Level (ppm)	Males		Females	
	U/g	% of Control	U/g	% of Control
0	1.53±0.27	100	1.00±0.16	100
2	1.36±0.20	89	1.02±0.14	102
10	1.20±0.09*	78	1.06±0.36	106
50	0.76±0.31**	50	0.37±0.08**	37

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

TABLE 8. Plasma and Erythrocyte Cholinesterase Levels in Rats Fed E 605-Methyl for 2 Years

Dietary Level (ppm)	Erythrocyte Cholinesterase Level[a] at Week				
	1	4	13	52	105
Males					
0	(2.45)	(2.41)	(2.56)	(2.55)	(2.89)
2	98	91	93	83**	94
10	91	76**	73**	75**	78**
50	74*	62**	66**	69**	68**
Females					
0	(2.48)	(2.49)	(2.63)	(2.43)	(2.83)
2	101	94	89**	84**	94
10	90	83**	73**	79**	78**
50	79*	73**	58**	71**	65**
	Plasma Cholinesterase Level[a] at Week				
	1	4	13	52	105
Males					
0	(0.47)	(0.36)	(0.39)	(0.62)	(0.74)
2	100	97	85	92	108
10	81*	83*	79	73*	111
50	26**	36**	36**	32**	41**
Females					
0	(0.58)	(1.20)	(1.57)	(1.95)	(1.97)
2	103	86	86	89	104
10	76	63**	61*	75	73
50	19**	9**	14**	12**	13**

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

[a]Values in parentheses for controls are U/mL, mean of five animals; and for dosed animals the values are percent of control.

TABLE 9. Selected Mean Organ Weights (g) and Organ-to-Body Weight Ratios (percent) in Rats Fed E 605-Methyl for 2 Years[a]

Organ	Males/ Dietary Level (ppm) 0 (N = 76)	Males/ Dietary Level (ppm) 50 (N = 44)	Females/ Dietary Level (ppm) 0 (N = 71)	Females/ Dietary Level (ppm) 50 (N = 29)
Heart	1.240±0.156 (0.300±0.047)	1.161±0.156* (0.304±0.038)	0.956±0.116 (0.372±0.048)	0.873±0.108* (0.387±0.42)
Lung	1.757±0.305 (0.428±0.101)	1.559±0.295* (0.411±0.082)	1.226±0.175 (0.478±0.079)	1.119±0.139* (0.500±0.88)
Liver	14.951±2.167 (3.609±0.507)	13.024±1.954** (3.397±0.480)	9.161±1.244 (3.550±0.439)	8.110±1.229** (3.587±0.433)
Spleen	0.824±0.228 (0.199±0.053)	0.693±0.107** (0.181±0.027)	0.533±0.112 (0.207±0.039)	0.443±0.93** (0.195±0.029)
Kidney	2.783±0.293 (0.673±0.077)	2.565±0.298** (0.673±0.066)	1.890±0.193 (0.734±0.065)	1.725±0.172** (0.768±0.097)
Adrenal	0.061±0.035 (0.015±0.08)	0.047±0.007** (0.012±0.002)	0.066±0.20 (0.026±0.008)	0.066±0.19 (0.030±0.010)

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

[a]The values are means ± SD; the values in parentheses are percent of body weight. The mean organ-to-body weight ratios were calculated and statistically analyzed by our reviewers using ratio data on individual animal organs provided in the report.

Gross Pathology: Individual animal observations were recorded, but summary tables for the findings were not presented nor were findings discussed in the report.

Histopathology: Table 10 summarizes the incidence of neoplastic findings. The majority of tumors were endocrine in origin and benign (pituitary adenomas, adrenal pheochromocytomas, and thyroid adenomas). Thyroid adenomas were significantly increased ($p \leq 0.05$) in males receiving 50 ppm and nonsignificantly increased ($p \leq 0.05$) in females receiving 10 ppm, and pituitary adenomas were increased in females receiving 10 ppm. There was also nonsignificant increases when compared to controls ($p > 0.05$) in Leydig cell tumors in 50-ppm males and uterine adenocarcinomas in 10- and 50-ppm females. Although the authors indicated that the increases were within the range of variability found spontaneously, no data on the historical incidence of tumor were provided. Tumors not presented in Table 10 occurred spontaneously at a group incidence of 2 percent or less. One glioma occurred in control males; in control females there was one subcutaneous epithelial carcinoma and one alveolar carcinoma. In the 2-ppm group, one lipoma was recorded for a male and a subcutaneous fibroma and granulosa theca tumor of the ovary were found in one female. Males receiving 10 ppm had a subcutaneous sarcoma, a fibrosarcoma, and a lymph node hemangioma; in females receiving 10 ppm, fibrosarcoma, keratoacanthoma, and bile duct cystadenoma were found. At 50 ppm, there was a squamous cell carcinoma and salivary gland hemangioma in males and a salivary gland adenoma in a female.

