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WASHINGTON, D.C. 20460

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

013048

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicrotophos - Review of an Acute Neurotoxicity Study in Rats (MRID 43759801), a Subchronic Neurotoxicity Study in Rats (MRID 43980201), a Chronic Dog Study (MRID 44328401) and a Reproduction study in Rats (MRID 44296101)

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TO: Jess Rowland, Branch Chief
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THRU: Stephen C. Dapson, Ph.D., Branch Senior Scientist
Toxicology Branch II
Health Effects Division (7509C) Stephen C. Dapson 12/16/98

CC: Arnold Layne/Stephanie Willett, PM Team #51
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CONCLUSIONS: The acute neurotoxicity study in rats (MRID 43759801), subchronic neurotoxicity study in rats (MRID43980201), chronic toxicity study in dogs (MRID 44328401) and reproduction study in rats (MRID 44296101) are Acceptable (guideline) and satisfy the requirements for guideline series §81-8 acute neurotoxicity study in rats, §82-7 subchronic toxicity study in rats, §83-1 chronic toxicity study in dogs and §83-4 reproduction study in rats.

ACTION REQUESTED: SRRD has requested that TOX II review an acute neurotoxicity study in rats, a subchronic neurotoxicity study in rats, a chronic toxicity study in dogs and a reproduction study in rats.

DISCUSSION: The results of the studies are listed below:

CITATION: Rattray N.J. (2/22/95) Dicrotophos: Acute Neurotoxicity Study in Rats. Zeneca Central Toxicity Laboratory. Alderley Park, Macclesfield, Cheshire, UK. Study No: AR5795, February 22, 1995 (MRID 43759801). Unpublished

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 43759801) dicrotophos (87.65% ai) was administered by gavage to 10/sex/group Sprague-Dawley CD rats (20/sex/group) at dose levels of 0, 0.5, 5 or 10 mg/kg (in water, 10 ml/kg). The rats were evaluated for reactions in functional observations and motor activity measurements (3 hours, and 8 and 15 days postdosing). Two satellite groups (5/sex/group) were also included for the purpose of measuring plasma ChE and erythrocyte and brain AChE on days 1 and 8.

At 0.5 mg/kg, dicrotophos inhibited brain (21% in ♀ and 22% in ♂) and erythrocyte AChE (16% in ♀ and 19% in ♂) and plasma ChE (46% in ♀ and 38% in ♂) on day 1 (all depressions were statistically significant). Brain AChE and plasma ChE reached about 90% inhibition at 10 mg/kg. RBC AChE never exceeded 50% inhibition. Brain AChE remained inhibited (7%-14%) for males at all doses by day 16. **The NOEL and LOEL for ChE/AChE inhibition is < 0.5 mg/kg.**

At 5 mg/kg and above, dicrotophos produced clinical signs (principally decreased activity, upward curvature of spine, pinched in sides, flaccid appearance and decreased pupil response in most or all animals and several other signs in some animals. Overall some 25 in males and 24 in females FOB parameters were reported affected by treatment in the 5 and/or 10 mg/kg dose levels of dicrotophos. Motor activity was decreased. At 10 mg dicrotophos/kg produced deaths (7 ♀ and 1 ♂) within 3 hours after dosing. Most signs regressed after one day but some persisted for 3 or 4 days. **The LOEL for neurotoxicity is 5 mg/kg based on clinical signs. The NOEL is 0.5 mg/kg.**

This acute neurotoxicity study is classified Acceptable-Guideline and satisfies the guideline requirement for an acute neurotoxicity study (81-8)in rats.

CITATION: Horner S. (1995) Dicrotophos: Subchronic Neurotoxicity Study in Rats. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory Report No. CTL/P/4692, Study No. PR0997, November 6, 1995. MRID 43980201. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity feeding study (MRID 43980201), 12 Alpk:APfSD rats/sex/group were fed diets containing 0, 0.5, 5, or 25 ppm dicrotophos (87.65% w/w, Batch No. 403001B) for 13 weeks. Two satellite groups, each with 6 Alpk:APfSD rats/sex/dose, were similarly treated and included for the purpose of measuring cholinesterase activity (ChE) at week 5 (Group A) and week 9 (Group B). The average consumption of test material in the 0, 0.5, 5, and 25 ppm dose groups for males was 0, 0.04, 0.39, and 2.03 mg/kg/day and for females was 0, 0.04, 0.45, and 2.38 mg/kg/day.

There was no treatment-related effect on mortality. Effects on body weights were minimal: weights were statistically significantly lower than controls at the high dose (≤ 10.4%, $p \leq 0.05$ or 0.01) during weeks 2-6 in males and 2-4 in females, and were correlated with decreased food consumption during weeks 1 and 2 (and decreased food efficiency in males). No notable changes were seen in behavior or appearance upon clinical examination. Treatment-related effects on motor activity were seen only at 25 ppm in both sexes: forelimb grip strength was decreased at weeks 9 and 14 in females (13-14%, $p \leq 0.05$ or 0.01) and at week 5 in males (14%, $p \leq 0.05$). Hindlimb grip strength was decreased only in females at week 9 (16%, $p \leq 0.05$). The mean overall motor activity was decreased at week 9 in males (34%, $p \leq 0.01$) and at weeks 9 and 14 in females (23 and 33%, respectively; $p \leq 0.05$; $p \leq 0.01$). The neurotoxic effects, as well as the statistically significant increase in brain weight in 25 ppm males (≤ 5.2%), had no histopathological correlates. **Based on the motor activity alterations and marginal body weight decreases in both sexes of rats, 25 ppm is identified as the LOEL and 5 ppm is the NOEL for systemic toxicity/neurotoxicity.**

Brain, plasma, and erythrocyte ChE activities were inhibited by dicrotophos treatment in all groups of rats, most markedly in the brain: 11-20% at 0.5 ppm, 56-63% at 5 ppm, and 87-90% at 25 ppm ($p \leq 0.01$). ChE was inhibited similarly among the sexes except plasma ChE was inhibited slightly more in females. **Based on the dose-related inhibition in brain, plasma, and erythrocyte ChE activity, 0.5 ppm is the LOEL for ChE inhibition; a NOEL cannot be assigned because 0.5 ppm was the lowest dose tested.**

This study is classified as Acceptable-Guideline, satisfying the guideline requirement for a subchronic oral neurotoxicity feeding study (82-7) in the rat.

CITATION: Horner S. (1997) Dicrotophos: 1 year oral toxicity study in dogs. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory Report No. CTL/P/5103, Study No. PD1008, June 27, 1997. MRID 44328401. Unpublished.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 44328401), dicrotophos (87.65% w/w, Batch No. 403001 B) in corn oil was administered to 4 beagle dogs/sex/dose by capsule at doses of 0

(corn oil only), 0.025, 0.1, or 1.0/0.5 mg/kg/day for 1 year (high-dose dogs received 1.0 mg dicrotophos/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day from week 15-52).

No animals died during the study. Treatment-related clinical signs were seen primarily at the high dose before it was lowered to 0.5 mg/kg/day, and were correlated with the inhibition of plasma and erythrocyte cholinesterase (ChE) activity. Signs included shaking and subdued behavior (2/4 or more dogs/sex during weeks 13-14 and in one female during week 24); unsteady gait (one female at week 13) and a slight increase in the incidence/frequency of fluid feces, regurgitation, and vomiting (both sexes). High-dose dogs also resisted dosing and males had reddening and peeling of the scrotal skin with sores primarily during the first 14 study weeks.

Weekly body weights of high-dose males were lower than those of controls ($\leq$ 8.4%; $p < 0.05$) several times between weeks 5 and 14, and their week 1-14 body weight gain was 48% of controls. After the high dose was lowered, the body weights improved and their overall weight gain was greater than of the controls. Weekly body weights of mid- and high-dose females were up to 10.3% lower than controls throughout the study ($p < 0.05$ for weeks 6, 7, 9, 14, 47, and 49-53 at one or both doses), and their overall weight gain and food efficiency were 74-78% of controls. There were no toxicologically significant differences from the controls for hematology, clinical chemistry, urinalysis, or ophthalmoscopic parameters, organ weights, or gross and microscopic pathology. No neoplastic lesions were reported.

Based on the 26% lower overall (week 1-52) body weight gain (and food efficiency) in mid-dose females, 0.1 mg/kg/day is the LOEL under the conditions of this study; the corresponding NOEL is 0.025 mg/kg/day. The reviewer disagrees with the study author that the body weight changes at the mid-dose were not toxicologically significant and that the high dose is the LOEL.

The plasma, RBC, and brain ChE activities were inhibited by dicrotophos treatment throughout most of the study in both sexes. The degree of inhibition was clearly dependent on the dose but not on the exposure duration. At the high dose, the plasma, RBC, and brain ChE values were 36-64% of controls ($p \leq 0.01$) and neurologic impairment was clinically evident. At the mid-dose, plasma ChE activity was 55-61% of controls ($p \leq 0.01$) in both sexes, RBC ChE activity was 83-91% of controls ($p \leq 0.05$ or 0.01 for males only), and brain ChE was 81-88% of controls ($p \leq 0.05$ for females only). Although there were no visible neurological effects at the mid-dose, **because the parameter most germane to neurotoxicity, i.e. brain ChE, was statistically significantly inhibited in females (19%, $p \leq 0.05$), and plasma ChE was substantially inhibited in both sexes, the LOEL for ChE inhibition is 0.1 mg/kg/day, and the NOEL is 0.025 mg/kg/day.** The reviewer disagrees with the study author that the high dose is the LOEL for ChE inhibition.

This study is classified as Acceptable-Guideline and satisfies the guideline requirement for a chronic oral toxicity study (83-1b) in the dog.

CITATION: Moxon, M.E. (1997) Dicrotophos: Multigeneration study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No. CTL/P/5129, Study No. RR0689. EPA MRID Number 44296101. Unpublished.

EXECUTIVE SUMMARY: In a multigeneration reproduction study (MRID # 44296101), Dicrotophos (87.65% a.i.; Batch No. 403001 B) was administered to groups of 26 male and 26 female Alpk:AP$_f$SD rats (from the Rodent Breeding Unit, Alderley Park) in the diet at concentrations of 0, 0.5, 5.0, or 10/15/25 ppm for two generations. Two litters were produced in the first generation and one litter was produced in the second generation. Premating doses for the adult F_0 males were 0.5, 0.49, and 2.53 mg/kg/day and for the F_0 females were 0.5, 0.53, and 2.79 mg/kg/day, respectively. Premating doses for the adult F_1 males were 0.5, 0.56, and 1.15 mg/kg/day and for the F_1 females were 0.6, 0.59, and 1.25 mg/kg/day, respectively. **Due to a high mortality in the F_{1a} pups in the 25 ppm group, the dietary concentration of dicrotophos was lowered to 10 ppm for four dams from lactation day 8 through termination of the litter.** During mating, gestation, and lactation of the F_{1b} litters, high-dose animals were given diets containing 15 ppm. The control, low-, and mid-dose F_1 pups were weaned onto the same diets as their parents. The F_1 pups in the high dose group were weaned onto diets containing 10 ppm dicrotophos. Animals were given test or control diet for 10 weeks then mated within the same dose group. All animals were exposed to test material either in the diet or during lactation until sacrifice.

Clinical signs of toxicity were observed during premating weeks 2-5 as involuntary shaking of the limbs in 5/26 F_0 males ($p<0.05$) and 11/26 F_0 females ($p<0.01$) given 25 ppm. No dose- or treatment-related clinical signs of toxicity were observed in the parental F_0 or F_1 animals given diets containing less than 25 ppm. No dose- or treatment-related gross abnormalities were observed in the F_0 or F_1 adults at necropsy; histopathological evaluations were not performed.

Mid and high-dose F_0 males had significantly lower mean body weights as compared to controls ($p<0.05$ or 0.01). Food consumption was significantly ($p<0.01$) reduced in the high-dose F_0 males as compared with controls during premating. Food utilization was significantly ($p<0.05$ or 0.01) lower in the mid- and high-dose F_0 males as compared with the controls.

Mid and high-dose F_0 females had significantly lower mean body weights than the controls during premating ($p<0.05$ or 0.01). Food consumption by the high-dose F_0 females was significantly ($p<0.01$) less at the beginning of premating, but was significantly ($p<0.05$ or 0.01) greater than the controls during latter premating. High-dose F_0 females also had significantly ($p<0.01$) lower food

utilization at the beginning of premating and then significantly ($p<0.05$) higher food utilization during latter premating weeks as compared with the controls.

Mean body weights of the mid- and high-dose F_1 males were significantly ($p<0.01$) lower than the controls during week 1 of the premating period. Food consumption by the F_1 males was similar between the treated and control groups throughout the premating period. Food utilization was significantly ($p<0.05$) reduced in the mid- and high-dose groups during early premating.

Mean body weights of all treated F_1 female groups were significantly ($p<0.05$ or 0.01) lower than the controls during week 1 of the premating period. No statistically significant differences in body weights occurred during the remainder of the premating period. In the high-dose F_1 females, food consumption was significantly ($p<0.05$ or 0.01) greater than the controls during most of the premating period and food utilization was significantly ($p<0.05$) greater during weeks 8-10.

The Systemic Toxicity NOEL is 0.5 ppm and the Systemic Toxicity LOEL is 5.0 ppm based on lower body weights in the F_0 and F_1 males and females and reduced food utilization in F_0 males and females and F_1 males.

High-dose F_0 females had significantly ($p<0.05$ or 0.01) lower body weights than the controls during gestation of litter A. No differences in body weights occurred between treated and control groups during gestation of litter B. During lactation of litter A, maternal body weights of the mid- and high-dose animals were significantly ($p<0.05$ or 0.01) lower than the controls. During lactation of litter B, maternal body weights of the mid- and high-dose animals were significantly ($p<0.05$ or 0.01) lower than the controls. Food consumption by the treated F_0 groups was greater than the controls during gestation of both the A and B litters with occasional statistical significance in the mid- and high-dose groups. In contrast, food consumption by the high-dose F_0 females was significantly ($p<0.05$ or 0.01) less than the controls throughout lactation of both litters (68-80%).

No treatment-related differences in body weights were observed in the F_1 females during gestation. However, all treated F_1 groups had significantly ($p<0.05$ or 0.01) lower maternal body weights as compared with the controls during lactation. Food consumption was significantly ($p<0.01$) greater than the controls by the high-dose F_1 females throughout gestation and once by the mid-dose F_1 females. In contrast, food consumption during lactation was occasionally significantly ($p<0.05$ or 0.01) less than the controls in all treated dams.

For the control, low-, mid-, and high-dose F_0 adults in production of litter A, the proportion of successful matings was 70%, 67.9%, 85.7%, and 33.3% ($p<0.01$), respectively; the per cent of live born pups was 97%, 99.2%, 98%, and 90.4% ($p<0.05$), respectively; and

whole litter losses were 4/21, 0/19, 5/24, and 8/12 ($p<0.05$), respectively. Whole litter losses during production of litter B were 4/22, 2/18, 6/23, and 10/19 ($p<0.05$), respectively. Whole litter losses by the F_1 generation were 0/20, 1/23, 2/21, and 6/23 ($p<0.05$), for the control, low-, mid-, and high-dose groups, respectively.

During lactation, no dose- or treatment-related clinical signs of toxicity or differences in pup body weights were observed in the offspring of either generation. For both litters produced by the F_0 animals, there was a significant ($p<0.05$ or 0.01) decrease in the number of F_1 pups/litter in the high-dose group after day 1 of lactation. Pup deaths in the high-dose group resulted in significantly lower lactation indices of 56.6% ($p<0.01$) vs 88.0% for the controls for litter A and 75.4% ($p<0.05$) vs 92.7% for the controls for litter B. The number of F_2 pups/litter was significantly ($p<0.01$) decreased due to treatment in the mid- and high-dose groups as compared to controls after lactation day 1. Lactation indices for the control, low-, mid-, and high-dose F_2 litters were 96.8%, 94.1%, 83.8% ($p<0.05$), and 75.6% ($p<0.01$), respectively.

The Reproductive Toxicity NOEL is 0.5 ppm and the Reproductive Toxicity LOEL is 5.0 ppm based on a reduced number of F_2 pups/litter during lactation.

This study is classified as Acceptable-Guideline and satisfies the guideline requirement for a reproduction study (§83-4) in rats.

DATA EVALUATION RECORD 013048

DICROTOPHOS

Study Type: 81-8: Acute Neurotoxicity Study -Rats
Work Assignment No. 2-7A (MRID No. 43759801)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Review Section IV , Toxicology Branch II (7509C)

013048

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Oral Gavage Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: §81-8

DP BARCODE: D219351 SUBMISSION CODE: S494085
P.C. CODE: 035201 TOX. CHEM. NO.: 900

TEST MATERIAL (PURITY): Dicrotophos, (87.65% ai)

SYNONYMS: bidrin, dimethyl phosphate ester with 3-hydroxy-N,N-dimethyl-cis-crotonamide.

CITATION: Rattray N.J. (2/22/95) Dicrotophos: Acute Neurotoxicity Study in Rats. Zeneca Central Toxicity Laboratory. Alderley Park, Macclesfield, Cheshire, UK. Study No: AR5795, February 22, 1995 (MRID 43759801). Unpublished

SPONSOR: AMVAC Chemical Corporation

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 43759801) dicrotophos (87.65% ai) was administered by gavage to 10/sex/group Sprague-Dawley CD rats (20/sex/group) at dose levels of 0, 0.5, 5 or 10 mg/kg (in water, 10 ml/kg). The rats were evaluated for reactions in functional observations and motor activity measurements (3 hours, and 8 and 15 days postdosing). Two satellite groups (5/sex/group) were also included for the purpose of measuring plasma ChE and erythrocyte and brain AChE on days 1 and 8.

