IMAGE

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

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

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013975

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Oxamyl: Review of Acute- and Subchronic Neurotoxicity Studies, 21-Day Dermal Toxicity and other Chronic and Subchronic Toxicity Studies in Support of Reregistration (MRID Nos. 44254401, 44420301, 44740701, 44628701 - 44628703, 44660601, 44504901, 44751201, 44737501 & 44737503)

PC Nos.: 103801
Tox. Chem. No.: 561A
Project Nos.: D241653, D241654, D246742, D249642, D250235, D253117, D253122 & D253414
Submission Nos.: S534905, S544071, S548879, S550051, S556445, S556451 & S556996

To: Betty Shackleford/Carmelita White
Project Manager 53
Reregistration Branch III
Special Review and Reregistration Division (7508W)

From: Guruva B. Reddy
Reregistration Review Branch II
Health Effects Division (7509C)
1/12/2000

Thru: Robert Fricke
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1/13/2000

I. CONCLUSIONS:

Reregistration Review Branch II reviewed acute- (MRID Nos. 44254401, 44420301, 44740701, 44628701 - 446287030 & 44660601) and subchronic neurotoxicity studies in rats (MRID 44504901)

and 21-Day rabbit dermal toxicity study (MRID 44751201) and the studies are **Acceptable**. Chronic toxicity studies in dog (MRID 41697901, 42052701 & 44737503) were reevaluated in the light of Du Pont's response (MRID 44737503) and the studies have been upgraded to **Acceptable**, however, the NOAEL of 0.930 mg/kg/day was based on 20% decrease in brain cholinesterase inhibition in male dogs at 1.56 mg/kg/day (LOAEL). Further, the HIARC (07/15/1999) reviewed the existing data base on oxamyl and agreed with the registrant (MRID 44737501) that there is no increased susceptibility to fetuses because decrease in maternal weight gain seen at 0.5 mg/kg/day in the rat developmental toxicity study (40859201) at which decreased fetal body weights also occurred. The RRB2 also updated the original DER (HED Doc. No. 005858 & 000369; MRID 00063009) to conform to the current standards.

Copy of the reviews listed in Table are provided.

II. ACTION REQUESTED

The DuPonte de Nemours and Company submitted Acute- (MRID Nos. 44254401, 44420301, 44740701, 44628701 - 446287030 & 44660601) and subchronic neurotoxicity studies (MRID 44504901) in rat and 21-Day dermal toxicity study in rabbit (MRID 44751201), in support of reregistration of Oxamyl for terrestrial food use on field crops, vegetables, fruits and ornamentals. In addition, the registrant submitted a position paper (MRID 44737501) to address the fetal sensitivity issues in the rat developmental study and unresolved issues (MRID 44737503) regarding lack of NOAEL in male dogs in the chronic dog studies.

III. STUDIES REVIEWED:

STUDY/CLASSIFICATION	RRB2 COMMENTS
81-8ss Acute Neurotoxicity - Oral Species: rat Haskell Lab. Tox. and Industrial Medicine Lab. Report #1118-96; April 10, 1997 MRID #s 44254401, 44420301, 44740701, 44628701 - 446287030 & 44660601 **Acceptable**	In an acute oral neurotoxicity study (MRID Nos: 44254401, 44420301, 44740701, 44628701 - 446287030 & 44660601), single gavage doses of oxamyl (98.3% a.i.; Sample No.: 16995-02) in deionized water were administered to groups of Crl:CD rats (42/sex/dose). Males received 0, 0.1, 1.0, or 2.0 mg/kg and females received 0, 0.1, 0.75, or 1.5 mg/kg. Twelve rats/group were designated as neurotoxicity subgroup animals. All twelve of these rats/group were used for Functional Observational Battery (FOB) and Motor Activity (MA) assessments on days 1, 8, and 15. Body weight and clinical signs were also recorded for these animals. Six of the rats/group were euthanized for *in situ* perfusion. Thirty rats/group were designated as the clinical pathology group and were utilized for blood and brain collection for evaluation of cholinesterase levels. One high-dose male died on day 1. High-dose males had significantly ($p<0.05$) decreased mean body weight gain for days 1-2. Similarly, mid-dose males and high-dose females also exhibited lower (n.s.) body weight gains. Decreased food consumption (n.s.) was also observed in mid- and high-dose males. Statistically ($p<0.05$) and biologically significant dose related decreases in blood and brain cholinesterase activity were observed in mid- and high-dose males and females on day 1. Mean decreases were generally ≥40%. By day 2, decreases in cholinesterase activity were no longer biologically significant. No toxicologically significant decreases in cholinesterase activity were observed in any animals after day 1 or in low-dose males and females at any time point. Clinical signs and FOB effects, consistent with decreased cholinesterase activity, were observed 30-60 minutes post-exposure in the mid- and high-dose males and females. Observations included soiled fur, lacrimation, salivation, slow righting reflex, abnormal gait, tremors, impaired locomotion, no response to tail pinch, limb splay, incoordination, labored breathing, and decreased forelimb and hindlimb grip strength. Other effects, including posture, palpebral closure, docile behavior, and decreased motor activity, were likely due to lethargy and malaise secondary to decreased cholinesterase activity. No treatment-related clinical signs or FOB effects were observed in low-dose animals or after day 1. Validation of test method was conducted using the positive controls (MRIDs 44628701 - 446287030& 44660601). **Therefore, the RBC, plasma and brain ChE inhibition NOAEL = 0.1 mg/kg/day for males and females and LOAEL = 1.0 mg/kg/day in males and 0.75 mg/kg/day in females.** No treatment-related gross effects or histopathology were observed. **Under the conditions of this study, the systemic/neurotoxicity LOAEL is 1.0 mg/kg for male rats and 0.75 mg/kg for female rats based on clinical signs, FOB effects, and decreased blood and brain cholinesterase activity. The NOAEL is 0.1 mg/kg.** This acute oral neurotoxicity study is classified **acceptable (guideline)**. This study does satisfy the guideline requirement for an acute oral neurotoxicity study (81-8) in rats.

82-7 90-Day feeding Species: rat Haskell Lab. Tox. and Industrial Medicine Lab. Proj. # HL-1998-00708; Feb. 23, 1998 MRID No. 44504901 **Acceptable**	In a subchronic oral neurotoxicity study (MRID 44504901), 42 Crl:CD^R(SD)BR rats/sex/exposure group were administered Oxamyl Technical (purity, 98.3%; Haskell sample number 16995-02) at concentrations of 0, 10, 30, or 250 ppm (equivalent to 0, 0.564, 2.10, or 14.9 mg/kg/day for male rats and 0, 0.679, 2.40, or 19.9 mg/kg/day for female rats, respectively) in the diet. The 30 and 250 ppm concentrations were reduced from 100 and 300 ppm, respectively, on day 7 of administration due to toxic effects including tremors and weight loss. Twelve rats/sex/exposure group were assigned to the neurotoxicity group and underwent functional observational battery (FOB) and motor activity (MA) testing prior to dietary administration and during weeks 4, 8, and 13. Ten rats/sex/exposure group were sacrificed on days 27 and 55 and at termination of the study for cholinesterase activity determinations. Six rats/sex/exposure group (from the neurotoxicity group) were perfused for neuropathology at study termination. All animals survived to scheduled termination. At the end of 90 days, body weights of male and female rats receiving 250 ppm in the diet were significantly depressed by 24% and 10%, respectively ($p<0.05$). Decreases in body weights correlated with decreased food consumption in males and decreased food efficiency in both sexes. Exposure-related clinical signs (tremors, abnormal gait or mobility, hunched-over posture, exophthalmus, ptosis, hyperactivity, piloerection, colored discharge from the eyes, hyperactivity and lacrimation) were present in one or both sexes administered 250 ppm in the diet but not in animals administered 30 or 10 ppm. During the FOB, significant changes in incidences of ptosis, piloerection, abnormal gait, pupillary response to light, and hindlimb grip strength were observed in either male and/or female rats administered 250 ppm. At the end of the study, the mean plasma, red blood cell and cortical (brain) ChE levels were decreased by 24, 48 and 40%, respectively in males and 60, 55, and 51%, respectively in females, compared to controls. Decreases in brain and blood cholinesterase activity correlated with the presence of clinical signs and changes in FOB parameters in the 250 ppm group. Generally, the magnitude of ChE inhibition was greater in females than males; and there was no cumulative effect with time. Motor activity - duration and number of movements - was not significantly affected at any concentration. No Oxamyl-related neuropathological changes were observed in any exposure group. Under the conditions of this study, the **Systemic Toxicity LOAEL is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively), based on decreases in body weights and food efficiency of both sexes. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively).** **The LOAEL for neurobehavioral effects is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively) based on decreases in plasma, RBC and brain ChE activity, clinical signs consistent with cholinesterase inhibition, and changes in incidences of FOB parameters such as increases in ptosis, piloerection, and abnormal gait and decreases in pupillary response to light and hindlimb grip strength. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively.** This study is classified **acceptable** and satisfies the guideline requirement for a subchronic oral neurotoxicity study (82-7) in rats.

82-2 21-Day Dermal Toxicity Species: rabbit Haskell Lab. Tox. and Industrial Medicine Lab. Proj. # Dupont-1599; Jan 21, 1999 MRID No. 44751201 **Acceptable**	In a 21-day dermal toxicity study (MRID 44751201), groups of 6 male and 6 female HM:(NZW)fBR rabbits were treated with oxamyl technical (96.9%, a.i.) moistened with deionized water by dermal occlusion at doses of 0, 25, 40, 50 or 75 mg/kg/day, for 6 hours a day, 7 days/week. The dosages and the peak time (1 hour post unwrapping) for blood collection was based on pilot studies. No mortality was recorded, and there were no clinical signs indicative of systemic toxicity at any treatment level. No treatment-related dermal irritation was produced. There were no treatment-related effects on body weight, food consumption or food efficiency in either sex of rabbits. Significant inhibition of plasma (29%) and brain (10.7%) cholinesterase was observed in female rabbits at 75 mg/kg/day. In addition, there was inhibition of red blood cell (RBC) cholinesterase in female rabbits treated with 75 mg/kg/day was noticed; although inhibition was statistically not significant, but considered biologically relevant because of magnitude of depression (24%) accompanying statistically significantly plasma and brain cholinesterase depression. In males rabbits, there were no treatment-related changes in the plasma, red blood cell or brain cholinesterase at any dose levels during the study. The female plasma, RBC or brain cholinesterase activity was not statistically significantly changed at 25, 40, or 50 mg/kg/day. Systemic toxicity was not observed in this study. The **Systemic Toxicity NOAEL = 75 mg/kg/day.** The **Cholinesterase NOAEL = 50 mg/kg/day, and LOAEL = 75 mg/kg/day,** based on decreased plasma, red blood cell and brain ChE inhibition in females rabbits. In male rabbits, the **NOAEL = 75 mg/kg/day, and LOAEL > 75 mg/kg/day.** The study is classified as **Acceptable/non-guideline.**

83-1b 1- Year feeding Species: dog Haskell Lab. Tox. and Industrial Medicine Lab. Proj. #HLR 381-90 and HLO 555-90; 1990 MRID Nos. 41697901, 42052701 & 44737503 **Acceptable**	In a 1-Year Chronic Feeding Study (MRID 41697901, 42052701 & 44737503) oxamyl (99%) was administered in diet to groups of male and female beagle dogs (5/dose) at dose levels of 0, 50, 150, or 250 ppm (equivalent to 0, 1.56, 4.60, or 8.0 mg/kg/day, for males and 0, 1.46, 4.50, or 7.84 mg/kg/day, for females, respectively). The dogs were offered the food once daily. . In this study a NOAEL for male dogs was not established due to depression of cholinesterase in plasma and brain at all dose levels. Subsequently, a second one-year study (MRID 42052701) was repeated in male dogs (5/dose) at dose levels of 0, 12.5, 20, 35 or 50 ppm (equivalent to 0, 0.372, 0.577, 0.930 or 1.364 mg/kg/day). In the later study food was offered *ad libitum*. Administration of oxamyl did not produce any adverse effects in urinalysis, ophthalmological examinations, and gross pathology at any dose levels. At 50 ppm, in males, the plasma ChE was inhibited ($P < 0.05$) at 6, 9, and 12 months by 33, 34, and 32%, respectively, compared to the controls; females exhibited slight depression during the study. At this dose, only in males, brain ChE was depressed 17% compared to controls. At doses 150 and above, increased incidence of clinical signs including tremors and vomiting in males and females were observed. Mean body weight gains/body weight gains in 250 ppm dose males and females decreased 23%/81% and 17%/49%, respectively, compared to controls and the decreases were statistically significant ($P < 0.05$). The body weight changes were accompanied by decreases food consumption food efficiency. The food consumption/food efficiency of 250 ppm males decreased 11%/75% ($P < 0.05$) in comparison to the controls. In 250 ppm females food consumption and food efficiency decreased 7% and 42%, respectively, compared to control, but the decreases were not significant. Plasma cholesterol of 150 ppm and 250 ppm decreased males and females. Histologically, 3/5 high dose males exhibited increased regenerative renal tubular epithelial alterations. Second study (MRID 42052701) conducted to establish a NOAEL in male dogs treated with oxamyl. At 50 ppm, by study termination, the plasma, RBC and brain (cerebellum+medulla) ChE was not statistically significantly depressed 11, 4, and 20%, respectively, compared to controls. Although, marked (20%) brain ChE inhibition may not be statistically significant, but considered biologically relevant, since tremors were observed at 150 and 250 ppm in males and at all doses in females in the previous study. At the 35 ppm, the plasma, RBC and brain ChE levels were depressed 18%, 5% and 2%, which appears to be true NOAEL. Even though, the study was not replicated as previous study to establish the NOAEL in male dogs, but the results do suggest that the plasma ChE levels are comparable and the information from this study could be utilized to derive a NOAEL in male dogs. **The Systemic Toxicity NOAEL = 50 ppm (1.56 mg/kg/day for males and 1.46 mg/kg/day for females) and LOAEL = 150 ppm (4.60 mg/kg/day for males and 4.50 mg/kg/day for females),** based on decreased body weights and body weight gains. **The Cholinesterase NOAEL = 35 ppm (0.930 mg/kg/day) for males and 50 ppm (1.56 mg/kg/day) for females, and LOAEL = 50 ppm (1.36 mg/kg/day) for males and 4.50 mg/kg/day) for females,** based on brain cholinesterase levels in males and vomiting, tremors, plasma and brain ChE inhibition in females. CLASSIFICATION: This study (MRID 41697901) in combination with the second 1-Year chronic dog study (MRID 42052701) is **Upgraded from Core-Supplementary to Acceptable/guideline** and satisfy the data requirements for a chronic toxicity study in dogs (83-1b).

83-3 Developmental toxicity Species: rat Haskell Lab. Tox. and Industrial Medicine Lab. Proj. #HLR 473-88; Oct. 3,1990 MRID Nos. 40859201 & 44737501 **Acceptable**	In a developmental toxicity study (MRIDs 40859201 & 44737501) oxamyl (97.2%) was administered by gavage to groups of pregnant Charles River (CD) BR rats (25/group) at dose levels of 0, 0.2, 0.5, 0.8, and 1.5 mg/kg from gestation days 7 to 16. On day 22, the fetuses were removed, and the dams were sacrificed. There were no mortalities or treatment-related gross abnormalities were reported. Maternal toxicity was observed at the 0.8 mg/kg/day, as decreased body weight gain (21%; $P < 0.05$), decreased food consumption (10%; $P < 0.05$) and increased incidence of tremors (4/25) associated with cholinesterase inhibition. The decreased body weight gain and food consumption and increased incidence of tremors were dose-related. At 1.5 mg/kg/day dose the body weight gains and food consumption decreased 30% and 16% ($P < 0.05$), respectively, compared to controls. At this dose increased number of dams showed statistically significant ($P < 0.05$) increase in signs of diarrhea, eye discharge, salivation, tremors, and wet legs, perineal and underbody. Treatment had no effect on the reproductive parameters and/or fetal malformations or variations. A dose-related decrease in fetal body weights was seen and decrease was statistically significant ($P < 0.05$) at doses 0.5 mg/kg and above. The fetal weights at 0.2, 0.5, 0.8, and 1.5 mg/kg, decreased 1.6%, 3.9%, 6.75% and 6.9%, respectively, compared to the controls. The HIARC (07/15/99) noticed the apparant quantitative fetal susceptibility to oxamyl and concluded that there is no fetal susceptibility due to decrease in maternal weight gain of 9% seen at 0.5 mg/kg/day at which decreased fetal body weights also occurred. Under the conditions of this study, **Maternal Toxicity NOAEL = 0.5 mg/kg/day and the LOAEL = 0.8 mg/kg/day**, based on decreased body weight gains, decreased food consumption and increased incidence of tremors. The **Developmental Toxicity NOAEL = 0.2 mg/kg/day and the LOAEL = 0.5 mg/kg/day,** based on dose-related decreased in the fetal body weight. CLASSIFICATION: The study is classified as Acceptable/Guideline and satisfies the data requirements for developmental toxicity study (83-3) in rat.

83-3 Developmental toxicity Species: rabbit Hazleton Labs., Vienna, VA. Study #201-405; Oct. 3,1980 MRID No. 00063009 **Acceptable**	In a developmental toxicity study (MRID 00063009), 17 pregnant New Zealand White rabbits per group were administered Oxamyl (97.1% a.i.; Lot No. H-10, 963-02, IND-1410-196) by gavage at doses of 0, 1, 2, or 4 mg/kg/day on gestation days (GD) 6-19, inclusive. On GD 29, all surviving does were sacrificed and all fetuses were weighed, measured for crown-rump distance, and examined for external malformation/variations. Each fetus was examined viscerally by fresh dissection and the sex determined. The heads from one-third of the fetuses were removed, fixed in Bouin's solution, and sectioned by Wilson's freehand razor technique. All carcasses were eviscerated and processed for skeletal examination. One doe each in the low- and high-dose groups died prior to scheduled sacrifice; these deaths were attributed to gavage error. All other animals survived to terminal sacrifice. Necropsy was unremarkable. No treatment-related clinical signs of toxicity were observed in any animal in any treated group. Maternal absolute body weights and food consumption were comparable between the treated and control groups throughout the study. However, during the treatment interval (day 6 - 19), the mid- and high-dose groups had significantly ($p \leq 0.05$) reduced body weight gains as compared with the controls. Body weight gains by the mid- and high-dose groups during treatment were 38.5% and 32.8%, respectively, of the control levels. Recovery was apparent during the postdosing interval when body weight gains by these groups were 113.5% and 157.9%, respectively, of the controls. **Therefore, the maternal toxicity LOAEL was 2 mg/kg/day based on reduced body weight gains and the maternal toxicity NOAEL is 1 mg/kg/day.** No treatment-related differences were observed between the treated and control groups for number of corpora lutea/doe, implantations/doe, preimplantation loss, fetal body weights and lengths, or fetal sex ratios. Dose-related increased resorption rates for the mid- and high-dose groups resulted in increased postimplantation losses and decreased litter sizes, but statistical significance was not reached for any parameter. For the control, low-, mid-, and high-dose groups, the mean resorptions/doe were 0.8, 0.5, 1.0, and 1.2, respectively, resulting in postimplantation losses of 10.4%, 8.7%, 15.9%, and 24.8%, respectively. The number of live fetuses/litter was 6.6, 6.6, 5.9, and 5.8. Resorptions in the mid-dose group consisted of both early (0.7/doe) and late (0.3/doe) resorptions which were not considered treatment-related. In the high-dose group only early resorptions were observed and two animals had whole litter resorption consisting of early resorptions of 1 and 7 implantation sites, respectively, are not considered treatment-related since complete resorptions in rabbits is not uncommon. Furthermore, resorptions in the high-dose group may have been a consequence of the maternal toxicity at this dose. The number of fetuses(litters) examined in the 0, 1, 2, and 4 mg/kg/day groups was 113(17), 90(15), 89(15), and 75(13), respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses. **Therefore, the developmental toxicity NOAEL was 4 mg/kg/day and the developmental toxicity was not observed.** This study is classified as **Acceptable/guideline** and satisfies the requirements for a developmental toxicity study (83-3b) in rabbits. Several deficiencies were noted in the conduct of this study, however, this study was performed prior to implementation of the current guidelines.

013975

DATA EVALUATION REPORT

OXAMYL

STUDY TYPE: ACUTE ORAL NEUROTOXICITY - RAT (81-8ss)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Toxicology and Risk Analysis Section
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Oak Ridge National Laboratory
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Task Order No. 98-04

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Date: 1-12-98

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Date: 1-12-98

Disclaimer

This Data Evaluation Report may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D. Date 7/8/99
Reregistration Branch 2 (7509C)
EPA Work Assignment Manger:
J. Rowland, MS. Date 7/8/99
Science Analysis Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity – Rat [§81-8ss]

DP BARCODE: D241653, D241654, D249642, D250235 & D253117
SUBMISSION CODE: S534905, S548879, S550051 & S556445
P.C. CODE: 103801 TOX. CHEM. NO.: 561A

TEST MATERIAL: Oxamyl Technical (98.3%)

SYNONYMS: Ethanimidothioic acid, 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-, methyl ester; methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-ethanmidothioate; methyl N', N'-dimethyl-N-[(methylcarbomoyl)oxy]-1-thiooxamimidate; IN-D1410; DPX-D1410 Technical; DPX-D1410-196

CITATION: Malley, L. (1997) Acute Oral Neurotoxicity Study of Oxamyl Technical in Rats. E. I. Du Pont de Nemours and Company. Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Haskell Laboratory Report No. 1118-96, Medical Research Project No. 10730-001, April 1, 1997. MRID 44254401. Unpublished.

Malley, L. (1997) Acute Oral Neurotoxicity Study of Oxamyl Technical in Rats. E. I. Du Pont de Nemours and Company. Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Haskell Laboratory Report No. 1118-96, Medical Research Project No. 11268-001, September 24, 1997. MRID 44420301. Unpublished.

Marshall, C., K. Mikles (1995) Neurotoxicity Evaluation of Trimethyltin in Rats (Positive Control Study). Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Haskell Laboratory Report No. 266-95, May 24, 1995. MRID 44628701. Unpublished.

Mikles, K. (1997) Neurotoxicity Evaluation of Carbaryl in Rats (Positive Control Study). Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Medical Research Project No. 5764-001, August 25, 1997. MRID 44628702. Unpublished.

Mikles, K. (1997) Neurotoxicity Evaluation of Amphetamine in Rats (Positive Control Study). Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Medical Research Project No. 11240-001, October 27, 1997. MRID 44628703. Unpublished.

Mikles, K. (1996) Neurotoxicity Evaluation of Acrylamide in Rats (Positive Control Study). Haskell Laboratory for Toxicology and Industrial Medicine. Elkton Road, P. O. Box 50. Newark, Delaware 19714. Medical Research Project No. 5764, January 3, 1996. MRID 44660601. Unpublished

Van Pelt, CS (1999) DuPont,s Position on the NOEL in Rats following Acute Neurotoxicity Testing with Oxamyl. E.I. du Ponte de Nemours and Company, Welmington, Delaware. Laboratory Project ID: Dupont-2020, January 21, 1999. MRID 44740701. Unpublished.

SPONSOR: E. I. Du Pont de Nemours and Company, Wilmington, Delaware. Dupont Agricultural Products.

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID Nos: 44254401, 44420301, 44740701, 44628701 - 446287030 & 44660601), single gavage doses of oxamyl (98.3% a.i.; Sample No.: 16995-02) in deionized water were administered to groups of Crl:CD rats (42/sex/dose). Males received 0, 0.1, 1.0, or 2.0 mg/kg and females received 0, 0.1, 0.75, or 1.5 mg/kg. Twelve rats/group were designated as neurotoxicity subgroup animals. All twelve of these rats/group were used for Functional Observational Battery (FOB) and Motor Activity (MA) assessments on days 1, 8, and 15. Body weight and clinical signs were also recorded for these animals. Six of the rats/group were euthanized for *in situ* perfusion. Thirty rats/group were designated as the clinical pathology group and were utilized for blood and brain collection for evaluation of cholinesterase levels.

One high-dose male died on day 1. High-dose males had significantly ($p<0.05$) decreased mean body weight gain for days 1-2. Similarly, mid-dose males and high-dose females also exhibited lower (n.s.) body weight gains. Decreased food consumption (n.s.) was also observed in mid- and high-dose males.

Statistically ($p<0.05$) and biologically significant dose related decreases in blood and brain cholinesterase activity were observed in mid- and high-dose males and females on day 1. Mean decreases were generally ≥40%. By day 2, decreases in cholinesterase activity were no longer biologically significant. No toxicologically significant decreases in cholinesterase activity were observed in any animals after day 1 or in low-dose males and females at any time point.

Clinical signs and FOB effects, consistent with decreased cholinesterase activity, were observed 30-60 minutes post-exposure in the mid- and high-dose males and females. Observations included soiled fur, lacrimation, salivation, slow righting reflex, abnormal gait, tremors, impaired locomotion, no response to tail pinch, limb splay, incoordination, labored breathing, and decreased forelimb and hindlimb grip strength. Other effects, including posture, palpebral closure, docile behavior, and decreased motor activity, were likely

due to lethargy and malaise secondary to decreased cholinesterase activity. No treatment-related clinical signs or FOB effects were observed in low-dose animals or after day 1. Validation of test method was conducted using the positive controls (MRIDs 44628701 - 446287030& 44660601). **Therefore, the RBC, plasma and brain ChE inhibition NOAEL = 0.1 mg/kg/day for males and females and LOAEL = 1.0 mg/kg/day in males and 0.75 mg/kg/day in females.**

No treatment-related gross effects or histopathology were observed.

Under the conditions of this study, the systemic/neurotoxicity LOAEL is 1.0 mg/kg for male rats and 0.75 mg/kg for female rats based on clinical signs, FOB effects, and decreased blood and brain cholinesterase activity. The NOAEL is 0.1 mg/kg.

This acute oral neurotoxicity study is classified **acceptable (guideline)**. This study does satisfy the guideline requirement for an acute oral neurotoxicity study (81-8) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not included.

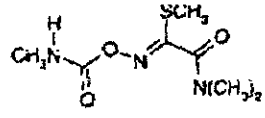
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Oxamyl Technical

   Description: Off-white solid
   Chemical Name: Ethanimidothioic acid, 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-, methyl ester
   CAS No.: 23135-22-0
   Sample No.: 16995-02
   Purity: 98.3%
   Contaminants: "none considered to be of toxicological significance at this time"

Structure:



2. Vehicle

   deionized water

3. Test animals

Species: rat
Strain: Crl:CD®BR
Age and mean group weights at study initiation: males - approx. 42 days old, 247.0-248.9 g for neurotoxicity study, 207.0-209.1 for clinical pathology study; females - approx 42 days old, 163.3-165.7 g for neurotoxicity study, 165.5-167.6 for clinical pathology study
Source: Charles River Breeding Laboratories, Inc., Raleigh, North Carolina
Housing: individually in suspended stainless steel wire mesh cages
Diet: Purina® Certified Rodent Chow #5002, *ad libitum*, except for overnight fast and during neurobehavioral assessment
Water: tap water (United Water Delaware), *ad libitum* except during neurobehavioral assessment
Environmental conditions:
Temperature: 23 ± 1 °C
Humidity: 50 ± 10%
Air changes: not provided
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 6-7 days

B. STUDY DESIGN

1. In life dates

Start: September 18, 1996; end: October 24, 1996

2. Animal assignment

Animals were assigned to treatment groups (Table 1) using a computer-based randomization that ensured homogeneity of group means for body weight.

TABLE 1. Animal assignment

Test group	Dose	Number assigned	
	mg/kg	Male	Female
Control	0	42	42
Low	0.1	42	42
Mid (for females)	0.75	0	42
Mid (for males)	1.0	42	0
High (for females)	1.5	0	42
High (for males)	2.0	42	0

Data taken from p. 17, MRID 44254401.

Twelve rats/group were designated as neurotoxicity subgroup animals. All twelve of these rats/group were used for FOB and MA assessments and six of the twelve rats/group were euthanized for *in situ* perfusion. Thirty rats/group were designated as the clinical pathology group and were utilized for blood and brain collection for evaluation of cholinesterase levels.

3. Validation of test methods

Positive control data were provided for FOB, MA, and neuropathology endpoints. Positive control chemicals included acrylamide, carbaryl, DDT, d-amphetamine, and trimethyl tin.

4. Dose selection rationale

Dose levels were based on previously determined rat oral LD_{50} values (3.1 mg/kg for males; 2.5 mg/kg for females) and a pilot study. In the pilot study, groups of 6 male rats were administered single gavage doses of oxamyl at levels of 0, 0.1, 0.75, 1.5, or 2.0 mg/kg. Female rats (6/group) received dose levels of 0, 0.1, 0.25, 0.75, or 1.5 mg/kg. Males and females administered 0.1 mg/kg and females administered 0.25 mg/kg did not exhibit compound-related clinical signs or effects on motor activity. Males and females administered 0.75 mg/kg and above exhibited dose-related increases in the incidence of clinical signs, including abnormal gait or mobility, salivation, tremors, ocular discharge, and wet perineum. The time for peak effect for clinical signs was 30-60 minutes after oxamyl administration. (Due to the fact that motor activity monitors could not accommodate all test animals, only groups of animals exhibiting no signs of clinical toxicity were assessed for motor activity effects.)

5. Test solution preparation and analysis

A concentrated stock solution of approximately 2 mg oxamyl/mL in deionized water was prepared on the day of dosing. Aliquots of the stock solution were then diluted to nominal concentrations of 0.01, 0.075, 0.1, 0.15, or 0.2 mg/mL for the 0.1, 0.75, 1.0, 1.5, and 2.0 mg/kg groups, respectively. Nominal concentrations reflected adjustments for purity. Dosing volumes were 10 mL/kg. Two samples from each test solution were analyzed for concentration. One sample each of the 0.01, 0.075, and 0.15 mg/mL test solutions was analyzed after storage at room temperature to verify stability. Analyses were carried out via liquid chromatography.

Results -

Homogeneity analysis: Homogeneity analysis was not performed.

Stability analysis: Measured concentrations of test samples ranged from 93.3-106% of nominal after five hours at room temperature, indicating acceptable stability.

Concentration analysis: Analytical measurements indicated that actual dose levels were 91.3 to 106% of nominal.

6. Estimated time of peak effect

In the range-finding study, peak effects for clinical signs occurred from 30-60 minutes after test substance administration.

Based on the results of the preliminary study, clinical signs were recorded for the clinical pathology rats approximately 30-60 minutes post-dosing on test day 1, and on either test day 2 or 15. FOB and MA tests were conducted prior to compound administration and on test days 1, 8, and 15.

7. Statistical analysis

Clinical sign incidence data and descriptive FOB data were analyzed by the Cochran Armitage Trend test. If significant, the test was repeated sequentially dropping off the highest dose level until no significance was found. If a significant lack of fit was found, a Fisher's Exact test with a Bonferroni correction was used.

Body weight, body weight gain, food consumption, grip strength, and foot splay data were evaluated as parametric data. For grip strength and foot splay, Bartlett's test for homogeneity of variance was used. If Bartlett's test was not significant, these data and the body weight and food consumption data were analyzed by a univariate ANOVA, with Dunnett's test used to determine test groups significantly different from controls.

Cholinesterase data were evaluated by a one-way ANOVA. Dunnett's test was used for pairwise comparisons between test and control groups. If the Shapiro-Wilk test for normality was significant, the Kruskal-Wallis test and Dunn's Multiple Comparisons were used. If the Shapiro-Wilk test was not significant, but Levene's test was significant, a robust version of Dunnet's was used.

Motor activity data were evaluated with the Kruskal-Wallis test followed by Dunn's multiple comparison procedure.

C. METHODS

1. Observations

Clinical signs were recorded for neurotoxicity animals once daily through day 15. Clinical signs were recorded for clinical pathology animals before dosing on test day 1, approximately 30-60 minutes post-dosing, and on either day 2 or 15.

2. Body weight

Body weights of neurotoxicity rats were recorded immediately before dosing and on days 2, 8 and 15. Clinical pathology rats were weighed immediately before dosing.

3. Food consumption and food efficiency

Food consumption was determined for neurotoxicity rats for test day intervals 1-2, 2-8, and 8-15. Food consumption was not determined for clinical pathology rats. Food efficiency was not determined.

4. Functional observational battery

Animals were tested one week prior to treatment, and on days 1, 8, and 15. For FOB testing, the "home cage" was defined as the cage on the transport rack since the actual home cages were not amenable to transport between the housing room and the laboratory. The FOB home cage assessments began after the rats were acclimated to the transport "home cage" for approximately 30 minutes.

a. Home cage observations

Posture, palpebral closure, writhing, circling, and biting.

b. Measurements during handling

Ease of removal, ease of handling, muscle tone, vocalizations, piloerection, bite marks on tail and/or paws, palpebral closure, fur appearance, lacrimation, salivation, exophthalmus.

c. Open field measurements

Righting reflex, labored breathing, convulsions/tremors, coordination, grooming, gait, locomotion, vocalizations, arousal, palpebral closure, defecation, and urination.

d. Reflex/physiologic observations and measurements

Approach response, touch response, auditory response, pinch tail response, forelimb grip strength, hindlimb grip strength, foot splay.

5. Motor activity

Following FOB testing, the animals were individually tested in one of 30 automated activity monitors (Coulbourn® Instruments). The infrared monitoring device enables measurement of time spent moving and number of movements. A continuous movement, regardless of duration, was counted as one movement. This measurement system is such that duration of movement is analogous to "counts" in devices that monitor interruption of light beams. Duration of movement and number of movements were evaluated in six

consecutive 10 minute blocks as well as for the total 60 minute session.

6. Clinical Chemistry (Cholinesterase)

Blood was collected from the orbital sinus of the first ten rats in the clinical pathology group prior to oxamyl administration for red blood cell and plasma cholinesterase determination. Blood was collected on day 1 approximately 30-60 minutes post-dosing (the first ten rats/dose in the clinical pathology group), test day 2 (the second ten rats/dose in the clinical pathology group), and test day 15 (the third ten rats/dose in the clinical pathology group). Rats were under light CO_2 anesthesia during blood collection. After blood collection on days 1, 2, and 15, rats were sacrificed and brains were removed and dissected into hippocampus, cerebral cortex, half brain, cerebellum, and midbrain. The brain sections were frozen at -70°C until cholinesterase activity was measured on a Boehringer Mannheim/Hitachi 717 clinical chemistry analyzer.

7. Sacrifice/necropsy/neurohistopathology

Six neurotoxicity group males from the 0.1, 1.0, and 2.0 mg/kg dose groups and six neurotoxicity group females from the 0.1, 0.75, and 1.5 kg/kg dose groups were randomly selected for perfusion and collection of tissues. Six unexposed control animals/sex served as negative controls. Fifteen days following oxamyl administration, rats were killed by pentobarbital anesthesia followed by exsanguination and whole body perfusion fixation. Tissues were saved from all groups; however, only tissues from control and high-dose animals were processed for histopathology and examined. The following tissues were evaluated microscopically:

X	Brain	X	Spinal Cord	X	Peripheral Nerves
X	Olfactory region	X	Cervical	X	Optic nerves
X	Forebrain		Thoracic	X	Spinal nerve root fiber & ganglion
X	Midbrain	X	Lumbar	X	Dorsal & ventral, cervical
X	Pons			X	Dorsal & ventral, lumbar
X	Cerebellum			X	Gasserian ganglion
X	Medulla Oblongata			X	Sciatic nerve
				X	Tibial nerve
					Sural nerve
					Other
					Eyes
				X	Gastrocnemius muscle
				X	Gross findings

Sections of the brain, spinal cord, and skeletal muscle were embedded in paraffin, sectioned at thickness of 5 μm and stained with hematoxylin and eosin. Additional

sections of brain and spinal cord were sectioned and stained with Luxol fast blue and periodic acid Schiff. Sections of sciatic and tibial nerves, gasserian ganglia, dorsal root fibers and ganglia, and ventral root fibers were embedded in glycol methacrylate, sectioned at thickness of 3 μm and stained with hematoxylin and eosin.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

One male in the 2.0 mg/kg group was found dead on day 1. This rat exhibited low posture, tremors, and salivation during clinical observation. There were no other treatment-related deaths.

Treatment-related clinical effects indicative of cholinesterase inhibition were observed on day 1 (30-60 minutes post-dosing) in males given 1.0 or 2.0 mg/kg oxamyl and in females given 0.75 or 1.5 mg/kg. No treatment-related clinical signs were observed at lower doses or after day 1. Summary data for the clinical pathology group rats are presented in Table 2. Similar clinical observations were also noted among FOB group rats on day 1; however, data for these animals were not tabulated.

TABLE 2. Clinical signs observed on day 1 in rats administered Oxamyl by gavage

	Males				Females			
	0 mg/kg	0.1mg/kg	1.0 mg/kg	2.0 mg/kg	0 mg/kg	0.1 g/kg	0.75 mg/kg	1.5 mg/kg
Gasping	0/30	0/30	0/30	2/30	0/30	0/30	0/30	0/30
Clear ocular discharge	0/30	0/30	2/30	1/30	0/30	0/30	0/30	1/30
Salivation	0/30	0/30	**22/30***	**30/30***	0/30	0/30	**4/30***	**24/30***
Tremors	0/30	0/30	**30/30***	**30/30***	0/30	0/30	**29/30***	**30/30***
Low posture	0/30	0/30	**21/30***	**28/30***	0/30	0/30	**13/30***	**30/30***
Wet perineum	0/30	0/30	0/30	0/30	0/30	0/30	**4/30***	**7/30***

Data taken from pp. 48-49, MRID 44254401. *p<0.05.

B. BODY WEIGHT, BODY WEIGHT GAIN and FOOD CONSUMPTION

There were no significant differences in group mean body weight for any treated animals compared

to controls. However, toxicologically significant effects on body weight gain were observed. Mean body weight gains are presented in Table 3.

Table 3. Mean body weight gain [a]

Test Day	Males				Females			
	0 mg/kg	0.1 mg/kg	1.0 mg/kg	2.0 mg/kg	0 mg/kg	0.1 mg/kg	0.75 mg/kg	1.5 mg/kg
1 - 2	28.1±3.8	26.3±5.1	23.5±4.5	**13.9±6.0***	18.1±3.8	18.3±4.5	17.9±2.5	15.9±1.8
2 - 8	42.3±8.1	41.7±8.6	43.0±9.6	46.7±7.1	20.4±5.7	20.5±4.9	15.3±3.2*	21.1±5.8
8 - 15	39.9±7.1	40.7±5.9	41.7±8.8	40.7±5.5	14.7±3.5	16.2± 4.6	15.5±7.7	40.7±5.5
1 - 15	110.3±14.0	108.7±12.8	108.7±16.8	101.3±11.4	53.2 ±6.7	55.0 ±9.6	48.7 ±8.7	52.8 ±5.2

a Data copied from pp. 52, MRID 44254401. *P≤ 0.05

Males administered 2.0 mg/kg oxamyl had a 51% decrease ($p<0.05$) and males administered 1.0 mg/kg showed a 16% decrease (n.s.) in mean body weight gain over days 1-2 compared to controls. Corresponding decreases ($p<0.05$) in food consumption of 13% and 4.1% were observed in the 1.0 and 2.0 mg/kg males, respectively. There were no treatment-related effects on body weight gain or food consumption in lower doses or for days 2-15.

Females administered 1.5 mg/kg oxamyl had a 12% decrease (n.s.) in mean body weight gain over days 1-2 compared to controls. However, no corresponding decrease in food consumption was observed. In fact, food consumption was increased 15% for these rats. Females administered 0.75 mg/kg exhibited a 21% decrease ($p<0.05$) in body weight gain for the day 2-8 interval. However, in the absence of a dose-response, this effect is of questionable toxicological significance. No other treatment-related effects on body weight gain or food consumption were observed in female rats.

C. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Table 4. Mean fore- and hindlimb grip strength (kg) and foot splay (cm)a

Test Day	Males (mg/kg)				Females (mg/kg)			
	0	0.1	1.0	2.0	0	0.1	0.75	1.5
Forelimb Grip Strength (kg)								
Baseline	0.54	0.57	0.57	0.54	0.47	0.42	0.46	0.46
1	0.60	0.64	0.50	**0.38***	0.49	0.47	0.45	0.43
8	0.69	0.75	0.70	0.72	0.58	0.59	0.52	0.61
15	0.71	0.67	0.79	0.75	0.52	0.50	0.47	0.50
Hindlimb Grip Strength (kg)								
Baseline	0.44	0.45	0.42	0.46	0.41	0.37	0.45	0.41
1	0.46	0.43	0.43	**0.36***	0.41	0.48	0.41	0.35
8	0.65	0.66	0.62	0.67	0.61	0.57	0.57	0.56
15	0.63	0.57	0.58	0.60	0.52	0.52	0.47	0.49
Foot Splay (cm)								
Baseline	7.6	7.9	7.4	7.1	5.7	7.0	6.0	6.6
1	7.5	7.1	7.7	8.1	6.2	6.6	6.2	6.9
8	8.2	7.7	7.7	7.0	6.2	6.1	5.7	6.3
15	8.7	8.6	9.0	7.7	6.9	7.3	6.7	7.1

a Data copied from pp.54 - 56, MRID 44254401; $P \leq 0.05$

Summary of fore- and hindlimb grip strength and foot splay are provided above (Table 4). Treatment-related effects were observed approximately 30-60 minutes post-exposure on day 1 in males administered 1.0 or 2.0 mg/kg oxamyl and in females administered 0.75 or 1.5 mg/kg. No treatment-related FOB effects were observed at lower doses or on days 8 or 15. Many of the observed effects, including soiled fur, lacrimation, salivation, pupillary response, slow righting reflex, abnormal gait, tremors, impaired locomotion, no tail pinch response, limb splay, foot splay, labored breathing, increased urination, and decreased forelimb and hindlimb grip strength, are consistent with decreased cholinesterase activity.

On day 1, forelimb and hindlimb grip strength were decreased ($p<0.05$) 37% and 22%, respectively, in males administered 2.0 mg/kg oxamyl. Similarly, forelimb and hindlimb grip strength were decreased (n.s.) 12% and 15%, respectively, in females administered 1.5 mg/kg oxamyl. Hindlimb foot splay was increased (n.s.) in high-dose males compared to both the control and baseline values on day 1. This effect is consistent with the observed decrease in grip strength. No other treatment-related effects were observed concerning grip strength and foot splay.

A summary of other selected day 1 FOB data are presented in Table 5.

TABLE 5. Selected fob results on day 1 in male and female rats administered Oxamyl

Home Cage	Dose (mg/kg)	Males				Females			
		0	0.1	1.0	2.0	0	0.1	0.75	1.5
	Curled up	7/12	3/12	10/12	**11/12***	2/12	2/12	**8/12***	**9/12***
Removal from home cage	No resistance to removal	0/12	0/12	1/12	**5/12***	0/12	0/12	0/12	**3/12***
	Soiled fur	0/12	0/12	**7/12***	**11/12***	0/12	0/12	2/12	**7/12***
	Slight lacrimation	0/12	0/12	**4/12***	**8/12***	0/12	0/12	0/12	**5/12***
	Dilated pupils	0/12	0/12	**4/12***	**6/12***	0/12	0/12	1/12	**4/12***
	Severe salivation	0/12	0/12	**3/12***	**10/12***	0/12	0/12	0/12	**4/12***
Open Field	Decreased righting reflex	0/12	0/12	**3/12***	**5/12***	0/12	0/12	**5/12***	**3/12***
	Tremors	0/12	0/12	**11/12***	**12/12***	0/12	0/12	**8/12***	**12/12***
	Uncoordinated	0/12	0/12	2/12	**7/12***	0/12	0/12	1/12	**5/12***
	Abnormal gait	0/12	0/12	**3/12***	**9/12***	0/12	0/12	**2/12***	**8/12***
	Impaired locomotion	0/12	0/12	**3/12***	**9/12***	0/12	0/12	0/12	**5/12***
	Low arousal	0/12	0/12	**3/12***	**3/12***	0/12	0/12	0/12	1/12
	Limb splay	0/12	0/12	**6/12***	**11/12***	0/12	0/12	**4/12***	**9/12***
	Absent tail pinch response	0/12	1/12	**6/12***	**9/12***	0/12	0/12	1/12	**9/12***

Data taken from pp. 58-72, MRID 44254401; *P≤ 0.05

D. MOTOR ACTIVITY

Treatment-related effects on motor activity were observed in day 1 in males administered 1.0 and 2.0 mg/kg and in females administered 0.75 and 1.5 mg/kg oxamyl. Mean duration of movements was decreased ($p<0.05$) in both mid- and high-dose males and females during the first and second 10-minute intervals. Additionally, 1.5 mg/kg females had decreased ($p<0.05$) duration of movement during the third 10-minute interval. Corresponding with these results, total duration

of movement was decreased in both mid- (n.s.) and high-dose ($p<0.05$) males and females. Data are summarized in Table 6.

TABLE 6. Motor Activity: Mean duration of movement (sec.) On day 1 in male and female rats administered Oxamyl

Males							
Dose (mg/kg)	Successive 10-minute Interval						
	1	2	3	4	5	6	Total
0	309±45	158±71	61±70	43±61	14±33	29±66	614±277
0.1	333±54	220±64	98±64	37±78	19±33	22±24	729±253
1.0	**104±88***	**48±67***	38±43	30±49	57±62	100±90	378±267
2.0	**44±32***	**27±29***	24±27	28±18	25±24	21±23	**169±103***
Females							
	Successive 10-minute Interval						
Dose (mg/kg)	1	2	3	4	5	6	Total
0	364±39	225±72	113±76	34±39	54±68	52±62	842±140
0.1	337±42	202±55	106±60	97±44	96±73	86±77	925±235
0.75	**188±116***	**105±92***	72±72	88±73	66±78	72±85	590±369
1.5	**38±24***	**14±13***	**23±27***	41±50	48±52	36±40	**200±142***

Data taken from, pp.73-74, MRID 44254401.
*P≤ 0.05

Changes in the mean number of movements paralleled changes observed in mean duration of movements. Mid- and high-dose males showed statistically significant ($p<0.05$) decreases in the mean number of movements on day 1 during the first and second 10-minute intervals. Although the number of movements for the 2.0 mg/kg males was significantly higher during the fifth 10-minute interval, the total number of movements for the entire 60-minute period was lower ($p<0.05$) than controls. The mean number of movements was lower ($p<0.05$) for 1.5 mg/kg females during the first, second, and third 10-minute intervals and for the total 60-minute period. Data are summarized in Table 7.

TABLE 7. Motor Activity: Mean number of movements on day 1 in male and female rats administered Oxamyl

Males							
	Successive 10-minute interval						
Dose (mg/kg)	1	2	3	4	5	6	Total
0	143±21	101±34	45±41	34±42	11±22	24±38	357±147
0.1	136±14	129±20	67±39	24±35	18±25	25±25	398±102
1.0	**58±32***	**36±34***	28±24	24±31	38±36	61±50	**245±134***
2.0	**50±37***	**39±36***	31±34	36±27	**33±25***	30±31	**220±151***
Females							
	Successive 10-minute Interval						
Dose (mg/kg)	1	2	3	4	5	6	Total
0	142±14	113±29	76±38	33±33	44±54	40±39	448±143
0.1	147±17	119±22	76±37	77±32	75±49	68±52	562±130
0.75	101±37	63±48	49±43	61±48	44±45	46±46	364±183
1.5	**39±21***	**20±14***	**30±29***	39±35	46±37	38±34	**212±109***

Data taken from, pp.75-76, MRID 44254401.
*P≤ 0.05

E. CHOLINESTERASE ACTIVITY

Statistically ($p<0.05$) and biologically significant dose related decreases in blood and brain cholinesterase activity were observed in mid- and high-dose males and females on day 1. Mean decreases were generally ≥40%. By day 2, decreases in cholinesterase activity were no longer biologically significant. No toxicologically significant decreases in cholinesterase activity were observed in low-dose males and females. Observed decreases in erythrocyte cholinesterase activity at day 15 in low-dose females and in midbrain activity at day 15 in low-dose males are not considered toxicologically significant due to the absence of cholinergic signs in these animals. Cholinesterase activity data are summarized in Tables 8 (males) and 9 (females).

TABLE 8. Mean cholinesterase activity in male rats administered Oxamyl (% change)

Day	Dose (mg/kg)	Erythrocyte	Plasma	Cortex	Hippocampus	Midbrain	Cerebellum	Half-Brain
1	0	2138	428	10.19	13.03	11.63	4.83	11.13
	0.1	2000 (↓6.5)	387 (↓9.6)	8.23 (↓19.2)	11.69 (↓10.3)	11.96 (↑2.8)	5.04 (↑4.3)	10.96 (↓1.5)
	1.0	**914 (↓57.2)***	**170 (↓60.3)***	**4.53 (↓55.5)***	8.06 (↓38.1)	**6.95 (↓40.2)***	3.59 (↓25.7)	**5.89 (↓47.1)***
	2.0	**800 (↓62.6)***	**99 (↓76.9)***	**2.98 (↓70.8)***	**3.40 (↓73.9)***	**4.64 (↓60.1)***	**1.43 (↓70.4)***	**3.76 (↓66.2)***
2	0	1894	401	11.27	17.19	15.23	5.72	12.21
	0.1	1684 (↓11.1)	453 (↑13.0)*	10.82 (↓4.0)	15.12 (↓12.0)	14.58 (↓4.3)	8.00 (↑39.9)	11.65 (↓4.6)
	1.0	1738 (↓8.2)	448 (↑11.7)	11.69 (↑3.7)	19.41 (↑12.9)	15.43 (↑1.3)	6.19 (↑8.2)	12.32 (↑0.9)
	2.0	1816 (↓4.1)	414 (↑3.2)	11.05 (↓2.0)	24.62 (↑43.2)	14.00 (↓8.1)	6.18 (↑8.0)	11.67 (↓4.4)
15	0	2024	406	9.83	10.91	11.83	4.43	11.35
	0.1	1740 (↓14.0)	380 (↓6.4)	8.91 (↓9.4)	9.74 (↓10.7)	**10.57 (↓10.7)***	4.07 (↓8.1)	11.32 (↓0.3)
	1.0	1614 (↓20.3)	377 (↓7.1)	9.20 (↓6.4)	10.62 (↓2.7)	11.27 (↓4.7)	4.23 (↓4.5)	10.87 (↓4.2)
	2.0	1872 (↓7.5)	284 (↓5.4)	9.27 (↓5.7)	10.07 (↓7.7)	10.82 (↓8.5)	4.31 (↓2.7)	10.89 (↓4.1)

Data taken from, pp.36 & 38, MRID 44254401.
*P≤ 0.05

TABLE 9. Mean cholinesterase activity in female rats administered Oxamyl (% Change)								
Day	Dose (mg/kg)	Eryhtrocyte	Plasma	Cortex	Hippocampus	Midbrain	Cerebellum	Half-Brain
1	0	2010	994	10.03	10.15	13.60	5.61	11.97
	0.1	2270 (↑12.9)	1082 (↑8.9)	8.56 (↓14.7)	8.71 (↓14.2)	12.48 (↓8.2)	**4.23 (↓24.6)***	11.89 (↓0.7)
	0.75	**914 (↓54.5)***	**613 (↓38.3)***	**4.16 (↓58.5)***	**6.09 (↓40.0)***	**6.82 (↓49.9)***	**2.77 (↓50.6)***	**6.43 (↓46.3)***
	1.5	**606 (↓69.9)***	**279 (↓71.9)***	**3.18 (↓68.3)***	**2.97 (↓70.7*)**	**4.09 (↓69.9)***	**1.45 (↓74.2)***	**3.94 (↓67.1)***
2	0	1954	1314	9.5	8.01	13.73	4.69	11.99
	0.1	1768 (↓9.5)	991 (↓24.6)	10.01 (↑5.4)	9.57 (↑19.5)	12.62 (↓8.1)	5.15 (↑9.8)	12.30 (↑2.6)
	0.75	1982 (↑1.4)	921 (↓29.9)	10.34 (↑8.8)	8.82 (↑10.1)	12.26 (↓10.7)	4.44 (↓5.3)	12.35 (↑3.0)
	1.5	1692 (↓13.4)	1030 (↓21.6)	9.21 (↓3.1)	8.87 (↑10.7)	12.42 (↓9.5)	4.85 (↑3.4)	11.96 (↓0.3)
3	0	1886	1129	10.87	7.55	11.25	3.71	11.49
	0.1	**1428 (↓24.3)***	1531 (↑35.6)	10.09 (↓7.2)	9.17 (↑21.5)	11.80 (↑4.9)	4.10 (↑10.5)	10.83 (↓5.7)
	0.75	1734 (↓8.1)	1612 (↑42.8)*	10.29 (↓5.3)	9.99 (↑32.3)	12.40 (↑10.2)	4.60 (↑24.0)*	11.13 (↓3.1)
	1.5	1818 (↓3.6)	1283 (↑13.6)	11.03 (↑1.5)	10.21 (↑35.2)	11.20 (↓0.4)	4.43 (↑19.4)*	11.18 (↓2.7)

Data taken from, pp. 37 & 39, MRID 44254401.
*P≤ 0.05

F. SACRIFICE/NECROPSY/NEUROHISTOPATHOLOGY

No treatment-related gross or histopathological lesions were observed.

III. DISCUSSION

A. DISCUSSION

Oxamyl is a carbamate insecticide the mechanism of which is to inhibit cholinesterase activity. Statistically ($p<0.05$) and biologically significant decreases in blood and brain cholinesterase activity were observed 30-60 minutes post-exposure in male rats administered 1.0 or 2.0 mg/kg and in female rats administered 0.75 or 1.5 mg/kg oxamyl. These decreases were generally ≥40% and occurred in a dose-dependent manner. Other observed decreases in the general range of 20-

30% (erythrocyte, day 15, 1 mg/kg males; erythrocyte, day 15, 0.1 mg/kg females; plasma, day 2, all treated females; cortex, day 1 0.1 mg/kg males) are considered toxicologically and biologically insignificant since no clear dose-response exists and since no corresponding cholinergic signs were observed in these animals. In 0.1 mg/kg females rats day 1 mean cerebellar ChE activity was inhibited by approximately 25% ($P < 0.05$) compared to controls by Dunnett's test. This decrease in ChE levels in the cerebellum is considered equivocal since there was no test substance-related neurobehavioral effects seen either in males or females at this dose. Further there is great variability in ChE levels in female cerebellum on test day 1, 2, and 15 (5.61 ± 0.36, 4.69 ± 0.94, and 3.71 ± 0.91 U/g, respectively) and the mean values for the 0.1 mg/kg females (4.23 U/g) was well within the control range of this study. The registrant claimed that the variability in brain ChE levels in different anatomical regions was due to physical factors (MRID 44740701). The HIARC (June 8, 1999) did not concur with the registrants explanation, however, the committee considered that increased ChE inhibition in single compartment is not appropriate as regulatory endpoint. **Therefore, the RBC, plasma and brain ChE inhibition NOAEL = 0.1 mg/kg/day for males and females and LOAEL = 1.0 mg/kg/day in males and 0.75 mg/kg/day in females.**

Clinical signs and FOB effects were observed in mid- and high-dose males and females only on day 1 and are consistent with decreased cholinesterase activity or general lethargy secondary to decreased cholinesterase activity.

One high-dose male died during the study. Mid- and high-dose males and high-dose females had decreased mean body weight gain for days 1-2. Decreased food consumption was also observed in mid- and high-dose males.

No treatment-related gross effects or histopathology were observed.

Under the conditions of this study, the systemic/neurotoxicity LOAEL is 1.0 mg/kg for male rats and 0.75 mg/kg for female rats based on clinical signs, FOB effects, and decreased blood and brain cholinesterase activity. The NOAEL is 0.1 mg/kg.

Additional data on brain cholinesterases (MRID 44420301) to establish a reference range for the testing laboratory supports the study findings that the mid- and high-doses were adverse effects.

B. DEFICIENCIES: None identified.

C. CLASSIFIACTION: **Acceptable**.

DATA EVALUATION REPORT

013975

OXAMYL

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY – RAT (82-7)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Date 12/2/98

Date 12/2/98

013975

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity – Rat [§82-7]

DP BAR CODE: D246742
P.C. CODE: 103801

SUBMISSION CODE: S544071
TOX. CHEM. NO.: 561A

TEST MATERIAL: Oxamyl Technical

SYNONYMS: Ethanimidothioic acid, 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-, methyl ester; DPX-D1410-196; DPX-D1410-196 Technical; methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxo-ethanimidothioate; emthyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate

CITATION: Malley, L.A. (1998) Oxamyl technical: subchronic oral neurotoxicity study in rats. E.I. duPont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, Delaware. Laboratory project ID HL-1998-00708, February 23, 1998. MRID 44504901. Unpublished.

SPONSOR: E.I. duPont de Nemours and Company, Wilmington, Delaware 19898.

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 44504901), 42 Crl:CD^{R}(SD)BR rats/sex/exposure group were administered Oxamyl Technical (purity, 98.3%; Haskell sample number 16995-02) at concentrations of 0, 10, 30, or 250 ppm (equivalent to 0, 0.564, 2.10, or 14.9 mg/kg/day for male rats and 0, 0.679, 2.40, or 19.9 mg/kg/day for female rats, respectively) in the diet. The 30 and 250 ppm concentrations were reduced from 100 and 300 ppm, respectively, on day 7 of administration due to toxic effects including tremors and weight loss. Twelve rats/sex/exposure group were assigned to the neurotoxicity group and underwent functional observational battery (FOB) and motor activity (MA) testing prior to dietary administration and during weeks 4, 8, and 13. Ten rats/sex/exposure group were sacrificed on days 27 and 55 and at termination of the study for cholinesterase activity determinations. Six rats/sex/exposure group (from the neurotoxicity group) were perfused for neuropathology at study termination.

All animals survived to scheduled termination. At the end of 90 days, body weights of male and female rats receiving 250 ppm in the diet were significantly depressed by 24% and 10%, respectively ($p<0.05$). Decreases in body weights correlated with decreased food consumption in males and decreased food efficiency in both sexes. Exposure-related clinical signs (tremors, abnormal gait or mobility, hunched-over posture, exophthalmus, ptosis, hyperactivity, piloerection, colored

discharge from the eyes, hyperactivity and lacrimation) were present in one or both sexes administered 250 ppm in the diet but not in animals administered 30 or 10 ppm. During the FOB, significant changes in incidences of ptosis, piloerection, abnormal gait, pupillary response to light, and hindlimb grip strength were observed in either male and/or female rats administered 250 ppm. At the end of the study, the mean plasma, red blood cell and cortical (brain) ChE levels were decreased by 24, 48 and 40%, respectively in males and 60, 55, and 51%, respectively in females, compared to controls. Decreases in brain and blood cholinesterase activity correlated with the presence of clinical signs and changes in FOB parameters in the 250 ppm group. Generally, the magnitude of ChE inhibition was greater in females than males; and there was no cumulative effect with time. Motor activity - duration and number of movements - was not significantly affected at any concentration. No Oxamyl-related neuropathological changes were observed in any exposure group.

Under the conditions of this study, the **Systemic Toxicity LOAEL is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively), based on decreases in body weights and food efficiency of both sexes. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively).**

The LOAEL for neurobehavioral effects is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively) based on decreases in plasma, RBC and brain ChE activity, clinical signs consistent with cholinesterase inhibition, and changes in incidences of FOB parameters such as increases in ptosis, piloerection, and abnormal gait and decreases in pupillary response to light and hindlimb grip strength. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively.

This study is classified **acceptable** and satisfies the guideline requirement for a subchronic oral neurotoxicity study (82-7) in rats.

COMPLIANCE: Signed and dated Quality Assurance and Good Laboratory Practice Compliance statements were present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Oxamyl Technical

   Description: off-white solid
   CAS No.: 23135-22-0
   Batch No.: 16995-02 (Haskell sample number)
   Purity: 98.3%
   Contaminants: none considered to be of toxicological significance

2. Vehicle

   None; substance was administered in the diet.

3. Test animals

Species: rat
Strain: Crl:CD^{R}(SD)BR
Age and weight at study initiation: 52 days old; males: 201.5 - 291.7 g, females: 150.8 - 211.5 g
Source: Charles River Laboratories, Inc.
Housing: individually, in suspended wire mesh stainless steel cages
Environmental conditions:
Temperature: 23±1°C
Humidity: ~50%
Air changes: not given
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 6 days of quarantine followed by 10 days of acclimation

B. STUDY DESIGN

1. In life dates

Start: April 4, 1997; end: July 10, 1997

2. Animal assignment

All animals were received on March 18, 1997. The number of animals assigned to the exposure groups is listed in Table 1. Animals were separated by sex and then a computer-generated randomization list was used to assign animals to groups based on body weight (excess rats were first culled based on lowest and highest weights). The first 12 rats/sex/group were assigned to the neurotoxicity subgroup (six rats/sex/group were sacrificed for neuropathology at study termination) and the remaining 30 rats/sex/group were assigned to the clinical pathology subgroup. Of these, 10 rats/sex/group were sacrificed for clinical studies during weeks 4, 8, and 13.

TABLE 1. Study Design					
Test group	Dietary Concentration (ppm)[a]	Dose Level (mg/kg/day)[b]		Number of animals	
		Males	Females	Males	Females
Control	0	0	0	42	42
Low	10	0.564	0.679	42	42
Mid	30	2.10	2.40	42	42
High	250	14.9	19.9	42	42

Data taken from Tables 12 and 13, pp. 71-72, MRID 44504901.
[a] For test days 0-7, the dietary concentrations were 0, 10, 100, and 300 ppm. Due to clinical signs of toxicity and severe body weight loss, the intermediate and high dietary concentrations were reduced to 30 and 250 ppm, respectively, on test day 7.
[b] Values based on dietary concentrations of 0, 10, 100, and 300 ppm for test days 0-7 and dietary concentrations of 0, 10, 30, 250 ppm for test days 7-90.

The control, low, intermediate, and high dose groups initially received concentrations of 0, 10, 100, or 300 ppm in the diet. Due to clinical signs of toxicity and severe body weight loss, the intermediate and high dose groups were reduced to 30 and 250 ppm, respectively, on day 7.

3. Validation of test methods

Studies with male or female Crl:CD^{R}BR rats were previously conducted to establish the sensitivity, reliability, and validity of the test methods in accordance with EPA Neurotoxicity Testing Guidelines Subdivision F, Addendum 10. An acute study with carbaryl (HL-1997-00361) and a four-week study with acrylamide (HRL 293-95) established the sensitivity, reliability, and validity of the FOB procedures and the observation of clinical signs. Locomotor activity changes were validated by studies with rats treated with substances that decrease (carbaryl) or increase (amphetamine; HL-1997-00686) motor activity. The micropathologic procedure for detecting lesions in the peripheral and central nervous systems was validated by studies with acrylamide and trimethyltin chloride (HLR 266-95), respectively. The studies with carbaryl and amphetamine were conducted within six months of the study with Oxamyl. The studies with acrylamide and trimethyltin were conducted earlier.

4. Rationale for dose selection

The exposure concentrations were based on the results of two earlier studies which were described. In a two-week range-finding study, dietary concentrations of 0, 25, 50, 250, and 350 ppm were administered to 5 female rats/group. Concentrations of 250 and 350 ppm resulted in reduced food consumption and reduc-

ed body weight gains (during the first week of the study); clinical signs of toxicity (diarrhea, exophthalmus, tremors, stained/wet inguinal areas and underbody, and colored discharge on the face); and lowered blood, plasma, and brain acetylcholinesterase activity. There were no gross lesions.

In a two-year study conducted at concentrations of 0, 25, 50, 100, or 150 ppm, the following effects were observed in one or both sexes: significantly lowered body weight, body weight gain, and food efficiency (both sexes, 100 and 150 ppm); hyperactivity (both sexes, 150 ppm); alopecia and skin sores (females, 100 and 150 ppm); swollen legs and/or paws (females, 150 ppm; males, 100 and 150 ppm); and significantly decreased plasma cholinesterase (males, 100 and 150 ppm). There were effects on red cell or brain ChE activity. There were no gross lesions.

Based on these study results, dietary concentrations of 0, 10, 100, and 300 ppm were initially selected for the subchronic study. As previously noted, the intermediate and high concentrations were lowered to 30 and 250 ppm, respectively, on day 7.

5. Diet

Diet (Certified Rodent Diet™ #5002) and water were available *ad libitum*.

6. Preparation and analysis of diet

Oxamyl Technical, weight-adjusted for purity, was added to the diet and thoroughly mixed for 3 minutes in a high-speed mixer. On day 7, the mixing time for the 10 and 30 ppm dietary concentrations was increased to 5 minutes. Control diet was treated similarly. Diets were initially prepared weekly; based on the stability study, the diets were later prepared every other week. All diets were refrigerated.

Concentrations were analyzed by taking 10 g samples on test day 5 (250 ppm, reduced from 300 ppm), and test days 41 and 83 (10, 30, and 250 ppm). Homogeneity was analyzed on test day 1 (10, 100, and 300 ppm), test day 5 (10 and 30 ppm), and test day 27 (10 and 250 ppm). Samples were collected from the top, middle, and bottom of the mixer; control diet samples were taken from the middle of the mixer. All samples were frozen until analyzed. For stability, samples were taken on test day 1 (10 and 300 ppm) and test day 5 (10 ppm). Storage was under the following conditions: 0, 7, 14, or 21 days at room temperature and 21 days under refrigeration, all followed by freezing. Samples stored at room temperature were taken from feeders.

7. Statistical analysis

Body weights, body weight gains, and food consumption data were analyzed by Dunnett's analysis of variance for differences from the control group. For grip strength and foot splay data, Barnett's test for homogeneity of variances was used to compare groups. If Barnett's test was not significant, the data were then analyzed with Dunnett's test. If Barnett's test was significant, non-parametric analyses were used.

Clinical sign incidence data and descriptive FOB parameters were evaluated by the Cochran-Armitage test for Trend. For positive trends in the intermediate and high concentration groups, the low concentration group was compared to the control group with a Fisher's exact test.

Cholinesterase data were tested for homogeneity of variances (Levene's test) and normality (Shapiro-Wilk) followed by Dunnett's test. If the test for normality was significant, nonparametric procedures (Kruskal-Wallis and Dunn's Multiple Comparisons) were used.

Motor activity data were similarly tested for normality and equality of variance. Because Levene's test for homogeneity of variance indicated that the data were not homogeneously distributed, the Kruskal-Wallis nonparametric test and Dunn's test were used. Data for males and females were treated separately.

All data were tested at the $p<0.05$ level. No statistical tests were performed on the neuropathology incidence data.

C. METHODS

1. Observations

Animals in all groups were examined once daily for clinical signs during the first two weeks. Daily observations continued for the 250 ppm groups, but the other groups were observed once weekly (with daily cage-side observations). Starting with week five, the 250 ppm groups were observed weekly.

2. Body weight

Body weights were recorded on day 0 and weekly thereafter.

3. Food consumption and food efficiency

Food consumption and food efficiency were determined weekly.

4. Functional observational battery (FOB)

Twelve rats/sex/group were subjected to a FOB prior to exposure and during weeks 4, 8, and 13. Animals were randomized into four groups and tested over a two-day period. The following observations were performed by trained technicians:

a. Home cage observations

Posture, palpebral closure, writhing, circling, biting, ptosis (drooping eyelids). (The "home cage" was the transport cage to which the rats had been acclimated and undisturbed for at least 10 minutes.)

b. Removal from cage

Ease of removal, ease of handling, muscle tone, vocalizations, piloerection, bite marks on tails/paws, palpebral closure, fur appearance, lacrimation, salivation, exophthalmus, ptosis.

c. Open field observations

Activity level, righting reflex, labored breathing, convulsions, coordination, grooming, gait, locomotion, arousal, vocalizations, palpebral closure, defecation, urination, approach and touch, auditory stimulus, tail pinch.

d. During motor activity monitoring

Defecation, urination, pupillary response.

e. Quantitative neuromuscular observations

Forelimb and hindlimb grip strengths and hindlimb splay.

5. Motor activity

Motor activity measurements were assessed for each animal following the FOB observations (prior to exposure and during weeks 4, 8, and 13). Individual animals were placed in one of 30 Coulbourn^{R} activity monitors and activity was measured for 6 consecutive 10-minute intervals over a one-hour period. Duration of movement and number of movements were monitored.

6. Sacrifice/necropsy/neurohistopathology

At study termination, six rats/sex/group were euthanized by pentobarbital anesthesia followed by exsanguination and whole-body perfusion fixation. Animals were examined grossly for lesions when perfused and dissected. The brain, spinal cord, and nerves, listed in the table below, were examined histo-

logically. Only the control (0 ppm) and high concentrations groups (250 ppm) were examined.

X	Brain	X	Spinal Cord	X	Peripheral nerves
X	Cerebellum	X	Cervical (C3-C6)	X	Sciatic nerve
X	Pons	X	dorsal root gang.		proximal,left and right
X	Medulla oblongata	X	dorsal root		Sural nerve
X	Olfactory nerve	X	ventral root	X	Tibial nerve
X	Frontal lobe	X	Lumbar (L1-L4)		
X	Parietal lobe	X	dorsal root gang.		
X	Midbrain	X	dorsal root		
	Pituitary gland	X	ventral root		
		X	Gasserian ganglion		

7. Clinical chemistry

Blood was collected for determination of red blood cell (RBC) and plasma cholinesterase activity prior to exposure and during week 4 of exposure (first 10 rats/ clinical pathology subgroup). Blood was collected from the second 10 rats of the clinical pathology subgroup during week 8 and from the third 10 rats of the subgroup during week 13. Animals were sacrificed after blood collection and brain tissue was collected and frozen (-70°C) for later cholinesterase activity measurements. Tissue ChE levels were determined using Hitachi 717 chemistry analyzer according to the Clinical Pathology SOP #CP070-P-002. Blood samples were stored on ice and were analyzed within 20 minutes of collection. Red blood cells were diluted !:20 in 0.5% Triton just before analysis (personal communication).

II.RESULTS

A. ANALYTICAL/EXPOSURE DATA

Analyzed concentrations of the 0, 10, and 250 ppm nominal dietary concentrations were non-detectable, 93.9-102.0%, and 96.7-106.0% of the target concentrations, respectively.

For homogeneity analyses, the mean percent of nominal concentrations of the diet samples on three different test days ranged from 90.7 to 106.4%. The coefficient of variation (standard deviation/mean x 100) ranged from 3.0 to 12.8% and was highest for the low concentration (i.e., 8.25, 8.56, and 10.4 ppm for the top middle and bottom samples on April 3, resulting in a coefficient of variation of 12.8%). Some of the samples were reanalyzed and reported values were means of the original and reanalysis.

The average percent in a sample analyzed at the beginning of the study contained 96.9% of the active ingredient and the average percent of active ingredient in a sample analyz-

ed near the end of the study was 97.9%. The sponsor reported an active ingredient of 98.3%. The percent of nominal concentration in samples stored at room temperature and refrigerated ranged from 81.9 to 110.7% and appeared unrelated to storage conditions. Differences among samples were attributed to homogeneity variability.

B. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived until termination of the study. Clinical signs indicative of cholinesterase inhibition were observed in male and/or female rats at concentrations of 100, 250, and 300 ppm. During days 0-7, 26/42 males and 37/42 females receiving the 300 ppm dietary concentration exhibited tremors. During this time, animals of both sexes in this group exhibited abnormal gait or mobility and females exhibited hunched-over posture. Clinical signs such as abnormal gait or mobility, exophthalmus, hunched-over posture, ptosis, tremors, and hyperactivity (both sexes), piloerection, and colored discharge from the eyes (males), and hyperactivity and lacrimation (females) continued over one or more test intervals (days 8-28, 29-56, and 57-97) when the concentration was reduced to 250 ppm. Other clinical signs (wet chin, stained fur, and lacrymation in males and stained perineum in females) were increased but not significantly. The incidences of clinical signs decreased over the duration of the study.

Males receiving the 100 ppm dietary concentration for test days 0-7 did not exhibit clinical signs of toxicity whereas females had a significantly higher incidence of tremors (12/42 compared to 0/42 in the control group). Following reduction of the dietary concentration to 30 ppm, no signs associated with cholinesterase inhibition were observed in rats of either sex. No test substance-related signs were observed in rats administered the 10 ppm diet.

C. BODY WEIGHTS

During the first week of the study, significant decrements in body weight and body weight gains occurred in males and females at dietary concentrations of 100 and 300 ppm. When concentrations were lowered to 30 and 250 ppm, respectively, mean body weights of males and females administered 30 ppm recovered by day 90, but males and females receiving 250 ppm did not. However, females recovered by day 97. There were no decreases in body weight or weight gain in either sex at dietary concentrations of 30 ppm or below. Mean body weights for representative weeks are listed in Table 2.

When the test interval of days 7-90 is considered, recovery in terms of weight gain took place in the 250 ppm groups, but the weight gain of males by day 90 was 14% lower than that of the control group whereas the weight gain of females in this group was 30% greater than that of the control group (data not shown).

D. FOOD CONSUMPTION AND FOOD EFFICIENCY

Mean daily food consumption and mean food efficiency for male and female rats are summarized in Table 3. During tests days 0-7, when the dietary concentrations were 0,

10, 100, and 300 ppm, food consumption for males was significantly reduced ($p<0.05$) in all treatment groups compared to the control group (by 8, 21, and 71%, respectively). The decreases were dose related. Although food consumption was slightly lower for all treated groups throughout the study, decreases were significant only for males in the 250 ppm group at all test intervals. Mean food efficiency was significantly lower for males in the 300/250 ppm group during days 0-7 and 0-90, but was higher than that of controls during several weekly intervals. After the first week, there were no effects on food consumption at dietary concentrations of 10 or 30 ppm. After the first week, food efficiency in the 30 ppm group was significantly higher than that of controls during several weekly intervals but was the same as that of the control group over the 90-day period.

TABLE 2. Mean body weights (g) of male and female rats administered Oxamyl in the diet (selected weeks)				
Days on test	Male Dietary Concentration Groups (ppm)[a,b]			
	0	10	30	250
0	259.7±13.6	256.7±17.8	255.2±17.2	256.4±20.2
7	309.7±18.6	302.2±22.3	275.1±20.3*	210.7±20.8*
14	347.5±23.8	337.1±26.1	323.0±25.2*	249.1±25.6*
28	406.2±27.9	397.6±29.9	388.9±32.2	306.9±31.2*
56	482.1±34.8	479.2±36.8	466.6±46.4	372.1±38.0*
84	532.9±41.3	533.0±43.9	519.5±55.9	405.6±44.3*
90	541.0±41.9	542.4±47.0	528.4±59.0	409.7±49.4*
Days on test	Female Dietary Concentration Groups (ppm)[a,b]			
	0	10	30	250
0	184.9±12.4	185.7±11.1	184.2±13.4	180.7±13.8
7	199.5±14.5	202.7±13.9	179.4±14.5*	146.1±14.7*
14	217.8±16.2	220.9±16.9	210.3±16.1	178.6±15.2*
28	241.0±18.6	244.6±19.1	232.8±18.1	204.8±13.5*
56	267.8±24.3	272.4±22.1	263.0±23.2	234.3±16.5*
84	281.7±30.8	293.5±24.3	283.4±22.7	254.8±17.7*
90	283.6±32.2	294.6±27.6	285.8±22.6	256.1±16.6*
97	280.6±42.1	302.9±31.6	283.5±23.6	250.6±11.2

Data taken from Tables 4 and 5, pp. 63-64, MRID 44504901.
[a]42 animals per dose group at start of study.
[b]For test days 0-7, the dietary concentrations were 0, 10, 100, and 300 ppm.
*$p<0.05$.

TABLE 3. Mean daily food consumption and mean food efficiency for male and female rats administered Oxamyl in the diet (selected weeks)

Days on test	Male Dietary Concentration Groups (ppm)[a]			
	0	10	30	250
0-7[b]	24.1±2.1[c] (0.295)[d]	22.2±4.1* (0.306)	19.0±2.5* (0.146)	7.1±1.9* (-1.072)*
7-14	24.6±2.3 (0.218)	23.4±2.5 (0.210)	24.8±2.6 (0.276)*	19.5±3.2* (0.282)*
14-21	25.2±1.9 (0.196)	24.4±2.3 (0.195)	24.6±2.6 (0.213)	21.3±2.2* (0.201)
21-28	24.7±2.0 (0.132)	24.4±2.1 (0.136)	23.9±2.4 (0.161)*	21.1±3.0* (0.176)*
56-63	25.4±2.3 (0.087)	25.1±2.5 (0.077)	24.4±2.5 (0.088)	22.0±2.5* (0.089)
84-90	25.3±2.6 (0.053)	24.9±2.7 (0.059)	23.7±3.5 (0.058)	21.0±5.0* (-0.072)
0-90	25.2±2.0 (0.124)	24.8±2.2 (0.127)	24.2±2.4 (0.124)	20.3±1.9* (0.085)*
Days on test	**Female Dietary Concentration Groups (ppm)[a]**			
	0	**10**	**30**	**250**
0-7[b]	16.4±1.9 (0.125)	16.9±1.7 (0.142)	12.1±2.7* (-0.073)*	7.1±2.4* (-0.833)*
7-14	17.5±1.7 (0.147)	17.8±1.7 (0.147)	18.4±1.7 (0.240)*	15.8±2.2* (0.295)*
14-21	17.4±1.6 (0.109)	17.9±2.0 (0.111)	17.6±1.6 (0.096)	17.0±2.0 (0.098)
21-28	16.8±1.9 (0.083)	17.0±1.9 (0.077)	16.4±1.8 (0.090)	17.8±1.8* (0.117)*
56-63	16.6±2.0 (0.031)	17.7±1.4 (0.046)	16.4±1.7 (0.040)	18.5±2.0* (0.033)
84-90	16.4±1.6 (0.017)	17.0±2.6 (0.000)	16.9±2.1 (0.019)	18.6±2.1* (0.011)
0-90	17.1±1.8 (0.064)	17.5±1.2 (0.069)	16.9±1.2 (0.063)	17.5±1.7 (0.049)*

Data taken from Tables 8-11, pp. 67-70, MRID 44504901.

[a]42 animals per dose group on days 0-28, 32 animals on test days 29-56, and 22 animals on test days 57-90.

[b]For test days 0-7, the dietary concentrations were 0, 10, 100, and 300 ppm.

[c]food consumption in g.

[d]food efficiency (g weight gain/g food consumed).

*$p<0.05$.

During test days 0-7, when the dietary concentrations were 0, 10, 100, and 300 ppm, mean daily food consumption for females was significantly reduced in the 100 ppm (by 26%) and 300 ppm (by 57%) treatment groups (both $p<0.05$). After the first week, mean daily food consumption in the 250 ppm group was higher than that of controls during some weekly intervals. After the first week, food consumption in the 10 and 30 ppm groups did not differ significantly from that of the control group throughout the test for both weekly intervals and weeks 0-90. Mean food efficiency in the 250 ppm group was significantly higher than that of the control group at several weekly intervals, but remained significantly lower than that of the control group over the 90-week period. With the exception of a significant increase in food efficiency during the second week in the 30 ppm group, increases and decreases in food efficiency in the 10 and 30 ppm groups appeared to be random and not treatment related.

E. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

The following neurobehavioral changes were considered treatment related. All statistical significance is at the $p<0.05$ level.

In the 250 ppm concentration group, ptosis was exhibited by 2/12, 1/12, and 1/12 males in the home cage during weeks 4, 8, and 13, respectively (not statistically significant). Five of 12 males in the 250 ppm group exhibited ptosis upon removal from the home cage during week 4 (statistically significant). In the open field, 11/12, 9/12 and 5/12 males in this group exhibited ptosis during weeks 4, 8, and 13, respectively (all statistically significant). One to two animals in the lower treatment and control groups also exhibited ptosis at various times. Upon removal from the home cage, one female in the 250 ppm group exhibited ptosis during weeks 4, 8, and 13 (not statistically significant). One of 12 females in this group exhibited ptosis in the open field during weeks 4 and 13 (not statistically significant) and 4/12 females exhibited ptosis during week 8 (statistically significant).

Males in the 250 ppm group had a statistically significantly higher incidence of piloerection (5/12) during week 4. Piloerection was present in 1/12 females in this group during week 4 (not statistically significant).

Abnormal gait was observed in 2/12, 1/12, and 2/12 males in the 250 ppm group during weeks 4, 8, and 13, respectively (not statistically significant). Incidences in females were 2/12 and 1/12 during weeks 4 and 8, respectively (not statistically significant).

Pupillary response was absent in 9/12, 10/12, and 10/12 males in the 250 ppm group during weeks 4, 8, and 13, respectively (all statistically significant). Incidences in females in this group during these respective observation times were 10/12, 10/12, and 7/12 (all statistically significant).

Forelimb grip strength for male and female rats was not affected by treatment. Hindlimb grip strength was statistically significantly reduced for males in the 250 ppm treatment group during week 4 and for females in the 250 ppm treatment group during

weeks 8 and 13. Mean hindlimb foot splay was statistically significantly lower in males in the 250 ppm group during week 13 and for females during weeks 8 and 13.

Increased or decreased incidences of arousal, urination, and defecation in males in different treatment groups at various intervals were not considered treatment related. Compared to the control group, there were no treatment related neurobehavioral differences in males in the 10 and 30 ppm treatment groups.

Increases and decreases in vocalizations in females in different groups during different time intervals were not treatment related. Compared to the control group, there were no treatment related neurobehavioral differences in females in the 10 and 30 ppm treatment groups.

F. MOTOR ACTIVITY

Total mean duration of movement and mean number of movement for successive 10-minute intervals for male and female rats in the treated groups did not differ significantly from that of control groups. However, total mean duration of movement was slightly lower for males in the 250 ppm dietary group at weeks 4 (by 28%), 8 (by 13%), and 13 (by 14%). For females in the 250 ppm dietary group, total mean duration of movement was similar to controls at the 4 and 8-week tests and lower than controls by 24% (not statistically significant) at the 13-week evaluation. For females, this activity was decreased by 20% in the 30 ppm group at 13 weeks (not statistically significant).

With the single exception of the fifth 10-minute interval during the 13-week evaluation of males receiving 250 ppm, the mean number of movements in treated male rats was not significantly different than that of the control group. The mean number of movements during the 5th 10-minute interval decreased by 56% ($P < 0.05$), compared to the controls. Females that received 250 ppm had a 20% lower mean number of movements (not statistically significant) at the 13 week evaluation.

G. CHOLINESTERASE ACTIVITY MEASUREMENTS

Significant decreases in plasma, RBC, and brain (all regions) cholinesterase activity occurred during one or more sampling times for both males and females in the 250 ppm groups (Table 4). At the end of the study, the mean plasma, red blood cell and cortical (brain) ChE levels were decreased by 24, 48 and 40%, respectively in males and 60, 55 and 51%, respectively in females, compared to the controls. Changes were generally greater in females than in males. These changes in plasma, red cells, or brain ChE activity correlated with clinical and neurobehavioral changes which were indicative of excessive cholinergic stimulation. Decreases were consistent over time, indicating no accumulative effect. Changes of >10% that occurred in the 10 and 30 ppm concentration groups of both sexes occurred in a random fashion and are not considered treatment related.

TABLE 4. Cholinesterase activity (% of the concurrent control group mean) in male and female rats administered Oxamyl in the diet

Cholinesterase activity	Male Dietary Concentration Groups (ppm)[a,b]			
	0	10	30	250
Plasma (U/L)				
week 4	429	400	466 (109%)	281 (66%)*
week 8	380	395 (104%)	399 (105%)	272 (72%)
week 13	378	385 (102%)	398 (105%)	289 (76%)
RBC (U/L)				
week 4	2036	1904 (94%)	2410 (118%)	1212 (60%)*
week 8	1326	1474 (111%)	1456 (110%)	978 (74%)
week 13	2434	1962 (81%)	2286 (94%)	1264 (52%)*
Cortex (U/g)				
week 4	9.57	9.01 (94%)	8.41 (88%)	5.15 (54%)*
week 8	8.41	7.41 (88%)	7.52 (89%)	5.46 (65%)*
week 13	8.46	8.48 (100%)	7.88 (93%)	5.05 (60%)*
Hippocampus (U/g)				
week 4	10.17	10.87 (107%)	11.04 (109%)	6.97 (69%)*
week 8	11.23	10.10 (90%)	9.13 (81%)*	7.37 (66%)*
week 13	10.13	10.64 (105%)	11.18 (110%)	7.39 (73%)*
Midbrain (U/g)				
week 4	11.51	12.48 (108%)	13.41 (117%)	8.08 (70%)
week 8	10.72	10.84 (101%)	10.31 (96%)	6.86 (64%)*
week 13	10.61	10.53 (99%)	9.38 (88%)	7.28 (69%)*
Cerebellum (U/g)				
week 4	4.63	4.46 (96%)	4.60 (99%)	3.20 (69%)
week 8	4.19	4.45 (106%)	4.03 (96%)	3.17 (76%)
week 13	4.18	4.16 (100%)	4.18 (100%)	2.86 (68%)*
Halfbrain (U/g)				
week 4	13.58	13.28 (98%)	13.51 (99%)	8.39 (62%)@
week 8	10.59	10.98 (104%)	10.68 (101%)	6.88 (65%)
week 13	10.10	10.03 (99%)	9.56 (95%)	5.90 (58%)@
Cholinesterase activity	**Female Dietary Concentration Groups (ppm)[a,b]**			
	0	**10**	**30**	**250**
Plasma (U/L)				
week 4	1616	1563 (97%)	1306 (81%)	526 (33%)*
week 8	1809	1815 (100%)	1663 (92%)	805 (44%)*
week 13	2383	1949 (82%)	2146 (90%)	964 (40%)*
RBC (U/L)				
week 4	2294	1902 (83%)	2188 (95%)	802 (35%)*
week 8	1588	1340 (84%)	1494 (94%)	934 (59%)*
week 13	2432	1892 (78%)	1862 (77%)	1086 (45%)@

TABLE 4. Continued

Cholinesterase activity	Male Dietary Concentration Groups (ppm)[a,b]			
	0	10	30	250
Cortex (U/g)				
week 4	8.99	8.31 (92%)	8.54 (95%)	4.02 (45%)@
week 8	7.11	8.18 (115%)	7.76 (109%)	4.54 (64%)*
week 13	8.91	8.40 (94%)	8.40 (94%)	4.33 (49%)*
Hippocampus (U/g)				
week 4	11.56	11.56 (100%)	10.08 (87%)	4.64 (40%)*
week 8	10.66	9.45 (89%)	9.87 (93%)	6.40 (60%)*
week 13	9.34	9.45 (101%)	9.68 (104%)	5.38 (58%)*
Midbrain (U/g)				
week 4	11.89	12.38 (104%)	11.68 (98%)	7.11 (60%)@
week 8	8.01	8.14 (102%)	8.81 (110%)	6.41 (80%)
week 13	11.64	11.13 (96%)	10.52 (90%)	5.73 (49%)@
Cerebellum (U/g)				
week 4	4.00	4.40 (110%)	4.60 (115%)	2.53 (63%)
week 8	4.18	3.97 (95%)	4.24 (101%)	2.86 (68%)
week 13	3.84	3.66 (95%)	4.14 (108%)	2.34 (61%)@
Halfbrain (U/g)				
week 4	11.07	10.89 (98%)	10.87 (98%)	4.89 (44%)@
week 8	10.65	10.70 (100%)	10.72 (101%)	6.33 (59%)
week 13	9.82	9.45 (96%)	8.92 (91%)	5.01 (51%)*

Data taken from text Tables 2 and 3, pp. 43-44, MRID 44504901.
[a]10 animals per dose group.
[b]For test days 0-7, the dietary concentrations were 0, 10, 100, and 300 ppm.
*$p<0.05$ (parametric analysis).
@$p<0.05$ (non-parametric analysis).

H. SACRIFICE/NECROPSY/NEUROHISTOPATHOLOGY

No gross or microscopic lesions related to exposure were observed.

III. DISCUSSION

A. DISCUSSION

Administration of Oxamyl Technical at dietary concentrations of 0, 10, 100, or 300 ppm to Crl:CD^{R}(SD)BR rats resulted in clinical signs of toxicity and significant decreases in body weight gains at the 300 and 100 ppm concentrations for one or both sexes. Therefore, dietary concentrations in the mid- and high-dose groups were reduced to 30 and 250 ppm, respectively, on day 7.

During days 0-7, males and females that received 300 ppm and females that received 100 ppm lost weight. These weight losses were accompanied in most cases by significantly reduced food consumption and significantly decreased food efficiency. Despite increased food efficiency of males in the 250 ppm group (over that of controls) during some intervals, weight gain for males remained significantly below that of controls by the end of the study (90 days). The mean body weight of females was significantly lower than that of controls at 90 days but not at 97 days. At the end of 90 days, mean daily food consumption, food efficiency, and mean body weights were similar among controls and the 10 and 30 ppm groups for both males and females.

Because Oxamyl Technical is a carbamate insecticide, clinical signs resulting from cholinesterase inhibition would be expected. Clinical signs indicative of cholinesterase inhibition (cholinergic stimulation) were observed in male and/or female rats at concentrations of 100, 250, and 300 ppm. During days 0-7, both sexes receiving 300 ppm exhibited tremors, abnormal gait or mobility and females exhibited hunched-over posture. These and additional signs - exophthalmus, ptosis, hyperactivity, piloerection, colored discharge from the eyes, hyperactivity and lacrimation - continued in either or both sexes over one or more test intervals when the concentration was reduced to 250 ppm. However, the incidences of some clinical signs decreased slightly over the duration of the study, indicating the development of some tolerance to Oxamyl Technical. Females, but not males receiving the dietary concentration of 100 ppm during days 0-7 also exhibited tremors. The dietary concentration of 30 ppm was a NOEL for clinical signs indicative of cholinesterase inhibition. A

During the FOB, statistically significant increases or decreases in incidences of ptosis, piloerection, abnormal gait, pupillary response to light, and hindlimb grip strength were observed in either males or females or both sexes receiving the dietary concentration of 250 ppm. These signs/effects are considered treatment related. These signs/effects were not present/significant in the 10 and 30 ppm groups.

Although motor activity was lowered in the treatment groups (by up to 28% for males in the 250 ppm group), the decreases were generally not statistically significant for either males or females in any group at any interval.

Decreases in cholinesterase activity (in the 250 ppm group) correlated with clinical signs and changes in FOB parameters that were indicative of cholinergic stimulation in this group. No signs of cholinergic stimulation were present in the 30 ppm groups; these groups had cholinesterase activity similar to that of the control group.

Neurohistopathology examinations did not indicate any exposure-related effects.

Under the conditions of this study, the **Systemic Toxicity LOAEL is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively), based on decreases in body weights and food efficiency of both sexes. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively).**

Based on clinical signs consistent with neurotoxicity, **the LOAEL for neurobehavioral effects is 250 ppm (14.9 mg/kg/day and 19.9 mg/kg/day for male and female rats, respectively) based on decreases in plasma, RBC and brain ChE activity, clinical signs consistent with cholinesterase inhibition, and changes in incidences of FOB parameters such as increases in ptosis, piloerection, and abnormal gait and decreases in pupillary response to light and hindlimb grip strength. The NOAEL is 30 ppm (2.10 mg/kg/day and 2.40 mg/kg/day for male and female rats, respectively.**

Classification: **Acceptable.**

B. STUDY DEFICIENCIES

No study deficiencies were identified.

[Oxamyl Developmental Toxicity Study - Rat (83-3)]

Supplement to Document No. 007099 - DER for MRID No.40859201& 44737501: Oxamyl, Developmental Toxicity Study in Rat. This amendment provides an Revised Executive Summary to the original DER.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D., [signature], Date 5/21/99
Reregistration Branch 2
Health Effects Division (7509C)

Secondary Reviewer: Robert Fricke, Ph.D., R Fricke, Date 26 May 99
Reregistration Branch 2
Health Effects Division (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity Study - Rat

OPP Number: 83-3
DP BARCODE: D253122
PC CODE: 103801

OPPTS Number: 870.3700
SUBMISSION CODE: S556451
TOX CHEM NO: 561A

TEST MATERIAL (PURITY): Oxamyl Technical (99%, a.i.)

SYNONYMS: Ethanimidothioicacid,2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-, methyl ester; DPX-D1410-196; DPX-D1410-196 Technical; methyl2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-ethanimidothioate; emethyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate

CITATIONS:

1. Rickard, L. B. (1988) Teratogenicity Study of IN D1410-196 in the rat. Haskell Laboratory for Toxicology & Industrial Medicine. Medical Research No. 8424-001; Lab. Project. No. 473-88. October 3, 1988. MRID 40859201. Unpublished.

2. Munley, S.M. (1998) DuPont's Position on Fetal Weight Changes in Rats Following Developmental Toxicity Testing with Oxamyl. E.I. du Ponte de Nemours and Company, Newark, Delaware. Medical Research No. 8428-001. December 16, 1998. MRID 44737501. Unpublished.

[Oxamyl **Developmental Toxicity Study - Rat (83-3)]**

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, DE 19880-0038

EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 40859201 & 44737501) oxamyl (97.2%) was administered by gavage to groups of pregnant Charles River (CD) BR rats (25/group) at dose levels of 0, 0.2, 0.5, 0.8, and 1.5 mg/kg from gestation days 7 to 16. On day 22, the fetuses were removed, and the dams were sacrificed.

There were no mortalities or treatment-related gross abnormalities were reported. Maternal toxicity was observed at the 0.8 mg/kg/day, as decreased body weight gain (21%; $P < 0.05$), decreased food consumption (10%; $P < 0.05$) and increased incidence of tremors (4/25) associated with cholinesterase inhibition. The decreased body weight gain and food consumption and increased incidence of tremors were dose-related. At 1.5 mg/kg/day dose the body weight gains and food consumption decreased 30% and 16% ($P < 0.05$), respectively, compared to controls. At this dose increased number of dams showed statistically significant ($P < 0.05$) increase in signs of diarrhea, eye discharge, salivation, tremors, and wet legs, perineal and underbody. Treatment had no effect on the reproductive parameters and/or fetal malformations or variations. A dose-related decrease in fetal body weights was seen and decrease was statistically significant ($P < 0.05$) at doses 0.5 mg/kg and above. The fetal weights at 0.2, 0.5, 0.8, and 1.5 mg/kg, decreased 1.6%, 3.9%, 6.75% and 6.9%, respectively, compared to the controls.

The HIARC (07/15/99) noticed the apparant quantitative fetal susceptability to oxamyl and concluded that there is no fetal susceptability due to decrease in maternal weight gain of 9% seen at 0.5 mg/kg/day at which decreased fetal body weights also occurred.

Under the conditions of this study, **Maternal Toxicity NOAEL = 0.5 mg/kg/day and the LOAEL = 0.8 mg/kg/day**, based on decreased body weight gains, decreased food consumption and increased incidence of tremors.

The **Developmental Toxicity NOAEL = 0.2 mg/kg/day and the LOAEL = 0.5 mg/kg/day**, based on dose-related decreased in the fetal body weight.

CLASSIFICATION: The study is classified as Acceptable/Guideline and satisfies the data requirements for developmental toxicity study (83-3) in rat.

1. Action Requested

The DuPonte de Nemours and Company on January 19, 1999, submitted comments (MRID 44737501) on the rat developmental toxicity review (HED Doc. No. 007099, March 23, 1989). In this study, maternal toxicity was observed at 0.8 mg/kg/day and above as significant decrease in maternal body weight and food consumption and increased adverse clinical observations (tremors).

The maternal toxicity NOAEL was 0.5 mg/kg/day. Developmental toxicity was observed at 0.5 mg/kg/day and above as dose-related statistically significant ($P < 0.05$) decrease in fetal body weight. The developmental toxicity NOAEL was 0.2 mg/kg/day. The study author concluded that oxamyl was "unlikely to be a hazard to the conceptus unless exposure occurs at levels or near those that were also toxic to the dam." The registrant contends that fetal weight effect seen at 0.5 mg/kg/day is marginal and occurred in the presence of a slight, non-statistically significance, non-adverse effect on maternal weight gain seen at that level. Further, results of historical control data from 15 studies conducted at Haskell Laboratory from January 1997 to April 1989, show the fetal weight control data ranged from 4.97 to 5.43 grams. The control mean from the rat developmental study was 5.33g which was high end of historical control data. The mean fetal weight in the study was 5.12 grams is well within the control range. Therefore, the registrant is requesting that no additional safety factor to protect children and infants should be applied. Fetal and maternal weight data are summarized below:

Table.1 Fetal weight Data from HLR 473-88

Group	Mean fetal weight (g)	% Relative to control
0 mg/kg	5.33	-
0.20 mg/kg	5.24	98.3
0.50 mg/kg	**5.12***	96.1
0.80 mg/kg	**4.97***	93.2
1.5 mg/kg	**4.96***	93.1

Table.2 Maternal Weight Gain Data from HLR 473-88

Group	Mean maternal weight (g)	% Relative to control
0 mg/kg	57.3	-
0.20 mg/kg	57.1	99.7
0.50 mg/kg	52.4	91.4
0.80 mg/kg	**45.2***	78.9
1.5 mg/kg	**40.2***	69.8

Agency Conclusions: The HIARC (07/15/99) reviewed the existing data base on oxamyl and agreed with the registrant that there is no increased susceptality to fetusues because decrease in maternal weight gain of 9% seen at 0.5 mg/kg/day at which decreased fetal body weights also occurred.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D.
Review Section: Reregistration Action Branch 2 (7509C)
EPA Secondary Reviewer: Robert Fricke, Ph.D.
Review Section: Reregistration Action Branch 2 (7509C)

Date 4/13/99

Date 4/13/99

013975

DATA EVALUATION RECORD

STUDY TYPE: 21-Day dermal toxicity - rabbit (OPPTS 870.3200) (§82-2)

DP BAR CODE: D253414
P.C. CODE: 103801

SUBMISSION CODE: S556996
TOX. CHEM. NO.: 561A

TEST MATERIAL (PURITY): Oxamyl Technical (96.9%, a.i.), batch DPX-D1410-196 (Haskel ID # H-16995-02)

SYNONYMS: Ethanimidothioicacid,2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-, methyl ester; DPX-D1410-196; DPX-D1410-196 Technical; methyl2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-ethanimidothioate; emethyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate

CITATION: Linda, A.M. 1999. 21-Day repeated dose dermal toxicity study in rabbits. E. I. Du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware 19714-0050. Laboratory report No. Dupont-1599, January 19, 1999. MRID 44751201. Unpublished.

SPONSOR: E.I. du Ponte de Nemours and Company
Wilmington, Delaware 19898

EXECUTIVE SUMMARY:

In a 21-day dermal toxicity study (MRID 44751201), groups of 6 male and 6 female HM:(NZW)fBR rabbits were treated with oxamyl technical (96.9%, a.i.) moistened with deionized water by dermal occlusion at doses of 0, 25, 40, 50 or 75 mg/kg/day, for 6 hours a day, 7 days/week. The dosages and the peak time (1 hour post unwrapping) for blood collection was based on pilot studies. No mortality was recorded, and there were no clinical signs indicative of systemic toxicity at any treatment level. No treatment-related dermal irritation was produced. There were no treatment-related effects on body weight, food consumption or food efficiency in either sex of rabbits. Significant inhibition of plasma (29%) and brain (10.7%) cholinesterase was observed in female rabbits at 75 mg/kg/day. In addition, there was inhibition of red blood cell (RBC) cholinesterase in female rabbits treated with 75 mg/kg/day was noticed; although inhibition was statistically not significant, but considered biologically relevant because of magnitude of depression (24%) accompanying statistically significantly plasma and brain cholinesterase depression. In males

rabbits, there were no treatment-related changes in the plasma, red blood cell or brain cholinesterase at any dose levels during the study. The female plasma, RBC or brain cholinesterase activity was not statistically significantly changed at 25, 40, or 50 mg/kg/day.

Systemic toxicity was not observed in this study. The **Systemic Toxicity NOAEL = 75 mg/kg/day.**

The **Cholinesterase NOAEL = 50 mg/kg/day, and LOAEL = 75 mg/kg/day,** based on decreased plasma, red blood cell and brain ChE inhibition in females rabbits. In male rabbits, the **NOAEL = 75 mg/kg/day, and LOAEL > 75 mg/kg/day.**

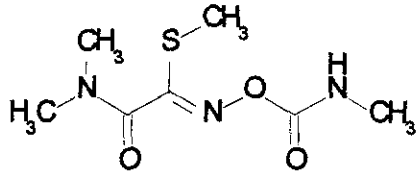
The study is classified as **Acceptable/non-guideline.**

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Oxamyl Technical
   Description: off-white solid; appear to be stable during course of the study based on color.
   Lot/Batch #: DPX-D1410-196
   Purity:96.9% a.i.
   Stability of compound: paste prepared for administration was not analyzed for homogeneity and stability.
   CAS #: 23135-22-0



2. Vehicle and/or positive
   Vehicle: deionized water
   Positive control: none

3. Test animals:
   Species: rabbits
   Strain: HM:(NZW)fBR
   Age and weight at study initiation: ≈ 10 weeks; 1584 - 2083g

Source: Hare Marland, Hewitt, NJ
Housing: individually in suspended wire-mesh, stainless cages
Diet: PMI Certified High Fiber Rabbit Lab Diet® 5325 ≈ 125 g
Water: tap water ad libitum
Environmental conditions:
Temperature: 20°C ± 1°C
Humidity: 50% ± 10%
Air changes: not given
Photo period: 12 hr light/dark
Acclimation period: 8 days

B. STUDY DESIGN:

1. In life dates - start: October 22, 1998; end: November 12, 1998

2. Animal assignment

Apparently healthy animals were assigned to one of 5 groups (6/sex/group) using a computerized stratified randomization procedure based on pretest plasma cholinesterase activity within a sex. The study design is detailed in Table 1.

Table 1. Experimental Design

Dosage (mg/kg/day)	No. of Animals	
	Male	Female
0	6	6
25	6	6
40	6	6
50	6	6
75	6	6

Data taken from p. 15, MRID 44751201

3. Dose selection rationale

Doses were selected based on the previous 21-day dermal study (MRID 40827601) and 2 pilot studies which were conducted in response to Agencys' recommendations (HED Doc. # 012857; Sep. 15, 1998). In the 21-day dermal study the LOAEL was 50 mg/kg/day and the NOAEL was 2.5 mg/kg/day, based on plasma, RBC and brain cholinesterase (ChE) inhibition. The two pilot studies showed that plasma ChE was inhibited at 50 mg/kg/day and no inhibition at 25 mg/kg/day; and peak time for plasma

cholinesterase activity occurred at 7 hours post exposure i.e., 1 hour after removal of wrapping. These information were used to select doses of 0, 25, 40, 50, and 75 mg/kg/day for this study. Below are provided the Agency recommendations and a brief summary of pilot studies:

a. HED recommendations

i. CONCLUSIONS:

Reregistration Review Branch II reviewed the E.I. Du Pont de Nemours and Company July 28, 1998 protocol for 21-Day Dermal Toxicity Study in rabbits using carbamate insecticide oxamyl. The protocol is acceptable. This is a standard protocol consistent with EPA guideline (82-2) for evaluating toxicity due to repeat dermal exposure, except that in this proposed study only cholinesterase inhibition will be measured. Since cholinesterase inhibition is readily reversible and is influenced by several factors such as dilution, temperature, time etc., EPA suggest that blood samples should be stored on ice and cholinesterase levels should be measured within 30 minutes of sampling, and that the sample not be diluted until the actual time of analysis [for details see comment iii(b)].

ii. ACTION REQUESTED:

E.I. Du Pont de Nemours and Company submitted a 21-Day Dermal Toxicity Study protocol in rabbits for Oxamyl. The purpose of this study is to evaluate the toxicity of test substance in rabbits and establish a NOAEL and LOAEL below 2.5 and 50 mg/kg/day, respectively, based on blood and brain cholinesterase inhibition established in earlier study (MRID 40827601). The proposed study will utilize 6 rabbits/sex at dose levels of 0, 25, 50 or 75 mg/kg, 6 hours a day for a total of 21 applications. This is a standard protocol consistent with EPA guideline (82-2) for evaluating toxicity due to repeat dermal exposure with the following exceptions:

ii(a) Only cholinesterase determinations will be performed. Hematology and other clinical chemistry determinations will not be determined, since in the earlier 21-day dermal toxicity study no systemic toxicity was observed. The NOEL of 2.5 mg/kg/day and LOAEL of 50 mg/kg/day, was based on plasma, red cell and brain ChE inhibition.

ii(b) All tissues prescribed under the US EPA Pesticide Assessment Guidelines, Subdivision F, 82-2 will not be collected. Only tissues with gross lesions will be collected at the discretion of the pathologist or study director, processed to slides and evaluated microscopically.

iii COMMENTS:

iii(a) This is standard protocol for evaluating toxicity due to repeat dermal exposure. The deviation cited above would not effect the validity of the study because no effects were observed in parameters suggestive of systemic toxicity. Only plasma, red cell and brain ChE levels were effected. The dose range proposed in this protocol covers the dose levels at which ChE levels were affected.

iii(b) Spectrophotometric methods (i.e. modification of the Ellman method with and without the use of an autoanalyzer) for the measurement of cholinesterase inhibition by carbamates have been neither validated nor standardized in previous round-robin efforts initiated by EPA. Since cholinesterase inhibition by the carbamate oxamyl is readily reversible, special care needs to be taken in sample handling, processing and measurement to minimize reversibility. Factors which can influence spontaneous reactivation of enzyme include excessive tissue dilution, pre-incubation/incubation temperature and time, and sample storage temperature. It is therefore suggested that tissues be assayed quickly after sampling (i.e. within 30 minutes), that tissues not be homogenized or diluted until just prior to assay, that tissue dilution be kept to a minimum (probably no higher than 1:10 for blood and no higher than 1:15 for brain), that samples (including blood) be kept on ice prior to imminent assay and that if longer tissue storage (e.g. brain) is required that temperatures of approaching -80 C be used. It is also suggested the pre-incubation/incubation time should be minimized. A lower reactivation temperature (i.e. 23 C vs 37 C appears to retard spontaneous reactivation (adjustment may not be possible with some autoanalyzers). It is further suggested that the following literature article by D.L. Hunter, et. al. (Including references and methods cited therein) be consulted for discussion on these issues: Toxicology Methods, Vol. 7, No. 1, pp. 43-53 (1997).

Tissues should be sampled at the peak of inhibition following dosing with oxamyl by the dermal route in the rabbit and evidence should be provided to support this determination. (Data was provided by the registrant to support peak time of inhibition of 30-60 minutes post -dosing with oxamyl by the oral route (gavage) in the rat).

Detailed methods for sample collection, handling, processing and measurement of cholinesterase activity should be submitted with the final report of the study.

iii(c) The proposed protocol is acceptable to provide the additional information requested by August 27, 1998, Hazard Identification SARC.

b. Pilot studies - In one study, one male and one female rabbit each treated dermally with 25 or 50 mg/kg/day oxamyl and blood was collected 1 hour post removal of wrapping. Plasma ChE was inhibited at 50 mg/kg/day and no inhibition was observed at 25 mg/kg/day. There was no RBC or brain ChE inhibition at any dose. The study results also support collaring the rabbits post exposure. In the second pilot study eight rabbits received a single dermal application of 150 mg/kg/day oxamyl in water and equal number of controls received deionized water. Cholinesterase determinations were done on plasma and RBC samples collected at 3, and 6 (post application, still wrapped), and 30 minutes, and 1, 2, and 3 hours, after removal of wrapping and washing the test site with Ivory® soap and water. The animals were euthanized and discarded after last sample collection. Maximum plasma ChE inhibition was noted 1 hours after removal of wrapping.

4. Test substance preparation and analysis

Calculated amount of test substance was moistened with enough deionized water to form a paste just prior to treatment.

Results -

Homogeneity analysis - Homogeneity analysis was not performed and is not required.

Stability analysis - No information on test substance stability was supplied in the study.

Concentration analysis - Concentration analysis was not performed and is not required. The test substance was administered as a paste in deionized water.

5. Dose application

One day prior to the initial application, an area of fur approximately 25 square centimeters (≈ 1% of the total body surface area) was clipped from the back and trunk. The area used in this study was only 1/10 of the EPA testing guideline recommendations, which could affect the absorption of the test material due to dilution. However, the study authors explained that the highest dose applied was too small to cover a surface area larger than 1%. The clipping was repeated during the study but did not specify frequency of clipping. The appropriate amount of oxamyl technical was moistened with deionized water to form a visually uniform paste and applied evenly and thinly to the shaved intact skin of treated rabbits. The treated areas were then covered with 2-ply porous gauze, and followed by several layers of stretch gauze and adhesive bandage. Application sites were occluded for approximately 6 hr/day, 7 days/week, for a total 21 days, except 40 mg/kg group

rabbit # 32283 received 20 exposures. Following each 6-hour exposure, the dressings were removed and the treated areas were washed with Ivory® soap solution, wiped with dry swab. Control animals were clipped, wrapped, and washed in same manner, but were dosed with deionized water based on the volume of water used in the 75 mg/kg group. All animals wore neck collars during the study.

4. Statistics

Dunnett's multiple comparison test (two-tail) was employed to evaluate statistically significant differences in body weights and body weight gain measurement. Clinical observations data were evaluated by the Cochran-Armitage test for trend and Fisher's Exact for the fit. Clinical pathology data was analyzed by parametric and non-parametric pairwise comparison between test and control groups. Levine's test for homogeneity and Shapiro-Wilk test for normality of data was employed. If Shapiro-Wilk test was significant the data were analyzed with Kruskal-Wallis test and pairwise comparison with Dunnett's test. If the Shapiro-Wilk test not significant, the data was analyzed by one-way ANOVA.

C. METHODS:

1. Observations:

Animals were inspected at least once daily for signs of toxicity and dermal effects after removal of test substance. Any skin effects were noted prior to each dosing. A detailed clinical signs inventory was conducted at each weighing.

2. Body weight

Animals were weighed twice weekly during the study, five times at 2 - 3 day intervals pre-treatment, and prior to necropsy at terminal sacrifice.

3. Food consumption

Group mean food consumption was determined weekly during the study. Food efficiency was calculated using food consumption and body weight

4. Ophthalmoscopic examination

Ophthalmology was not conducted. This study was designed to establish NOAEL and LOAEL based on plasma, red cell and brain ChE determinations. Data exists for other clinical effects from previous 21-Day dermal study (MRID 40827601; HED Doc. 006944) which was done at much higher doses.

5. Blood was collected from auricular vein of all rabbits pretest period, and from all surviving animals on last study day 7 hours post exposure (1 hour after removal of neck collars) for plasma and red cell ChE determinations only. The CHECKED (X) parameters were examined.

   a. Hematology

      Hematology was not done, since study design was for ChE determinations only.

   b. Clinical Chemistry was not done. Only ChE was determined.