Nonneoplastic lesions were not tabulated in summary tables. It was reported that almost all males and several females exhibited some degree of chronic glomerulonephritis but it was not associated with dosing. Examination of composite summary tables on pathologic findings for individual animals indicated no increase in preneoplastic lesions of the thyroid or adrenals in dosed groups. Nonneoplastic lesions found were reported to be related to aging and not dosing.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. There was no indication of an oncogenic response; all neoplastic changes noted were within the range of variability found spontaneously in aging male and female rats of this strain.

At 50 ppm E 605-methyl, growth was slightly retarded in male and female rats, and at some intervals the symptoms of cholinesterase inhibition occurred. Mortality was increased in the 50-ppm group in the first 2 weeks of the study from deaths (six females, three males) due to cholinergic effects.

There were no toxicologically important effects of dosing on hematologic or clinical chemistry parameters. Although decreases in Hct value and Hb level and decreases in plasma protein in females receiving 50 ppm may have been compound related, these effects were marginal.

TABLE 10. Neoplastic Lesions in Rats Fed E 605 Methyl for 2 Years

Organ/Neoplasm	Males/Dietary Level (ppm) 0	2	10	50	Females/Dietary Level (ppm) 0	2	10	50
Pituitary	(47)[a]	(43)	(45)	(46)	(42)	(44)	(49)	(38)
Adenoma	10[b]	13	11	4	6	7	16*	3
Adenocarcinoma	1	0	0	0	0	0	0	1
Adrenal	(50)	(50)	(48)	(50)	(50)	(50)	(50)	(49)
Ganglioneuroma	1	0	0	0	0	0	0	0
Pheochromocytoma (B)[c]	8	3	4	3	3	0	0	0
Pheochromocytoma (M)[c]	1	1	0	0	0	0	0	0
Cortical adenoma	0	1	0	0	0	1	2	0
Cortical cystadenoma	0	0	0	0	0	0	0	1
Thyroid	(49)	(48)	(46)	(48)	(49)	(47)	(50)	(40)
Adenoma	4	3	3	11*	3	5	9	3
Cystadenoma	2	0	0	1	0	0	0	0
Adenocarcinoma	1	0	0	0	0	0	0	1
Cystadenocarcinoma	1	0	0	0	0	0	0	0
Testes	(50)	(47)	(50)	(50)				
Leydig cell tumor	1	2	0	4				
Uterus					(47)	(50)	(50)	(46)
Adenocarcinoma					4	3	9	8
Adenoma					0	1	0	0
Hemangioma					1	0	0	0
Mammary Gland[d]								
Adenoma/Fibroadenoma					4	1	1	1
Reticuloendothelial[d]								
Lymphoma	0	0	0	1	0	0	1	0
Lymphoma, histiocytic	2	0	1	0	0	0	0	0
Leukemia, myeloid	0	0	1	0	0	0	0	1
Leukemia, lymphatic	0	1	0	0	0	0	1	0

*Significantly different from control value ($p < 0.05$), by Fisher's exact test; analysis by our reviewers.

[a]Numbers in parentheses are the numbers of tissues examined histologically; determined by our reviewers.

[b]The values are the number of animals with neoplasm, not numbers of neoplasms.

[c]B = benign; M = malignant.

[d]Number examined could not be determined.

Cholinesterase was not affected at 2 ppm E 605-methyl. However, at 10 and 50 ppm, there was a dose-related inhibition of plasma and erythrocyte cholinesterase in both males and females throughout the study. At 24 months, brain cholinesterase was inhibited for males receiving 10 or 50 ppm and females receiving 50 ppm E 605-methyl.

The LOEL, based on cholinesterase inhibition, is 10 ppm; based on all other parameters, the LOEL is 50 ppm.

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The oncogenic potential of E 605-methyl could not be adequately evaluated from the data provided since historical control incidence of neoplasms was not indicated, neoplasms were not adequately described, summary tables of neoplasms could not be validated, and the completeness of the histologic examination could not be determined.