At 0.5 mg/kg, dicrotophos inhibited brain (21% in ♀ and 22% in ♂) and erythrocyte AChE (16% in ♀ and 19% in ♂) and plasma ChE (46% in ♀ and 38% in ♂) on day 1 (all depressions were statistically significant). Brain AChE and plasma ChE reached about 90% inhibition at 10 mg/kg. RBC AChE never exceeded 50% inhibition. Brain AChE remained inhibited (7%-14%) for males at all doses by day 16. **The NOEL and LOEL for ChE/AChE inhibition is < 0.5 mg/kg.**

At 5 mg/kg and above, dicrotophos produced clinical signs (principally decreased activity, upward curvature of spine, pinched in sides, flaccid appearance and decreased pupil response in most or all animals and several other signs in some animals. Overall some 25 in males and 24 in females FOB parameters were reported affected by treatment in the 5 and/or 10 mg/kg dose

levels of dicrotophos. Motor activity was decreased. At 10 mg dicrotophos/kg produced deaths (7 ♀ and 1 ♂) within 3 hours after dosing. Most signs regressed after one day but some persisted for 3 or 4 days. **The LOEL for neurotoxicity is 5 mg/kg based on clinical signs. The NOEL is 0.5 mg/kg.**

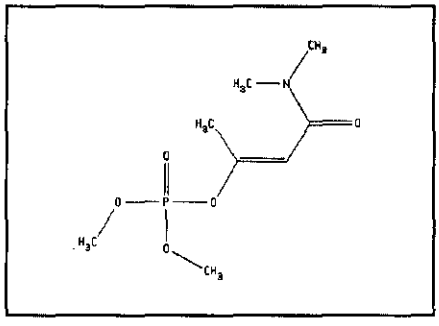
This acute neurotoxicity study is classified **acceptable** and satisfies the guideline requirement for an acute neurotoxicity study (81-8)in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dicrotophos
   Description: Clear brown liquid
   Lot/Batch #: 403001B
   Purity: 87.65 % ai.
   Stability of compound: stable
   CAS #: 141-66-2
   Structure:



2. Vehicle: Distilled water; Lot/Batch # Y04517/015

3. Test animals: Species: Rat
   Strain: Sprague-Dawley Alpk:APfSD
   Age and weight at study initiation: 42 days, males weighed 155-200g and females 127-154g
   Source: Zeneca Pharmaceuticals SPF Barriered Animal Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK
   Housing: 5/cage main groups and 3/cage satellite groups
   Diet: CT1 Diet, Special Diets Services Limited, Stepfield, Witham, Essex, UK ad libitum
   Water: Municipal water via automated watering system, ad libitum
   Environmental conditions: Temperature: 19-23 degrees C
   Humidity: 40-94%
   Air changes: 25-30/hour
   Photoperiod: 12 hour light/dark via automatic timer
   Acclimation period: 14 days

B. STUDY DESIGN:

1. In life dates - Start: 07/12/94 End: 07/28/94
(Reviewer estimated)

2. Animal assignment

Animals were assigned using computerized generated sequences of random numbers to the groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Dose (mg/kg)	Main Groups [a]		Satellite Groups[b]	
		Males	Females	Males	Females
Control	0	10	10	5/5	5/5
Low (LDT)	0.5	10	10	5/5	5/5
Mid (MDT)	5.0	10	10	5/5	5/5
High (HDT)	10.0	10	10	5/5	5/5

[a]Main group animals were sacrificed on day 16;
[b] Satellite groups were for cholinesterase determinations on days 1 and 8, five/sex/group sacrificed at each interval.

Rats were orally administered, via gastric intubation, a single dose of a test material dissolved in distilled water at a dosing volume of 1 ml/100 grams bodyweight (10/ml/kg).

No information was provided to justify the selection of doses in this study.

3. Dosing Solutions preparation and analysis: The test article was stored in the dark under refrigeration (4°C) until ready for use. For each dose concentration, an appropriate amount of test article was dissolved in distilled water and samples of each preparation formulation were analyzed to verify the concentration of test article in the vehicle.

Homogeneity: Was not assessed since the test material was completely soluble in the distilled water vehicle.

Concentration Analysis: The mean achieved concentrations of test article in the vehicle were within 6% of nominal levels (Table 2, pages 32 and 33 of report).

4. Statistics: Body weight data for each sex were subjected to analysis of covariance. Feed consumption, motor activity measurements, cholinesterase activity, time to tail-flick, landing foot splay and grip strength data

for each sex were subjected to analysis of variance. Brain weight, brain length and brain width for each sex were subjected to analysis of variance and analysis of covariance on final body weights. Differences from control were tested statistically by comparing each treatment group least squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

C. METHODS:

1. Observations:

Animals were inspected once daily for signs of toxicity and mortality.

2. Body weight:

Animals were weighed 7 days prior to dosing, immediately before dosing and 2-3 hours after dosing (day 1), day 7 and prior to scheduled termination (days 8 and 15).

3. Feed consumption:

Feed consumption for each animal was recorded continuously throughout the study and calculated on a weekly basis.

4. Neurobehavioral Studies:

Motor Activity - Motor activity of all animals was measured pretest (7 days before dosing) and on days 1 (approximately 3 hours after dosing), 8 and 15 of study. Motor activity was assessed in a separate room to minimize disturbances and each observation period was divided into 10 scans of five-minute durations. Animals were randomized and treatment groups counterbalanced across test times and monitors and when trials were repeated they were assigned to the same activity monitor at the same time of day as in the previous trial.

Functional Observational Battery - A functional observational battery (landing foot splay, sensory perception [tail-flick test], and muscle weakness (fore and hind limb grip strengths) was performed on all animals pretest (7 days before dosing) and on study days 1 (2-3 hours post dosing), 8, and 15. All evaluations were performed without the observer knowing the identity of the animal's dose group and, where appropriate, observations were coded and the degree of condition noted (slight, moderate or extreme). The following parameters

were evaluated:

- Assessment for signs of autonomic function: lacrimation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function and ptosis
- Description, incidence and severity of any convulsions, tremors or abnormal motor function, abnormal behavior
- Reactivity to stimuli
- Changes in level of arousal
- Sensorimotor responses
- Alterations in respiration

5. Cholinesterase Activity

At the termination of the satellite groups and the main study animals not required for neuropathology, 1.3 ml of blood was placed in heparinized tubes for plasma and erythrocyte cholinesterase activity determinations. The whole brain from these animals was also subjected to cholinesterase determination. The method of Ellman (1961) was used to assess for CHE/ACHE.

6. Sacrifice and Pathology

At the scheduled termination 5 designated rats/sex/group were selected for neuropathology (anesthetized with an ip injection of sodium pentobarbitone anesthesia and killed by whole body perfusion fixation with modified Karnovsky's solution) and the following tissues were taken from the control and high-dose animals for histological examination:

- brain (hippocampus, medulla, pons, cerebellar cortex and cerebral cortex)
- spinal cord (cervical and lumbar segments)
- Gasserian ganglion
- dorsal root ganglia
- spinal roots (cervical and lumbar segments)
- gastrocnemius muscle
- sciatic nerve
- sural nerve
- tibial nerve

All tissues except for the sciatic nerve were sectioned in the transverse plane. The sciatic nerve was sectioned in both the transverse and longitudinal planes.

III. RESULTS

A. Mortality: Seven deaths (1 male and 6 females) occurred within 3 hours after dosing which were considered due to direct administration of the test article. Clinical signs of toxicity in these animals included signs of salivation, chromodacryorrhea and urinary incontinence.

B. Body weight: Slight reductions in body weight were noted in both sexes in the high dose group at study days 8 and 15 (males 2.8-4.1%, females 1.2-2.7%). Male means were statistically significant.

C. Feed consumption: Feed consumption was slightly reduced for both sexes in the high dose group (males 15%, females 9%) and for males in the mid dose group (6.5%) during the first week of study. The mean feed consumption values for males were statistically significant.

D. Functional Observational Battery: Clinical signs of toxicity occurred in males and females after dosing (3 hours) on day 1 and only a few signs persisted until day 3 or 4. Overall some 25 parameters in males and 24 parameters in females were reported affected by treatment in the 5 and/or 10 mg/kg dose levels of dicrotophos (refer to Table 6 of the study report[1]). Some of the more important signs are shown in Table 2 and are described as follows. Decreased activity was observed in all mid-dose males (slight/moderate) and females (predominantly moderate), in all surviving high dose males (predominantly moderate) and females (predominantly extreme). Approach response was reduced in all survivors at 10 mg/kg and 6 males and 6 females at 5 mg/kg. Most animals at the two highest doses were subdued and flaccid and displayed a reduced foot withdrawal reflex, slight to moderate at 5 mg/kg and moderate to extreme at 10 mg/kg. Tip toe gait was seen in 6 males and 9 females at 5 mg/kg and in 4 males and 7/7 surviving females at 10 mg/kg. In the high-dose females, it persisted for 2 days in three animals and 3 days in one. Upward curvature of the spine was predominantly slight in all males at 5 mg/kg and was slight to moderate in 9 males at 10 mg/kg, persisting for 3 days in one and 2 days in another rat. In females at 5 mg/kg, upward curvature of the spine (moderate) was seen in all on day 1 and four rats on day 2. In the 10 mg/kg females, it was present in all survivors and persisted for up to 3 days. Pinched sides, shaking, and chromodacryorhea were seen in most high-dose animals; ataxia was seen in one male and four females at 10 mg/kg.

[1]Table 6 apparently has the data for females for the parameter "reduced splay reflex in the wrong columns with respect to the dose. This apparent discrepancy is noted but does not impact the conclusions of the study.

TABLE 2. Neurotoxic Signs Observed in Mid- and High-Dose Rats Administered Dicrotophos[1]

Observation	Males (mg/kg)		Females (mg/kg)	
	5	10	5	10
Died[2]	0	1	0	3
Animals Observed[a]	10[a]	9[a]	10[a]	7[a]
Decreased activity	10	9	10	7
Dec. approach response	6	9	6	7
Ataxia	0	1	0	4
Flaccid	9	9	8	7
Foot withdrawal dec.	7	9	8	7
Salivation	0	4	0	7
Splayed gait	1	3	1	1
Sides pinched in	9	9(12)	10	7(16)
Tip toe gait	6	4	9	7(16)
Upward curvature spine	10	9(12)	10(14)	7(18)
Decrease pupil response	6	7	9	1

[1] The incidence of these signs was 0 or in the control and mid dose groups.
[2] The number of animals that died is for the groups designated for FOB and motor activity. Additional females died in the high dose group in the satellite dose groups.
[a] The number of rats available for FOB and motor activity.
The numbers in parentheses are number of occurrences (days 1-4).

Rats in the mid and high dose groups exhibited statistically significant, dose response-increases in average time to tail flick measurements on study day 1 (males 2.7-4.2 fold, females 2.3-2.7 fold).

Forelimb grip strength was 29% and 51% decreased compared to controls ($p<0.01$) in males at 5 mg/kg and 10 mg/kg, respectively, and was 40% lower than in controls ($p<0.01$) in high dose females. Hindlimb grip strength was 17% and 19% lower than in controls in mid-dose males and females ($p< 0.05$ in females) and 46% and 37% lower ($p<0.01$) in males and females at the high dose. At day 8, hindlimb grip strength in females at 5 and 10 mg/kg were 32% and 41% higher than in controls.

E. Motor Activity Measurements: Motor activity measurements were significantly reduced for both sexes in the mid and high dose group approximately 3 hours after

dosing. Overall motor activity (ten 5-min sessions) was 48% and 32% of control activity in males at 5 and 10 mg/kg (i.e. reduced 52% and 68%), respectively, and 21% and 24% of controls in females at the same doses (i.e. reduced 79% and 76%). Figure 2 (photocopied from the study report is attached to illustrate the motor activity data. By day 8 of the study, motor activities in dosed rats were similar to the controls.

F. Cholinesterase Activity: Both sexes in the low-, mid- and high-dose groups exhibited dose response-related, statistically significant inhibition in brain, erythrocyte and plasma cholinesterase at the time of the peak clinical effects on the day of dosing. Brain cholinesterase in males at 10 mg/kg remained about 30% lower than control activity after 8 days and was 15% lower than control at 16 days. The statistically significant depressions (percentages of the control groups) in cholinesterase activity are summarized in Table 3.

TABLE 3. Percent Depression in Cholinesterase Activities in Brain, Erythrocyte and Plasma Tissue of Rats Administered Dicrotophos

Tissue/Study Day	Dose Level (mg/kg)					
	Males			Females		
	0.5	5	10	0.5	5	10
Brain AChE day 1	**22***	**81***	**90***	**21***	**81***	**91***
day 8	6	15*	**30***	-	8*	14*
day 16	7*	14*	13*	-	13	10*
Erythrocyte AChE day 1	19*	**37***	**48***	16*	**46***	**47***
day 8	-	16*	**24***	5	**20***	**24***
day 16	-	6	**15***	5	5	17*
Plasma ChE day 1	**38***	**84***	**90***	**46***	**86***	**89***
day 8	-	1	2	5	7	1

These data were calculated from values extracted from Table 11 (pages 59-61) of Report CTL/P/4486.

* Indicated as statistically significant p=0.05 or 0.01 in study. The bolded values are considered of by the contractor reviewer to be of biologic importance, e.g. > 20% inhibition.

G. Sacrifice and Pathology: The study author asserts that there were no treatment-related macroscopic or microscopic findings reported for this study. Only the control and high dose animals were assessed histopathologically. The pathology summary report (Table 14) consisted of a single table showing the data for the sciatic nerve. Appendix 8 and 9 present the individual animal findings. The data table for the sciatic nerve indicated that there was one female with nerve fiber degeneration (minimal). TB-I notes the presence of this lesion but does not consider that it is conclusively associated with treatment.

III. DISCUSSION

Author's Conclusions:

The author concluded that a single oral dose of dicrotophos at 10 mg/kg produced unequivocal evidence of toxicity in the form of mortality, adverse clinical signs of toxicity, marked inhibition of cholinesterase activities, acute depression of the central nervous system and reduced growth in both sexes. At a dose of 5 mg/kg, dicrotophos produced changes in the functional observation battery and inhibition of cholinesterase activities on day 1. At a dose of 0.5 mg/kg, dicrotophos produced small reductions in cholinesterase activities on day 1. The study author asserted that the no observed adverse effect was 5 mg/kg for this study.

Reviewer's Conclusions:

At a dose of 0.5 mg/kg, no clinical signs of neurotoxicity were observed but day 1 brain AChE was reduced about 22% in both sexes and activity in plasma was 38% (males) and 46% (females) reduced compared to controls.

At a dose of 5 mg/kg, especially at the time of peak effect at 2-3 hours postdosing, dicrotophos produced clinical signs of neurotoxicity, inhibition of AChE in brain and erythrocyte and plasma ChE. Day 8 erythrocyte AChE in both sexes and day 8 brain AChE. A single oral dose of 10 mg dicrotophos/kg produced evidence of toxicity in the form of mortality (7 deaths in females), clinical signs of neurotoxicity, and inhibition of cholinesterase activities at days 1 and 8 in brain and erythrocytes and at day 1 in plasma. Slightly reduced mean body weights (males 2.8-4.1%, females 1.2-2.7%) and decreased week 1 feed consumption values (males 15%, females 9%) may also be related to dosing.

Most of the clinical signs of neurotoxicity were reversed on the day after dosing. A decrease in activity was seen on the day of dosing in all males and females that received 5 or 10 mg/kg; a reduced approach response was observed in 60% of males and females at 5 mg/kg and all survivors of both sexes at 10 mg/kg. Ataxia was seen at day 1 in 1/9 males and 4/7 females at 10 mg/kg. A decreased pupil response to light was seen in 6/10 and 7/9 males at the mid- and high dose and in 9/10 females at 5 mg/kg, but in only one high-dose female. Tip to toe gait was seen in 6/10 and 4/9 males at 5 and 10 mg/kg and in 9 females at 5 mg/kg and did not persist after day 1; however, it was seen in all the surviving females at 10 mg/kg and persisted up to 3 days. Other findings that persisted after 1 day were upward curvature of the spine (mid-dose females and high dose rats of both sexes) and sides pinched in (males and females at 10 mg/kg).

The inhibition of plasma ChE was reversed more rapidly than AChE in the brain. There was not a particularly good correlation between brain cholinesterase inhibition and clinical signs of neurotoxicity. Quantitative FOB parameters (forelimb and hindlimb strength, time to tail flick), and motor activity were only affected during day 1 and at 5 and 10 mg/kg when brain AChE inhibition was above 80%. No clinical signs or changes in motor activity or FOB parameters indicative of neurotoxicity were apparent at day 8 when brain AChE was inhibited up to 30% (males) and erythrocyte AChE was inhibited 24%. No histologic changes in the nervous system were seen.

Study Deficiencies:

Although no positive control data were provided by the laboratory to document the reliability of the FOB methods or the motor activity measurements in the testing laboratory, such documentation for this laboratory was presented previously (refer to HED Document #011013).

ATTACHMENTS

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

1. Figure 2 (photocopied from the study report) entitled "Histograms of Group Mean Motor Activity - Day 1".

DATA EVALUATION REPORT

013048

Dicrotophos

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

Prepared for

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

Prepared by

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Toxicology and Risk Analysis Section
Life Sciences Division
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Oak Ridge, TN 37831
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Date: 8-11-97

Disclaimer

This review 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: Kathleen Raffaele, Ph.D. Kathleen C. Raffaele Date 4/22/98
Toxicology Branch 2 (7509C)
EPA Secondary Reviewer:
Steve Dapson, Ph.D. Stephen C. Dapson, Date 4/22/98
Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

013048

STUDY TYPE: Subchronic Oral Neurotoxicity - Rat
OPPTS 870.6200 [§82-7]

DP BARCODE: D228049
P.C. CODE: 035201

SUBMISSION CODE: S508539
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): Dicrotophos (87.65% w/w)

SYNONYMS: Bidrin; 3-(dimethylamino)-1-methyl-3-oxo-1-propenyl dimethyl ester phosphoric acid

CITATION: Horner S. (1995) Dicrotophos: Subchronic Neurotoxicity Study in Rats. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory Report No. CTL/P/4692, Study No. PR0997, November 6, 1995. MRID 43980201. Unpublished.

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Boulevard, Los Angeles, CA.

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity feeding study (MRID 43980201), 12 Alpk:APfSD rats/sex/group were fed diets containing 0, 0.5, 5, or 25 ppm dicrotophos (87.65% w/w, Batch No. 403001B) for 13 weeks. Body weight, food consumption, and clinical signs were recorded weekly. Behavioral testing (Functional observation battery [FOB] and motor activity) was conducted one week prior to start of treatment and during weeks 5, 9, and 14 of dosing. At sacrifice, 6 rats/sex/group were perfused and neuropathological evaluation was conducted for the control and high dose groups; for the remaining 6 rats/sex/group, cholinesterase activity in plasma, red blood cells (RBC), and whole brain was determined. Two satellite groups, each with 6 Alpk:APfSD rats/sex/dose, were similarly treated and included for the purpose of measuring cholinesterase activity (ChE) at week 5 (Group A) and week 9 (Group B). The average consumption of test material in the 0, 0.5, 5, and 25 ppm dose groups for males was 0, 0.04, 0.39, and 2.03 mg/kg/day and for females was 0, 0.04, 0.45, and 2.38 mg/kg/day.

There was no treatment-related effect on mortality. Effects on body weights were minimal: weights were statistically significantly lower than controls at the high dose (≤ 10.4%, $p \leq 0.05$ or 0.01) during weeks 2-6 in males and 2-4 in females, and were correlated with decreased food consumption during weeks 1 and 2 (and decreased food efficiency in males). For females only, body weights, food consumption, and food efficiency were significantly increased at multiple time points starting at week 4. No notable changes were seen in behavior or appearance upon clinical examination.

Treatment-related effects on FOB and motor activity were seen only at 25 ppm in both sexes. Forelimb grip strength was decreased at weeks 9 and 14 in females (13-14%, $p \leq 0.05$ or 0.01) and at week 5 in males (14%, $p \leq 0.05$). Hindlimb grip strength was decreased only in females at week 9 (16%, $p \leq 0.05$). The mean overall motor activity was decreased at week 9 in males (34%, $p \leq 0.01$) and at weeks 9 and 14 in females (23 and 33%, respectively; $p \leq 0.05$; $p \leq 0.01$). The neurotoxic effects, as well as the statistically significant increase in brain weight in 25 ppm males (≤ 5.2%), had no histopathological correlates.

Brain, plasma, and erythrocyte ChE activities were inhibited by dicrotophos treatment in all groups of rats, most markedly in the brain: 11-20% at 0.5 ppm, 56-63% at 5 ppm, and 87-90% at 25 ppm ($p \leq 0.01$). RBC ChE inhibition range was 0-17%, 38-51%, and 46-60% at 0.5, 5, and 25 ppm, respectively. Plasma ChE inhibition varied among males and females: the range was 10-12%, 34-39%, 68-71% for males and 11-28%, 66-74%, and 84-88% for females at 0.5, 5, and 25 ppm, respectively.

Based on the decreased grip strength in females, decreased motor activity in both sexes, and marginal body weight decreases in both sexes of rats, 25 ppm (2.03 mg/kg/day for males, 2.38 mg/kg/day for females) is identified as the LOEL and 5 ppm (0.39 mg/kg/day for males, 0.45 mg/kg/day for females) is the NOEL for systemic toxicity/neurotoxicity. Based on the dose-related inhibition in brain, plasma, and erythrocyte ChE activity, 0.5 ppm (0.04 mg/kg/day for males and females) is the LOEL for ChE inhibition; a NOEL cannot be assigned because 0.5 ppm was the lowest dose tested.

This study is classified as acceptable, pending submission of requested information, satisfying the guideline requirement for a subchronic oral neurotoxicity feeding study (82-7) in the rat.

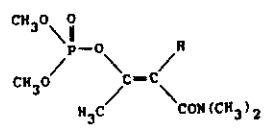
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided. A Flagging statement was not present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dicrotophos

Description: clear brown liquid
Lot/Batch #: Batch No. 403001B
Purity: 87.65% w/w (purity level stated by sponsor [see study report, p. 14]; no documentation of the purity or of stability of test substance was included in the report)
Stability of compound: stated to be stable for period of study (see above); stored under argon and refrigeration at 4°C in the dark
CAS #: 141-66-2
Structure:



2. Vehicle and/or positive control:

None - Dicrotophos was given in the feed; no positive controls were used because positive historical control data was available (validation positive control study is HED Doc. No. 011988).

3. Test animals

Species: Rat
Strain: Alpk:APfSD
Age and/or weight at start of treatment: About 42 days (not specified); males, 178-266 g; females, 146-198 g (note that baseline behavioral testing occurred one week prior to start of treatment)
Source: Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, U.K.
Acclimation period: about 2 weeks prior to start of dosing (about one week prior to initial behavioral testing)
Diet: CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, U.K.)
Water: tap water pre-experiment; filter-sterilized water during treatment, *ad libitum*
Housing: Groups of 3-4 of same sex in stainless-steel, wire mesh cages with one solid side.
Environmental conditions:
Temperature: 18.5 - 23°C

Humidity: 26-70%
Air changes: 25-30 per hour
Photoperiod: 12 hour light/dark cycle

B. STUDY DESIGN AND METHODS

1. In life dates

Treatment Start: January 17-20, 1995 (animals were received on January 3, behavioral pre-testing occurred one week prior to start of treatment); end: April 1995

2. Animal assignment and treatment

Animals were assigned to test groups after ensuring that any unhealthy rats, rats that failed a pre-randomization tail-flick test, or rats at the extremes of weight were excluded. The rats were sorted according to weight (sexes separately) and allocated to the experimental groups using a Latin Square until each cage in the main study contained 4 rats and in the satellite study contained 3 rats. The two satellite groups were included for measuring plasma, erythrocyte, and brain cholinesterase activity during the study. Satellite group A rats were sacrificed after 5 weeks and Satellite Group B rats after 9 weeks of treatment. The study design is shown in Table 1.

TABLE 1. Study design

Test Group	Dietary Conc. (ppm)	Mean daily dose received over 13 weeks (mg/kg/day)		Number of Animals					
				Main group (kill week 14)		Satellite group A (kill week 5)		Satellite group B (kill week 9)	
		Male	Female	Male	Female	Male	Female	Male	Female
Control	0	0	0	12	12	6	6	6	6
Low-Dose	0.5	0.04	0.04	12	12	6	6	6	6
Mid-Dose	5	0.39	0.45	12	12	6	6	6	6
High-Dose	25	2.03	2.38	12	12	6	6	6	6

Data taken from pp. 17, 97, and 98, MRID 43980201.

Dosing was initiated over a four-day period, by replicate, with sex and treatment group balanced across days (except that for the two satellite groups, treatment groups were balanced across days but sex was not).

3. Validation of test methods

Studies were previously conducted to establish the sensitivity, reliability, and validity of the FOB, motor activity, and pathology test methods (HED Doc. No. 011988, corresponding to MRID 43013301-43013305). Reference to these was not made in this document, however, but was obtained by the reviewer from the EPA. Acceptability of these studies may be reevaluated pending receipt of requested procedural information for the current study (see below).

4. Rationale for dose selection

Dose levels were selected on the basis of results from previously conducted studies in the same laboratory using Alpk:APfSD rats. A description of the studies was not provided.

5. Diet preparation and analysis

The diets were prepared in 30 kg batches. The concentration was adjusted for purity of the test substance (87.65% w/w). For the 5 and 25 ppm levels, 0.5 kg premix (test substance + diet ground together) was added to 29.5 kg CT1 diet and mixed thoroughly. For the 0.5 ppm level, 0.3 kg premix with a target concentration of 50 ppm was mixed with 29.7 kg diet. Because the first batch of the 0.5 ppm diet was 16-20% below the target concentration (it was fed to the rats during the first study week only), the premix concentration was increased to 55 ppm. The diets were stored frozen at -20°C and used within 30 days of preparation. Fresh aliquots of the diets were given every other day (except the 0.5 ppm diet was given every day during the first week) after being thawed about 1 hour.

The concentration of dicrotophos in 10 g portions of the diet was determined for all dose levels periodically throughout the study by gas chromatography. The homogeneity was assessed in 0.5 and 25 ppm samples from the bottom, middle, and top. The chemical stability of dicrotophos in the diet (at room temperature and at -20°C) was analyzed in 0.5 and 50 ppm diet samples from a concurrent long-term feeding study in the same laboratory (CTL Study Numbers PR0986 and PM0992).

Results -

Homogeneity Analysis: The mean concentration measured at the top, middle, and bottom of the samples varied from the overall mean by ≤ 5%. In the 0.5 ppm samples, values ranged from 74-88% of the nominal concentration, and at 25 ppm, values ranged from 98-103% of the nominal concentration.

Stability Analysis: After 1, 2, 3, or 4 days at room temperature, 0.5 ppm samples were 88.5, 80.8, 75.0, and 69.2%, respectively, of the initial concentration, whereas 50 ppm samples were 88.8, 93.1, 83.8, and 79.9% of the initial concentration. At -20°C, 0.5 ppm samples were within 86.5% of the initial concentration for up to 51 days, and 50 ppm samples were within 96.9% for up to 35 days.

Concentration Analysis: As % of target concentration: 90-108% at 0.5 ppm if the week 1 batch was excluded (week 1 batch was 82-86%); 88-98% at 5 ppm; 90-104% at 25 ppm.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

6. Statistical analysis

An analysis of covariance was used to assess differences from controls in body weight (comparing to initial body weight) and brain weight, length, and width (comparing to final body weight). Analysis of variance was used to assess differences from controls in weekly food consumption, food utilization, motor activity measurements, time to tail-flick, landing foot splay, limb grip strength, and cholinesterase activity. All statistical analyses were performed separately on males and females. The analyses of variance and covariance were conducted with the GLM procedure in SAS, by comparison of the least-squares means of the treatment and control groups using a two-sided Student's t-test.

B. METHODS

1. Clinical observations and mortality

Animals were observed daily for changes in clinical condition and behavior. Once a week, each rat was removed from its cage and physically examined for changes in general health status.

2. Body weights

All animals (main and satellite groups) were weighed immediately before feeding of the test diet began (designated week 1), then on the same day of each subsequent week, and at termination. For the main study animals, the body weight of each rat was recorded in replicate order in week -1 as well.

3. Food consumption and efficiency

Food consumption (g/rat/day) was recorded every two days (or as required) for each cage of rats, being calculated on a weekly basis. The food efficiency/cage was calculated as the body weight gain/cage per 100 grams food consumed by the rats.

4. Functional observational battery (FOB)

An FOB which included detailed clinical observations and quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind-limb grip strength) was conducted on the main study animals (12 rats/sex/dose) in weeks -1, 5, 9, and 14. Only the detailed clinical observations were also made for the 6 rats/sex/dose in satellite groups A (at week 5) and B (at weeks 5 and 9). FOB tests were conducted in a room separate from the animals' cages by one observer "blind" with respect to the animals' treatment. The FOB included, but was not limited to, the following parameters:

a. Autonomic function

Lacrimation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis, alterations in respiration.

b. Clinical observations

Convulsions, tremors, abnormal motor function, abnormal behavior.

c. Sensorimotor observations

Tail-flick test, reactivity to stimuli, changes in level of arousal, sensorimotor responses

d. Neuromuscular observations

Landing foot splay, hindlimb and forelimb grip strength

A description of the procedures used for conducting these assessments (both the detailed clinical observations and the quantitative measurements) was not provided. This information should be supplied by the sponsor.

5. Locomotor activity

Locomotor activity was measured in all main study animals at weeks -1, 5, 9, and 14 using an automated activity recording apparatus (the type of apparatus and calibration procedures were not specified; this information should be provided by the sponsor). Each observation period consisted of ten 5-minute scans performed in a room separate from the animals' cages. The different treatment groups were counter-balanced across test times and across devices and when the trials were repeated, each animal was returned to the same activity monitor at about the same time of day.

6. Cholinesterase activity measurement

Plasma, red blood cell, and whole brain cholinesterase activity was measured at final sacrifice in 6 animals/sex/dose of the main study and from all the satellite group A and B animals (6 rats/sex/dose in each satellite group). Blood samples were collected during the terminal exsanguination under halothane vapor anesthesia. The whole brains were rapidly removed and processed for cholinesterase activity according to the method of Ellman et al. (1961). The method used for determining cholinesterase activity in plasma and red blood cells was not stated; this information should be provided by the sponsor.

7. Necropsy examinations

In addition to the scheduled necropsy examination of brain measurements and neuropathology, gross post-mortem examinations were performed on animals which died or were killed prior to terminal sacrifice within 24 hours of death. Necropsy was not performed on animals used for cholinesterase measurements.

a. Brain measurements

The weight, length, and width of whole brains of 6 rats/sex/dose from the main study (presumably those designated for neuropathology) were measured. However, details of the methods used were not provided in the study report.

b. Neuropathology

Six animals/sex/group of the control and 25 ppm dose group animals of the main study were designated for neuropathology examination at terminal sacrifice. These rats were deeply anesthetized with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following (x) tissues were embedded in either Araldite (cervical and lumbar spinal cord, gasserian ganglion, sciatic nerve, sural nerve, and tibial nerve) or paraffin (all other tissues), sectioned in the transverse plane, and stained with hematoxylin and eosin (paraffin-embedded tissues) or with toluidine blue (Araldite-embedded tissues) [the sciatic nerve was sectioned in both the transverse and longitudinal plane]:

X	Brain (7 levels)	X	Spinal Cord	X	Peripheral nerves
X	Cerebrum	X	Cervical	X	Sciatic nerve
X	Cerebellum	X	Lumbar	X	Sural nerve
	Forebrain		Thoracic	X	Tibial nerve
	Hypothalamus	X	Lumb.Dors.root gang		Peroneal nerve
X	Medulla oblongata	X	Cerv.Dors.root gang		Optic nerve
	Pituitary gland	X	Gasserian ganglion/		
	Midbrain		trigeminal nerves		Other
X	Pons	X	Cervical roots	X	Gastrocnemius muscle
X	Hippocampus	X	Lumbar roots		

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

Two males from satellite group B that had exhibited no previous clinical signs were found dead: one treated with 0.5 ppm (day 3) and one treated with 25 ppm (day 1). Additionally, one control male was killed for humane reasons during week 8 due to reduced hindlimb function, lacrimation, subdued behavior, and hunched appearance. This male had a mass in the

brain and firm areas in the lungs. Detailed clinical observations are described in the functional observational battery (FOB) section below (II D.).

B. BODY WEIGHTS

Changes approaching biological significance were only seen in the 25 ppm groups: statistically significant decreases ($p \leq 0.05$ or 0.01) occurred in the adjusted mean body weights of males at weeks 2-6 (3.3-10.4%) and females at weeks 2-4 (4.2-7.8%). The greatest decrease was at week 2 in both sexes. Mid-dose males had a slightly decreased body weight relative to controls at week 2 (1.4%, $p \leq 0.05$). Body weights were occasionally up to 6.2% greater than controls in 5 and 25 ppm females between weeks 9-14 (5 time points for 5 ppm, 3 time points for 25 ppm; $p \leq 0.05$ or 0.01). The overall body weight gains (calculated by the reviewer) for treated males were within $\pm$ 6% of controls and for females were 2.4, 19.4, and 16.4% greater than controls for low, mid, and high dose groups, respectively.

C. FOOD CONSUMPTION AND EFFICIENCY

The food consumption of males and females given 25 ppm was significantly lower than that of controls only during weeks 1-2 (8.6-10.1% for males; 7.2-12.8% for females; $p \leq 0.01$). Females given 5 or 25 ppm, however, ate more food than controls for most weeks throughout the treatment period. For females in the 5 ppm group, the increase was significant in weeks 1 [4.5%], 9-11 [8.5-12.9%], and 13 [10%]; for females in the 25 ppm group, the increase was significant for every week starting at week 4 [8-18.5%] ($p \leq 0.05$ or 0.01).

Overall food efficiency (mean for weeks 1-13) was lower (6.5%; $p \leq 0.05$) than controls in males given 25 ppm; it was comparable to controls in 0.5 and 5 ppm males and in all female treated groups. For 4-week time periods within the study, transient deviations in the food efficiency occurred in 25 ppm males (7.9% lower than controls for weeks 1-4, $p<.01$) and 5 and 25 ppm females (30 and 53% greater than controls, respectively, at weeks 5-8; $p<.01$).

D. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

The incidence of most clinical observations was similar in treated and control rats of both sexes, i.e. no more than 3 animals from any one dose group exhibited the sign. The exception is males given 25 ppm dicrotophos, who had a slightly greater incidence of decreased pupil response to

light (1/17 vs. 5/17) and scabs (1/11 vs. 5/11)($p > 0.05$; calculated by reviewer using Fisher's exact test).

Landing foot splay measurements made at 5, 9, and 14 weeks in treated (main study) rats were not significantly different from controls; the 14.7% increase ($p \leq 0.05$) in 0.5 ppm females at week 5 was not dose-related. The time to tail-flick was similar in treated and control groups; there were no statistically significant changes. Forelimb grip strength was slightly decreased in 25 ppm females at weeks 9 and 14 (13-14%, $p \leq 0.05$ or 0.01); other statistically significant grip strength decreases were transient (week 5 males at 25 ppm; 14.3%, $p<.05$) or not dose-related (week 14 males at 5 ppm; 12.0%, $p<.05$). Hindlimb grip strength was decreased only in 25 ppm females at week 9 (16%, $p \leq 0.05$).

E. MOTOR ACTIVITY

The mean overall (i.e. 1-50 minutes total; ten 5-minute increments) group motor activity counts in 0.5 and 5 ppm males were comparable to controls. There were one or two 5-minute time periods of decreased activity at each dose ($p \leq 0.05$ or 0.01), and week 14 activity was increased from 41-50 minutes at 0.5 ppm ($p \leq 0.05$ or 0.01). In 25 ppm males, overall activity was decreased at week 9 (34%, $p \leq 0.01$), with the decreases at minutes 26-45 being statistically significant. The overall activity at week 14 in 25 ppm males was also lowered (22%), though not statistically significantly (there was a significant decrease in the 16-20 minute subsession for this group [33%, $p<.05$]).

Females given 0.5 or 5 ppm dicrotophos had activity measurements similar to controls. At 25 ppm, overall activity was lowered at weeks 9 and 14 (23 and 33%, respectively; $p \leq 0.05$; $p \leq 0.01$). The 5-minute periods statistically significantly different from the controls at 9 weeks were minutes 21-25 and at 14 weeks were minutes 21-50. Note that control females failed to decrease their activity with time during the week 14 test session, such that activity during the first 5 minute subsession (59.4 counts) is almost identical to that during the 46-50 minute subsession (53.1 counts) [By contrast, for week -1, activity decreased from 57.9 in the first subsession to 2.2 in the final subsession).

The results are summarized in Table 2.

TABLE 2. Mean overall activity counts (1-50 minutes as ten 5-minute intervals) in groups of male and female rats fed dicrotophos for 13 weeks

Test week	Exposure concentration (ppm)			
	0	0.5	5	25
Males				
Week -1	169.9 (69.1)	148.3 (49.6)	207.8 (108.3)	177.9 (76.2)
Week 5	390.8 (94.2)	426.5 (111.9)	384.3 (133.0)	351.2 (139.4)
Week 9	506.0 (192.5)	498.9 (109.4)	508.8 (132.5)	333.3** (140.1)
Week 13	466.9 (146.2)	506.3 (151.8)	397.6 (119.8)	365.0 (165.1)
Females				
Week -1	225.0 (128.1)	288.0 (124.4)	239.5 (91.4)	226.8 (107.4)
Week 5	528.6 (87.2)	574.8 (110.0)	535.2 (114.4)	459.6 (125.8)
Week 9	496.1 (140.5)	524.5 (159.1)	486.6 (149.6)	380.3* (121.9)
Week 13	529.5 (123.5)	502.0 (137.7)	474.0 (113.7)	357.4** (108.6)

Data taken from Table 12, pp. 65-72, MRID 43980201.
Significantly different from controls, * $p \leq 0.05$; ** $p \leq 0.01$.
N=12, except that n=11 for high dose males at week -1 and for control males at week 14. Values in parentheses are standard deviations.

F. CHOLINESTERASE ACTIVITY MEASUREMENTS

Plasma, erythrocyte, and brain cholinesterase (ChE) activities were significantly inhibited by treatment with dicrotophos in all dose groups of both sexes of rats. Brain ChE activity was affected the most markedly, the decrease relative to controls in 0.5, 5, and 25 ppm groups being 11-20%, 56-63%, and 87-90% ($p \leq 0.01$ or 0.05), respectively. Plasma and erythrocyte ChE activities were statistically significantly decreased in both sexes at all weeks at 5 ppm (34-74%) or 25 ppm (46-88%); rats given 0.5 ppm had smaller decreases (5-28%) that were significant at one or more test weeks (5, 9, and/or 14 weeks). The decreases in brain and erythrocyte ChE activities were comparable in males and females; plasma ChE activity was more markedly decreased in females than males. The results are summarized in Table 3.

TABLE 3. Cholinesterase (ChE) activity ± standard deviation (and % inhibition relative to concurrent controls) in male and female rats fed dicrotophos for 13 weeks

Parameter	Exposure concentration (ppm)			
	0	0.5	5	25
Males				
Plasma ChE (U/L)				
week 5	566±51	508±52* (10)	348±41** (39)	164±19** (71)
week 9	531±12	470±34* (11)	336±49** (37)	171±39** (68)
week 14	515±100	454±57 (12)	339±26** (34)	167±26** (68)
RBC ChE (U/L)				
week 5	2082±198	1973±137 (5)	1208±224** (42)	1117±238** (46)
week 9	2162±330	1980±237 (8)	1297±190** (40)	1162±158** (46)
week 14	2358±230	1957±279* (17)	1163±246** (51)	1082±210** (54)
Brain ChE (IU/G)				
week 5	11.00±0.95	9.82±0.68** (11)	4.13±0.36** (62)	1.21±0.08** (89)
week 9	10.64±0.76	8.53±0.54** (20)	4.24±0.30** (60)	1.18±0.33** (89)
week 14	9.83±0.50	8.57±0.34** (13)	4.30±0.43** (56)	1.25±0.08** (87)
Females				
Plasma ChE (U/L)				
week 5	1201±201	1070±121 (11)	404±53** (66)	195±23** (84)
week 9	1468±152	1102±255** (25)	473±67** (68)	178±31** (88)
week 14	1660±225	1190±173** (28)	439±113** (74)	258±118** (84)
RBC ChE (U/L)				
week 5	2223±194	1885±220** (15)	1372±124** (38)	1210±151** (46)
week 9	2232±94	2100±267 (6)	1308±266** (41)	1113±185** (50)
week 14	2550±138	2602±896 (ND)	1388±124** (46)	1022±218** (60)
Brain ChE (IU/G)				
week 5	9.66±1.07	8.44±0.78* (13)	3.82±0.54** (60)	1.09±0.30** (89)
week 9	10.82±1.17	9.38±0.56** (13)	3.95±0.39** (63)	1.07±0.10** (90)
week 14	10.72±0.87	9.16±0.66** (15)	4.00±0.38** (63)	1.23±0.10** (89)

ND = no inhibition detected compared to concurrent controls
Data taken from Tables 13A and 13B, pp. 73-76, MRID 43980201.
Significantly different from control group, * $p \leq 0.05$; ** $p \leq 0.01$.
N=6 except that n=5 for males at week 9, 0.5 and 25 ppm groups and at week 14 for control and 25 ppm groups.

G. PATHOLOGY

Brain length and width measurements were similar for control and all treated rats of both sexes. Brain weight was unaffected by treatment in all females and in 0.5 and 5 ppm males, but was statistically significantly increased in males fed 25 ppm dicrotophos: there was a 3.7% increase in the absolute brain weight ($p \leq 0.05$) and a 5.2% increase in the brain weight adjusted for body weight ($p < 0.01$).

There were no treatment-related macroscopic or microscopic lesions in any group of animals. The only lesion found on

histopathological examination was minimal sciatic nerve degeneration; incidence was similar among control and treated groups.

III. DISCUSSION

A. DISCUSSION

There was no treatment-related effect on mortality. Effects on body weights at 25 ppm were of minimal toxicological significance (≤ 10.4% decrease); they were statistically significantly lower than controls during weeks 2-6 in males and 2-4 in females. The body weight changes were correlated with decreased food consumption during weeks 1 and 2 (and decreased food efficiency in males). For females at 5 and 25 ppm, there were significant increases in body weight gain, food consumption, and food efficiency during the later weeks of the study.

Detailed clinical examination of behavior and appearance revealed only minor, toxicologically non-significant findings (e.g. decreased pupil response to light and increased incidence of scabs in males; $p > 0.05$ calculated by reviewer using Fisher's exact test).

Forelimb grip strength in 25 ppm females was statistically significantly decreased at weeks 9 and 14 (13-14%, $p \leq 0.05$ or 0.01) and in 25 ppm males at week 5 (14%, $p \leq 0.05$). Hindlimb grip strength was statistically significantly decreased only in 25 ppm females at week 9 (16%, $p \leq 0.05$). Treatment-related changes were not seen in either the landing foot splay or the time to tail-flick measurements made at 5, 9, or 14 weeks.

Effects on motor activity were seen primarily at 25 ppm in both sexes of rats; the sporadic statistically significant effects at 5 ppm were not dose-related. The mean overall activity (i.e. 50 minute total) in 25 ppm males was decreased at week 9 (34%, $p \leq 0.01$) and slightly at week 14 (22%, $p > 0.05$). Overall activity in 25 ppm females was lowered at weeks 9 and 14 (23 and 33%, respectively; $p \leq 0.05$; $p \leq 0.01$).

There were no treatment-related macroscopic or microscopic lesions in any group of animals. It therefore appeared that the minor, statistically significant increase in 25 ppm males in the absolute and adjusted brain weight (3.7 and 5.2%, respectively) reflected biological variation. Brain length and width were similar for control and treated rats.

Based on the marginal body weight alterations, decreased fore- and/or hindlimb grip strength and decreased overall activity seen at 25 ppm in both sexes of rats, this dose is identified as the LOEL and 5 ppm is the NOEL for systemic toxicity/neurotoxicity.

Dicrotophos inhibited plasma, erythrocyte, and brain ChE activity at all test weeks in the 5 and 25 ppm treatment groups. Brain ChE was inhibited the most markedly, being inhibited by 56-63% at 5 ppm ($p \leq 0.01$) and 87-90% at 25 ppm ($p \leq 0.01$). In rats given 0.5 ppm, brain ChE was inhibited at all weeks tested (11-20%, $p \leq 0.01$) whereas plasma and erythrocyte ChE were significantly inhibited at one or more weeks. Because brain ChE was significantly decreased at 0.5 ppm in both sexes of rats at all test weeks ($p \leq 0.05$ or 0.01), 0.5 ppm was identified as the LOEL for ChE inhibition. Sex differences in ChE inhibition were only seen for plasma ChE, which was more inhibited in females than males. Because ChE inhibition occurred at the lowest dose tested, a NOEL cannot be identified from the study data.

The study author selected 5 ppm as the NOEL for "neurotoxic potential" of dicrotophos. A separate NOEL for ChE inhibition was not given, although the study author considered that the ChE inhibition at 0.5 and 5 ppm was "of no toxicological importance." While the reviewer agrees with the NOEL for systemic toxicity/neurotoxicity, a LOEL of 0.5 ppm is identified for ChE inhibition, particularly in the brain (a NOEL could not be identified because the LOEL was the lowest test dose).

B. STUDY DEFICIENCIES

Minor deficiencies that do not adversely affect the study results are: validation study results (i.e. positive controls) or reference to such studies conducted by the testing laboratory (Zeneca Central Toxicology Laboratory, UK) were not provided by the study author (this was obtained from the EPA by the reviewer); the studies on which the dose selection rationale was based were not described; the first batch of the 0.5 ppm diet (fed to the rats during the first study week only) was 16-20% below the target concentration; the animal body weight gains were not calculated and statistically analyzed; there was no statistical analysis of clinical observations; and details of the methods used to measure the weight, length, and width of whole brains were not given.

The following information should be provided by the sponsor: 1) details of the procedures used for the FOB (detailed clinical observations and quantitative measures) and the motor activity (the type of device used and calibration procedures), 2) the procedure used to evaluate cholinesterase inhibition in plasma and red blood cells, 3) the analytical data documenting the chemical stability and purity of the test substance.

Based on the decreased grip strength in females, decreased motor activity in both sexes, and marginal body weight decreases in both sexes of rats, 25 ppm (2.03 mg/kg/day for males, 2.38 mg/kg/day for females) is identified as the LOEL and 5 ppm (0.39 mg/kg/day for males, 0.45 mg/kg/day for females) is the NOEL for systemic toxicity/neurotoxicity. Based on the dose-related inhibition in brain, plasma, and erythrocyte ChE activity, 0.5 ppm (0.04 mg/kg/day for males and females) is the LOEL for ChE inhibition; a NOEL cannot be assigned because 0.5 ppm was the lowest dose tested.

This study is classified as acceptable, pending submission of requested information, satisfying the guideline requirement for a subchronic oral neurotoxicity feeding study (82-7) in the rat.

DATA EVALUATION REPORT 013048

Dicrotophos

STUDY TYPE: CHRONIC ORAL TOXICITY [CAPSULE] - DOG (83-1b)

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
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Disclaimer

This review 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.

DICROTOPHOS **Chronic Oral Study (83-1b)**

EPA Reviewer: I. Mauer, Ph.D.
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S. Diwan, Ph.D.
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I. Mauer, Date 04/16/98

Sanjiv Diwan, Date 04/16/98

013048

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [capsule] - Dog
OPPTS 870.4100 [§83-1b]

DP BARCODE: D242039
P.C. CODE: 035201

SUBMISSION CODE: S533410
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): Dicrotophos (87.65% w/w)

SYNONYMS: Not given; in another study (MRID 43980201) were given as: Bidrin; 3-(dimethylamino)-1-methyl-3-oxo-1-propenyl dimethyl ester phosphoric acid

CITATION: Horner S. (1997) Dicrotophos: 1 year oral toxicity study in dogs. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory Report No. CTL/P/5103, Study No. PD1008, June 27, 1997. MRID 44328401. Unpublished.

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Boulevard, Los Angeles, CA.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 44328401), dicrotophos (87.65% w/w, Batch No. 403001 B) in corn oil was administered to 4 beagle dogs/sex/dose by capsule at doses of 0 (corn oil only), 0.025, 0.1, or 1.0/0.5 mg/kg/day for 1 year (high-dose dogs received 1.0 mg dicrotophos/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day from week 15-52).

No animals died during the study. Treatment-related clinical signs were seen primarily at the high dose before it was lowered to 0.5 mg/kg/day, and were correlated with the inhibition of plasma and erythrocyte cholinesterase (ChE) activity. Signs included shaking and subdued behavior (2/4 or more dogs/sex during weeks 13-14 and in one female during week 24); unsteady gait (one female at week 13) and a slight increase in the incidence/frequency of fluid feces, regurgitation, and vomiting (both sexes). High-dose dogs also resisted dosing and males had reddening and peeling of the scrotal skin with sores primarily during the first 14 study weeks.

Weekly body weights of high-dose males were lower than those of controls (≤ 8.4%; $p < 0.05$) several times between weeks 5 and 14, and their week 1-14 body weight gain was 48% of controls. After the high dose was lowered, the body weights improved and their overall weight gain was greater than of the controls. Weekly body weights of mid- and high-dose females were up to 10.3% lower than controls throughout the study ($p < 0.05$ for weeks 6, 7, 9, 14, 47, and 49-53 at one or both doses), and their overall weight gain and food efficiency were 74-78% of controls. There were no toxicologically significant differences from the controls for hematology, clinical chemistry, urinalysis, or ophthalmoscopic parameters, organ weights, or gross and microscopic

pathology. No neoplastic lesions attributable to test article administration were reported.

Based on the 26% lower overall (week 1-52) body weight gain (and food efficiency) in mid-dose females, 0.1 mg/kg/day is concluded to be the LOEL under the conditions of this study; the corresponding NOEL is 0.025 mg/kg/day. The reviewer disagrees with the study author that the body weight changes at the mid-dose were not toxicologically significant and that only the high dose represents a LOEL for body weight changes.

Plasma, RBC, and brain ChE activities were inhibited by dicrotophos treatment throughout most of the study in both sexes. The degree of inhibition was clearly dependent on the dose but not on the exposure duration. At the high dose, the plasma, RBC, and brain ChE values were 36-64% of controls ($p \leq 0.01$) and neurologic impairment was clinically evident. At the mid-dose, plasma ChE activity was 55-61% of controls ($p \leq 0.01$) in both sexes, RBC ChE activity was 83-91% of controls ($p \leq 0.05$ or 0.01 for males only), and brain ChE was 81-88% of controls ($p \leq 0.05$ for females only). Although there were no visible neurological effects at the mid-dose, **because the parameter most germane to *neurotoxicity, i.e. brain ChE, was statistically significantly inhibited in* females (19%, $p \leq 0.05$), and plasma ChE was substantially inhibited in both sexes, the LOEL for ChE inhibition is considered to be 0.1 mg/kg/day, and the NOEL is 0.025 mg/kg/day.** The reviewer disagrees with the study author that the high dose is the LOEL for ChE inhibition.

This study is classified as acceptable and satisfies the guideline requirement for a chronic oral toxicity study (83-1b) in the dog.

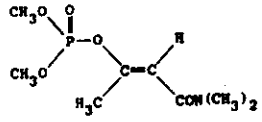
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dicrotophos

Description: Clear brown liquid
Lot/Batch #: Batch No. 403001 B
Purity: 87.65% w/w
Stability of compound: Stable for period of study; stored under argon and refrigeration at 4°C in the dark
CAS #: 141-66-2 Structure:



2. Vehicle and/or positive control

Vehicle and negative control: Corn oil (Kraft Wesson Corn Oil, CTL reference number Y00790/004). No positive control.

