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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Toxicology Chapter for the Reregistration Eligibility Document for ETHOPROP (Chemical 041101).

**TO:** Judy Loranger
Special Review and Reregistration Division (7508W)

RECEIVED
OPP PUBLIC DOCKET

**FROM:** Kit Farwell
Reregistration Branch 1
Health Effects Division (7509C)

K Farwell 4-17-98

**THRU:** Whang Phang, Senior Scientist
Reregistration Branch 1
Health Effects Division (7509C)

W Phang 4/17/98

**ACTION REQUESTED:** Prepare Toxicology RED Chapter for Ethoprop.

**SUMMARY:** The toxicology chapter for the ethoprop RED is attached. Ethoprop (O-ethyl S,S-dipropyl phosphorodithioate) is a nematicide and insecticide for use on fruit and vegetable crops and golf course turfgrass.

The toxicology database for ethoprop is essentially complete, with two exceptions. Cholinesterase determinations for the M1 metabolite of ethoprop in an acute study are ongoing, however, the remainder of this study (MRID 44472501) has already been reviewed (see Acute Toxicity section of this chapter). A neurotoxic esterase study has been requested which is "confirmatory" in nature (see Neurotoxicity Studies section of this chapter). These results will not significantly change the understanding of the toxicity of ethoprop and should not delay the reregistration process.

The endpoints for acute and chronic dietary exposure as well as short-, intermediate-, and long-term dermal occupational or residential exposure are presented in this chapter. The HED Cancer Assessment Peer Review Committee (10/2/97 document) classified ethoprop as a "likely" human carcinogen with a $Q_1$*(see Carcinogenicity Classification section of this chapter). Recommendations for uncertainty factors as required under FQPA are included in the Dose Response Assessment section of this chapter.

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## CONTENTS OF ETHOPROP TOXICOLOGY CHAPTER





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## B. DOSE RESPONSE ASSESSMENT.

1. SENSITIVITY OF INFANTS AND CHILDREN.

   a. 10X Factor for Protection of Infants and Children: Acute and Chronic Dietary Exposure.

   b. Assessment of Need for a Developmental Neurotoxicity Study.

2. REFERENCE DOSE (RfD).

3. OTHER TOXICOLOGICAL ENDPOINTS

   Table 4. Toxicological Endpoints

7. INHALATION EXPOSURE (any time period).

8. CARCINOGENIC CLASSIFICATION.

## A. HAZARD ASSESSMENT.

**1. ACUTE TOXICITY STUDIES.** The acute toxicity of ethoprop is due to inhibition of acetylcholinesterase. Ethoprop is in Toxicity Class I based on an oral study in rats and a dermal study in rabbits. **Ethoprop is also in Toxicity Class I due to the eye and dermal irritation studies in which all rabbits died. The dose-response curve for ethoprop is steep, clinical signs appear at slightly lower doses than a lethal dose .** Rabbits were approximately 50x more susceptible than rats in dermal $LD_{50}$ studies, likely due to increased dermal absorption.

**TABLE 2A. ACUTE TOXICITY: TECHNICAL ETHOPROP**

| GDLN | STUDY TYPE | MRID # YEAR | RESULTS | TOXICITY CATEGORY |
|---|---|---|---|---|
| 81-1 | Acute Oral - Rat[a] | 00078035 1965 | M $LD_{50}$ = 56.2 mg/kg F $LD_{50}$ = 30.2 mg/kg | I |
| 81-2 | Acute Dermal - **Rabbit** | 42979502 1987 | $LD_{50}$ = 8.5 mg/kg | I |
| 8-2 | Acute Dermal - **Rat** | 42979501 1987 | $LD_{50}$ = 1280 mg/kg M $LD_{50}$ = 424 mg/kg F | II |
| 81-3 | Acute Inhalation - Rat | 070060 1980 | $LC_{50}$ = 0.123 mg/L | II |
| 81-4 | Eye Irritation - Rabbit | 00078036 1965 | 0.1 mL killed all 3 rabbits | I |
| 81-5 | Skin Irritation - Rabbit | 00048774 1977 | 0.5 mL killed all 6 rabbits. | I |
| 81-6 | Dermal Sensitization[b] | N/A | N/A | N/A |
| 81-7 | Delayed Neuropathy - Hen | 40609401 1986 | Negative | N/A |
| 81-8 | Acute Neurotoxicity - Rat | 43442402 43197701 1994 | systemic NOEL/LOEL = 5/25 mg/kg (clinical signs), ChE NOEL <5 mg/kg | N/A |

[a]These $LD_{50}$ values are from the review; slightly different values were reported in 1988 Reregistration Document. [b]Requirement for a Dermal Sensitization study waived due to high acute dermal toxicity of ethoprop in rabbits.

All acute studies are acceptable and the database is complete, with the exception of a portion of an ongoing acute toxicity study in rats with ethoprop metabolites. In the acute study with ethoprop metabolites, cholinesterase inhibition for the M1 metabolite of ethoprop (O-ethyl-S-propylphosphorothioate) has yet to be determined because of depletion of test material. The rest of the study (MRID 44472501) has already been received by HED and reregistration should not be delayed.

The acute study with metabolites in rats (MRID 44472501) was conducted to determine if the M1 metabolite caused significant cholinergic toxicity. This study determined that the M1 metabolite was in Toxicity Category III and did not cause significant acute toxicity compared to parent ethoprop. M1 treatment required approximately 20x higher doses than ethoprop to cause similar mortality or clinical signs. Cholinesterase inhibition for M1 was not yet determined; test material was depleted due to the large quantities needed during $LD_{50}$ testing. SME treatment caused cholinesterase inhibition, clinical signs, and mortality at generally similar, but slightly lower doses than ethoprop. OME treatment caused clinical signs and mortality at approximately 1/3 to ½ the dose of ethoprop.

**This study also showed the steep dose-response curve for ethoprop.** Mortality at 50 mg/kg ethoprop was 3/10, at 60 mg/kg was 6/10, and at 75 mg/kg was 10/10. The number of animals with severe clinical signs (tremor or staggering gait) at 50 mg/kg ethoprop was 7/10 and at 60 mg/kg was 10/10. This study was successful in determining acute toxicity of the ethoprop metabolites and is classified **Acceptable/Guideline**.