6. Urinalysis* - was not required and was not done.

7. Sacrifice and Pathology

   All animals survived until scheduled termination, and were sacrificed at the end of the treatment period by iv injection of barbiturate into the auricle vein followed by exsanguination. Since no effects were observed in any of the organs in the previous study (MRID 40827601) at dosages up to 250 mg/kg/day, only organs or tissues with gross lesions were collected. **Brain and testes were collected.**

8. Cholinesterase methodology

   Cholinesterase activity in plasma, RBC, and brain was measured spectrophotometrically using a modified Ellman method. Boehringer Mannheim/Hitachi 717 clinical chemistry analyzer and Boehringer Mannheim Reagent was utilized for determining ChE activity in the above samples. Following table outlines the steps involved in the preparation and analysis of samples:

TABLE 2*

MEASUREMENT OF CHOLINESTERASE ACTIVITY

	RBC	Plasma	Brain
Collection	Blood is collected into heparinized syringe and placed in tube containing EDTA or collected directly into EDTA tube. Samples are placed immediately on ice.	Same as RBC	Brain tissues harvested, weighed, and placed immediately on ice until frozen.
Handling	Whole blood samples are held on ice and processed within a maximum of 15 minutes after collection	Same is RBC	Brains are frozen at -70°C within approximately 60 minutes.
Processing	The sample is separated into RBC and plasma by centrifugation (13000g for 4 minutes). The plasma (supernatant) is removed and analyzed. The white cell 'buffy coat' is removed and discarded, and 50 µl of the RBC fraction(without 'washing') is added to 950 µl 0.5% Triton X- 100 (1:20 dilution) to lyse the cells. The mixture is placed on ice for 30-90 seconds and the next pre-incubation step begins immediately thereafter.	Plasma is assayed immediately after separation (within approximately 10 minutes)	Tissue is thawed at room temperature and homogenized as soon as possible. 12 ml of buffer are added to each half-brain to make homogenate (homogenized according to SOP). A 1:3 dilution of homogenate is prepared (I part brain homogenate to 2 parts buffer)
Pre-incubation	At the start of pre-incubation the Hitachi analyzer immediately aliquots 6 µl of lysed RBC into 300µl of phosphate buffer containing 0.21 mM DTNB (chromophoric agent). The sample is incubated (37°C) for 5 minutes.	At the start of pre-incubation the Hitachi analyzer immediately aliquots 6 µl of plasma into 300µl of phosphate buffer containing 0.21 mM DTNB (chromophoric agent). The sample is incubated (37°C) for 5 minutes.	At the start of pre-incubation the Hitachi analyzer immediately aliquots 6 µl of diluted brain homogenate into 300 µl of phosphate buffer containing 0.21 mM DTNB (chromophoric agent). The sample is incubated (37°C) for 5 minutes.
Dilution ratio at incubation (initial sample volume/final volume)	1:1000 dilution	1:51 dilution	1:150 dilution
Temperature of reaction	37°C	37°C	37°C
Readings	Endpoint assay read at 2 minutes	Endpoint assay read at 2 minutes	Endpoint assay read at 2 minutes

* Data taken from p. 30, MRID44751201

II. RESULTS

A. Observations :

No treatment-related dermal effects. There were no mortalities or clinical signs indicative of toxicity at any treatment level. A few incidences such as diarrhea and hyperactivity, indicative of cholinergic activity, was observed post-treatment, however, lacked dose-response. Also one male each at 50 and 75 mg/kg/day, one female each at 25, and 75 mg/kg/day, exhibited pupillary constriction in one eye post-exposure. This occurred only unilaterally. Study authors explained this was probably due to flashing during washing off the test substance.

B. Body weight and weight gain

There were no statistically significant differences in body weight or body weight gain between controls and treated groups at any dose.

C. Food consumption

There were no statistically significant differences in food consumption or food efficiency of male or female rabbits at any dose.

D. Ophthalmoscopic examination: Ophthalmology was not done.

E. Blood work:

1. Hematology - was not done.

2. Clinical Chemistry -

Table 3 summarizes cholinesterase activity in blood and brain of rabbits. Dermal application of oxamyl 6 hours a day for 21 days resulted in biologically and toxicologically significant inhibition of plasma and brain cholinesterase in female rabbits at 75 mg/kg/day. The plasma and brain cholinesterase of females rabbits was inhibited 29% and 11%, respectively, compared to the controls and the inhibition was statistically significant ($P < 0.05$). In addition, the red blood cell cholinesterase was depressed 24% in female rabbits treated with 75 mg/kg/day; although statistically not significant, but considered biologically relevant since the plasma and brain cholinesterase were statistically significantly depressed compared to controls. In males rabbits, there were no treatment-related changes in the plasma, red blood cell or brain cholinesterase at any dose levels during the study. The females plasma, RBC or brain cholinesterase activity was not changed at 25, 40, or 50 mg/kg/day.

TABLE 3[a]

SUMMARY OF CHOLINESTERASE FINDINGS AT TERMINATION

	MALE		
Dose (mg/kg)	RBC (U/L)	Plasma (U/L)	Brain (U/L)
0	2373 ± 513[b] (-)	660 ± 129 (-)	7.2 ± 0.6 (-)
25	2837 ± 468 (-20)[c]	613 ± 108 (7)	7.5 ± 0.4 (-4.1)[c]
40	2317 ± 618 (2)	542 ± 176 (18)	7.7 ± 0.3 (-5.9)[c]
50	2350 ± 531 (1)	616 ± 122 (7)	7.4 ± 0.6 (-1.7)[c]
75	1967 ± 779 (17)	536 ± 226 (19)	6.8 ± 0.6 (5.9)
FEMALE			
0	2380 ± 549 (-)	639 ± 96 (-)	7.3 ± 0.3 (-)
25	2100 ± 487 (12)	676 ± 148 (-6)[c]	7.2 ± 0.5 (1.3)
40	2340 ± 560 (2)	609 ± 196 (5)	7.0 ± 0.6 (4.5)
50	2227 ± 466 (-5)[c]	638 ± 158 (0)	7.0 ± 0.4 (4.7)
75	1820 ± 520 (24)	**455* ± 92 (29)**	**6.5* ± 0.5 (10.7)**

a Data taken from p. 46, MRID 44751201; from Rabbit 33283 in the 40 mg/kg group samples were collected on Test day 20, all others were collected Test day 21
b Data presented as mean ± SD
c A negative number for % of control indicates the value was greater than the control value
() Values in parentheses are Percent inhibition from control
* Significant at 5% level by Dunnett's multiple comparison test

F. Urinalysis

Urinalysis was not required and was not performed.

G. Sacrifice and Pathology:

1. Organ weight

No treatment-related effects were observed in any of the organs in the previous study (MRID 40827601) at dosages up to 250 mg/kg/day, therefore, only organs or tissues with gross lesions were collected. Brain weights or gross morphology was not altered either in males or females at any dose level.

2. Gross pathology

Small testes one each in control and in the 25 mg/kg/day male was observed. This occurrence lacked a dose-response, and it was considered to be spontaneous occurrence.

3. Microscopic pathology

Histopathology was not reported on testes.

III. DISCUSSION

A. DISCUSSION

No treatment-related mortality was observed. One male in the 40 mg/kg/day group was sacrificed *in extremis* on test day 2 because of fractured lumbar vertebra. There were no clinical signs indicative of systemic toxicity at any dose. A significantly higher incidence of mild erythema was observed in males dosed at 25 and 75 mg/kg/day following daily applications; mild erythema was observed in all groups, however, it lacked dose-response, considered to be of no toxicological significance. Occasionally, diarrhea and hyperactivity, suggestive of cholinesterase effects, occurred with low incidence, but dose-response was not evident. One male each in the 50 and 75 mg/kg/day group and one female each in the 25 and 75 mg/kg/day group were noticed with pupillary constriction in one eye following unwrapping and washing of test substance. It was explained that pupillary constriction may have been due to splashing while cleaning with water. Body weights of both sexes and food consumption and/or efficiency were unaffected at any dose.

Significant inhibition of plasma and brain cholinesterase was observed in female rabbits at 75 mg/kg/day. In addition, there was a non-significant inhibition of RBC cholinesterase in female rabbits treated with 75 mg/kg/day was noticed; although inhibition was statistically not significant, but considered biologically relevant because of magnitude of depression (24%) accompanying statistically significantly plasma and brain cholinesterase depression. In males rabbits, there were no treatment-related changes in the plasma, red blood cell or brain cholinesterase at any dose levels during the study. The female plasma, RBC or brain cholinesterase activity was not changed at 25, 40, or 50 mg/kg/day.

Brain weights were not effected in either sex at any dose. Small testes one each in control and 25 mg/kg/day group was observed, not dose-related, therefore, considered to be a spontaneous occurrence.

Systemic toxicity was not observed in this study. The **Systemic Toxicity NOAEL = 75 mg/kg/day.**

The **Cholinesterase NOAEL = 50 mg/kg/day, and LOAEL = 75 mg/kg/day,** based on plasma, red blood cell and brain ChE inhibition in females rabbits. In male rabbits, the

NOAEL = 75 mg/kg/day, and LOAEL > 75 mg/kg/day.

B. Study deficiencies

Major deficiencies exists in the conduct of study i.e., compound homogeneity, stability, ophtholmology, hematology, clinical chemistry, detailed sacrifice report, organ weights and histopathology was not done; however, these deficiencies do not impact upon the outcome of the study, because, the study was designed to establish clearly the blood and brain cholinesterase NOAEL and LOAEL. This study in conjunction with previously conducted 21-day dermal toxicity study (MRID 40827601) is adequate to establish endpoints for cholinesterase inhibition.

C. Classification

The study by itself is **Unacceptable**, however, in conjunction with the previous 21-day dermal toxicity study (MRID 40827601), the study is classified as **Acceptable** (non-guideline) and adequate for intended purposes. This study **does not satisfy** the guideline requirement for a 21-day dermal study in rabbits (82-2b).

[Oxamyl **1-Year Feeding Study - Dog (83-1b)]**

Supplement to Document No. 009351 - DER for MRID Nos. 41697901, 42052701 & 44737503: Oxamyl, 1-Year Feeding Study in the Dog. This amendment provides an Revised Executive Summary to the original DER.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D., Date 8/4/99
Reregistration Branch 2
Health Effects Division (7509C)

Secondary Reviewer: Robert Fricke, Ph.D., R. Fricke, Date 26 May 99
Reregistration Branch 2
Health Effects Division (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Chronic (1-Year) Toxicity Study - Dog

OPP Number: 83-1b
DP BARCODE: D253122
PC CODE: 103801

OPPTS Number: 870.4100
SUBMISSION CODE: S556451
TOX CHEM NO: 561A

TEST MATERIAL (PURITY): Oxamyl Technical (99%, a.i.)

SYNONYMS: Ethanimidothioic acid, 2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-, methyl ester; DPX-D1410-196; DPX-D1410-196 Technical; methyl2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-ethanimidothioate; emethyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate

CITATIONS:

1. Mebus, C.A. (1990) Chronic Toxicity Study with Oxamyl (IN D 1410-196): One Year Feeding Study in Dogs. Haskell Lab. Study No. 381-90. MRID 41697901. Unpublished.

2. Dickrell, L. (1991) 52-Week Dietary Toxicity Study with IND-1410 (Oxamyl) in Male Dogs. Hazleton Laboratories America, Inc., Madison, WI. Study No. HLO 555-90. MRID 42052701. Unpublished.

3. Van Pelt, C.S. (1999) DuPont's Position on the NOEL in Male Dogs following

[Oxamyl **1-Year Feeding Study - Dog (83-1b)]**

Chronic Dietary Exposure to Oxamyl (DuPont Reports HLR 381-90 and HLO 555-90). Lab. Project ID. DuPont-2019. January 18, 1999. MRID 44737503. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, DE 19880-0038

EXECUTIVE SUMMARY: In a 1-Year Chronic Feeding Study (MRID 41697901, 42052701 & 44737503) oxamyl (99%) was administered in diet to groups of male and female beagle dogs (5/dose) at dose levels of 0, 50, 150, or 250 ppm (equivalent to 0, 1.56, 4.60, or 8.0 mg/kg/day, for males and 0, 1.46, 4.50, or 7.84 mg/kg/day, for females, respectively). The dogs were offered the food once daily. . In this study a NOAEL for male dogs was not established due to depression of cholinesterase in plasma and brain at all dose levels. Subsequently, a second one-year study (MRID 42052701) was repeated in male dogs (5/dose) at dose levels of 0, 12.5, 20, 35 or 50 ppm (equivalent to 0, 0.372, 0.577, 0.930 or 1.364 mg/kg/day). In the later study food was offered *ad libitum*.

Administration of oxamyl did not produce any adverse effects in urinalysis, ophthalmological examinations, and gross pathology at any dose levels. At 50 ppm, in males, the plasma ChE was inhibited ($P < 0.05$) at 6, 9, and 12 months by 33, 34, and 32%, respectively, compared to the controls; females exhibited slight depression during the study. At this dose, only in males, brain ChE was depressed 17% compared to controls. At doses 150 and above, increased incidence of clinical signs including tremors and vomiting in males and females were observed. Mean body weights/body weight gains in 250 ppm dose males and females decreased 23%/81% and 17%/49%, respectively, compared to controls and the decreases were statistically significant ($P < 0.05$). The body weight changes were accompanied by decreases food consumption food efficiency. The food consumption/food efficiency of 250 ppm males decreased 11%/75% ($P < 0.05$) in comparison to the controls. In 250 ppm females food consumption and food efficiency decreased 7% and 42%, respectively, compared to control, but the decreases were not significant. Plasma cholesterol of 150 ppm and 250 ppm decreased males and females. Histologically, 3/5 high dose males exhibited increased regenerative renal tubular epithelial alterations.

Second study (MRID 42052701) conducted to establish a NOAEL in male dogs treated with oxamyl. At 50 ppm, by study termination, the plasma, RBC and brain (cerebellum+medulla) ChE was not statistically significantly depressed 11, 4, and 20%, respectively, compared to controls. Although, marked (20%) brain ChE inhibition may not be statistically significant, but considered biologically relevant, since tremors were observed at 150 and 250 ppm in males and at all doses in females in the previous study. At the 35 ppm, the plasma, RBC and brain ChE levels were depressed 18%, 5% and 2%, which appears to be true NOAEL. Even though, the study was not replicated as previous study to establish the NOAEL in male dogs, but the results do suggest that the plasma ChE levels are comparable and the information from this study could

be utilized to derive a NOAEL in male dogs.

The **Systemic Toxicity NOAEL = 50 ppm (1.56 mg/kg/day for males and 1.46 mg/kg/day for females) and LOAEL = 150 ppm (4.60 mg/kg/day for males and 4.50 mg/kg/day for females),** based on decreased body weights and body weight gains.

The **Cholinesterase NOAEL = 35 ppm (0.930 mg/kg/day) for males and 50 ppm (1.56 mg/kg/day) for females, and LOAEL = 50 ppm (1.36 mg/kg/day) for males and 4.50 mg/kg/day) for females,** based on brain cholinesterase levels in males and vomiting, tremors, plasma and brain ChE inhibition in females.

CLASSIFICATION: This study (MRID 41697901) in combination with the second 1-Year chronic dog study (MRID 42052701) is **Upgraded from Core-Supplementary to Acceptable/guideline** and satisfy the data requirements for a chronic toxicity study in dogs (83-1b).

1. Action Requested

The DuPonte de Nemours and Company on January 19, 1999, submitted a response (MRID 44737503) to the HED comments on two 1-Year dog studies (HED Doc. No. 009351, March 10, 1992), and registrant's meeting with HED to resolve issues on February 24, 1993. The submission included the following information:

a. Impact of eating behavior on the NOAEL in Dogs. The manner in which food is offered to young dogs might impact on their food consumption habit and thus the compound consumption, in this case.

i) In the first study, dogs obtained from the Marshall Research Animals were accustomed to receiving food once a day. Therefore, they generally consumed entire offering in a single feeding. The consumption will have a bolus effect on compound intake. As a result, blood concentrations are likely to reach rapidly. The resulting compound intake was averaged to 1.56 mg/kg/day for male dogs.

ii) Animals in the second study were bred and raised at the Hazleton Laboratory, were used to *ad. libitum* intake. Even though, test diet have same concentration of the compound but smaller quantities over a long period of time tend to have lower blood levels. In this study, the mean daily intake at 50 ppm was 1.364 mg/kg/day and at 35 ppm was 0.930 mg/kg/day for male dogs. The 50 ppm diet dietary concentration is near the threshold level for male dogs and based on food consumption habits of juvenile dogs and mean compound intake, effects may or may not occur. The registrant feels in the second study there were no effects were observed, therefore, 1.364 mg/kg/day is the NOAEL for male dogs in this chronic feeding study.

b. Deficiencies noted in the second chronic dog study (DuPont study HLO 555-

90/MRID 420527-01)

1. Blood and brain tissue collection

Agency Concern: "One of the intents of the study was to determine if the inhibition of brain and plasma cholinesterase activity in male dogs by 50 ppm oxamyl could be reproduced. According to the report, the blood samples for cholinesterase activity determination were collected between 10:00 a.m. and 12:00 p.m. and the test animals were fed the test diet ad. libitum. This procedure was clearly different from that of the previous study which collected the blood sample 3 hrs post-feeding for plasma and whole blood cholinesterase activity determinations. In addition, the brain tissues for cholinesterase activity measurements were removed from dogs sacrificed 3 hours post dosing. Since many carbamates are known to reversibly inhibit the cholinesterase activity, the procedures for collecting the test samples should be carried out soon as possible after administrating the test compound."

DuPont Response: "Blood and brain samples were obtained at approximately the same time period following dosing in both studies. The blood samples and necropsy of the animals in the Hazleton study were performed approximately 3 hrs after fresh food was presented to the dogs (corresponding to 10:00 a.m. to 12:00 p.m.), which is essentially the same time period between feeding and sampling in the Haskell study. Hazleton Laboratories also used similar dissection and sampling techniques as was Haskell study. Thus, there were no significant differences in cholinesterase sampling procedures between the studies."

Agency Conclusions: The response is adequate.