3. Test animals

Species: Dog
Strain: Beagle
Age and weight at study initiation: 16-24 weeks old; study day 1 individual weights of males: 9.1-12.1 kg; females: 7.9-10.4 kg.
Source: Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, U.K.
Housing: By treatment groups (sexes separated) in indoor pens with heated sleeping platforms. The pens had interlinking gates so the dogs could be separated during feeding and dosing.
Diet: Male dogs were fed 400 g and females 350 g of an expanded dry diet (Laboratory Diet A from Special Diet Services Ltd, Witham, Essex, U.K.).
Water: Potable water was supplied *ad libitum*.
Environmental conditions:
- Temperature: 19±4°C
- Humidity: 40-70%
- Air changes: 15/hour
- Photoperiod: 12 hours light per 24 hours

Acclimation period: Approximately 4 weeks

B. STUDY DESIGN

1. In life dates

Start: May 2, 1995; end: May 3, 1996

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1, such that there was an even distribution according to body weight, and that litter mates were in different groups.

TABLE 1: Study Design

Test Group	Dose to Animal (mg/kg/day)	Number of Animals	
		Male	Female
1 (Control)	0	4	4
2 (Low)	0.025	4	4
3 (Mid)	0.1	4	4
4 (High)	1.0/0.5[1]	4	4

Data taken from p. 19, MRID 44328401.
[1]The high-dose dogs were given 1.0 mg dicrotophos/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day for weeks 15-52.

3. Dose selection rationale

The dose levels were selected based on results from previous dog studies performed in the laboratory [.... however, no further details were given]. In the present study, the high dose was lowered from 1.0 mg/kg/day to 0.5 mg/kg/day after 13 weeks of administration because shaking, depressed appetite, and marked inhibition of cholinesterase (ChE) activity were observed in more than half the animals at week 13.

4. Diet preparation and analysis

Animals were fed an expanded dry diet (see above) not containing any test material. The dicrotophos was administered in corn oil in 6 mL gelatin capsules immediately prior to feeding at approximately the same time each day. Stock dosing solutions were prepared fresh monthly as 0.5 mg/mL and 5 mg/mL solutions in corn oil, adjusted for dicrotophos purity of 87.65% w/w. The two stock solutions were stored at room temperature under argon, and were analyzed approximately every other month during the study for achieved concentration. The chemical stability of dicrotophos in corn oil was determined over a period of up to 47 days by extraction with ethyl acetate and gas chromatography. The amount (weight) of test compound in corn oil loaded into the capsules was determined by the animal's most recent body weight. Controls received gelatin capsules containing only corn oil (same volume as that received by the high-dose animals).

Results:
Homogeneity Analysis: not relevant; a weighed amount of dicrotophos in corn oil was given by capsule.

Stability Analysis: over a period of 5-47 days after preparation, the % of the initial concentration of the 0.5 mg/mL and 5 mg/mL stock solutions ranged from 89.4-110.6% and 87.7-107.6%, respectively.

Concentration Analysis: the achieved concentration of the 0.5 mg/mL and 5 mg/mL stock solutions ranged from 96.0-104.0% and 98.6-104.8% of the nominal concentrations, respectively.

5. Statistics

Statistical analyses were performed by calculating either variance or covariance using the GLM procedure in SAS (SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC: SAS Institute Inc, 1989). The differences from the controls of the treated groups were represented by the differences in their least-squares means, and were tested statistically using a two-sided Student's *t*-test. Body weight changes were analyzed by analysis of covariance from week 1 weights, separately for males and females. Hematology, blood clinical chemistry, and plasma/erythrocyte cholinesterase activity were evaluated by analysis of covariance of the sexes combined; a covariate adjustment was made based on separate sex pre-experimental group means. Food consumption, brain cholinesterase activity, urine clinical chemistry, and organ weight differences from controls were examined separately for males and females by analysis of variance. The presence of differential effects in left and right paired organs was investigated, and since no differences were found, the paired organ weights were combined for analysis of statistical significance.

Several outlying values were excluded from analysis: control female #5 plasma ChE activity at week 52 was very high and considered to be spurious, and organ weight data from two mid-dose females were excluded due to a congenitally missing right kidney (female #21) or right thyroid (female # 22).

C. METHODS

1. Observations

Animals were inspected at least thrice daily for signs of toxicity and mortality, and were given a thorough examination weekly. Fecal consistency was assessed daily for up to 5 hours after dosing for individual animals, and subsequently on a group basis, if necessary. The dogs were given a full clinical examination including cardiac and pulmonary auscultation pre-study and during weeks 13, 26, 39, and 52 (prior to termination).

2. Body weight

Animals were weighed weekly (before feeding) throughout the pre-treatment period, on treatment day 1, and at weekly intervals thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined daily for at least 2 weeks pre-study and throughout the treatment period. Consumption was determined by weighing the food residues approximately 4 hours after feeding; residual food was discarded. The compound was administered by gelatin capsules based on animal body weight, and was independent of food consumption. Food efficiency was not calculated by the study author but was calculated by the reviewer for each dose group as [(week 1-52 body weight gain/week 1-52 food consumption) X 100]).

4. Ophthalmoscopic examination

Eyes were examined by indirect ophthalmoscopy pre-study and during weeks 13, 26, 39, and 52 (prior to termination).

5. Blood was collected from the jugular vein of all surviving animals before the morning feeding (i.e. after overnight fast) at weeks -1, 4, 13, 26, and 52 for hematology and clinical analysis. Additionally, jugular vein blood samples were taken from male #11 (low-dose) during week 45 (because of inappetence and body weight loss). The CHECKED (X) parameters were examined.

Plasma and erythrocyte cholinesterase activity were measured using the method of Ellman et al. (Biochem. Pharmacol. 7:88-95, 1961). At scheduled termination, the brain of each dog was halved longitudinally after being weighed: the right half was used for measurement of cholinesterase activity (method of Ellman et al. 1961) and the left half was used for histopathology.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Kaolin-cephalin time)		
x	(Prothrombin time)		

* Required for chronic studies based on FIFRA Subdivision F Test Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
		x	Total serum protein (TP)*
	ENZYMES	x	Triglycerides
x	Alkaline phosphatase (ALK)		Serum protein electrophoris
x	Cholinesterase (ChE)		
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on FIFRA Subdivision F Test Guidelines

6. Urinalysis

Urine was collected by catheterization pre-treatment (week -1) and during weeks 26 and 52 [not specified whether animals were fasted]. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

* Required for chronic studies based on FIFRA Subdivision F Test Guidelines.

7. Sacrifice and pathology

All animals were sacrificed by sodium pentobarbitone anesthesia and exsanguination at study termination, and were subjected to gross pathological examination. The CHECKED (X) tissues were collected and examined histologically. The [XX] organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)T
x	Stomach*	x	Lymph nodes*		
x	*Duodenum**	x	*Spleen**	x	*Pituitary**
x	Jejunum*	x	Thymus*	x	Eyes (optic n.)T
x	Ileum*				
x	Cecum*		UROGENITAL		GLANDULAR
x	Colon*	xx	Kidneys*+	xx	Adrenal gland*
x	Rectum*	x	Urinary bladder*		Lacrimal glandT
xx	Liver*'	xx	Testes*'	x	Mammary glandT
x	Gall bladder*	xx	Epididymides	xx	Parathyroids*"
x	Pancreas*	x	Prostate	xx	Thyroids*"
			Seminal vesicle		OTHER
	RESPIRATORY	x	Ovaries		Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
x	Lung*	x	Cervix	x	Skin
	Nose			x	All gross lesions
	Pharynx			x	and masses* (i.e., all
	Larynx				abnormal tissue)

* Required for subchronic studies based on Subdivision F Guidelines
' Organ weight required in subchronic and chronic studies.
" Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Shaking, subdued behavior, salivation at immediately after dosing, unsteady gait, resistance to dosing and/or inappetence were seen during weeks 13-14 at the high dose in both sexes. These observations prompted the reduction of the high dose from 1.0 mg/kg/day to 0.5 mg/kg/day starting at week 15 (animals were not dosed during week 14, nor during the last 1-3 days of week 13). Once the high dose was reduced, these signs of toxicity largely disappeared. Slight tremors and/or subdued behavior were thereafter seen during week 24 in 2/4 high-dose females, during week 45 in one low-dose male, and at week 40 in one high-dose male (this male also had involuntary twitching on the left thigh and side of the neck and held its left front forelimb off the floor; these effects were likely due to an infection, since the animal was discovered to have minimal chronic meningitis).

In 2/4 high-dose males, reddening and peeling of the scrotal skin with wet and/or dry sores were seen frequently during weeks 3-14, and much less frequently after the high dose was reduced. The mid- and low-dose males (3/4) also occasionally had reddening of the scrotal skin (± scabbing) and wet or dry sores. A large firm swelling was seen under the jaw on the ventral neck of one high-dose female, and appeared to be due to an infection resulting from a dog bite. Slight increases in the incidence or frequency of fluid feces, blood and/or mucus in the feces, regurgitation, and vomiting occurred in the high-dose dogs, primarily during the first 14 weeks of the study. The results are summarized in Table 2.

TABLE 2: Frequency (and incidence) of selected clinical observations in dogs given dicrotophos by capsule for 1 year

Parameter	Dose (mg/kg/day)			
	0	0.025	0.010	1.0/0.5[1]
Males				
Shaking	0	0	0	3 (2)
Subdued	0	0	0	5 (4)
Scrotum: dry sores, skin	1 (1)	6 (3)	6 (2)	11 (2)
peeling,	0	0	0	15 (2)
reddened	0	2 (2)	9 (2)	32 (2)
wet sores	2 (1)	1 (1)	1 (1)	3 (1)
Resists dosing	0	0	0	3 (3)
Salivation at dosing	81 (4)	52 (4)	106 (4)	53 (4)
Fluid feces[2] - individual- group	3 (2) 1	3 (2) 7	2 (1) 6	8 (3) 26
Regurgitation	0	0	0	6(4)
Vomiting	0	0	0	1 (1)
Females				
Shaking	0	0	0	8 (4)
Subdued	0	0	0	4 (3)
Tremors	0	0	0	2 (2)
Unsteady gait	0	0	0	1 (1)
Resists dosing	0	0	0	2 (2)
Salivation at dosing	8 (2)	8 (2)	104 (3)	70 (4)
Fluid feces[2] - individual	2 (1)	1 (1)	2 (2)	10 (4)
- group	1	2	4	47
Regurgitation	0	0	0	8 (4)
Vomiting	0	0	0	3 (2)

Data taken from pp. 47-55 and pp. 63-77, MRID 44328401.
[1]Dogs were given 1.0 mg/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day for weeks 15-52.
[2]Fecal consistency was assessed daily for up to 5 hours after dosing for individual animals, and subsequently on a group basis, if necessary.

2. Mortality

There were no animal deaths during the study.

B. BODY WEIGHT

Statistically significant decreases in weekly body weights relative to the controls occurred at weeks 5, 7, 8, 9, and 14 in high-dose males (≤ 8.4% decrease; $p < 0.05$). However, the overall (week 1-53) body weight gains for all groups of treated males were greater than of controls. The high-dose males thus overcame their initial weight loss for weeks 1-13 (before the high dose was lowered from 1.0 to 0.5 mg/kg/day), for which period they had gained only 48% as much weight as controls.

Weekly body weights of mid- and high-dose females were up to 10.3% lower than of controls throughout the study; statistical significance

($p < 0.05$) was achieved at the mid dose for weeks 6, 9, and 51-53, and at the high dose for weeks 6, 7, 9, 14, 47, and 49-53. The overall weight gain of mid- and high-dose females was 74-75% of control values, compared to being 84% and 41% of controls, respectively, for weeks 1-13. Low-dose females' weight gain was similar to that of the controls. The results are summarized in Table 3.

TABLE 3. Group mean body weights (kg) and body weight changes (kg) of dogs given dicrotophos in corn oil by capsule for 1 year[1]

Week of study	Dose (mg/kg/day)			
	0	0.025	0.1	0.5/1.0[2]
Males - Mean body weight				
1	10.60	10.30	10.33	10.60
9	12.26	11.96 (98)	12.01 (98)	11.58* (94)
14	12.69	12.40 (98)	12.19 (96)	11.62* (92)
26	13.30	13.08 (98)	13.06 (98)	12.98 (98)
39	13.18	13.48 (102)	13.48 (102)	13.56 (103)
53	13.04	13.25 (102)	13.39 (103)	13.79 (106)
Males - Mean body weight change				
weeks 1-14	2.09	2.10 (100)	1.86 (89)	1.02 (49)
weeks 1-53	2.44	2.95 (121)	3.06 (125)	3.19 (131)
Females - Mean body weight				
1	9.20	9.40	8.98	8.95
9	10.39	10.50 (101)	9.98* (96)	10.00* (96)
14	10.65	10.73 (101)	10.20 (96)	9.55* (90)
26	11.18	11.52 (103)	10.72 (96)	10.58 (95)
39	11.75	12.26 (104)	10.90 (93)	10.86 (92)
53	11.94	12.07 (101)	11.00* (92)	11.01* (92)
Females - Mean body weight change				
weeks 1-14	1.45	1.33 (92)	1.22 (84)	0.60 (41)
weeks 1-53	2.74	2.67 (97)	2.02 (74)	2.06 (75)

Data taken from Table 8, pp. 80-93, MRID 44328401.
Significantly different from control group: *$p \leq 0.05$; **$p \leq 0.01$.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]Dogs were given 1.0 mg/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day for weeks 15-52.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

There were no clearly treatment-related effects on food consumption in either sex. The overall (week 1-52) food consumption of the dicrotophos-treated males and females was

within 2% and 4%, respectively, of the controls (see Table 4). At week 13, the food consumption of the high-dose males and females was about 16% and 29% lower than of the control groups, respectively; this was largely due to one dog in each group that had about 51-67% lower consumption at week 13 (food consumption of these two dogs improved once the high dose was lowered).

2. Compound consumption

The compound in corn oil was administered in 6 mL gelatin capsules immediately prior to feeding each day before feeding, the amount given determined by the most recent body weight measurement (see Table 1).

3. Food efficiency

The food efficiencies of the three groups of dicrotophos-treated males were 23-32% higher than of the controls. The food efficiency of the low-dose females was similar to that of the controls, whereas of the mid- and high-dose females was 22-26% lower than of the controls. The effects on food efficiency were a reflection of the females' lower weight gain; food consumption of all groups was comparable to that of the controls. The results are summarized in Table 4.

TABLE 4. Food efficiency (and percent of controls) of dogs given dicrotophos by capsule for 1 year[1]

Relevant parameters	Dose (mg/kg/day)			
	0	0.025	0.10	1.0/0.5[2]
Males				
Total weight gain (kg)	2.44	2.95	3.06	3.19
Total food consump. (kg)	143.75	141.30	142.16	141.60
FOOD EFFICIENCY (%)	**1.70 (100)**	**2.09 (123)**	**2.15 (126)**	**2.25 (132)**
Females				
Total weight gain	2.74	2.67	2.02	2.06
Total food consump.(kg)	125.71	124.67	125.27	121.58
FOOD EFFICIENCY (%)	**2.18 (100)**	**2.14 (98)**	**1.61 (74)**	**1.69 (78)**

[1]Values were calculated by the reviewer using data from Tables 8 and 9, pp. 80-105, MRID 44328401.
[2]Dogs were given 1.0 mg/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day for weeks 15-52.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects were seen at any dose in either sex.

E. BLOOD PARAMETERS

1. Hematology

No hematology parameters were affected by compound treatment. The occasional parameters that were statistically significantly different from the controls ($p \leq 0.05$ or 0.01) were not toxicologically relevant because their values were within 9% of controls, they were unrelated to dose, and/or were transient.

2. Clinical chemistry

A number of parameter alterations ($p < 0.05$ or 0.01) were judged to be incidental to treatment because they were not dose-related, and/or were transient. In males, these included decreased plasma creatinine at the low dose at week 52, and decreased alkaline phosphatase, GGT, SGOT, SGPT, or plasma sodium at one or more doses at weeks 4 and/or 13. High-dose females had decreased cholesterol at weeks 4 and 26, and low and/or mid-dose females had increased plasma urea, creatinine, total bilirubin, SGPT and/or plasma chloride and decreased cholesterol, triglycerides, or creatine kinase at weeks 4 and/or 52 (and week 26 for creatine kinase).

The decreases seen in the plasma albumin, total protein, and calcium in high-dose dogs may have been treatment-related. Although the decreases were small ($\leq 9.8\%$) and not statistically significant for all time points, these parameters are inter-related, the decreases were generally dose-related and were seen in both sexes. These results are summarized in Table 5.

TABLE 5: Selected clinical chemistry changes in dogs given Dicrotophos for 1 year[1]

Parameter	Week	Dose (mg/kg/day)			
		0	0.025	0.10	1.0/0.5[2]
Males					
Albumin (g/L)	-1	29.5	28.5	29.8	28.5
	4	30.1	28.5	29.7	27.0** (90)
	13	30.7	30.4	29.5	27.7** (90)
	26	30.6	29.6	29.1	28.3* (92)
	52	30.3	30.3	30.1	28.5
Total protein (g/L)	-1	55.5	54.0	55.5	54.3
	4	56.7	54.5	57.7	53.4* (94)
	13	56.1	56.6	55.8	53.5* (95)
	26	59.2	58.7	58.7	56.9
	52	60.2	59.8	60.7	57.0
Calcium (mmol/L)	-1	2.91	2.80	2.82	2.80
	4	2.85	2.77	2.85	2.63** (92)
	13	2.75	2.75	2.73	2.60* (95)
	26	2.64	2.68	2.67	2.62
	52	2.62	2.68	2.64	2.64
Females					
Albumin (g/L)	-1	29.8	30.5	30.3	29.8
	4	30.7	30.5	29.4	28.7* (93)
	13	31.5	31.2	31.1	29.2** (93)
	26	30.3	30.4	29.6	27.5* (91)
	52	30.6	30.3	29.2	28.4
Total protein (g/L)	-1	55.5	56.3	56.8	54.3
	4	57.3	57.7	55.7	55.2
	13	57.1	56.8	57.4	55.4
	26	58.9	59.0	58.5	55.4
	52	60.4	58.0	57.0* (94)	57.5
Calcium (mmol/L)	-1	2.84	2.91	2.90	2.84
	4	2.93	2.87	2.79* (95)	2.76** (94)
	13	2.84	2.74* (96)	2.80	2.70** (95)
	26	2.72	2.68	2.71	2.59* (95)
	52	2.69	2.61	2.62	2.59* (96)

Data taken from pages 146-149 and 172-173, MRID 44328401. Significantly different from controls: *p ≤ 0.05; **p ≤ 0.01.

[1]The values presented for treatment weeks 4, 13, 26, and 52 are ※adjusted means※ obtained by covariate adjustment of the separate sex pre-treatment group means; the numbers in parentheses are the percent of controls, calculated by reviewer.

[2]Dogs were given 1.0 mg/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day for weeks 15-52.

F. CHOLINESTERASE ACTIVITY

The levels of plasma ChE for the low-, mid-, and high-dose dogs of both sexes were lower than of concurrent controls throughout the study ($p \leq 0.01$). The decreases were dose-related, and the adjusted mean ChE values as a percentage of controls ranged from 63-79% at the low dose, 55-61% at the mid-dose, and 36-52% at the high-dose. RBC ChE levels were significantly lowered throughout the study for male and female high-dose dogs (51-64% of controls; $p \leq 0.01$) and for the mid-dose males at weeks 13-52 (79-83% of controls; $p \leq 0.05$ or 0.01). The degree of plasma and RBC ChE inhibition was constant over time. There was also a dose-related inhibition of brain ChE in both sexes; statistical significance was achieved in high-dose males and females ($p \leq 0.01$) and in the mid-dose females ($p \leq 0.05$). Results are shown in Table 6.

TABLE 6. Cholinesterase (ChE) activity in dogs given dicrotophos for 1 year: unadjusted (± standard deviation) and adjusted (for pre-treatment values)[1]

	Dose (mg/kg/day)							
	0		0.025		0.10		1.0/0.5[2]	
Week	Mean±SD	Adj. mean	Mean±SD	Adj. mean	Mean±SD	Adj. mean	Mean±SD	Adj. mean
Males								
Plasma ChE (U/L)								
-1	1854±180		1950±254		1726±99		1609±213	
4	1896±235	1846	1454±162	1333**(72)	1085±81	1127**(61)	660±116	788**(43)
13	1777±300	1715	1473±207	1325**(77)	959±47	1011**(59)	566±137	722**(42)
26	1924±372	1849	1438±291	1256**(68)	946±94	1010**(55)	705±97	898**(49)
52	2168±418	2076	1532±332	1310**(63)	1067±54	1145**(55)	780±188	1015**(49)
RBC ChE (U/L)								
-1	2975±1163		2645±1045		2703±643		2150±271	
4	2883±908	2595	2383±938	2361	2333±692	2265	1268±175	1644**(63)
13	3373±872	3115	3048±755	3028	2530±676	2469**(79)	1623±295	1960**(63)
26	2808±962	2521	2455±797	2433	2150±750	2082* (83)	1248±296	1623**(64)
52	2928±1027	2608	2640±1159	2616	2128±519	2052* (79)	1220±219	1639**(63)
Brain ChE (IU/G)								
52	5.55 ±1.02		5.37 ±0.39 (97)		4.90 ±0.58 (88)		2.58** ±0.94 (46)	
Females								
Plasma ChE (U/L)								

-1			1681±132		1964±96		1703±275	
4					1192±151			
13	1787±490		1332±132				822±251	887**(49)
26	1806±447	1803		1416**(79)	1099±81	1046**(58)	571±111	611**(36)
52	1722±390	1720	1227±154	1278**(74)		1009**(59)	865±143	922**(52)
	1767±441	1764		1297**(74)	1198±72	1070**(61)	932±256	1010**(48)
	2143±649	2107	1224±132	1378**(65)		1246**(59)		
					1391±102			
			1281±168					
RBC ChE (U/L)								
-1			2855±684		3415±466		2560±640	
4								
13			2675±597		2985±351		1290±268	
26	2818±489							
52	2555±720	2610	3118±618	2708	3083±270	2692	1518±24	1495**(57)
	3300±401	3347		3146		2833		1692**(51)
	2655±422	2711	2603±832	2636	2770±397	2470	1478±160	1688**(62)
	3168±487	3237		2787		2855		1736**(54)
			2745±870		3225±382		1478±178	
Brain ChE (IU/G)								
52	5.96 ±0.77		5.60 ±0.48 (94)		4.85* ±1.13 (81)		3.49** ±0.37 (59)	

Data taken from Tables 15 and 16, pp. 185-190, MRID 44328401.
Significantly different from control group: *p ≤ 0.05, **p ≤ 0.01
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]Dogs were given 1.0 mg/kg/day for 13 weeks, after which they were not dosed for 7 days, and were then given 0.5 mg/kg/day from week 15-52.

G. URINALYSIS

There were no notable differences in urinalysis parameters between dicrotophos-treated and control animals.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

Organ weights of dicrotophos-treated males were similar to those of the controls (only absolute organ weights were given, and paired organ weights were combined because there were no differential effects between the two sides). Females had several organ weights that were statistically different from the controls ($p \leq 0.05$), but were not dose-related: there was a 13% decrease in kidney weight at the mid-dose and a 14-15% decrease in liver weight at the low and mid-dose.

2. Gross pathology

The incidence of all findings was similar in dicrotophos-treated and control groups.

3. Microscopic pathology

a) Non-neoplastic: There were no treatment-related findings. In both sexes, the incidence of a number of microscopic lesions differed in only one animal (1/4) between the dicrotophos-treated and control groups. Several lesions were present in 3/4 dicrotophos-treated animals vs. in 1/4 controls, and therefore a clear difference from the controls was not evident (minimal mixed inflammatory cell liver infiltration and minimal lung pneumonitis in high-dose females; alveolar hemorrhage and parathyroid cysts in high-dose males).

b) Neoplastic: No neoplastic lesions were reported in any animals.

III. REVIEWER'S DISCUSSION AND CONCLUSIONS

A. DISCUSSION

In this chronic toxicity study (MRID 44328401) dicrotophos was administered to 4 beagle dogs/sex/dose by capsule at doses of 0, 0.025, 0.1, or 1.0/0.5 mg/kg/day for 1 year (high-dose animals were given 1.0 mg dicrotophos/kg/day for 13 weeks, not dosed for 7 days, after which they were given 0.5 mg/kg/day from week 15-52).

No animals died during the study. Clinical signs were predominantly observed at the high dose in both sexes, their frequency greatly diminishing after the high dose was lowered from 1.0 to 0.5 mg/kg/day. Shaking and subdued behavior were seen in 2/4 or more high-dose animals/sex during weeks 13-14 and in one female during week 24; one female had unsteady gait at week 13.

This was consistent with the concurrently observed inhibition of plasma and erythrocyte cholinesterase activity, and suggestive of a neurological effect. The slight increases in the incidence/frequency of fluid feces, regurgitation, and vomiting in both sexes could also possibly have been neurologically-based. High-dose dogs resisted dosing (2-3 dogs/sex vs. 0 for controls) and males had reddening and peeling of the scrotal skin with sores (also seen occasionally in mid- and low-dose males) primarily during the first 14 study weeks. The relationship of the latter effects to treatment is unclear.

Weekly body weights of high-dose males were lower than those of controls (≤ 8.4%; $p < 0.05$) on several occasions between weeks 5 and 14, and the group gained only 48% as much weight during weeks 1-14. Once the high dose was lowered to 0.5 mg/kg/day, however, the dogs' weights improved such that their overall weight gain (weeks 1-52) was actually greater than that of controls (as was their food efficiency). The mid- and high-dose females had lower body weight gains than controls throughout the study (≤ 10.3%), statistically ($p < 0.05$) for weeks 6, 7, 9, 14, 47, and 49-53 at one or both doses. Their overall weight gain was 74-75% of controls, indicating that both the mid and high doses were toxic to females. Consistent with this, the week 1-51 food efficiency of the mid- and high-dose females was 22-26% lower than of controls (food consumption was unaffected). Body weight gains and food efficiencies were unaffected at the low dose.

Statistically significant differences from the controls ($p \leq 0.05$ or 0.01) were seen occasionally in various hematology and clinical chemistry parameters, but the changes were either not dose-related, transient, and/or too small to be considered toxicologically significant. The small (≤ 9.8%) decreases in plasma albumin, total protein, and calcium at several time points in high-dose males and females were generally dose-related and possibly related to dicrotophos treatment, but again were not considered toxicologically relevant.

There were no notable differences between dicrotophos-treated and control animals in urinalysis or ophthalmoscopy parameters, organ weights, or gross and microscopic pathology. No neoplastic lesions were reported.

Based on the 26% lower overall (week 1-52) body weight gain (and food efficiency) in mid-dose females, 0.1 mg/kg/day is concluded to be the LOEL under the conditions of this study; the corresponding NOEL is 0.025 mg/kg/day. The reviewer does not agree with the study author that the body weight changes at the mid-dose were not toxicologically significant and that only the high dose is the LOEL for both sexes.

The plasma, RBC, and brain ChE activities were inhibited by dicrotophos treatment throughout most of the study in both sexes of dogs. The degree of inhibition was clearly dependent on the dose but not on the exposure duration. At the high dose, the plasma, RBC, and brain ChE values were 36-64% of controls ($p \leq$

0.01) and were correlated with clinical signs (shaking and subdued behavior at week 13-14; possibly fluid feces and regurgitation) and were obviously both statistically and biologically significant. At the mid-dose, plasma ChE activity was 55-61% of controls ($p \leq 0.01$) in both sexes, RBC ChE activity was 83-91% of controls ($p \leq 0.05$ or 0.01 for males only), and brain ChE was 81-88% of controls ($p \leq 0.05$ for females only). Although the significance of the ChE inhibition at the mid-dose was somewhat equivocal because there were no visible signs of neurological impairment, the facts that the ChE inhibition was clearly dose-related in the plasma, erythrocytes, and brain; that the plasma inhibition was substantial in both sexes; and that the parameter most germane to toxicity, i.e. brain ChE inhibition, was inhibited by nearly 20% in females and was statistically significant, indicate that the ChE activity decreases at the mid-dose were toxicologically significant. **Therefore, the LOEL for cholinesterase inhibition is concluded to be 0.1 mg/kg/day and the NOEL is 0.025 mg/kg/day for both sexes.** The reviewer disagrees with the study author that the ChE activity inhibition at the mid-dose was not toxicologically significant, and that the high dose is the LOEL for both sexes.

B. STUDY DEFICIENCIES

There were no major deficiencies that would alter the classification of this study as acceptable. Minor deficiencies included (1) the inability to define precisely the value of the high dose since it was changed from 1.0 to 0.5 mg/kg/day after 13 weeks, (2) the lack of historic control data for most of the parameters examined (which would have helped establish the significance of the clinical chemistry and ChE activity changes), (3) the lack of details of the dose selection rationale, and (4) failure of the authors to calculate the food efficiencies, which were needed since body weight gain was affected.

DATA EVALUATION REPORT 013048

DICROTOPHOS

STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT (83-4)

Prepared for

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013048

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat
OPPTS 870.3800 [§83-4]

EPA DP BARCODE: D242036
EPA SUBMISSION BARCODE: S533927
EPA PESTICIDE CHEMICAL CODE: 035201

TEST MATERIAL (PURITY): Dicrotophos (87.65%)

SYNONYMS: (*E*)-2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate

CITATION: Moxon, M.E. (1997) Dicrotophos: Multigeneration study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Report No. CTL/P/5129, Study No. RR0689. EPA MRID Number 44296101. Unpublished.

SPONSOR: AMVAC Chemical Corporation

EXECUTIVE SUMMARY: In a multigeneration reproduction study (MRID # 44296101), Dicrotophos (87.65% a.i.; Batch No. 403001 B) was administered to groups of 26 male and 26 female Alpk:AP_fSD rats (from the Rodent Breeding Unit, Alderley Park) in the diet at concentrations of 0, 0.5, 5.0, or 10/15/25 ppm for two generations. Two litters were produced in the first generation and one litter was produced in the second generation. Premating doses for the adult F_0 males were 0.5, 0.49, and 2.53 mg/kg/day and for the F_0 females were 0.5, 0.53, and 2.79 mg/kg/day, respectively. Premating doses for the adult F_1 males were 0.5, 0.56, and 1.15 mg/kg/day and for the F_1 females were 0.6, 0.59, and 1.25 mg/kg/day, respectively. **Due to a high mortality in the F_{1a} pups in the 25 ppm group, the dietary concentration of dicrotophos was lowered to 10 ppm for four dams from lactation day 8 through termination of the litter.** During mating, gestation, and lactation of the F_{1b} litters, high-dose animals were given diets containing 15 ppm. The control, low-, and mid-dose F_1 pups were weaned onto the same diets as their parents. The F_1 pups in the high dose group were weaned onto diets containing 10 ppm dicrotophos. Animals were given test or control diet for 10 weeks then mated within the same dose group. All

animals were exposed to test material either in the diet or during lactation until sacrifice.

Clinical signs of toxicity were observed during premating weeks 2-5 as involuntary shaking of the limbs in 5/26 F_0 males ($p<0.05$) and 11/26 F_0 females ($p<0.01$) given 25 ppm. No dose- or treatment-related clinical signs of toxicity were observed in the parental F_0 or F_1 animals given diets containing less than 25 ppm. No dose- or treatment-related gross abnormalities were observed in the F_0 or F_1 adults at necropsy; histopathological evaluations were not performed.

Mid and high-dose F_0 males had significantly lower mean body weights as compared to controls ($p<0.05$ or 0.01). Food consumption was significantly ($p<0.01$) reduced in the high-dose F_0 males as compared with controls during premating. Food utilization was significantly ($p<0.05$ or 0.01) lower in the mid- and high-dose F_0 males as compared with the controls.

Mid and high-dose F_0 females had significantly lower mean body weights than the controls during premating ($p<0.05$ or 0.01). Food consumption by the high-dose F_0 females was significantly ($p<0.01$) less at the beginning of premating, but was significantly ($p<0.05$ or 0.01) greater than the controls during latter premating. High-dose F_0 females also had significantly ($p<0.01$) lower food utilization at the beginning of premating and then significantly ($p<0.05$) higher food utilization during latter premating weeks as compared with the controls.

Mean body weights of the mid- and high-dose F_1 males were significantly ($p<0.01$) lower than the controls during week 1 of the premating period. Food consumption by the F_1 males was similar between the treated and control groups throughout the premating period. Food utilization was significantly ($p<0.05$) reduced in the mid- and high-dose groups during early premating.

Mean body weights of all treated F_1 female groups were significantly ($p<0.05$ or 0.01) lower than the controls during week 1 of the premating period. No statistically significant differences in body weights occurred during the remainder of the premating period. In the high-dose F_1 females, food consumption was significantly ($p<0.05$ or 0.01) greater than the controls during most of the premating period and food utilization was significantly ($p<0.05$) greater during weeks 8-10.

The Systemic Toxicity NOEL is 0.5 ppm and the Systemic Toxicity LOEL is 5.0 ppm based on lower body weights in the F_0 and F_1 males and females and reduced food utilization in F_0 males and females and F_1 males.
High-dose F_0 females had significantly ($p<0.05$ or 0.01) lower body

weights than the controls during gestation of litter A. No differences in body weights occurred between treated and control groups during gestation of litter B. During lactation of litter A, maternal body weights of the mid- and high-dose animals were significantly ($p<0.05$ or 0.01) lower than the controls. During lactation of litter B, maternal body weights of the mid- and high-dose animals were significantly ($p<0.05$ or 0.01) lower than the controls. Food consumption by the treated F_0 groups was greater than the controls during gestation of both the A and B litters with occasional statistical significance in the mid- and high-dose groups. In contrast, food consumption by the high-dose F_0 females was significantly ($p<0.05$ or 0.01) less than the controls throughout lactation of both litters (68-80%).

No treatment-related differences in body weights were observed in the F_1 females during gestation. However, all treated F_1 groups had significantly ($p<0.05$ or 0.01) lower maternal body weights as compared with the controls during lactation. Food consumption was significantly ($p<0.01$) greater than the controls by the high-dose F_1 females throughout gestation and once by the mid-dose F_1 females. In contrast, food consumption during lactation was occasionally significantly ($p<0.05$ or 0.01) less than the controls in all treated dams.

For the control, low-, mid-, and high-dose F_0 adults in production of litter A, the proportion of successful matings was 70%, 67.9%, 85.7%, and 33.3% ($p<0.01$), respectively; the per cent of live born pups was 97%, 99.2%, 98%, and 90.4% ($p<0.05$), respectively; and whole litter losses were 4/21, 0/19, 5/24, and 8/12 ($p<0.05$), respectively. Whole litter losses during production of litter B were 4/22, 2/18, 6/23, and 10/19 ($p<0.05$), respectively. Whole litter losses by the F_1 generation were 0/20, 1/23, 2/21, and 6/23 ($p<0.05$), for the control, low-, mid-, and high-dose groups, respectively.

During lactation, no dose- or treatment-related clinical signs of toxicity or differences in pup body weights were observed in the offspring of either generation. For both litters produced by the F_0 animals, there was a significant ($p<0.05$ or 0.01) decrease in the number of F_1 pups/litter in the high-dose group after day 1 of lactation. Pup deaths in the high-dose group resulted in significantly lower lactation indices of 56.6% ($p<0.01$) vs 88.0% for the controls for litter A and 75.4% ($p<0.05$) vs 92.7% for the controls for litter B. The number of F_2 pups/litter was significantly ($p<0.01$) decreased due to treatment in the mid- and high-dose groups as compared to controls after lactation day 1. Lactation indices for the control, low-, mid-, and high-dose F_2 litters were 96.8%, 94.1%, 83.8% ($p<0.05$), and 75.6% ($p<0.01$), respectively.

The Reproductive Toxicity NOEL is 0.5 ppm and the Reproductive Toxicity LOEL is 5.0 ppm based on a reduced number of F_2 pups/litter during lactation.

This study is classified as Acceptable-Guideline and satisfies the guideline requirement for a reproduction study (§83-4) in rats.

COMPLIANCE: A signed and dated STATEMENT OF DATA CONFIDENTIALITY CLAIM (no confidentiality claims were made), STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION, EPA FLAGGING CRITERIA (according to the study authors, the study neither meets nor exceeds any of the applicable criteria), and a QUALITY ASSURANCE STATEMENT were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Dicrotophos

Description: clear, brown liquid
Batch No.: 403001 B
Purity: 87.65% a.i.
Stability of compound: responsibility of the sponsor; not reported
Storage: refrigerated and under an inert atmosphere
CAS No.: 141-66-2
Structure:

2. Vehicle

CT1 diet was used as the vehicle.

3. Test animals

Species: Rat
Strain: Alpk:AP$_f$SD
Age and weight at start of study: 36 days; males: 109-159 g; females: 93-151 g
Source: Rodent Breeding Unit, Alderley Park
Housing: Rats were housed in litters initially then 2/cage, sexes separate, during the premating period. Females were housed individually during gestation and lactation.
Diet: CT1 diet supplied by Special Diet Services Limited, Stepfield, Witham, Essex, U.K., was available *ad libitum*.
Water: Domestic mains water was available *ad libitum*.
Environmental conditions:
Temperature: 21 ± 2°C
Humidity: 40-70%
Air Changes: 25-30/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 2 weeks

B. PROCEDURES AND STUDY DESIGN

The protocol with amendments was provided (APPENDIX I of the study report).

1. In life dates

Start: May 8, 1995; end: April 8, 1996

2. Mating procedure

For mating, F_0 animals of the same dose group were paired one male to one female for up to 21 days. If evidence of mating was not detected after this time, the female was mated with a different male following a 3 day separation period. Different pairings within treatment groups were used to produce the F_{1b} litters and remates were not allowed for production of the B litters. The F_1 adults were selected from the F_{1b} litters at lactation day 29. After 10 weeks on test diets, the F_1 adults were mated to produce the F_{2a} litters. Sibling matings were avoided and remates were not allowed for production of the F_2 litters. Day 1 of gestation was designated as the day sperm were observed in a vaginal smear.

3. Study schedule

F_0 males and females were fed control or treated diets for 10 weeks prior to mating and continued throughout mating, gestation, and lactation of their litters. Due to a high mortality in the F_{1a} pups in the 25 ppm group, the dietary concentration of dicrotophos was lowered to 10 ppm for four dams from lactation day 8 through termination of the litter on lactation day 29. Because of the low number of F_{1a} surviving pups, the F_0 adults were mated to produce the F_{1b} litters; during mating, gestation, and lactation of the F_{1b} litters, high-dose males and females were given diets containing 15 ppm. The control, low-, and mid-dose F_1 pups were weaned onto the same diets as their parents. The F_1 pups in the high dose group were weaned onto diets containing 10 ppm dicrotophos. After weaning, the F_1 animals were maintained on treatment for 10 weeks prior to mating.

4. Animal assignment

Because whole litters were received from the supplier, animals were randomly allocated to treatment groups by litter to assure that littermates were evenly

distributed across groups. Cards were numbered corresponding to the number of litters of each sex, shuffled, and one card placed on each cage of litters to give the order of allocation of the litters to the replicates. The sequence of groups within each replicate was determined by a computer-generated random number sequence. Allocation from within litters was done by numbering cards 1-4 to correspond to the number of groups in a replicate. The cards were shuffled and a card was picked at the same time a rat was picked out of the first litter at random. This procedure was carried out until all the 'male' cages contained one rat and then was repeated to allocate the second male to each cage. The same procedure was used to allocate females. The adults of the second generation were selected from animals in the B litter of the first generation on lactation day 29. Animal assignment is given in Table 1.

TABLE 1. Animal assignment

Dose Group	Conc. in Diet[a] (ppm)	No. of parental animals per group			
		F_0 Generation		F_1 Generation	
		Male	Female	Male	Female
0 (Control)	0	26	26	26	26
1 (Low)	0.5	26	26	26	26
2 (Mid)	5	26	26	26	26
4 (High)	25/15/10[b]	26	26	26	26

Data taken from p. 20, MRID 44296101.

[a]Diets were administered from the beginning of the study until the animals were sacrificed.

[b]Due to a high level of toxicity to the F_{1a} pups, dietary concentrations were reduced as described in Section I.B.3.

5. Dose selection rationale

The dietary levels were based on the results of a two year feeding study, in the same strain of rat (CTL study number PR0986), ongoing at the testing facility. Details were not provided with the current report.

6. Diet preparation and analysis

Experimental diets were prepared in 30 kg batches by

direct addition of the test substance to the diet. The test substance was ground with a small quantity of milled diet in a mortar using a pestle. This premix was ground using an automatic pestle and mortar for 15 minutes at minimum speed and then mixed using a Kenwood Chef mixer for 15 minutes at minimum speed. The premix was added to the remainder of the diet and mixed in a Pharma Matrix Blender Model PMA 100S (TK Fielder) for 6 minutes at speed 3. The diets were either used immediately or stored at -20°C. The diets were utilized within 2 days of the preparation date or time of removal from the freezer. The concentration of the test article was measured in samples from all dietary levels at intervals throughout the study. Samples were taken from the top, middle, and bottom of the lowest and highest dietary levels for homogeneity analysis. The stability of the test article in the diet was determined for dietary levels of 0.5 and 50 ppm, at room temperature and at -20°C; stability data were obtained from concurrent long term studies.

Results -

Homogeneity analysis: Samples from the top, middle, and bottom of the 0.5 and 25 ppm diets ranged were 112-115% and 96-102% of nominal, respectively.

Stability analysis: After 1, 2, and 4 days at room temperature, the concentrations of test article in the 0.5 ppm diet were 88.5, 80.8, and 69.2%, respectively of the initial concentration and the concentrations in the 50 ppm diet were 88.8, 93.1, and 79.9%, respectively. Following storage at -20°C, the test article concentration in the 0.5 ppm diet was 98.1% of the initial concentration after 51 days and in the 50 ppm diet was 96.9% of the initial concentration after 35 days.

Concentration analysis: Absence of test article was confirmed in the control diet. Concentrations of the 0.5, 5, 10, 15, and 25 ppm diets ranged from 88-120%, 78.4-97.2%, 89-100%, 100-102.7%, and 98.4-102.4%, respectively, of nominal. For the 0.5 ppm diet, all measurements were within 12% of nominal with the exception of one sample. Three of the seven samples from the 5 ppm diet were below 15% of nominal.

Results of the dietary analyses indicated that the test article was adequately mixed in the diet and that administered concentrations were within an acceptable range with the exception of the 5 ppm diet which was occasionally below 15% of nominal.

C. OBSERVATIONS

1. Parental animals

All animals were observed daily for changes in clinical condition and behavior, and a detailed examination was made at the same time that the rats were weighed. Body weights and food consumption of the F_0 and F_1 adults were recorded weekly throughout the study. Food consumption was calculated and reported as g/animal/week. After mating, the males were weighed every two weeks until termination. Females were weighed on days 1, 8, 15, and 22 of gestation and on days 1, 5, 11, 15, 22, and 29 of lactation. Reproductive function tests were not performed (not required at this time); however, reproductive and offspring viability indices were calculated.

2. Litter observations

Litter observations were made as shown in Table 2. All females were allowed to litter normally and the number of live and dead pups was determined within 24 hours. Litters were examined for dead and moribund pups at least once daily and any such pups were subjected to a macroscopic examination. A count of live and dead pups was made, individual body weights were recorded, and the sex of each pup was recorded on lactation days 1, 5, 8, 11, 15, 22, and 29. Standardization of litters was not performed.

TABLE 2: F_1 and F_2 litter observations

Observation	Lactation day						
	Day 1	Day 5	Day 8	Day 11	Day 15	Day 22	Day 29
Dead/moribund pups	Daily						
No. live pups	X	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X	X
Sex of each pup	X	X	X	X	X	X	X
No. dead pups	X	X	X	X	X	X	X

3. Postmortem observations

1) **Parental animals**: Rats were killed by exsanguination while under halothane Ph. Eur. anesthesia. The F_0 adults were sacrificed and necropsied soon after weaning of the F_{1b} litters. F_1 adults were sacrificed and necropsied soon after weaning of the F_2 litters. F_0 and F_1 parental animals were subjected to gross necropsy consisting of external and internal examinations. The presence or absence of implantation sites in the uterus was recorded for females. The following tissues (X) were preserved and processed for microscopic examination. Tissues from suspect infertile adult animals only were subject to histopathological evaluations. Epididymides and testes were weighed from adult F_0 and F_1 males selected for necropsy.

X	Testes	X	Ovaries
X	Epididymides	X	Uterus
X	Prostate	X	Vagina
X	Seminal vesicle	X	Cervix
	including	X	Mammary gland (adult
	coagulating gland		females only)
X	Gross lesions	X	Pituitary

2) **Offspring**: F_{1a} pups were killed by over exposure to carbon dioxide on lactation day 29 and discarded. F_{1b} pups not selected to be F_1 adults and all F_2 pups were killed on lactation day 29. Pups killed moribund or found dead through lactation day 18 were subjected to gross necropsy and discarded. Pups killed moribund or found dead after lactation day 18 were subjected to gross necropsy and tissues submitted for microscopic examination. At termination, 5 male and 5 female F_{1b} pups from the control, low-, and mid-dose groups, one male F_{1b} pup from the high-dose group, and 10 male and female F_2 pups per group were randomly selected for full necropsy. An addition, two pups per sex per litter (where possible) and clinically abnormal pups were examined grossly. Epididymides and testes were weighed from male pups selected for necropsy.

D. DATA ANALYSIS

1. Statistical analyses

Males and females were analyzed separately. Adult and pup body weight data were analyzed by Analysis of

Covariance (ANCOVA) on initial body weight. Food consumption and food utilization during premating were analyzed by Analysis of Variance (ANOVA). Male organ weights were considered by ANOVA and ANCOVA on final body weight. Litter size, mean gestation length, mean precoital interval, mean pup weight on lactation day 1, and total litter weight were analyzed by ANOVA. Proportion data were analyzed by Fisher's Exact test. Percentages of pups born live and percentages of pups surviving were analyzed by ANOVA following double arcsine transformation of Freeman and Tukey. Analyses of lactation weight, lactation food consumption, litter size, and pup survival to day 22 excluded data from whole litter losses.

2. **Indices**

Reproductive indices: The day of birth was designated as day 1 of lactation. Males and females were considered fertile if mating produced at least one live pup on day 1. The precoital interval and length of gestation were recorded. Reproductive indices were not calculated by the study authors.

Offspring viability indices: The following indices were calculated:

Live birth index = (No. liveborn pups/No. live + dead pups born) x 100

Lactation index = (No. live pups at lactation day 22/No. live pups on day 1) x 100

3. **Historical control data** for F_{2a} litter sizes were included.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

All F_0 males survived to termination. The incidence of intercurrent deaths in the F_0 females was 1, 2, 0, and 1 in the control, 0.5, 5, and 25 ppm groups, respectively. The control female and a low-dose female were killed moribund during weeks 29 and on GD 4 (first mating), respectively. Another low-dose female and the high-dose female were killed during weeks 14 and 24, respectively, with dystocia.

The incidence of intercurrent deaths in the F_1 adults was 1, 1, 1, and 1 in the control, 0.5, 5, and 10 ppm groups, respectively. A control female was found dead during week 4, a low-dose female was killed during week 15 with dystocia, and a mid-dose male was killed in poor condition (swollen limbs, piloerection, swollen penis, dry sores, desquamation, scaly tail) during week 6. In addition, a high-dose female was killed on GD 25 having failed to litter.

None of these deaths were considered due to treatment with dicrotophos.

Transient clinical signs of toxicity were limited to involuntary shaking of the limbs in 5/26 F_0 males ($p< 0.05$) and 11/26 F_0 females ($p<0.01$) given 25 ppm. This was observed during weeks 2-5 of the premating period. Involuntary shaking was not observed in any animals of either generation receiving diets containing less than 25 ppm.

2. Body weight and food consumption

a. Premating

Body weights were analyzed using the adjusted mean weights which were based on initial body weights and accounted for weight gains at each interval. Body weight, food consumption, and food utilization data for the F_0 males are given in Table 3. High-dose males had significantly ($p<0.01$) lower adjusted mean body weights as compared to controls during weeks 2-11. At week 2, body weights of the high-dose males were 84% and body weight gains were 45% of the control values. However, final body weights were 95% with overall weight gains 92% of the control level. Mid-dose males had significantly ($p<0.05$ or 0.01) lower body weights than the controls during weeks 2-5 (about 97%), and showed recovery during the remainder of the premating period with overall weight gains 98% of the control level. Food consumption was significantly ($p<0.01$) reduced in the high-dose males as compared with controls during weeks 1-3 of the premating period. Food utilization was significantly ($p<0.05$ or 0.01) lower in the mid- and high-dose males as compared with the controls during weeks 1-4 and overall.

Body weight, food consumption, and food utilization data for the F_0 females are given in Table 4. High-dose females had significantly ($p<0.01$) lower adjusted mean body weights than the controls during

weeks 2-5 of the premating period and mid-dose females had significantly ($p<0.05$) lower body weights during premating week 2. At week 2, body weights of the high-dose females were 88% and body weight gains were 42% of the control values . However, these groups showed recovery during the second half of the premating period with both final premating body weights and overall premating weight gains 100-104% of the controls. Food consumption by the high-dose females was significantly ($p<0.01$) less than the controls during weeks 1 and 2, but was significantly ($p<0.05$ or 0.01) greater than the controls during weeks 4-10 of the premating period. Food consumption by the low- and mid-dose females was occasionally less than or greater than the controls. Although the overall food utilization was not different between any treated groups and the control group, high-dose females had significantly ($p<0.01$) lower food utilization during weeks 1-4 and significantly ($p<0.05$) higher food utilization during weeks 5-7 as compared with the controls.

TABLE 3. F_0 males: Adjusted mean body weights, food consumption, and food utilization during the premating period				
Week of Treatment	Treatment Group			
	0 ppm	0.5 ppm	5.0 ppm	25 ppm
Body weight (g)				
1	139.5	138.8	139.2	139.0
2	193.2	191.5	188.6**	162.9**
5	338.3	332.1	328.9*	306.9**
11 (end of premating)	473.8	466.9	467.6	449.9**
Overall weight gain weeks 1-11[a]	334.3	327.3 (98)	328.6 (98)	310.5 (93)
Food consumption prior to mating (g/rat/day)				
1	27.3	27.3	27.3	22.8**
2	29.6	29.2	29.5	25.8**
5	31.8	30.6	32.0	32.2
10	28.3	27.7	28.8	29.0
Food utilization weeks 1-4 (g growth/100 g food)	23.59	23.21	22.64**	21.68**
Food utilization weeks 1-10 (g growth/100 g food)	15.73	15.80	15.36*	14.95**

Data taken from Tables 8, 14, and 16, pp. 95-96, 109, and 113, respectively, MRID 44296101.
[a]Body weight gains calculated by reviewer from absolute body weights for weeks 1 and 11; number in parentheses is per cent of control.
Significantly different from control; *p<0.05; **p<0.01.

TABLE 4. F_0 females: Adjusted mean body weights, food consumption, and food utilization during the premating period

Week of treatment	Treatment group			
	0 ppm	0.5 ppm	5.0 ppm	25 ppm
Body weight (g)				
1	122.6	119.6	121.7	120.9
2	151.4	151.4	147.3*	132.9**
5	212.0	210.9	208.8	203.3**
11 (end of premating)	264.1	258.6	264.7	269.6
Overall weight gain weeks 1-11[a]	142.9	137.4 (96)	143.6 (100)	148.4 (104)
Food consumption (g/rat/day)				
1	21.4	21.7	21.6	18.8**
2	22.0	21.1*	22.0	19.9**
5	22.2	21.4	22.3	24.2**
10	19.0	18.4	19.2	20.7**
Food utilization weeks 1-4 (g growth/100 g food)	14.81	14.91	14.20	13.90**
Food utilization weeks 1-10 (g growth/100 g food)	9.53	9.47	9.48	9.53

Data taken from Tables 8, 14, and 16, pp. 97-98, 110, and 113, respectively, MRID 44296101.
[a]Body weight gains calculated by reviewer from absolute body weights for weeks 1 and 11; number in parentheses is per cent of control.
Significantly different from control, *$p<0.05$, **$p<0.01$.

