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

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Methomyl: 21-Day Dermal Toxicity Study in Rabbits**

FROM: Yung G. Yang, Ph.D. Yung G. Yang 3/4/98
Toxicology Branch 1
Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D. Alberto Protzel 3/5/98
Branch Senior Scientist
Toxicology Branch 1, HED (7509C)

TO: Tom Myers
PM 52
Reregistration Division (7508W)
and
Felecia Fort
RRB1
Health Effects Division (7509C)

DP Barcode:	D241634
Case:	819319
Submission:	S534860
Chemical:	Methomyl
Caswell No.:	549C
PC No.:	090301
Registrant:	E.I. du Pont de Nemours & Co.

ACTION REQUESTED: Review a 21-day dermal toxicity study in rabbits for methomyl which was submitted to re-define a NOEL for the calculation of a new MOE for mixer/loader/applicators and reentering workers.

RESPONSE: The 21-day dermal toxicity study in rabbits (MRID# 44436301) has been reviewed and was found to be acceptable/non-guideline. The Data Evaluation Record (DER) has been reviewed by the HED Hazard Identification Assessment Review Committee on February 19, 1998. The attached DER is a revised one. An executive summary is as follows.

Methomyl

EXECUTIVE SUMMARY

In a 21-day dermal toxicity study (MRID 44436301), technical grade methomyl (98.6%, a.i.) was administered dermally to New Zealand White rabbits (6/sex/group) at dose levels of 0, 15, 30, 45, or 90 mg/kg/day for 21 consecutive days.

No treatment-related deaths or clinical signs were observed. Body weight and food consumption were not affected by the treatment. Hematology, clinical chemistry parameters were not measured. Gross pathological examination did not reveal significant effect by the treatment. No microscopic histopathology was examined.

There was no statistically or biologically significant differences in plasma or RBC cholinesterase inhibition. Statistically significant decrease of brain ChE activity was observed in males exposed to methomyl at doses of 30, 45, or 90 mg/kg/day (90%, 88%, or 90% of the control, respectively) and in females at the dose of 90 mg/kg/day (94% of the control). The HED Hazard Identification Assessment Review Committee evaluated the data and determined that the NOEL should be 90 mg/kg/day (HDT) for brain ChE inhibition and that a LOEL was not established in this study based on the following reasons: 1) lack of dose-response in either sex; 2) the values approached the level of sensitivity of the assay itself; 3) there was concern about inherent variability; 4) lack of convincing evidence in the other two compartment (RBC abd plasma) at this dose; 5) lack of clinical signs in this dermal study as opposed to the observeance of clinical signs in the oral study in the same species; and 6) lack of toxicity via the dermal route (LD_{50}=2000 mg/kg) when compared to the oral route (NOEL=16 mg/kg/day) in rabbits (Memorandum, J. Rowland to A. Protzel, March 3, 1998).

Under the conditions of this study and a consideration of a previous 21-day dermal toxicity study in rabbits (HED DOC# 007599), **the NOEL is estimated to be 90 mg/kg/day (the highest dose tested)** based on brain, plasma, and RBC ChE inhibition.

This study is classified as **Acceptable/Non-guideline.** This study was not designed to fulfill guideline requirements.

EPA Reviewer: Yung G. Yang, Ph.D. Yung G. Yang 3/4/98
Toxicology Branch I (7509C)
EPA Secondary Reviewer: P. V. Shah, Ph.D. P.V.Shah 3/4/98
Toxicology Branch I (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: 21-Day Dermal Toxicity- rabbits;
OPPTS 870.3200 [§82-2]

DP BARCODE: D241634 SUBMISSION CODE: S534860
P.C. CODE: 090301 ID NO.: 090301
CASE: 819319 CAS No: 16752-77-5

TEST MATERIAL (PURITY): Methomyl (98.6% a.i.)
SYNONYMS: Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-,methyl ester
CITATION: Finlay, C. (1997) Methomyl Technical: 21-Day Repeated Dose Dermal Toxicity Study in Rabbits. Haskell Laboratory for Toxicology and Industrial Medicine, E.I.du Pont de Nemours and Company. Lab ID HL-1997-00913. November 14, 1997. MRID 44436301. Unpublished.
SPONSOR: E.I.du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY

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in this dermal study as opposed to the observeance of clinical signs in the oral study in the same species; and 6) lack of toxicity via the dermal route (LD_{50}=2000 mg/kg) when compared to the oral route (NOEL=16 mg/kg/day) in rabbits (Memorandum, J. Rowland to A. Protzel, March 3, 1998).

Under the conditions of this study and a consideration of a previous 21-day dermal toxicity study in rabbits (HED DOC# 007599), **the NOEL is estimated to be 90 mg/kg/day (the highest dose tested)** based on brain, plasma, and RBC ChE inhibition.

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COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methomyl; Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-,methyl ester
   Description: White solid
   Lot/Batch #: H22577/DPX-X1179-512
   Purity: 98.6%
   Stability of compound: Stable
2. Vehicle: Deionized water
3. Test animals: Species: Rabbits
   Strain:HM:(New Zealand White)fBR
   Age & weight, at study initiation:
   Male: 1603-1974, age: 10 weeks
   Female: 1639-1972 g, 10 weeks
   Source: Hare Marland, Hewitt, NJ
   Housing: Caged individually, stainless steel, wire meshed cages
   Diet: Purina Certified High Fiber Rabbit Chow#5325, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions: Temperature: 20±1°C
   Humidity: 50±10%
   Air changes: Not provided
   Photoperiod: 12 hours light/dark.
   Acclimation period: 2 weeks.

B. STUDY DESIGN:

1. In life dates - start: 8/26/97 End: 9/16/97

   Animals were assigned randomly allocated by weight to test groups (6/sex/group) (Table 1).

Table 1. Animal Assignment

Test Group	Males	Females
1. 0 mg/kg	6	6
2. 15 mg/kg	6	6
3. 30 mg/kg	6	6
4. 45 mg/kg	6	6
5. 90 mg/kg	6	6

3. Test Material Preparation and Administration
The test materials (specifically weighed) were moistened with approximately 1 mL of deionized water just prior to the treatment and applied directly to the shaved, intact skin of the rabbit (190 cm^2 or 10% of a body surface). A 2-ply gauze pad was placed onto the test site and wrapped with successive layers of a porus dressing. Control animals were similarly treated with 1 mL of deionized water only. After an exposure period of 6 hours, the bandages were removed and the test sites were washed with Ivory soap and warm tap water, and the skin was patted dry.

4. Statistics
Body weights, body weight gains, and pathology data were analyzed by a one-way analysis of variance. Pairwise comparisons between test and control groups were made with Dunnett's test. Cholinesterase measurements were analyzed by the Jonckheere test for trend ($p<0.05$). Increases in the incidences of clinical observations were evaluated by the Cochran-Armitage test for trend.

C. METHODS:

1. Observations:
Animals were observed for mortality and clinical signs and dermal irritation before treatment and after test material removed each day.

2. Body weight
Body weights were measured twice each week.

3. Food consumption
Food consumption was determined on a weekly basis.

4. Ophthalmoscopic examination
No ophthalmoscopic examination was performed.

5. Blood collection
Blood was collected on day 21 from the jugular vein of all study rabbits and evaluated for cholinesterase activity in plasma and red blood cells. Cholinesterase activity was measured spectrophotometrically using a modified Ellman method. No hematology or clinical chemistry parameters were measured.

6. Urinalysis*: No urinalysis was performed.

7. Sacrifice and Pathology
Following the collection of blood samples, all animals were

anesthetized with an injection of barbiturate into the auricular blood vessel and then exsanguinated. The rabbits were given a gross pathological examination. Brains were collected and weighed, frozen at approximately -70 C and analyzed for brain cholinesterase activity as described above. The remaining tissues were discarded without microscopic evaluation.

II. **RESULTS**

A. Observations
1. Mortality - No deaths occurred during the study.
2. Toxicity - No treatment-related clinical signs were observed.

B. Body weight and body weight gain
No significant effects in body weights or body weight gains were observed.

C. Food consumption:
No significant compound-related effect was observed.

D. Ophthalmoscopic examination: Not performed.

E. Urinalysis - No urinalysis was performed.

F. Blood work:
1. Hematology - No hematology parameters were measured.
2. Clinical Chemistry - No clinical chemistry parameters were measured.

G. Sacrifice and Pathology:
1. Organ weight - No organ weight was measured.
2. Gross pathology - No significant gross pathological findings were observed.
3. Histopathology
No histopathological examination was performed.

H. Cholinesterase (ChE) activity

Brain ChE activity

Statistically significant decreases of mean brain ChE activity were observed in males of the 30, 45, and 90 mg/kg/day groups (Table 2). The mean values in these groups were 90%, 88%, and 90% of the control value, respectively. In females, the statistically significant decrease of brain ChE activity was observed only in the 90 mg/kg/day group (94% of the control).

RBC ChE activity

There was no statistically significant difference in RBC ChE activity between treated and control males and females although the RBC ChE activity in males of the 15, 30, 45, or 90 mg/kg/day group was 70%, 78%, 87%, or 77% of the control, respectively (Table 2). Individual animal data indicated a wide variation of the results (see attachment).

Plasma ChE activity

There has no statistically significant difference in plasma ChE activity between treated and control animals (Table 2).

Table 2. Summary of cholinesterase activity for methomyl

Parameter	Males / Dose (mg/kg/day)					Females / Dose (mg/kg/day)				
	0	15	30	45	90	0	15	30	45	90
Brain ChE	14.3	14.2 (99%)	12.9* (90%)	12.7* (88%)	12.8* (90%)	13.6	13.8 (101%)	13.6 (100%)	12.7 (94%)	12.7* (94%)
RBC ChE	2600	1823 (70%)	2020 (78%)	2270 (87%)	1997 (77%)	2493	2197 (88%)	2563 (103%)	2450 (98%)	2233 (90%)
Plasma ChE	611	603 (99%)	594 (97%)	570 (93%)	570 (93%)	614	635 (103%)	526 (86%)	580 (94%)	526 (86%)

* Significantly different from the control ($p<0.05$).
Data were extracted from Table 14-15, pages 46-47 of the report.

III. DISCUSSION AND CONCLUSION

New Zealand White rabbits (6/sex/group) were dermally exposed to 0, 15, 30, 45, or 90 mg/kg/day of methomyl technical for 21 days.

No deaths or significant treatment-related clinical signs were observed. Body weight or food consumption was not affected by the treatment. No urinalysis, hematology or clinical chemistry parameters were measured. Gross pathology did not reveal significant treatment-related effect. No microscopic histopathological examination was performed.

There were no statistically or biologically significant differences between treated and control animals for plasma ChE activity.

Decreased RBC ChE activity was observed in males at the 15, 30, 45, or 90 mg/kg/day group which was 70%, 78%, 87%, or 77% of the control value, respectively. However, these mean values were not statistically different from controls and, also, they did not exhibit a dose-dependent relationship. In addition, individual animal data indicated a wide variation. The inhibition of RBC ChE activity was not biologically significant.

Statistically significant decrease of brain ChE activity was observed in males exposed to methomyl at doses of 30, 45, or 90 mg/kg/day (90%, 88%, or 90% of the control) and females at a dose of 90 mg/kg/day (94% of the control, respectively). The study report concluded that "since these mean values did not exhibit a dose-response relationship, the statistically significant changes were considered to be spurious and indicative of the normal biological variation that occurs in cholinesterase levels." This reviewer disagreed with the conclusion. A dose-dependent relationship was shown by evaluation of the individual animal brain ChE activity which indicated that the incidence of brain ChE values that were more than 10% below the control group mean value were 1/6, 2/6, 4/6, and 4/6 in the 15, 30, 45, and 90 mg/kg/day groups, respectively. In addition, evaluation of the individual animal brain ChE activity in males (see attached Figure 1) demonstrated that a suppression of brain ChE activity was seen at doses equal to or above 30 mg/kg/day. The results were consistent with the previous study which showed an inhibition of brain ChE activity was observed at doses equal to or above 50 mg/kg/day (Figure 1).

On February 19, 1998, the Data Evaluation Record (DER) was submitted to the Health Effects Division Hazard Identification Assessment Review Committee (HIARC) for evaluation. The Committee determined that the NOEL should be 90 mg/kg/day (HDT) for plasma, RBC and brain ChE inhibition and that a LOEL for ChE inhibition was not established in this study. The HIARC did not attribute to treatment the statistically significant decreases observed in brain ChE inhibition for the following reasons: 1) lack of dose-response in either sex; 2) the values approached the level of sensitivity of the assay itself; 3) there was concern about inherent variability; 4) lack of convincing evidence in the other two compartment (RBC abd plasma) at this dose; 5) lack of clinical signs in this dermal study as opposed to the observeance of clinical signs in the oral study in the same species; and 6) lack of toxicity <u>via</u> the dermal route (LD_{50}=2000 mg/kg) when compared to the oral route (NOEL=16 mg/kg/day) in rabbits (Memorandum, J. Rowland to A. Protzel, dated March 3, 1998).

No clinical pathology or microscopic examinations were evaluated in this study. However, a previous submitted 21-day dermal toxicity study

(MRID# 41251501, HED DOC# 007599) showed that the clinical pathology and histopathological examination did not show significant differences between control and the high dose (500 mg/kg/day) groups. Considering findings in these two studies, the NOEL for the 21-day dermal toxicity study is estimated to be 90 mg/kg/day (HDT) based on RBC, brain, and plasma ChE inhibition.

IV. CLASSIFICATION

This study is classified as **Acceptable/Non-guideline**. The study was not designed to fulfill guideline requirements.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
SEE THE FILE COPY.

Figure 1-6. Individual cholinesterase activity.

Appendix: Individual cholinesterase measurement's data.

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Page _____ is not included in this copy.

Pages 12 through 21 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.

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