2. Method for Analysis of Cholinesterase Activity

Agency Concern: "The report did not indicate what method was used in determining cholinesterase activity.:

DuPont Response: "A similar and acceptable method of analysis of Cholinesterase activity was used by both testing facilities. Hazleton Laboratories used modified Ellman technique for assessing cholinesterase activity and commercial reagent kit form Boehringer Mannheim Diagnostics specifically developed for the Hitachi 704 clinical chemistry analyzer. Hazleton collected and maintained blood and brain samples on ice or "snap" froze these samples to minimize decarbamylation cholinesterase. Haskell Laboratory did not have an Hitachi 704 clinical chemistry analyzer at the time the study was conducted, but used an Encore System II (Baker Instruments, Allentown, PA) at a wavelength of 405nm. Similar precautions were taken to minimize decarbamylation at

Haskell Laboratory as well. Thus, the analytical methods for assessing cholinesterase activity were essentially equivalent in the two studies."

Agency Conclusions: The response is adequate.

3. Determination of RBC Cholinesterase Activity

Agency Concern: "The report did not indicate how the values of RBC cholinesterase activity were derived."

DuPont Response: "In the study conducted by Haskell Laboratory, a hematocrit correction was used to calculate cholinesterase activity in RBCs. Hazleton Laboratories' method assessing RBC cholinesterase activity did not use a hematocrit correction. Cholinesterase activity was measured directly on a lysate of packed red blood cells. After centrifugation of whole blood, 10 ml of packed RBCs were lysed with Triton X-100 and analyzed for cholinesterase activity. Because the RBCs were sampled directly from the packed cells (and not from the whole blood), a hematocrit correction was not necessary.

Agency Conclusion: The response is adequate.

2. **Conclusions**

Based on the registrant's response the two 1-Year chronic feeding dog studies were conducted similarly and the results are comparable between the studies. The second study clearly establishes NOAEL in the male dog that was not established in the previous study. These two studies in combination provide adequate information on chronic toxicity in dog and fulfill guideline requirements for chronic toxicity study in dog (83-1b).

The **Systemic Toxicity NOAEL = 50 ppm (1.56 mg/kg/day for males and 1.46 mg/kg/day for females) and LOAEL = 150 ppm (4.60 mg/kg/day for males and 4.50 mg/kg/day for females),** based on decreased body weights and body weight gains.

The **Cholinesterase NOAEL = 35 ppm (0.930 mg/kg/day) for males and 50 ppm (1.56 mg/kg/day) for females, and LOAEL = 50 ppm (1.36 mg/kg/day) for males and 4.50 mg/kg/day) for females,** based on brain cholinesterase levels in males and vomiting, tremors, plasma and brain ChE inhibition in females.

DATA EVALUATION REPORT

013975

OXAMYL

STUDY TYPE: DEVELOPMENTAL TOXICITY– RABBIT (83-3B)

MRID 00063009; DP BARCODE: none

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 99-46

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

013975

Supplement to Document No. 005858 & 000369 - DER for MRID No. 00063009: Teratology Study in Rabbits. This amendment provides an Update of Original Data Evaluation Record. This review supersedes the original Document (005858 & 000369).

EPA Reviewer: G. Reddy, Ph.D. Date: 10/1/93
Reregistration Branch 2
EPA Work Assignment Manager: S. Diwan, Ph.D. Date: 10/1/93
Toxicology Branch 1

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit
OPPTS 870.3700 [§83-3b]

DP BARCODE: none
P.C. CODE: 10801

SUBMISSION CODE: none
TOX. CHEM. NO.: 561A

TEST MATERIAL (PURITY): Oxamyl Technical (97.1% a.i.)

SYNONYMS: Ethanimidothioic,2-(dimethylamino)-N-{[(methylamino)carbonyl]oxy}-2-oxo-, methyl ester

CITATION: Hobermam, A.M.; Mossburg, P.A.; Wolfe, G.W.; et al. (1980) Teratogenicity Study in Rabbits. Hazleton Laboratory of America, Inc., Vienna, VA. Study No. 201-405. October 1980. MRID 00063009. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Inc., Wilmington, DE 19880

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 00063009), 17 pregnant New Zealand White rabbits per group were administered Oxamyl (97.1% a.i.; Lot No. H-10, 963-02, IND-1410-196) by gavage at doses of 0, 1, 2, or 4 mg/kg/day on gestation days (GD) 6-19, inclusive. On GD 29, all surviving does were sacrificed and all fetuses were weighed, measured for crown-rump distance, and examined for external malformation/variations. Each fetus was examined viscerally by fresh dissection and the sex determined. The heads from one-third of the fetuses were removed, fixed in Bouin's solution, and sectioned by Wilson's freehand razor technique. All carcasses were eviscerated and processed for skeletal examination.

One doe each in the low- and high-dose groups died prior to scheduled sacrifice; these deaths were attributed to gavage error. All other animals survived to terminal sacrifice. Necropsy was unremarkable. No treatment-related clinical signs of toxicity were observed in any animal in any treated group. Maternal absolute body weights and food consumption were comparable between the treated and control groups throughout the study. However, during the treatment interval (day 6 - 19),

the mid- and high-dose groups had significantly ($p \leq 0.05$) reduced body weight gains as compared with the controls. Body weight gains by the mid- and high-dose groups during treatment were 38.5% and 32.8%, respectively, of the control levels. Recovery was apparent during the postdosing interval when body weight gains by these groups were 113.5% and 157.9%, respectively, of the controls.

Therefore, the maternal toxicity LOAEL was 2 mg/kg/day based on reduced body weight gains and the maternal toxicity NOAEL is 1 mg/kg/day.

No treatment-related differences were observed between the treated and control groups for number of corpora lutea/doe, implantations/doe, preimplantation loss, fetal body weights and lengths, or fetal sex ratios. Dose-related increased resorption rates for the mid- and high-dose groups resulted in increased postimplantation losses and decreased litter sizes, but statistical significance was not reached for any parameter. For the control, low-, mid-, and high-dose groups, the mean resorptions/doe were 0.8, 0.5, 1.0, and 1.2, respectively, resulting in postimplantation losses of 10.4%, 8.7%, 15.9%, and 24.8%, respectively. The number of live fetuses/litter was 6.6, 6.6, 5.9, and 5.8. Resorptions in the mid-dose group consisted of both early (0.7/doe) and late (0.3/doe) resorptions which were not considered treatment-related. In the high-dose group only early resorptions were observed and two animals had whole litter resorption consisting of early resorptions of 1 and 7 implantation sites, respectively, are not considered treatment-related since complete resorptions in rabbits is not uncommon. Furthermore, resorptions in the high-dose group may have been a consequence of the maternal toxicity at this dose.

The number of fetuses(litters) examined in the 0, 1, 2, and 4 mg/kg/day groups was 113(17), 90(15), 89(15), and 75(13), respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses.

Therefore, the developmental toxicity NOAEL was 4 mg/kg/day and the developmental toxicity was not observed.

This study is classified as **Acceptable/guideline** and satisfies the requirements for a developmental toxicity study (83-3b) in rabbits. Several deficiencies were noted in the conduct of this study, however, this study was performed prior to implementation of the current guidelines.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Data Confidentiality, and Flagging statements were not included.

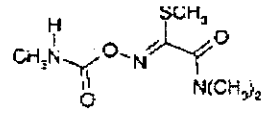
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Oxamyl

   Description: off-white, crystalline powder
   Lot No.: H-10, 963-02, IND-1410-196

Purity: 97.1% a.i.
Stability of compound: not stated
CAS No.: not stated
Structure:



2. Vehicle and/or positive control

The vehicle and negative control was Borden™ Crystal Distilled water (Polar Water Company, Pittsburgh, PA). No positive control was used in this study.

3. Test animals

Species: rabbit
Strain: New Zealand White
Age and weight at study initiation: age not given; 2940-3945 g
Source: Dutchland Laboratory Animals, Inc., Denver, Pennsylvania
Housing: Animals were housed individually in elevated metal cages.
Diet: Purina Lab Rabbit Chow® was available *ad libitum.*
Water: Tap water was available *ad libitum.*
Environmental conditions:
Temperature: 70°F to 80°F
Humidity: 32% to 92%
Air changes: not stated
Photoperiod: 12 hour light/dark
Acclimation period: approximately 5 weeks

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Oxamyl when administered by gavage to rabbits on gestation days 6 through 19, inclusive.

1. In life dates

Start: May 27 and 29, 1980; end: June 25 and 27, 1980

2. Insemination

Ovulation was induced in each doe by injection of 250 IU of human chorionic gonadotropin into the marginal ear vein. Four to five hours after injection, each female was artificially inseminated with semen from male New Zealand White rabbits maintained as breeding stock by the testing facility. The day of insemination was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Females were assigned to groups by a computerized randomization process.

TABLE 1. Animal assignment

Group	Dose (mg/kg/day)	Number of Does
Control	0	17
Low Dose	1	17
Mid Dose	2	17
High Dose	4	17

Data taken from text table p. 21, MRID 00063009.

4. Dose selection rationale

The dose selection rationale was not given in the study.

5. Dosing

Doses were administered in a volume of 1 mL/kg based on the most recently recorded individual body weight.

6. Dose solution preparation and analysis

The amount of test article required for each dosing solution was weighed, an appropriate amount of distilled water was added, and the mixture was placed on a magnetic stirrer until in solution. The report did not state the frequency of preparation or whether the dosing solutions were analyzed for concentration, homogeneity, or stability.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were observed daily for mortality, moribundity and clinical signs of toxicity. Maternal body weights were recorded on GD 6, 11, 15, 19, and 29. Individual food consumption was measured on days 7-29. On GD 29, all surviving does were killed by an intravenous injection of T-61® Euthanasia Solution (Taylor Pharmacal Co., Decatur, IL) via the lateral ear vein and subjected to gross necropsy. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. Gravid uteri were opened and the number of live and dead fetuses, resorptions, and the number and placement of implantations were recorded. Cesarean sections were also performed on does that were found dead and the number of corpora lutea, implantations, resorption, and live or dead fetuses were recorded if applicable. The ovaries and uterus from each doe was preserved in 10% neutral buffered formalin.

2. Fetal evaluations

All fetuses were weighed, examined for external malformations/variations, and the crown-rump distance recorded. Each fetus was opened by a longitudinal incision, examined viscerally, and the sex determined. The heads from one-third of the fetuses were removed, fixed in Bouin's solution, and sectioned by Wilson's freehand razor technique. All carcasses were eviscerated and processed for skeletal examination.

D. DATA ANALYSIS

1. Statistical analysis

Mean fetal body weights and lengths, maternal body weight changes, gravid uterine and ovarian weights, and maternal food consumption data were analyzed with a one-way analysis of variance (ANOVA) with Bartlett's test for homogeneity. If both the Bartlett's and the ANOVA were significant, a multiple pairwise comparison procedure was used to compare the group mean values. If the ANOVA was significant, but Bartlett's test was not, Scheffe's multiple pairwise comparison procedure was used. The reproduction and viability indices were analyzed by a chi-square method or by Wilcoxon's nonparametric comparison of group means. The litter was used as the experimental unit. Statistical significance was set a the 5% probability level.

2. Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

One low-dose and one high-dose animal were found dead during the study but no relationship to treatment was indicated. All other does survived to terminal sacrifice. No treatment-related clinical signs of toxicity were observed in any animal.

2. Body weight

Selected maternal body weight data are given in Table 2. No statistically significant differences in absolute body weights occurred between the treated and control groups during the study. However, during the treatment interval, the mid- and high-dose groups had significantly ($p \leq 0.05$) reduced body weight gains as compared with the controls. Body weight gains by the mid- and high-dose groups during treatment were 38.5% and 32.8%, respectively, of the control levels. Recovery was apparent during the postdosing interval when body weight gains by these groups were 113.5% and 157.9%, respectively, of the controls.

TABLE 2: Selected maternal body weights and body weight changes during gestation (g)				
GD	0 mg/kg/day	1 mg/kg/day	2 mg/kg/day	4 mg/kg/day
0	3362.9	3416.7	3349.3	3290.4
6	3420.9	3482.7	3409.0	3403.5
11	3478.8	3535.3	3394.0	3390.4
15	3554.7	3588.7	3481.0	3455.0
19	3590.4	3603.4	3474.2	3459.1
29	3728.8	3747.5	3631.3	3677.7
Wt. Gain (0-6)	58.0	66.0	59.7	113.1
Wt. Gain (6-19)	169.5	154.4	65.2* (38.5)[a]	55.6* (32.8)
Wt. Gain (19-29)	138.4	144.1	157.1 (113.5)	218.6 (157.9)
Wt. Gain (0-29)	365.9	368.7	282.0 (77.1)	387.3 (105.8)

Data taken from Table 2, p. 31, MRID 00063009.
[a]Numbers in parentheses are per cent of control; calculated by reviewer.
Significantly different from control: *p ≤ 0.05.

3. Food consumption

No statistically significant differences in food consumption were seen between the treated groups and the control group at any time during the study.

4. Gross pathology

No treatment-related findings were observed in any animals at necropsy. Findings in the two animals that died intercurrently were consistent with gavage error.

5. Cesarean section data

Cesarean section data are summarized in Table 3. No treatment-related differences were observed between the treated and control groups for number of corpora lutea/doe, implantations/doe, preimplantation loss, fetal body weights or lengths, or fetal sex ratios. The slight (n.s.) increase in mean resorptions/doe in the mid- and high-dose groups resulted in corresponding increases (n.s.) in postimplantation loss for these groups and decreases (n.s.) in litter size. However, in the mid-dose group, the increases were due to both early and late resorptions while in the high-dose group, only early resorptions occurred. Because the numbers of early and late resorptions for the mid-dose group were similar to the controls, the slight increase in mean resorptions for this group is not

considered treatment-related. In the high-dose group, two animals had whole litter resorption consisting of early resorptions of 1 and 7 implantation sites, respectively.

TABLE 3. Cesarean section observations

Observation	0 mg/kg/day	1 mg/kg/day	2 mg/kg/day	4 mg/kg/day
No. Assigned	17	17	17	17
No. Pregnant (%)	17 (100)	15 (88)	15 (88)	13 (76)
No. Died	0	1	0	1
No. Aborted	0	0	0	0
Corpora Lutea/Doe	11.1	9.9	11.1	9.8
Implantations/Doe	7.4	7.0	7.0	7.0
Preimplantation loss (%)	31.1	27.1	34.8	25.8
Postimplantation loss (%)	10.4	8.7	15.9	24.8
Mean Ovarian and Gravid Uterine Wts. (g)	418.7	416.6	384.9	413.5
Live Fetuses/litter	6.6	6.6	5.9	5.8
Mean Fetal Weight (g)				
Males	44.49	45.08	45.04	43.08
Females	43.35	43.83	43.26	44.76
Crown-Rump Length (cm)				
Males	9.24	9.33	9.37	9.28
Females	9.31	9.27	9.32	9.45
Sex Ratio (% Male)	63.2	50.7	60.3	54.5
Mean Resorptions/Doe	0.8	0.5	1.0	1.2
Early Resorptions/Doe[a]	0.7	0.4	0.7	1.2
Late Resorptions/Doe[a]	0.1	0.1	0.3	0.0
Does with all Resorptions	0	0	0	2
Total Dead Fetuses	0	0	1	0

Data taken from Table 5 and Appendix 3, pp. 35 and 53-56, respectively, MRID 00063009.
[a]Calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

The number of fetuses(litters) examined in the 0, 1, 2, and 4 mg/kg/day groups was 113(17), 90(15), 89(15), and 75(13), respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses.

1. External examination

Findings from external examination of the fetuses were not presented in either the text or tables of the report.

2. Visceral examination

No treatment-related visceral malformations/variations were observed. A significantly ($p \leq 0.05$) greater number of litters in the low-dose group contained fetuses with visceral abnormalities as compared with the control group (8/14 vs 3/17, respectively). However, no overall pattern of defects was observed and most litters contained only one or two affected fetuses.

3. Skeletal examination

No treatment-related skeletal malformations/variations were observed. The incidence rates of skeletal abnormalities was similar between the control, low-, and mid-dose groups. No skeletal malformations/variations were observed in fetuses from the high-dose group.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that treatment of pregnant rabbits with Oxamyl resulted in maternal toxicity and embryotoxicity. Decreases in maternal body weight gains were observed at the mid- and high-doses and a slight, but not statistically significant, increase in resorptions occurred at the high dose. Treatment did not result in teratogenic effects. NOAELs for maternal and developmental toxicity were not specified by the study author.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Maternal toxicity was evident as reduced body weight gains by the mid- and high-dose groups during the treatment interval. Because reduced weight gains were not accompanied by decreases in food consumption or associated with any other clinical sign of toxicity, they are considered a direct effect of the test article. Recovery was apparent during the post dosing interval such that overall weight gains were not affected.

Therefore, the maternal toxicity LOAEL was 2 mg/kg/day based on reduced body weight gains and the maternal toxicity NOAEL is 1 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

A dose-related increase in mean resorptions occurred in the mid- and high-dose groups although statistical significance was not reached. The increased resorption rates resulted in increased postimplantation losses and decreased mean litter sizes for these groups as compared with the controls. However, because the mid-dose group had both early and late resorptions, with late resorptions only slightly greater than the control group, the effect at the mid-dose is not considered treatment-related. Complete litter resorptions in two high-dose does is considered not related to treatment since total litter resorptions is not uncommon in the rabbit. Furthermore, increased resorptions in the high-dose group may have been a consequence of the maternal toxicity observed at this dose.

b. Altered growth

No evidence of altered growth of the fetuses was seen in this study. Fetal body weights and lengths were comparable between the treated and control groups.

c. Developmental variations

Variations common to rabbit fetuses were observed at a similar incidence rate in both treated and control litters.

d. Malformations

Treatment with the test article did not result in an increased incidence of fetal malformations. The significant increase in the number of low-dose litters containing fetuses with abnormalities is not considered a treatment-related effect because no consistent pattern of defects was observed, none of the individual observations occurred in a dose-related manner, and generally only one or two fetuses per litter were affected.

Therefore, the developmental toxicity NOAEL was 4 mg/kg/day. Developmental toxicity was not observed at the highest dose (4 mg/kg/day).

C. STUDY DEFICIENCIES

Several deficiencies were noted in the conduct of this study. The dosing solutions were not analyzed for concentration, homogeneity, or stability; individual fetal external, visceral, and skeletal observations were not included; and, a dose selection rationale was not given. However, this study was conducted prior to the implementation of the current guidelines. The deficiencies do not prevent assessment of the developmental toxicity potential of Oxamyl because dosing was adequate to cause maternal toxicity.

D. CLASSIFICATION

This study is classified as **Acceptable/guideline** and satisfies the requirements for a developmental toxicity study (83-3b) in rabbits.

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103801 0013000 020300 TX013975 R000911

Chemical:	**Oxamyl**
PC Code:	**103801**
HED File Code	**13000 Tox Reviews**
Memo Date:	**02/03/2000**
File ID:	**TX013975**
Accession Number:	**412-01-0045**

HED Records Reference Center
11/14/2000