**TABLE 2B. COMPARATIVE ACUTE ORAL TOXICITY: ETHOPROP AND METABOLITES.** (1998, MRID 44472501)

| COMPOUND[1] | RESULTS | TOXICITY CATEGORY |
|---|---|---|
| ETHOPROP | $LD_{50}$ = 55.8 mg/kg | II |
| SME | $LD_{50}$ = 50.0 mg/kg | I |
| OME | $LD_{50}$ = 22.4 mg/kg | I |
| M1 | $LD_{50}$ = 1608 mg/kg | III |

[1]SME = (O-ethyl-S-methyl-S-propylphosphorodithioate)
OME = (O-ethyl-O-methyl-S-propylphosphorothioate)
M1 = (O-ethyl-S-propylphosphorothioate)

**2. SUBCHRONIC TOXICITY STUDIES.** The database for subchronic toxicity is complete; no additional studies are required at this time. Principal toxicity in the subchronic dietary dog study and 21-day rabbit dermal study was inhibition of plasma, rbc, and brain cholinesterase (ChE) activity. Erythema also occurred in the 21-day rabbit dermal study. The ChE NOEL in the dietary dog study was 0.025 mg/kg/day. The ChE NOEL in the 21-day dermal rabbit study was 1 mg/kg/day. A subchronic neurotoxicity study is described in the Neurotoxicity Studies section of this document.

**82-1(b) Subchronic Dietary Toxicity Study in Dogs (1967, MRID 00075240):** In a subchronic dietary study, technical ethoprop (% a.i. unknown) was administered to groups of 3 Beagle dogs/sex/dose group in the diet at dose levels of 0, 1.0, 3.0, or 100 ppm (0, 0.025, 0.075, or 2.5 mg/kg/day). No mortality was reported nor were changes noted in hematology, necropsy, histopathology, or organ weights. The only clinical sign which may have been related to treatment was emesis, seen once in two high-dose dogs. Plasma and rbc ChE activity was reported as % of pre-treatment value, statistical significance was not reported. Brain ChE activity was not determined. Plasma ChE inhibition occurred at 0.075 and 2.5 mg/kg/day on day 2, the earliest post-treatment determination. Mean plasma ChE activity for the 0.075 mg/kg/day male group was 75% of the pre-treatment value on day 2 and was 60% on day 4. Females in the 0.075 mg/kg/day group had 94% and 72% of pre-treatment activity on days 2 and 4. Males and females at 2.5 mg/kg/day had 32% and 35% of pre-treatment values for plasma cholinesterase on day 2. At termination, male and female values for plasma cholinesterase activity were 80% and 91% of pre-treatment values at 0.025 mg/kg/day, 60% and 63% at 0.075 mg/kg/day, and 40% and 35% at 2.5 mg/kg/day. At termination, male and female values for rbc cholinesterase activity were 106% and 112% of pre-treatment values at 0.025 mg/kg/day, 100% and 82% at 0.075 mg/kg/day, and 56% and 57% at 2.5 mg/kg/day. The **NOEL** is 1 ppm (0.025 mg/kg/day) and the **LOEL** is 3.0 ppm (0.075 mg/kg/day) based on decreases in plasma cholinesterase activity. This study is **Acceptable.**

**82-2 21-day Dermal Toxicity Study in Rabbits (1989, MRID 41304404):** Ethoprop technical (95.6%) was applied to the shaved dorsal skin of groups of 10 Hra:(NZW)SPF rabbits of each sex at dose levels of 0, 0.03, 0.1, or 1 mg/kg/day, (5 days/week for 6 hr/day) for 3 weeks; controls received vehicle alone (4% carboxymethylcellulose in distilled water). The principal test compound-related effects were significant decreases, compared with controls, in the activities of plasma, erythrocyte, and brain cholinesterase in males and females dosed at 1 mg/kg/day. Cholinesterase activity for males and females in the 1 mg/kg/day group was 58% and 65% for plasma, 58% and 58% for erythrocyte, and 51% and 51% for brain when compared to respective controls. Compared with controls, other effects that may have been test compound-related in rabbits dosed at 1 mg/kg/day were significantly lower mean body weights of females at weeks 2 and 4, and lower absolute weights of the kidneys of females (significant) and males (nonsignificant) at the end of the treatment period. Incidences of erythema at

application sites were elevated, compared with controls, in high-dose animals of both sexes at day 9, and in mid- and high-dose animals of both sexes at later times of observation; the incidence of erythema was also marginally elevated in low-dose males at day 13. Mean scores for the severity of the erythema were elevated at all dose levels during the latter part of the treatment periods and, in general, were dose-related and increased with increasing time of animal exposure. There were no treatment-related effects upon mortality, clinical signs, body weight, food consumption, hematologic parameters, or gross or microscopic lesions in males or females, or body weights of males. The **NOEL** is 0.1 mg/kg/day and the **LOEL** is 1 mg/kg/day based on plasma, erythrocyte, and brain cholinesterase inhibition. On the basis of increased erythema at all doses, the 3-week repeated NOEL of dermal irritation was not established. Deficiencies included not reporting the stability, homogeneity, and concentrations of test material in dosing suspensions. Experimental animals were not healthy, 4 low-dose rabbits and 2 high-dose rabbits died or were sacrificed moribund due to illness with mucoid enteritis. The study is classified **Core Minimum**.

**82-7 Subchronic Neurotoxicity Study in Rats (1994, MRID 43424001):** This study is described in the Neurotoxicity Studies section of this chapter.

**3. NEUROTOXICITY STUDIES.** Acceptable acute and subchronic rat neurotoxicity studies and an acute delayed neurotoxicity study in hens were available. Although the hen study was negative for delayed neurotoxicity, a neurotoxic esterase study was requested by the HED RfD Committee (5/8/96) because of structure-activity concerns. This study is confirmatory in nature and should not delay the reregistration process.

Clinical signs indicative of ChE inhibition were seen as low as 25 mg/kg/day in the acute rat neurotoxicity study with a systemic NOEL of 5 mg/kg/day. The LOEL for ChE inhibition was 2.6 mg/kg/day with a NOEL of 0.26 mg/kg/day in the subchronic rat neurotoxicity study..

**81-7 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS (MRID 40609401:** In an acute delayed neurotoxicity study, no clinical or histopathological signs of neurotoxicity were seen in hens given doses causing high mortality (6.5 mg/kg initially followed by a second oral dose of 5.3 mg/kg 21-days later).

**81-8 ACUTE NEUROTOXICITY STUDY IN RATS (1994, MRID 43197701):** In an acute neurotoxicity screening study, groups of 17 male and 17 female rats received a single gavage doses of Ethoprop in corn oil (males: 5, 50, or 75 mg/kg; females: 5, 25, or 50 mg/kg). Control rats received only vehicle. Twelve rats per group were subjected to functional observation battery (FOB) tests and motor activity tests at predose, 2 hours postdose (time-to-peak effect), and at 8 and 15 days postdose. Plasma cholinesterase (ChEP) and red blood cell cholinesterase (ChER) activities were determined at predose,



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and days 2, 8, and 15 for five rats/sex/group not used for FOB tests. Neuropathology examinations of all appropriate tissues were conducted on day 15 in 6 rats/sex/group not used for cholinesterase determinations. At 25 mg/kg, two females exhibited salivation, lip smacking, ataxia, negative pupillary response and/or tremors. At 50 mg/kg in females and 75 mg/kg in males, the incidence and frequency of these signs increased and in addition, negative corneal response, negative air drop reflex, negative startle response, increased latency until first step, paralytic gait abnormalities, reduced activity, prostration and labored or gasping respiration were observed in both sexes (incidence ranged from 4-9 animals affected). Motor activity was reduced in both sexes at 50 mg/kg (-50%, males and -79%, females). A total of 6 females died on day 1 or 2. At 75 mg/kg (males only), 2 males died on day 3. Two additional deaths (a mid-dose male, day 1 and a low-dose female, day 8) were not considered treatment-related. The **neurotoxicity LOEL** is 25 mg/kg, based on transient neurobehaviorial signs in females related to cholinesterase inhibition. The **NOEL** is 5 mg/kg. In males at day 2, plasma ChE activity showed a dose-dependent inhibition at all doses (-45 to -94%; $p \leq 0.05$) and RBC activity was inhibited at 50 and 75 mg/kg. In females, plasma ChE decreased dose-dependently at day 2 (-49 to -94%; $p \leq 0.05$). At day 2, RBC ChE in females of all dose groups exhibited significant decreases (-32 to -49%; $p \leq 0.05$) although there was no dose relationship. Recovery was observed for plasma and RBC ChE although RBC values tended to be lower ($p < 0.05$, RBC ChE of high dose males at day 15). Brain ChE, measured only on day 15, was unaffected. Neuropathologic examinations revealed no remarkable findings in any of the treatment groups. The **cholinesterase LOEL** is 5 mg/kg, based on inhibition of plasma cholinesterase in both sexes and RBC cholinesterase in females. The **NOEL** is <5 mg/kg. This study is classified **Acceptable/Guideline** for an acute neurotoxicity study in rats and satisfies guideline requirements for 81-8SS.

**81-8 SPECIAL ACUTE NEUROTOXICITY STUDY IN RATS (time-course study, 1994, MRID 43442402):** In an acute gavage study groups of 24 Sprague-Dawley rats/sex (approx. 6 weeks old) received a single doses of Ethoprop (95.7%) in corn oil (males: 0, 30 or 60 mg/kg target; 0, 24.2 or 52 mg/kg actual; females: 0, 20 or 40 mg/kg target; 0, 15.7 or 33 mg/kg actual). Animals were observed twice daily for mortality and clinical signs. Plasma, red blood cell and brain (caudate/putamen, hippocampus, frontal cortex and cerebellum) cholinesterase activities were determined on days 1, 3, 8 and 15 for 6 rats/sex/group. At 40 mg/kg, several females exhibited tremors, excessive salivation and low carriage (Day 1). One 20 mg/kg female was cold to the touch. At 60 mg/kg, 1 male was sacrificed moribund on day 3. Some (1-4) males in the 60 mg/kg groups exhibited tremors of the head, limbs and body; hunched posture; yellow feces; labored and irregular breathing; rough and stained coat; red, clear, and cloudy ocular discharge; uncoordination; hypoactivity; excessive salivation; and were cold to the touch (Days 1-3). No treatment-related effects on body weight were observed. The **LOEL for clinical signs** in this study is 33 mg/kg (40 mg/kg/dose), based on cholinergic symptoms in females. The **NOEL** is 15.7 mg/kg (20 mg/kg dose). Statistically significant inhibition of plasma, RBC and brain ChE activity was observed in all treatment groups



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and was time-, tissue-and dose-dependent. In females at 20 mg/kg, inhibition of ChE relative to controls on day 1 (2-hr post-dosing) was 90% for plasma, 44% for RBC and from 50 - 72% for brain regions. In males at 30 mg/kg, inhibition of ChE relative to controls on day 1 was 83% for plasma, 43% for RBC and 45 - 48% for brain regions. At 40 mg/kg, females and 60 mg/kg, males, inhibition was slightly more pronounced for plasma and RBC and more sharply increased in brain (72 - 93% inhibition). Caudate/putamen ChE activity showed the greatest inhibition among the brain regions. Animals showed recovery from inhibition, but degree of recovery varied. By Day 15 plasma ChE levels were recovered but RBC levels remained marginally inhibited in males at 30 and 60 mg/kg (22 - 23%). In brain on Day 15, marginal but statistically significant inhibition was observed in the hippocampus in both sexes at high dose (17 - 18%) and frontal cortex ChE was marginally but significantly inhibited in males (19 - 27%). Caudate/putamen ChE activity remained slightly lower, but not significantly, in males at 60 mg/kg (32% inhibition) and females at 20 and 40 mg/kg (25% and 40%). The **cholinesterase LOEL** for this study is <15.7 mg/kg (20 mg/kg dose), based on inhibition of plasma, RBC and brain ChE in females. A **NOEL** was not determined. This acute oral cholinesterase study is classified **Acceptable/Non-guideline**. This study was not intended to fulfill a guideline requirement but was designed specifically to supplement the acute neurotoxicity screening study in rat (81-8ss) by investigating time-related effects of Ethoprop on ChE activities in plasma, red blood cells and four brain regions.

**82-7 SUBCHRONIC NEUROTOXICITY STUDY IN RATS (1994, MRID 43442401):** In a subchronic neurotoxicity study, 27 CD BR VAF/Plus rats/sex/dose were fed Ethoprop (tech., 95.7% a.i.) in the diet for 13 weeks at 0, 4, 40 or 400 ppm (0, 0.260, 2.648 or 27.113 mg/kg/day for males and 0, 0.306, 2.989 or 31.311 mg/kg/day for females, respectively). Twelve rats/sex/ dose were selected for functional observational battery (FOB) and motor activity (MA) testing, 15 animals/sex/dose for cholinesterase (ChE) analysis and 6/sex/dose were perfused for neuropathology. At 400 ppm, body weights were lowered by 13.1 to 16.2% for males and 5.7 to 8.3% for females; body weight gains were lowered by 64.2 to 24.7% for males and 44.4 to 10.2% for females. Food consumption was lowered at Week 1 by 21.6% for males and 7.9% for females. In males the following decreases were observed: hindlimb grip strength (28.4, 26.9 and 27.9% at Weeks 4, 8 and 13, respectively); analgesic reflex at Week 4 (6.8 sec less than controls) and MA (35.9, 30.0 and 26.0% at Weeks 4, 8 and 13, respectively; marginal decrease observed in females at Week 13). One male (week 4) and 1 female (week 8) showed an array of ChE-related symptoms, considered a possible marginal effect of treatment. No effects were observed at or below 40 ppm and no ethoprop-related neuropathological changes were observed. The **LOEL for systemic/neurobehaviorIal findings** is 400 ppm (27.113 mg/kg/day) based on de-creased body weight gain/food consumption, decreased hindlimb grip strength, motor activity and analgesic response time in males, and possible cholinergic signs. The **NOEL** is 40 ppm (2.648 mg/kg/day). Plasma ChE activities were decreased by 53.8 to 90.0% for males at 40 and 400 ppm and



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18.4 to 97.6% for females at 4, 40 and 400 ppm. Red blood cell ChE activities at 40 and 400 ppm were decreased by 23.4 to 40.3% in males and 19.2 to 39.9% (not significant) in females. Regional brain ChE activities were decreased by 22.6 to 82.3% at 40 and 400 ppm in males and 20.7 to 88.1% at 4, 40 and 400 ppm in females. The **LOEL for plasma and brain cholinesterase inhibition** is 4 ppm (0.306 mg/kg/day) based on decreases in females and the **LOEL for RBC ChE inhibition** is 40 ppm (2.648 mg/kg/day) based on decreases in both sexes. A **NOEL** for plasma and brain ChE was not determined and the **NOEL for RBC ChE inhibition** is 4 ppm. This subchronic neurotoxicity study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral study (82-7ss) in rats.

4. CHRONIC TOXICITY STUDIES. The database for chronic testing is complete; no additional studies are required at this time. Three chronic/carcinogenicity studies in rats were available. One study was acceptable while 2 studies were unacceptable, but upgradeable. In the rat studies, the NOEL for ChE inhibition was 0.04 mg/kg/day. Systemic effects included decreased weight gain, food consumption, and anemia with a systemic NOEL of 2.44 mg/kg/day.

Malignant adrenal pheochromocytomas were increased in the 1992 chronic rat study. Thyroid C-cell and/or parafollicular cell tumors were increased in all 3 rat studies. (See the Carcinogenic Classification section in the Dose Response Assessment section of this document.) Endometrial polyps were increased at the high dose in 2 studies.

The chronic dog study was followed up by a 5-month study to determine a NOEL for plasma ChE inhibition. Systemic effects at 1.0 mg/kg/day included anemia and evidence of liver injury with a systemic NOEL of 0.025 mg/kg/day. ChE inhibition occurred at 0.025 mg/kg/day and the ChE NOEL was 0.010 mg/kg/day.

**83-1(a) Combined Chronic Feeding/Carcinogenicity Study in Rats (1992, MRID 42530201):** In a combined chronic feeding/carcinogenicity study, ethoprop (95.6%) was administered to Crl:CD rats, 80/sex/dose, at dose levels of 0, 1, 60, or 600 ppm for 105 weeks. Doses corresponded to 0, 0.04, 2.44, or 18.38 mg/kg/day in males and 0, 0.06, 3.56, or 23.98 mg/kg/day in females. Interim sacrifice was conducted at 52 weeks with 10 rats/sex/dose. An additional 10/sex in control and high-dose groups were treated for 52 weeks and sacrificed after a 4-week recovery period. The high-dose group had received 600 ppm for the first 2 weeks of the study. This dose was reduced to 400 ppm after 2 weeks due to toxicity in females (weight gain depression, tremors, ataxia, and 2 mortalities). The **systemic toxicity NOEL** for both males and females was 60 ppm (2.44 mg/kg/day in males and 3.56 mg/kg/day in females) and the **LOEL** for both males and females was 400 ppm (18.38 mg/kg/day in males and 23.98 mg/kg/day in females), based on reduced body weight gain, reduced food consumption, reduced erythrocyte count, reduced hemoglobin, and reduced hematocrit. The **NOEL for plasma, red blood cell**



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**and brain cholinesterase inhibition** in both males and females was 1 ppm (0.04 mg/kg/day in males and 0.06 mg/kg/day in females). The **LOEL** for plasma, red blood cell and brain cholinesterase inhibition in both males and females was 60 ppm (2 44 mg/kg/day in males and 3.56 mg/kg/day in females). **Adrenal gland malignant pheochromocytomas** were increased in males (0/41, 2/16, 2/18, 5/60 in their respective dose groups). **Thyroid C-cell carcinomas** were increased slightly in males (0/61, 0/63, 1/64, 3/66 in their respective dose groups). This chronic toxicity study in the rat is **acceptable**, and satisfies the guideline requirement for a chronic oral study (83-1(a).

**83-1(a) Combined Chronic Feeding/Carcinogenicity Study in Rats (1985, MRID 40291801):** In a combined chronic feeding/carcinogenicity study (MRID 40291801) ethoprop (95%) was administered to F344 rats, 60/sex/dose, at dose levels of 0, 1, 10, or 100 ppm in the diet for 24 months. Doses corresponded to 0, 0.041, 0.40, or 4.19 mg/kg/day in males and 0, 0.052, 0.51, or 5.12 mg/kg/day in females. Interim sacrifices were conducted at 12 and 18 months with 10 rats/sex/dose in each interim sacrifice. The **systemic toxicity NOEL** for both males and females was ≥100 ppm (4.19 mg/kg/day in males and 5.12 mg/kg/day in females), the **highest dose level tested** in this study. The **NOEL for plasma and red blood cell cholinesterase inhibition** in both males and females was 1 ppm (0.041 mg/kg/day in males and 0.052 mg/kg/day in females). The **LOEL** for plasma and red blood cell cholinesterase inhibition in both males and females was 10 ppm (0.40 mg/kg/day in males and 0.51 mg/kg/day in females). The NOEL for brain cholinesterase inhibition in both males and females was 10 ppm (0.40 mg/kg/day in males and 0.51 mg/kg/day in females) with a LOEL of 100 ppm (4.19 mg/kg/day in males and 5.12 mg/kg/day in females). **Thyroid C-cell adenomas** were increased in high-dose males (8/49, 5/48, 5/50, 12/50 in the respective dose groups). Thyroid C-cell **carcinomas** were also increased slightly in high-dose males (0/49, 0/48, 1/50, 3/50 in the respective dose groups). Tumor incidence in females was comparable between controls and treated rats. This study was classified **supplementary, upgradeable.**

**83-1(a) Combined Chronic Feeding/Carcinogenicity Study in Rats by Feeding and Lactational Exposure (1983, MRID 00138636):** Test animals (F1 generation) were exposed to ethoprop ***in utero*, during lactation, and then by feeding.** This was accomplished by administering technical ethoprop (95.3%) in the diet to parental F344 rats (F0 generation) at dietary concentrations of 0, 60.5, 131, or 262 ppm. After weaning, 60 F1 pups/sex/group were fed diets containing 0, 4.5, 9.0, or 18 ppm for 12 weeks and then placed on diets containing 0, 49, 98, or 196 ppm of ethoprop. Ten of the 60 F1 rats/group/sex were sacrificed at 52 weeks. Male rats had increasing trends for thyroid C-cell adenomas (2/46, 4/43, 1/41, 10/40; $p<0.01$) as well for the pair wise comparison of the 196 ppm group with controls ($p<0.01$). Female rats had increasing trends for uterine endometrial polyps (0/44, 4/45, 8/37, 13/42; $p<0.01$) as well as for pair wise comparison of mid- and high-dose rats compared to controls ($p<0.01$). Combined endometrial and stromal polyps showed increasing trends (8/44, 6/45, 10/37, 16/42; $p<0.01$) as well as by pair-wise comparison of the high-dose group with controls ($p<0.05$). This study was

classified **Supplementary** because no NOEL was determined and the number of tissues examined microscopically was not evident to the reviewer. Other data requested included more data on analytical testing of diet, information on statistical methods used, and breeding and litter data. This study was **not evaluated by the RfD Committee** when it evaluated the ethoprop database on 5/9/96. The RfD Committee considered this study superseded by the 1992 toxicity study in rats (MRID 42530201). This study was evaluated by the Cancer Peer Review Committee on 6/25/97 and 8/20/97.

**83-1(b) Chronic Gavage Study in Dogs (1986, MRID 00160179 and 1990, MRID 41498601):**

In a 1-year capsule study in dogs (1986, MRID 00160179), groups of 4 female Beagle dogs per sex received capsules of ethoprop (96%) in peanut oil at doses of 0, 0.025, 1.0, or 10 mg/kg/day. Systemic effects at 1.0 mg/kg/day and above included decreases in red blood cell parameters in males and females and elevations in SGPT in males. At 10 mg/kg/day serum alkaline phosphatase was elevated in males, pathological changes occurred in the livers of males and females, and one treatment-related death occurred in one male. Plasma ChE was inhibited in all female treatment groups and in males at 1.0 and 10 mg/kg/day. Red blood cell ChE was inhibited in females at 1.0 and 10 mg/kg/day and in males at 10 mg/kg/day.

A 5-month study in dogs was later conducted to find a NOEL for plasma ChE inhibition. In the 5-month capsule study in dogs (1990, MRID 41498601), groups of 6 Beagle dogs per sex received capsules of ethoprop (95.6%) in corn oil at doses of 0, 0.01, 0.025, or 1.0 mg/kg/day. In this study, there were no systemic effects attributed to treatment. Plasma, rbc, and brain ChE were inhibited at 0.025, 1.0, and 10.0 mg/kg/day, respectively, for both males and females.

The **NOEL for systemic toxicity** was 0.025 mg/kg/day and the **LOEL** for systemic toxicity was 1.0 mg/kg/day based on decreases in red blood cell parameters in males and females and elevations in SGPT in males. The **combined NOEL values for plasma, red blood cell and brain cholinesterase inhibition** were 0.010, 0.025 and 1.0 mg/kg/day, respectively, and **combined LOEL values** were 0.025, 1.0, and 10.0 mg/kg/day, respectively, for both males and females. Together these 2 studies are classified **Acceptable/Guideline** and satisfy the requirement for a chronic toxicity study in the dog.

**5. CARCINOGENICITY STUDIES.** The database for carcinogenicity testing is complete; no additional studies are required at this time. The chronic/carcinogenic studies are described in the Chronic Toxicity section of this document. Also, see the Carcinogenic Classification section in the Dose Response Assessment section of this document.

**83-1(a) Carcinogenicity Studies in Rats (MRID 42530201, 40291801, 00138636):** These studies are described in the Chronic Toxicity section above.

**83-1(b) Carcinogenicity Study in Mice (1984, MRID 40356301 and 43326001):** Ethoprop technical (>99% purity) was administered in the diet to 50 B6C3F1 mice per sex per group for 104 weeks at 0, 0.2, 2.0, or 30 ppm (Males: 0, 0.026, 0.254, or 3.96 mg/kg/day; Females: 0, 0.032, 0.318, or 4.9 mg/kg/day) in a carcinogenicity study (83-2b, MRID 43326001 & 40356301). Additional animals (30/sex/group) were added for 3 interim sacrifices (10 mice per sex) at weeks 26, 52, and 78. Survival was unaffected at any dose level. Body weight was reduced compared to controls in males (4-6%, $p \leq 0.05$) and females (6-8%, generally statistically significant to week 36) at the 30 ppm dose level during the first year of the study. Body weight gain was also statistically significantly decreased weeks 0 to 26 (males 13% below control and females 15% below control) but not week 26 to week 104 at the 30 ppm dose level. The slightly reduced body weight was accompanied by reduced efficiency of food utilization the first year of the study, but neither body weight nor food efficiency was reduced the second year of the study. Plasma and erythrocyte cholinesterase activities were inhibited in a dose-dependent fashion at 2 and 30 ppm for both males and females at weeks 26, 52, 78, and 104. Most of the decreases were statistically significant relative to controls. At 2 ppm, plasma cholinesterase inhibition ranged from 10% to 24% (males and females) and ranged from 64-77% (males and females) at 30 ppm. A similar pattern was seen at 2 and 30 ppm for erythrocyte cholinesterase inhibition. Brain cholinesterase was clearly inhibited and statistically significant at 30 ppm only in both sexes at week 26 and 104. Inhibition ranged from 17% to 36%. A dose related response and statistically significant increase was noted in kidney basophilic change (in older reports referred to as regenerating epithelium) in males (29% at 0.2 ppm and 57% at 30 ppm) at ≥ 0.2 ppm in males and calcium deposits in males (31%) and females (16%) at 30 ppm. However, both the kidney basophilia and the calcium deposits in males were close to the historical control mean of 26% and within historical control range (0% to 72%) for kidney calcification and 49% (0% to 87%) for basophilic changes and the apparent dose relationship may been due to the low control values (4% and 1%, respectively) or a real test material effect. In females, significant kidney calcium deposits were seen only at 30 ppm (16%) (historical control mean of 4.2% and range of 0% to 17%). Brain calcium deposits were statistically significantly increased in males at 30 ppm (62%) (historical control mean of 39% and range 0% to 78%). In females, hyaline bodies in the brain were statistically significantly increased at 30 ppm (53%) (historical control mean of 42% and range of 0% to 97%). **No statistically significant dose related incidence of tumors were seen in males or females.** All nominally elevated tumor incidence was within historical control range, except liver carcinoma that was nominally increased at 30 ppm in females 6/43 (14%) vs. controls at 2/38 (5.3%). Historical control data indicated a mean of 2.5% (range of 0% to 10% for females. For the tumor data in the historical control, the mean and range are based on 50 animals/experiment (page 325 and 348 of MRID 43326001). The **cholinesterase NOEL/LOEL in males** were 0.026/0.254 mg/kg/day and in females were



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0.032/0.318 mg/kg/day based on decreased plasma and erythrocyte cholinesterase activity. The **systemic NOEL/LOEL in males** were 0.254/3.96 mg/kg/day and in females were 0.318/4.91 mg/kg/day based on body weight and body weigh gain decreases. Brain cholinesterase activity was statistically significantly decreased in both sexes at the high dose. The doses were adequate to test for the carcinogenicity of ethoprop. The carcinogenicity study report (MRID 403356301 and 43326001) is **acceptable** for a Guideline 83-2b carcinogenicity study in mice.

7. DEVELOPMENTAL TOXICITY STUDIES. The database for developmental toxicity testing is complete; no additional studies are required at this time. No developmental toxicity occurred in either the rat or rabbit developmental studies.

**83-3(a) Developmental Toxicity in Rats (1989, MRID 41304402):** In a rat developmental toxicity study (1989, MRID 41304402), groups of 25 female SD rats received doses of 0, 2, 9, or 18 mg/kg/day ethoprop (95.6%) by gavage in corn oil on gestation days 6-15. The **maternal NOEL** was 2 mg/kg/day and the **maternal LOEL** was 9 mg/kg/day based on decreased body weight gain and increased incidence of soft stool. The **developmental toxicity NOEL** was ≥ 18 mg/kg/day, the **highest dose tested.** This study was **acceptable.**

**83-3(b) Developmental Toxicity in Rabbits (1989, MRID 41304403):** In a rabbit developmental toxicity study (1989, MRID 41304403), groups of 20 NZW rabbits received doses of 0, 0.625, 1.25, or 2.5 mg/kg/day ethoprop (95.6%) in corn oil on gestation days 6-18. **Both the maternal and developmental NOELs were ≥ 2.5 mg/kg/day, the highest dose tested.** Although no maternal or developmental toxicity occurred in this study, dosing was considered adequate because the highest dose was close to a lethal dose. This study was classified **acceptable.**

8. REPRODUCTIVE TOXICITY STUDIES. The database for reproductive toxicity testing is complete; no additional studies are required at this time. No increased sensitivity of offspring compared to parents was noted in this study.

**83-4 Reproductive Toxicity in Rats (1991, MRID 41921201):** In a 2-generation reproduction study, groups of 28 male and 28 female Crl:Cd BR rats received dietary doses of 0, 1, 30, or 300/150 ppm ethoprop (95.3%). Doses were equivalent to 0, 0.08, 2.3, and 24/13 mg/kg/day. The high dose of 300 ppm was reduced to 150 ppm due to excess mortality in the F1a litter. Systemic **parental toxicity** at 150 ppm was limited to body weight decrements; at 300 ppm there were also tremors and loose stools. The **parental NOEL for systemic toxicity** is 30 ppm (2.3 mg/kg/day) and the parental **LOEL** for systemic toxicity is 150 ppm (13 mg/kg/day). Cholinesterase activity was determined in adults at termination. The **NOEL values for parental plasma and brain**



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**cholinesterase inhibition** were 1 ppm (0.08 mg/kg/day); **LOEL** values for plasma and brain were 30 ppm (2.3 mg/kg/day). The **NOEL for red blood cell cholinesterase inhibition** was ≥ 13 mg/kg/day, the highest dose tested. **Offspring toxicity** in both generations included pup body weight decrements after gestation day 4 at 150 ppm (13 mg/kg/day) and 300 ppm (24 mg/kg/day). The high dose of 300 ppm was reduced to 150 ppm after 19 weeks due to increased pup mortality in the 300 ppm group between days 21 and 28 postpartum. Although increased pup mortality occurred at a dietary concentration which only caused clinical signs of toxicity in parents, this was not an indication of increased sensitivity because the pups were receiving a greater dosage of ethoprop than parents: during the period of mortality between days 21 and 28 post partum young rats consume approximately twice the diet per unit body weight as an adult rat consumes, and during that time period the pups were receiving lactational as well as dietary exposure (Hazard ID document dated 11/10/97.) No reproductive toxicity was noted. The **NOEL for offspring toxicity** was 30 ppm and the **LOEL** for offspring toxicity was 150 ppm (13 mg/kg/day). This study is **acceptable.**

**9. MUTAGENICITY STUDIES.** Both the pre-1991 and the current mutagenicity initial testing battery guidelines are satisfied. No additional studies are required at this time. Ethoprop is an *in vitro* clastogen with metabolic activation required for genotoxicity. Due to severe toxicity, it could not be determined whether ethoprop is an *in vivo* clastogen. Additional mutagenicity testing is not required due to the limitations posed by toxicity. Results are in Table 3.

**TABLE 3. ACCEPTABLE MUTAGENICITY STUDIES**

| MUTA STUDY | RESULTS |
|---|---|
| Salmonella typhimurium reverse gene mutation assay (MRID 00160180) | NEGATIVE |
| Chinese hamster ovary (CHO) cell HGPRT gene mutation assay (MRID 00160181) | NEGATIVE |
| Mouse lymphoma L5178Y forward gene mutation assay (MRID 44065001) | NEGATIVE |
| In vitro CHO cell chromosome aberration assay (MRID 00160183) | POSITIVE only with S9 activation. |
| In vivo bone marrow cytogenetic assay (MRID 41211202) | NEGATIVE in SD rats. No apparent interaction with target tissue; severe toxicity at highest dose. |



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|---|---|
| Rat dominant lethal assay (MRID 40386901) | NEGATIVE in SD rats. No apparent interaction with target tissue; severe toxicity at highest dose. |
| In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID 00160182) | NEGATIVE |
| In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID 44038702) | NEGATIVE |
| In vitro CHO cell sister chromatid exchange assay (MRID 00160184) | POSITIVE only with S9 activation. |

**10. DERMAL ABSORPTION.** No *in vivo* dermal absorption study with ethoprop is available. For purposes of risk assessment, 100% dermal absorption will be assumed.

**11. METABOLISM.** The database for metabolism testing is complete; no additional studies are required at this time. Conclusions of the Health Effects Division Metabolism Assessment Review Committee (1/27/98) are in the next section of this chapter.

**85-1 METABOLISM STUDY IN RATS (1990, MRID 41804301):** In a metabolism study, ethoprop was administered to Crl:CD(SD)BR rats as a single IV bolus (males and females); single oral bolus (females, metabolism and pharmacokinetic studies; males, metabolism only); or by multiple oral doses. Following oral administration, ethoprop was completely absorbed and completely metabolized. Excretion was by urinary (≥50% administered dose), fecal (7-16%), and respiratory (11-19%) routes and was essentially complete by 48 hours. Terminal elimination $t_{1/2}$ in blood was 92-135 hours. Metabolism was by dealkylation of one or both S-propyl groups, followed by hydroxylation and probably conjugation. Two urinary metabolites were identified by HPLC while 3 others were believed to be possible conjugates of those metabolites. The TLC profiles of fecal metabolites were similar to the profiles for urinary metabolites. This study is **acceptable**.

**12. METABOLISM COMMITTEE CONCLUSIONS.** The Health Effects Division Metabolism Assessment Review Committee met on January 27, 1998 to determine residues of concern for risk assessment purposes.

Interim results from an acute study (MRID 44472501, Covance 6224-246) with ethoprop and metabolites were reviewed by the HED Metabolism Committee. Although

cholinesterase determinations for the M1 metabolite of ethoprop were not yet completed, the Metabolism Committee was able to determine residues of concern based upon interim toxicity testing results. (See Acute Toxicity section of this chapter.) Compounds evaluated by the Metabolism Committee were:

SME (O-ethyl-S-methyl-S-propylphosphorodithioate)
OME (O-ethyl-O-methyl-S-propylphosphorothioate)
M1 (O-ethyl-S-propylphosphorothioate)
S,S-dipropylphosphorodithioate

The Metabolism Committee's conclusions were:

For acute and chronic dietary non-cancer risk assessments, the residues of concern in crops are ethoprop, SME, and OME.

For water non-cancer risk assessments, the residues of concern are also ethoprop, SME, and OME.

For cancer risk assessments, the residues of concern in crops are ethoprop, SME, OME, and M1.

For cancer risk assessments, the residues of concern in water are ethoprop, SME, OME, M1, and S,S-dipropylphosphorodithioate.

**B. DOSE RESPONSE ASSESSMENT. An RfD value for ethoprop was selected by the HED Reference Dose Peer Review Committee on 5/9/96. Endpoints for acute dietary exposure and occupational/residential exposure (short-, intermediate-, and long-term exposure by dermal or inhalation routes) were selected by the HED Toxicology Endpoint Selection Committee on 5/21/96. The HED Hazard Identification Assessment Review Committee evaluated the reproductive, developmental, and neurotoxicity data for ethoprop to address the sensitivity of infants and children on 11/4/97.**

**1. SENSITIVITY OF INFANTS AND CHILDREN.** The Hazard Identification Committee (11/4/97) evaluated the toxicology data base of Ethoprop with special reference to the reproductive, developmental and neurotoxicity data. These data were re-reviewed specifically to address the sensitivity of infants and children from exposure to Ethoprop as required by the Food Quality Protecting Act (FQPA) of 1996.

**a. 10X Factor for Protection of Infants and Children.** For **acute and chronic dietary risk assessments**, the HED Hazard ID Committee determined that the



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**10x factor** to account for enhanced sensitivity of infants and children **(as required by FQPA) should be removed because a Margin of Exposure of 100 is adequate** to ensure protection of this population from acute dietary exposure to Ethoprop.

The Committee based its decision upon the following reasons: (a) No increased sensitivity of fetuses as compared to maternal animals following *in utero* exposure in developmental toxicity studies. (b) No increased sensitivity of pups as compared to adults in a multigeneration reproduction study. (c) There are no data gaps. (See the Hazard Identification Assessment Review Committee Report, dated 11/10/97.)

**b. Developmental Neurotoxicity.** Based upon a weight-of-the-evidence consideration of the data base, the HED Hazard ID Committee determined that a **developmental neurotoxicity study** in rats is **not required**. There were sufficient data available to adequately assess the potential for toxicity to young animals following pre-and/or post-natal exposure to Ethoprop including acceptable developmental toxicity studies in rats and rabbits as well as a 2-generation reproduction study in rats. In addition, no treatment-related neuropathology was seen in studies conducted in hen or rats.

**2. REFERENCE DOSE (RfD).** Using a weight-of-the evidence approach, the HED RfD Committee (5/9/96) assigned an **RfD of 0.0001 mg/kg/day** from a **NOEL of 0.01 mg/kg/day** in the combined chronic and 5-month toxicity studies in dogs (MRID 00160179 and 41498601); the LOEL was 0.025 mg/kg/day based on plasma cholinesterase inhibition. An **uncertainty factor of 100** was applied to account for inter-species extrapolation and intra-species variability.

The HED Hazard ID Committee (11/4/97) determined that the **10x factor** to account for enhanced sensitivity of infants and children **(as required by FQPA) should be removed. An uncertainty factor of 100 is adequate** to ensure protection of this population from chronic exposure to Ethoprop (See section on 10X Factor for protection of infants and children, above).

The NOEL used for the RfD was also supported by a rat chronic toxicity study (MRID 42530201) in which brain and red blood cell cholinesterase activities were inhibited at dose levels comparable to those causing plasma cholinesterase inhibition in dogs. In this rat study, the NOELs for plasma, red blood cell, and brain cholinesterase inhibition were 0.04 mg/kg/day with a LOEL of 2.44 and 3.56 mg/kg/day.

The FAO/WHO Joint Committee Meeting on Pesticide Residues assigned an acceptable daily intake of 0.0003 mg/kg/day for ethoprop in 1987.



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## 3. OTHER TOXICOLOGICAL ENDPOINTS.

TABLE 4. TOXICOLOGICAL ENDPOINTS

| EXPOSURE SCENARIO | NOEL (mg/kg/day) | ENDPOINT | STUDY MRID # | SAFETY FACTOR |
|---|---|---|---|---|
| ACUTE DIETARY | 0.025 | Plasma ChE Inhibition | Subchronic Dog 00075240 | 100 |
| SHORT-TERM DERMAL | 0.1 | Plasma, RBC, Brain ChE inhibition | 21-day Rabbit 41304404 | 100 |
| INTERMEDIATE-TERM DERMAL | 0.1 | Plasma, RBC, Brain ChE inhibition | 21-day Rabbit 41304404 | 100 |
| CHRONIC DERMAL | 0.1 | Plasma, RBC, Brain ChE inhibition | 21-day Rabbit 41304404 | 100 |

**7. INHALATION EXPOSURE (any time period).** Except for an acute inhalation study ($LC_{50}$ = 0.123 mg/L; Tox. Cat. II), no other inhalation studies with technical material are available. Therefore, for this risk assessment, the inhalation and dermal components should be added together in the calculation of the mixer/loader/applicator estimate of exposure. The per cent absorption should be 100% for inhalation (default value).

**8. CARCINOGENIC CLASSIFICATION:** The HED Cancer Assessment Peer Review Committee (10/2/97 document) classified ethoprop as a **"likely"** human carcinogen based on the following factors:

(a) presence of a rare and life-threatening (malignant) tumor (pheochromocytoma of the adrenal glands) in male Sprague-Dawley rats at the low dose in the absence of cholinesterase inhibition;

(b) occurrence of another type of tumor (C-cell carcinomas of the thyroid glands) in male rats in two strains (Sprague-Dawley and Fischer 344) in three different studies at doses that did cause cholinesterase inhibition; and

(c) evidence of clastogenicity *in vitro* mutagenicity testing.

The Committee recommended a linear low-dose approach for human risk characterization and extrapolation of risk should be based on the occurrence of malignant



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pheochromocytomas of the adrenal glands in male rats at all dose levels tested. This extrapolation is supported by: (I) lack of mode of action, (ii) evidence from the total data base [i.e., occurrence of other tumor types (C-cell carcinomas of the thyroid glands) at doses that caused cholinesterase inhibition], and (iii) confirmation of clastogenic activity in mutagenicity testing.

The $\mathbf{Q_1}$* for ethoprop, in the absence of a complete tumor count in low- and mid-dose groups, is calculated to be **$2.81 \times 10^{-2}$ mg/kg/day** (Hugh M. Pettigrew memo, 1/15/98).

The HED Cancer Assessment Peer Review Committee reconvened on 4/1/98 to re-assess the carcinogenicity of ethoprop. The registrant submitted new historical control data on adrenal pheochromocytomas in rats and also argued against the carcinogenic classification of ethoprop, principally because of low survival in controls and a new statistical analysis of the critical study. The Committee concluded that the carcinogenic classification of ethoprop should not be changed because the carcinogenicity could not be fully evaluated until all adrenals in the 1992 rat study (MRID 42530201) had been examined. (The report for this meeting is still in progress at the time of writing the toxicological chapter.)



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