For the control groups, there was incomplete gross and histologic examination. There were initially 100 male and 100 female controls. Histologic examination was performed on the first 50 males and females numerically. This resulted in histologic examination of 9 intercurrent deaths and 41 terminal sacrifices for males and 18 intercurrent deaths and 32 terminal sacrifices for females. Gross examination was not performed on all the same animals; 69 males were examined (26 that died and 43 sacrificed) and 50 females (19 that died and 31 sacrificed). Of the 50 males and 50 females examined histologically only 36 males and 32 females were examined grossly. Gross and microscopic correlations were not possible for controls and no rationale for choice of animals for gross examination was given.

Summary tables of neoplastic lesions (CBI vol. 2, pp. 6 and 7) did not indicate the number of tissues examined histologically, and there were no summary tables for data on nonneoplastic lesions. The summary table of neoplasms in the DER (Table 8) includes the number of tissues examined. This tissue inventory was prepared by our reviewers from the report's tabulation of individual histologic findings. For the pituitary and thyroid, particularly in high-dose females, several tissues were lost or not examined (16 to 20 percent). For the tables on neoplasm incidence, the report authors tallied bilateral tumors of the testes and bilateral pheochromocytomas as 2, whereas bilateral tumors of the thyroid appeared as 1. Table 8 in the DER indicates the number of animals with a tumor, correcting the report's inconsistency.

The incidence of thyroid adenomas in 50-ppm males (23 percent) and 10-ppm females (18 percent) was stated to be within the normal laboratory range; this incidence appears rather high for this strain,

and should have been supported by laboratory historical control data. Thyroids should also have been examined for all controls. The thyroid adenomas were not specified as either C-cell or follicular neoplasms or both. These tumors should not be combined for analysis.

We assess that in the first 2 weeks of the study the maximum tolerated dose was exceeded since there were deaths due to tremors in three males and six females receiving 50 ppm E 605-methyl. Compound intake for males and females receiving 50 ppm in week 1 were 9.43 and 11.31 mg/kg/day, respectively, while the average for the study was 2.61 and 4.91 mg/kg/day, for high-dose males and females, respectively. Overall mortality for the study, however, was not affected by dosing.

The table of mortality presented by the authors gives mortality at the end of week 105 (day 735). However, several animals died before sacrifice was completed (day 743): five control males, two each receiving 2 and 10 ppm and one male receiving 50 ppm.

Clinical observations were not presented in summary or for individual animals; therefore, the incidence of animals with cholinergic symptoms could not be determined. It was not indicated if detailed physical examinations including palpations for tissue masses were performed and ophthalmoscopic examinations were not conducted.

Body weights were decreased in both males and females receiving 50 ppm E 605-methyl throughout the study. These significant changes were not accompanied by a decreased food consumption. Food efficiency was probably affected but mean values were not presented in the report.

The number of animals used for clinical laboratory studies at the interim evaluations as well as the number for all cholinesterase evaluations (five/sex/group) was insufficient. In addition, brain cholinesterase should have been measured on the animals at 6- and 12-month interim sacrifices.

We assess that the changes in hematology parameters were marginal and may not have been related to dosing. Mean data at interim intervals are based on only five animals/sex/group. Changes in Hb, Hct, and reticulocytes in females receiving 50 ppm at 24 months would be within the normal range if data for one animal with severe anemia are omitted (see footnote to Table 3). We agree with the authors' conclusion that significant changes in clinical chemistry parameters were of doubtful toxicologic importance. There were no correlated histologic findings to indicate specific organ toxicity.

There was a clear inhibition of cholinesterase activity in brain, plasma and erythrocytes. However, there was no indication that nerve tissue was examined histologically. Nerve should have been included. It was also not indicated if coronal sections through the head were examined histologically.

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Evaluation of data on organ weights at the interim sacrifices was difficult since only five animals/sex/group were used, and there were variations in mean necropsy body weights of these animals when compared to the corresponding mean group body weights of the entire group at each interval. Statistical analysis of relative organ weights was not provided nor was the brain weighed.

Summary tabulations of gross pathologic findings and nonneoplastic findings were not presented. Animals sacrificed at 6 and 12 months were not assessed histologically. Another deficiency of the report was that a quality assurance statement was not provided.

Based on the data presented, we assess that the LOEL for systemic toxicity is 50 ppm and for cholinesterase inhibition is 10 ppm.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods