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

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OCT 5 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Developmental Toxicity Evaluation of E-120 Technical (Methyl-parathion) Administered by Gavage to Crl:CD BR Rats

TO: J. Edwards, PM-74
Registration Division (H-7508C)

FROM: David S. Liem, Ph.D. David Liem 9/19/90
Section II, Toxicology Branch II/HED

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 9/25/90
Section II, Toxicology Branch II/HED (H-7509C)
and
Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch/HED (H-7509C) M. van Gemert 10/1/90

EPA RECORD NO.: 247104 IDENTIFYING No.: 4787-4

MRID No.: 411361-01 CASWELL NO.: 372

ACTION REQUESTED:

To review a study on the Developmental Toxicity Evaluation of E-120 Technical Administered by Gavage to Crl:CD BR Rats submitted by Bayer AG.

CONCLUSIONS:

Four groups of 25 mated female Wistar/HAN rats were given oral administration of 0, 0.3, 1.0, and 3.0 mg/kg/day of methyl-parathion in distilled water mixed with Cremophor EL from day 6 to 15 of gestation, inclusive. Additionally, two groups of 10 mated female Wistar/HAN rats each, treated the same fashion as the main group above, were assigned to the vehicle control and the high dose subgroups.

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According to the data submitted, reduced body weight, body weight gains, and food consumption were observed in the high dose rats of the main group.

Compound related plasma, erythrocyte and brain cholinesterase activity inhibition was observed in the high dose (3.0 mg/kg/day) group.

Increased postimplantation loss and decreased fetal body weight gain were observed in the high dose group.

Since the data as submitted by the registrant lacked some critical information, the maternal and developmental toxicities can not be determined at this time. Therefore, the registrant should submit the following data with appropriate statistical analyses to aid in the determination of possible maternal and developmental toxicities of this compound:

- o Table of individual and summary clinical sign observation data (the number of rats with adverse clinical signs and duration of these signs observed)

  Summary table of fetal and litter incidences of skeletal observations, including the mean body weight values of fetuses/litter that showed adverse ossification together with appropriate statistical analyses.

- o Appropriate notations of statistical significance accompanied by the confidence level or probability.

This information is required in order for the Toxicology Branch to complete its evaluation of this developmental toxicity study. A copy of the DER is attached.

CLASSIFICATION:

The study as submitted is classified as core supplementary. This study may be upgraded upon satisfactory submission of the requested data.

Primary Reviewer: David S. Liem, Ph.D. David Liem 9/4/90
Section II, Toxicology Branch II/HED
Secondary Review: Stephen Dapson, Ph.D. Stephen C. Dapson 9/11/90
Section I, Toxicology Branch II/HED
Tertiary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 9/25/90
Section II, Toxicology Branch II/HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Crl:CD BR Rats Guideline:83-3

EPA Identification No.s: MRID (Accession) No.: 41136101
ID No.: 4787-4 Pack No.:9-1670
Record No.: 247104
Caswell No.: 372
HED Project No.:9-1670

Test Material: E 120 Technical

Synonyms: O, O-Dimethyl O-(4-Nitrophenyl)-phosphorothioate; Parathion-Methyl; Methyl-Parathion

Sponsor: Bayer AG, Institut Fuer Toxikologie (Pharma Forschungzentrum-Aprath), D 5600 Wuppertal 1, West Germany

Study Number: RCC 083553; Bayer T 3024665

Testing Facility: RCC Research and Consulting Company AG
P.O. Box, CH 4452 Itingen, Switzerland
and
RCC Umweltchemie AG, P.O. Box, CH 4452 Itingen, Switzerland

Title of Report: Embryotoxicity (Including Teratogenicity) Study with E 120 Technical (Common Name: Parathion-Methyl) in the Rat

Authors: H. Becker, D. Frei, H. Leutkemeier, W. Vogel, and Ch. Terrier

Report Issued: December 31, 1987

Conclusions: Four groups of 25 mated female Wistar/HAN rats were given oral administration of 0, 0.3, 1.0, and 3.0 mg/kg/day of methyl-parathion in distilled water mixed with Cremophor EL from day 6 to 15 of gestation, inclusive. Additionally, two groups of 10 mated female Wistar/HAN rats each, treated the same fashion as the main group above, were assigned to the vehicle control and the high dose subgroups.

According to the data submitted, reduced body weight, body weight gains, and food consumption were observed in the high dose rats of the main group.

Compound related plasma, erythrocyte and brain cholinesterase activity inhibition was observed in the high dose (3.0 mg/kg/day) group.

Increased postimplantation loss and decreased fetal body weight gain were observed in the high dose group.

Since the data as submitted by the registrant lacked some critical information, the maternal and developmental toxicities can not be determined at this time. Therefore, the registrant should submit the following data with appropriate statistical analyses to aid in the determination of possible maternal and developmental toxicities of this compound:

- o Table of individual and summary clinical sign observation data (the number of rats with adverse clinical signs and duration of these signs observed)
- o Summary table of fetal and litter incidences of skeletal observations, including the mean body weight values of fetuses/litter that showed adverse ossification together with appropriate statistical analyses.
- o Appropriate notations of statistical significance accompanied by the confidence level or probability.

This information is required in order for the Toxicology Branch to complete its evaluation of this developmental toxicity study.

CLASSIFICATION: Core Supplementary Data. This study may be upgraded upon satisfactory submission of the requested data.

Study Title: Embryotoxicity (Including Teratogenicity) Study with E 120 Technical (Common Name: Parathion-Methyl) in the Rat

Author: H. Recker, D. Frei, H. Leutkemeier, W. Vogel, and Ch. Terrier

Report Date: December 31, 1987

Study No.: RCC 083553; Bayer T 3024665

Study Period: June 15 to July 13, 1987

Testing Facility: RCC Research and Consulting Company AG
P.O. Box, CH 4452 Itingen, Switzerland
and
RCC Umweltchemie AG, P.O. Box, CH 4452 Itingen, Switzerland

Test Material: E 120 Technical; Parathion-Methyl

Test Animal: Wistar/HAN Rats (Kfm: Wist. Outbred, SPF Quality)

A. OBJECTIVE

According to the investigators, the objective of this study was to assess maternal cholinesterase activities, and developmental toxicity and teratogenic potential of E 120 Technical (Parathion-Methyl) following oral administration to pregnant rats during the period of major organogenesis.

B. MATERIALS AND METHODS

Materials and methods of this study are attached in Appendix A (copied from p. 12-18 of the study report).

Test Compound: A crystalline/liquid with a purity of about 97%
Batch No.: 230 606 003
Contaminants: Not presented in the report
Storage: At room temperature in the dark
Stability: Stable for at least 2 hours

Vehicle: Distilled water with 0.5% Cremophor EL (BASF) was used as the vehicle for the test material solution, and it was also used as the vehicle control article. The vehicle was stored at room temperature.

Test Animals: Species: Wistar/HAN Rat (Kfm: WIST, outbred SPF)
Source: KFM, Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf, Switzerland
Acclimation period: 7 days before mating
Age: Female and Male Rats were approximately 11 weeks of age when mated
Body Weight: Females from 179 to 228 g on day 0
Caging: Individual Makrolon Type-3 cages using standard softwood bedding
Feed: Pelleted standard Kliba 343 rat diet (from "Kliba" Batches nos. 72/87, 73/87 & 75/87) & water were provided ad libitum

Environmental Parameters: Temp. = 22 ± 3° C; Rel. Hum. 40-70%; 12 hrs light/dark cycle; 10-15 air exchanges/hr.

Study Design

This study was designed to assess maternal cholinesterase activities and the developmental toxicity potential of E 120 Technical (Parathion-Methyl) when administered by gavage to female rats on gestation day 6 through 15, inclusive.

Group Arrangement:

Dosages were selected from the results of a rangefinding study (RCC Project 083564). No study design nor the results of the rangefinding study were presented in the study report. The main group consisted of four dose groups of 25 mated female rats each; an additional 10 mated female rats each were assigned to vehicle control and high dose subgroups for cholinesterase activity measurements. Animals were assigned to the study using computer-generated randomization as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned	
		Main Group	Sub Group
Control	0	25	10
Low Dose	0.3	25	-
Mid Dose	1.0	25	-
High Dose	3.0	25	10

Mating:

Female rats were mated naturally; each female was caged with a male overnight; vaginal smears were taken daily during cohabitation. As soon as a copulatory plug or sperm in the vaginal smear was observed, this was considered evidence of mating and was designated as Day 0 post coitum.

Dose Suspension Preparations and Analyses:

The test material was mixed with the liquid vehicle using a homogenizer. The test material/vehicle mixtures were prepared daily prior to dosing.

Homogeneity, stability and concentration of the dosing suspensions were analyzed before the first day of dosing and once during the dosing period (no specific day was mentioned). Samples were taken immediately after mixture preparation and again after 2 hours. Analyses were performed by RCC Analytical Chemistry Laboratory.

Dosing:

All groups received a dose volume of 10 ml/kg body weight The appropriate dose was adjusted daily based on the most recent body weights.

Clinical Observations

The dams were checked a minimum of twice daily for mortality, moribundity and signs of toxicity from day 0 to 21. On day 21 post coitum all surviving dams were sacrificed using carbon dioxide gas and subjected to macroscopic examination. Any rat found dead was also subjected to macroscopic examination.

Maternal Body Weights

Individual body weights were taken daily, for the main group from day 0 to 21 post coitum and for the subgroup from day 0 to 16 post coitum. Body weight and net body weight gains were also computed.

Food Consumption

Food consumption data were collected on days 6, 11, 16, and 21 post coitum for the main group, and for days 6, 11, and 16 post coitum for the subgroup.

Cholinesterase Measurement Data

Blood samples for plasma and erythrocyte cholinesterase activity measurements were collected from all subgroup rats prior to the first day of dosing. On day 16 post coitum, 24 hours after the last dosing, blood samples were taken for plasma and erythrocyte cholinesterase activity measurements; in addition brain tissue samples were collected for brain cholinesterase activity measurement. Blood samples were drawn from the retro-orbital plexus using Lithium Heparin as anticoagulant.

Postmortem and Caesarian Section Examinations

Following blood collection, the subgroup rats were sacrificed on day 16 post coitum. Only the brain tissues were retained.

On day 21, all surviving dams of the main group were euthanized with carbon dioxide, and internal organ abnormalities evaluated macroscopically. The ovaries and other reproductive organs were evaluated for gross abnormalities. The uteri and ovaries were examined and the number of corpora lutea, implantation sites, resorptions, and live and dead fetuses were recorded. The uteri and their contents were weighed, and the fetuses were sexed, weighed, and examined for gross external abnormalities. Animals that died or were sacrificed in a moribund condition were also examined macroscopically as described above. All maternal tissues were discarded but all fetal tissues were retained.

Approximately one half of all fetuses from each litter were processed and examined for soft tissue anomalies using a modification of the Wilson technique. The remaining fetuses were eviscerated, processed, and examined for skeleton abnormalities. Fetal skeletal specimens were retained in glycerine.

Statistical analysis

A number of statistical methods were used for analyzing the data as follows:

Univariate one-way analysis of variance was used to assess the significance of intergroup differences. The Dunnett many-to t-test, based on a pooled variance estimate was used for intergroup comparisons (i.e. single treatment group against the control group).

A one-way univariate analysis of variance based on Wilcoxon ranks together with the Kruskall-Wallis test was applied to the reproduction data parameters.

Fisher's exact test for 2X2 tables was used if the variables could not be dichotomized without loss of information.

Compliance

- o A signed Statement of Confidentiality Claim was provided.
- o A signed Statement of compliance with EPA GLP's was provided
- o A signed Quality Assurance Statement was provided.

C. RESULTS AND DISCUSSIONS

I. Analyses of Dosing Suspensions

The homogeneity of dosing suspensions ranged from -15.2% to 14.3% of the mean concentration. The mean concentrations ranged from 74.7% to 109.4% of nominal concentrations. The dosing suspensions were stable for at least 2 hours after preparation. All these values were within the acceptable range.

II. Subgroup Animal Data

One dam of the subgroup control died during blood collection on day 16 post coitum. Results of clinical sign observations of the subgroup animals were not noted in the study report.

Two dams of the control and one dam of the high dose subgroups were not pregnant. The rat body weight and food consumption values of the high dose subgroup were comparable to the control subgroup. The body weight and food consumption data are presented in the attached Appendix B (copied from p. 114-117 of the study report).

Cholinesterase activity values are as follows:

Cholinesterase Activity

	Pretreatment		Day 16 Post Coitum	
	Control	High Dose	Control	High Dose
No. Rats Used	10	10	10	10
Plasma@	2.67	3.02	3.41	2.01*
Erythrocyte@	1.92a	1.87a	1.92	0.55*
Brain$	-	-	5.77	4.48*

* = significant $P < 0.05$; a = only 9 animals were used
@ = umol-SH/ml/min at 37° C; $ = umol-SH/g/min at 37° C.

As seen from the above table, the plasma, erythrocyte, and brain cholinesterase activities of the treated subgroup (3.0 mg/kg/day), measured on day 16 post coitum were significantly decreased as compared to the control subgroup. The cholinesterase activity inhibition in the treated subgroup is considered a treatment related effect.

III. Main Group Animal Data

a. Maternal Mortality

A total of five (20%) high dose dams died; one died after seven days of dosing (day 13 post coitum), two after nine days of dosing (day 13 post coitum), and two after ten days of dosing (day 16 post coitum). All other dams survived to day 21 post coitum.

b. Maternal Clinical Observations

In the study report, group summary and individual clinical sign observation data were not presented in tabular form. In the text, the investigators mentioned that significant clinical signs, such as slight somnolence, ataxia, dyspnea, salivation, ventral recumbency, repeated chewing and occasional whining (rales?) were observed in the majority (exact numbers not mentioned) of the high dose dams from day 13 to 16 post coitum. Slight somnolence, ataxia and dyspnea were observed until day 21 post coitum (it was not mentioned whether these clinical signs were observed in the same high dose animals noted above). These clinical signs appear to be compound related, but since there were no summary data tables and since no individual data were tabulated, this conclusion can not be verified.

c. Maternal Body weight data

Surviving dams with at least one live fetus on day 21 post coitum were included in body weight computations. Body weights of the subgroup dams were not presented in the report.

In the main group, the mean maternal body weights for the control, low and mid dose groups were comparable throughout the study, but the high dose mean maternal body weights were depressed starting from day 10 post coitum as compared to the control (see attached Appendix C, copied from p. 47-55 of the study report). Values which were statistically significant were not flagged on the tables in the study report.

The pre-, during, post-dosing, and for the day 6 to 21 period body weight changes, and the net percent body weights change for all surviving dams are summarized in the next page.

The body weight change during the predosing and postdosing periods was comparable among the four groups. In the high dose group, the body weight change during dosing and from the day 6 to 21 period was significantly decreased as compared to the control. The net percent body weight change for the high dose group was also significantly depressed as compared to control and this reduction is considered a dose-related effect. These data are summarized as follows:

Mean Body Weight Change(gm) and Mean Net % Body Weight Change

	Control	Low Dose	Mid Dose	High Dose
Treated Dams	25	25	25	25
# Pregnant	24	25	24	25
# Died	0	0	0	5
# Rats used for BW Calculations	24a	24b	24a	20c
BW Change(gm):				
Days 0 to 6	20	22	21	23
Days 6 to 16	45	42	40	14
Days 16 to 21	53	54	53	51
Days 6 to 21	98	96	93	65
Net % BW Change:				
Days 6 to 21	7.0±3.0	8.3±3.8	5.5±2.9	-3.9±6.4

BW = Body Weight; GUW = Gravid Uterine Weight; Corrected BW gain in gm = Day 21 BW - (Day 6 BW + GUW); Net % BW Change = Corrected BW gain in percent of Day 6 BW; a = one not pregnant dam was excluded; b = one dam with no live fetuses was excluded; c = five dams died

d. Food Consumption Data

Surviving dams with at least one live fetus on day 21 post coitum were included in food consumption computations. The maternal food consumption data are summarized as follows:

Mean Relative Food Consumption Data (g/rat/day)

	Control	Low Dose	Mid Dose	High Dose
Treated Dams	25	25	25	25
# Pregnant	24	25	24	25
# Died	0	0	0	5
# Rats used for FC Calculations	24a	24b	24a	20c
Relative FC:				
Days 0 to 6	19.2	19.8	19.4	19.7
Days 6 to 11	19.5	19.3	19.5	17.8
Days 11 to 16	23.0	23.9	22.7	16.3
Days 6 to 16	21.3	21.6	21.1	17.1
Days 16 to 21	23.6	24.3	23.2	20.0

FC = Food Consumption; a = one not pregnant dam was excluded; b = one dam with no live fetuses was excluded; c = five dams died

As seen from the table presented on the previous page, during the predosing period the food consumption values were comparable among all four groups. During the dosing and postdosing periods, reduction of food consumption values were observed in the high dose group as compared to the control. The investigators noted (p.20) that the food consumption values were significantly reduced from day 6 to 21 post coitum in the high dose group ($P < 0.05$). Food consumption reduction in the high dose group is considered treatment related.

e. Maternal Gross Pathological Observations

The uterus of one mid dose dam was filled with black brown fluid noted on day 21 post coitum. No other maternal gross abnormalities were observed in any other dam at necropsy (p.108-113 of the the report). The observed abnormality in the one mid dose dam noted above is not considered compound related.

f. Pregnancy Rates

The pregnancy rates were 100% (25/25) for the low and high dose groups and 96% (1/25) for the control and mid dose groups.

g. Caesarean Section Observations

The Caesarean-delivery observation data are presented in the attached Appendix D (copied from p. 39-40 of the study report). The number of corpora lutea, implantations per dam, preimplantation losses, live fetuses, mean percent of male and female fetuses, and fetal resorptions (except the high dose value), were comparable among the groups. The postimplantation loss and the mean number of embryonic resorptions (amorphous mass) in the high dose group were increased (7.4%) as compared to control (4.6%). Although the difference is not statistically significant, it is considered treatment related, because severe maternal toxicity was also observed.

The fetal M/F sex ratios in the low and mid dose groups were different as compared to control. Although the M/F sex ratios in this group are statistically different as compared to control, these sex ratio differences are within normal variation, and are not considered compound related.

h. Fetal Body Weight

The high dose group mean fetal body weight and the litter mean body weights were reduced as compared to the control (see attached Appendices D and E; copied from p.39-40 & p.71-74 of the study report). The reduction of litter mean body weight in the high dose group was statistically signifi[illegible] and was considered to be related to treatment. Statistical s[illegible]ficance notations were not indicated on Body Weight Tables [illegible]ne study report.

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i. Fetal External Observation and Fetal Soft Tissue Data

The investigators reported that no fetal external nor soft tissue abnormalities were observed in any fetus.

k. Fetal Skeletal Variations and Malformations

Some selected fetal skeletal malformation and/or anomaly data are summarized as follows:

Selected Fetal Skeletal Alterations (#Fetuses/#Litters)@

	Control	Low Dose	Mid Dose	High Dose
Fetuses/Litters Evaluated	156/24	141/24	151/24	125/20
Dumbbell-shaped Thoracic Vertebral Body				
#4	1/1	-	-	-
#9	1/1	-	-	-
#10	1/1	3/2	-	-
#12	1/1	-	-	4/4
#13	-	-	-	1/1
#9 & #10	-	-	1/1	-
Bipartite Thoracic Vertebra #12	-	-	1/1	-
Bipartite Lumbar Vertebral Body #1	-	-	-	1/1
Lumbar Vertebral Body #4 & 5 Fused	-	-	-	1/1
Wavy Ribs #5 to 13 (l & r)	-	-	-	1/1
#6 to 13 (l) & #7 to 13 (r)	1/1	-	-	-
#8 to 12 (l & r)	-	-	1/1	-
#8 to 13 (r)	-	-	1/1	-
#10 to 12 (r)	-	-	1/1	-
#11 to 12 (l) & #8 To 12 (r)	1/1	-	-	-
#9 to 12 (l) & #8 To 12 (r)	1/1	-	-	-

l = left side; r = right side; @ = extracted from p.41-43 of the study report

As seen from the table on the previous page, in general these selected skeletal anomaly and malformation observations were evenly distributed throughout all four dose groups. In the high dose group a slight increase of dumbbell-shaped thoracic vertebral body no.12 was observed (four fetuses in four litters), as compared to only one fetus in the control; both bipartite lumbar vertebral body #1 and fused lumbar vertebral body #4 & #5 were also observed in that one fetus. Since there was no clear trend and no significant differences were observed, and also since the types and frequencies of fetal skeletal findings were within the normal variation range of this rat strain, the differences observed are not considered related to treatment.

1. Skeletal Ossifications Data

The individual skeletal ossification data were presented on p. 146-296, but only the fetal skeletal ossification incidence data were summarized on p. 44-46 of the study report. The investigators concluded that "in the high dose group percentages of fetuses with incompletely ossified crania, cervical vertebrae, phalangeal nuclei and metatarsalia were slightly increased as compared to control; these findings were due to delayed fetal maturation due to reduced fetal body weight and therefore were not considered compound related effects". This reviewer can not evaluate these findings, since litter incidences and appropriate statistical analyses were not included in the summary data of the study report.

Therefore the registrant should submit summary data showing the fetal incidence together with the litter incidence (#fetuses/#litter/group) with appropriate notations of statistical significance flagged on the data, to aid in the determination of possible dose related effects.

CONCLUSIONS

According to the data presented in this study report, reduced body weight, body weight gains, and food consumption data were observed in the high dose group dams of the main group. A compound related plasma, erythrocyte and brain cholinesterase activity inhibitions were observed in the high dose (3.0 mg/kg/day) group dams (only dosed group measured).

Increased postimplantation loss and decreased fetal body weight gain were also observed in the high dose group.

Since the data as submitted by the registrant lacked some critical information, the maternal and developmental toxicities can not be determined at this time. Therefore, the registrant should submit the following data with appropriate statistical analyses to aid in the determination of possible maternal and developmental toxicities of this compound:

- o Table of individual and summary clinical sign observation data (the number of rats with adverse clinical signs and duration of these signs observed)

- o Summary table of the fetal and litter incidence of skeletal observations, including the mean body weight values of fetuses/litter that showed adverse ossification together with appropriate statistical analyses.

- o Appropriate notations of statistical significance accompanied by the confidence level or probability.

This information is required in order for the Toxicology Branch to complete its evaluation of this developmental toxicity study.

CLASSIFICATION: Core-supplementary Data. This study may be upgraded upon satisfactory submission of the requested data.

APPENDICES

APPENDIX A: Materials and Methods of the Study (copied from p. 12-18 of the report)

APPENDIX B: Maternal Body Weight and Food Consumption Data of the Subgroup (copied from p.114-117)

APPENDIX C: Maternal Body Weight Data of the Main Group (copied from p. 47-55 of the study report)

APPENDIX D: Caesarean Section Data (copied from p. 39-40 of the study report).

APPENDIX E: Fetal Body Weight Data (copied from p.71-74 of the study report).

Page _____ is not included in this copy.

Pages 17 through 41 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.