Body weight, food consumption, and food utilization data for the adult F_1 males are given in Table 5. Mean body weights of the mid- and high-dose groups were significantly ($p<0.01$) lower than the controls during week 1 of the premating period (90% and 88%, respectively of the controls). However, recovery was apparent during the remainder of the premating period with overall weight gains of both groups 97% of the control group level. Food consumption was similar between the treated and control groups throughout the premating period. Overall food utilization was significantly ($p<0.05$) reduced in the high-dose group due to a significant ($p<0.01$) reduction during weeks

1-4. Food utilization by the mid-dose group was also significantly ($p<0.01$) lower than the controls during weeks 1-4.

Body weight, food consumption, and food utilization data for the adult F_1 females are given in Table 6. Mean body weights of all treated groups were significantly ($p<0.05$ or 0.01) lower than the controls during week 1 of the premating period (89-94%). No statistically significant differences in body weights occurred between the treated and control groups during the remainder of the premating period. Overall body weight gains of the low-, mid-, and high-dose groups were 97%, 103%, and 103%, respectively of the control level. In the high-dose group, food consumption was significantly ($p< 0.05$ or 0.01) greater than the controls during most of the premating period and food utilization was significantly ($p<0.05$) greater during weeks 8-10.

TABLE 5. F_1 males: Adjusted mean body weights, food consumption, and food utilization during the premating period				
Week of treatment	Treatment group			
	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Body weight (g)				
1	84.9	80.7	76.4**	75.1**
2	129.1	131.1	130.4	130.9
5	294.1	292.8	289.1	288.5
11 (end of premating)	451.1	449.2	447.2	447.1
Overall weight gain weeks 1-11[a]	375.9	371.1 (99)	365.1 (97)	364.9 (97)
Food consumption prior to mating (g/rat/day)				
1	20.2	19.6	19.3	20.4
2	26.2	26.4	26.1	26.5
5	31.1	30.8	31.1	31.3
10	30.8	30.7	30.2	30.8
Food utilization weeks 1-4 (g growth/100 g food)	29.56	29.24	28.40**	27.43**
Food utilization weeks 1-10 (g growth/100 g food)	18.50	18.33	18.18	17.83*

[a]Body weight gains calculated by reviewer from absolute body weights for weeks 1 and 11; number in parentheses is per cent of control.
Data taken from Tables 9, 15, and 17, pp. 99-100, 111, and 114, respectively, MRID 44296101.
Significantly different from control: **p<0.01.

TABLE 6. F_1 females: Adjusted mean body weights, food consumption, and food utilization during the premating period

Week of treatment	Treatment group			
	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Body weight (g)				
1	78.0	73.2*	72.0*	69.2**
2	112.0	114.2	113.1	111.1
5	188.6	189.1	192.3	192.5
11 (end of premating)	257.0	250.3	259.8	264.5
Overall weight gain weeks 1-11[a]	182.2	177.2 (97)	187.1 (103)	192.6 (106)
Food consumption (g/rat/day)				
1	17.7	17.3	17.2	18.1
2	21.1	21.7	22.3*	23.0**
5	21.2	20.5	21.5	22.5*
10	20.5	19.1**	20.6	21.8**
Food utilization weeks 1-4 (g growth/100 g food)	20.14	20.47	20.30	19.59
Food utilization weeks 1-10 (g growth/100 g food)	12.62	12.60	12.66	12.55

[a]Body weight gains calculated by reviewer from absolute body weights for weeks 1 and 11; number in parentheses is per cent of control.
Data taken from Tables 9, 15, and 17, pp. 101-102, 112, and 114, respectively, MRID 44296101.
Significantly different from control, *p<0.05, **p<0.01.

b. Gestation and lactation

Body weights, body weight gains, and food consumption data for the F_0 adult females during gestation and lactation are given in Table 7. Due to few litters produced and animals with whole litter loss in the high-dose group, only 4 F_0 dams were included in the analysis for litter A and only 9 for litter B. High-dose F_0 females had significantly ($p<0.05$ or 0.01) lower body weights than the controls on GD 8, 15, and 22 during gestation of litter A with final body weights 94% of the controls. No differences in

body weights occurred between treated and control groups during gestation of litter B. During lactation of litter A, maternal body weights of the high-dose animals were significantly ($p<0.05$ or 0.01) lower than the controls on days 8, 11, 15, and 22 and mid-dose dams had significantly ($p<0.01$) lower body weights on days 15 and 22. During lactation of litter B, maternal body weights of the high-dose animals were significantly ($p<0.01$) lower than the controls on days 5, 8, 11, 15, 22, and 29 and mid-dose dams had significantly ($p<0.05$ or 0.01) lower body weights on days 5, 8, and 15. Food consumption by the treated groups was greater than the controls during gestation of both the A and B litters with statistical significance reached occasionally in the mid- and high-dose groups. In contrast, food consumption by the high-dose group was significantly ($p<0.05$ or 0.01) less than the controls throughout lactation of both litters (68-80%) except for week 4 of lactation of litter A (n.s., 82%).

Body weights, body weight gains, and food consumption data for the F_1 adult females during gestation and lactation are given in Table 8. No treatment-related differences in body weights were observed in the F_1 females during gestation. However, all treated groups had significantly ($p<0.05$ or 0.01) lower maternal body weights as compared with the controls during lactation: mid- and low-dose, days 8-29; high-dose, days 5-29. Maternal body weights at the end of lactation for the low-, mid-, and high-dose dams were 97%, 94%, and 96% of the control level. Food consumption was significantly ($p<0.01$) greater than the controls by the high-dose group throughout gestation and by the mid-dose group during week 3. In contrast, food consumption during lactation was significantly ($p<0.05$ or 0.01) less than the controls by the low-dose dams during week 2 (90%), by the mid-dose dams during weeks 2-4 (80-88%), and by the high-dose dams during weeks 1-4 (74-83%).

TABLE 7. F_0 Females: selected mean body weights during gestation and lactation and food consumption values during lactation

Observation	Treatment group			
	0 ppm	0.5 ppm	5.0 ppm	25 ppm
Litter A				
Adjusted mean body weight (g)				
Day 1 of gestation	266.6	258.0	264.3	273.3
Day 22 of gestation	390.9	393.9	394.9	369.2**
Day 1 of lactation	301.2	293.3	301.6	290.8
Day 22 of lactation	341.2	340.0	328.2**	324.5*
Day 29 of lactation	326.3	325.6	321.2	318.8
Food consumption during lactation (g/rat/day)				
Week 1 of lactation	35.4	35.1	36.0	27.4**
Week 3 of lactation	76.8	76.4	73.2	57.5**
Week 4 of lactation	133.7	134.2	133.9	109.1
Litter B				
	0 ppm	0.5 ppm	5.0 ppm	15 ppm
Adjusted mean body weight (g)				
Day 1 of gestation	315.6	310.9	316.0	304.3
Day 22 of gestation	435.9	441.0	442.4	436.2
Day 1 of lactation	351.9	344.7	351.4	338.3
Day 22 of lactation	366.3	372.2	361.4	342.0**
Day 29 of lactation	353.7	357.0	346.1	337.0**
Food consumption during lactation (g/rat/day)				
Week 1 of lactation	41.5	40.5	37.8	32.2**
Week 3 of lactation	83.2	83.8	76.8	56.2**
Week 4 of lactation	134.8	138.1	124.4	91.0**

Data taken from Tables 10, 12, and 20, pp. 103-104, 106-107, and 118-119, respectively, MRID 44296101.
Significantly different from control, *p<0.05; **p<0.01.

TABLE 8. F_1 females: selected mean body weights during gestation and lactation and food consumption values during lactation				
Observation	Treatment group			
	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Adjusted mean body weight (g)				
Day 1 of gestation	264.4	259.0	263.6	266.1
Day 22 of gestation	392.6	383.1*	388.3	388.4
Day 1 of lactation	290.8	291.8	299.8	298.5
Day 22 of lactation	329.7	319.7**	311.1**	308.6**
Day 29 of lactation	316.3	306.3**	297.0**	302.7**
Food consumption during lactation (g/rat/day)				
Week 1 of lactation	39.1	36.9	36.0	32.6**
Week 3 of lactation	80.6	75.6	71.1*	64.7**
Week 4 of lactation	152.5	137.6	121.4**	113.6**

Data taken from Tables 11, 13, and 21, pp. 105, 108, and 120, respectively, MRID 44296101.
Significantly different from control, *p<0.05; **p<0.01.

3. Test substance intake

Test substance intake is shown in Table 9. Doses were higher early in the premating period of both generations and declined as the animals grew. Also, during week 4 of lactation, the doses increased reflecting the increased consumption of diet by both dam and litter.

TABLE 9: TEST SUBSTANCE INTAKE IN RATS FED DICROTOPHOS FOR TWO GENERATIONS (mg/kg/day)			
Sex - Study interval	Concentration in diet		
	0.5 ppm	5 ppm	10/15/25ppm
F_0 Generation			
Males -Premating	0.05	0.49	2.53[a]
Females - Premating	0.05	0.53	2.79[a]
Females - Gestation of F_{1a}	0.04	0.43	2.15[a]
Females - Lactation of F_{1a}	0.11	1.15	2.22[a]/[b]
Females - Gestation of F_{1b}	0.04	0.42	1.29[b]
Females - Lactation of F_{1b}	0.11	1.02	2.46[b]
F_1 Generation			
Males -Premating	0.05	0.56	1.15[c]
Females - Premating	0.06	0.59	1.25[c]
Females - Gestation	0.04	0.44	0.89[c]
Females - Lactation	0.12	1.13	2.08[c]

[a] = 25 ppm, [b] = 15 ppm, [c] = 10 ppm
Data taken from Appendix G, pp. 219-222, MRID 44296101.

4. Reproductive function

Reproductive function tests for estrous cycle length, sperm measures, or sexual maturation were not conducted (not required at this time.

5. Reproductive performance

The reproductive performances of the F_0 and F_1 animals are summarized in Tables 10 and 11, respectively. For the F_0 adults in production of litter A, significant ($p < 0.05$ or 0.01) reductions in the proportion of successful matings (33.3% vs 70%) and per cent of live born pups (90.4% vs 97%) were observed in the high-dose group as compared to the controls. Because of the excessive loss of the F_{1a} pups in the 25 ppm group, the dietary concentration of dicrotophos was lowered to 10 ppm for the four remaining dams from lactation day 8 through termination of the litter. A second litter was

then produced during which time the high-dose animals were given diets containing 15 ppm. No effects on reproductive performance were observed in the F_0 animals during production of litter B or in the F_1 animals during production of the F_2 litters.

TABLE 10. F_0 generation reproductive performance

Observation	Dietary concentration			
	0 ppm	0.5 ppm	5.0 ppm	10/15/25ppm
Litter A				
Mean precoital interval (days)	3.14	3.30	3.07	3.29
Proportion of successful matings (%)[a]	21/30 (70.0)	19/28 (67.9)	24/28 (85.7)	12/36** (33.3)
Mean gestation length (days)	22.5	22.6	22.6	22.7
Number of litters	17	19	19	4
Total number of pups born	245	231	302	125
Number of live born pups (%)	237 (97)	229 (99.2)	296 (98)	111 * (90.4)
Per cent male pups	48	53	44	46
Litter B				
Mean precoital interval (days)	3.50	3.18	2.38	3.92
Proportion of successful matings (%)	22/26 (84.6)	18/24 (75.0)	23/26 (88.5)	19/26 (73.1)
Mean gestation length (days)	22.7	22.5	22.6	22.9
Number of litters	18	16	17	9
Total number of pups born	243	211	263	200
Number of live born pups (%)	241 (99.4)	204 (97.3)	261 (99.2)	197 (98.5)
Per cent male pups	45	49	50	47

Data taken from Tables 22-26 and Appendices 16 and 28, pp. 121-128, 493-547, and 780-832, respectively, MRID 44296101.
[a]Includes remates.
Significantly different from control: *p<0.05; **p<0.01.

TABLE 11. F_1 generation reproductive performance				
Observation	Dietary concentration			
	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Mean precoital interval(days)	2.80	3.42	4.15	4.46
Proportion of successful matings (%)	20/25 (80.0)	23/26 (88.5)	21/26 (80.8)	23/26 (88.5)
Mean gestation length (days)	22.5	22.7	22.8	22.7
Number of litters	20	22	19	17
Total number of pups born	258	257	223	263
Number of live born pups (%)	253 (97.9)	252 (98.3)	220 (98.8)	257 (97.9)
Per cent male pups	49	54	54	51

Data taken from Tables 22-26 and Appendix 50, pp. 121-128 and 1554-1626, respectively, MRID 44296101.
Significantly different from control: *p<0.05.

5. Parental postmortem results

a. Organ weights

No differences in testes or epididymes weights were observed between treated and control males of either generation.

b. Pathology

1) **Macroscopic pathology** - No dose- or treatment-related gross abnormalities were observed in the F_0 or F_1 adults. The control F_0 female killed moribund during week 29 had a distended uterus with discolored contents. Implantation sites and incomplete involution of the uterus were observed in the high-dose F_1 female that failed to litter.

2) **Microscopic pathology** - No dose- or treatment-related microscopic abnormalities were observed in the reproductive tracts of the F_0 or F_1 adults.

B. OFFSPRING

1. Viability and clinical signs

Viability data for the F_{1a} and F_{1b} litters are given in Tables 12 and 13, respectively. For both litters, there was a significant ($p<0.05$ or 0.01) decrease in the number of pups/litter in the high-dose group after day 1 of lactation. Whole litter losses by the control, low-, mid-, and high-dose groups were 4/21, 0/19, 5/24, and 8/12 ($p<0.05$), respectively, for litter A and 4/22, 2/18, 6/23, and 10/19 ($p<0.05$), respectively, for litter B. Pup deaths in the high-dose group resulted in significantly lower lactation indices of 56.6% ($p<0.01$) compared with 88.0% for the controls for litter A and 75.4% ($p<0.05$) compared with 92.7% for the controls for litter B. Most of the pup deaths occurred during lactation days 1-5.

Viability data for the F_2 litters are given in Table 14. The number of pups/litter was significantly decreased in all dose groups as compared to controls throughout lactation. However, the size of the control litters was greater than either of those produced by the F_0 generation and greater than that seen in the historical data provided. Because litter size of the low-dose group was comparable to the control values for the F_{1a} and F_{1b} litters and is similar to the historical control data, the effect is not considered treatment-related. In contrast, litter sizes for the mid- and high-dose groups were less (not statistically significant) than the F_{1a} and F_{1b} control litters and the historical litter sizes and were dose-related. Also, reductions in litter sizes for the mid- and high-dose groups were most pronounced during lactation days 1-5 similar to the effects seen in the F_1 high-dose litters. The reductions in F_2 litter sizes at the mid- and high-doses are, therefore, considered due to treatment. Whole litter losses by the control, low-, mid-, and high-dose groups were 0/20, 1/23, 2/21, and 6/23 ($p<0.05$), respectively.

During lactation, no dose- or treatment-related clinical signs of toxicity were observed in the offspring of either generation.

TABLE 12: Viability of F_{1a} litters during lactation				
Observation/study time	0 ppm	0.5 ppm	5.0 ppm	25/10 ppm
Number of litters	21	19	24	12
Whole litter losses	4	0	5	8*
Proportion of litters with all pups surviving[a]	8/17	8/19	3/19	0/4
Number of pups born alive	237	229	296	111
Mean # live pups/litter[a]				
Day 1	11.6 ± 2.5	12.1 ± 2.7	11.8 ± 3.7	11.5 ± 0.6
Day 5	10.4 ± 2.9	10.7 ± 2.9	10.7 ± 3.4	6.8 ± 2.8*
Day 15	10.1 ± 2.8	10.6 ± 2.9	10.5 ± 3.3	6.5 ± 3.1*
Day 29	10.1 ± 2.8	10.6 ± 2.9	10.5 ± 3.3	6.5 ± 3.1*
Number of litters weaned	17	19	19	4
Live birth index	97.0	99.2	98.0	90.4*
Lactation index (d 1-22)[a]	88.0	88.7	88.7	56.6**

Data taken from Tables 25-27 and 30, pp. 126-129 and 135, respectively, MRID 44296101.
[a]Excluding whole litter losses.
Significantly different from control: *p<0.05.

TABLE 13: Viability of F_{1b} litters during lactation				
Observation/study time	**0 ppm**	**0.5 ppm**	**5.0 ppm**	**15 ppm**
Number of litters	22	18	23	19
Whole litter losses	4	2	6	10*
Proportion of litters with all pups surviving[a]	8/18	9/16	6/17	2/9
Number of pups born alive	241	204	261	197
Mean # live pups/litter[a]				
Day 1	10.9 ± 3.1	12.6 ± 3.9	12.4 ± 3.7	9.3 ± 3.3
Day 5	10.2 ± 2.8	11.4 ± 3.5	10.4 ± 3.8	6.7 ± 2.5*
Day 15	10.1 ± 2.8	11.3 ± 3.4	10.2 ± 3.8	6.6 ± 2.7**
Day 29	10.1 ± 2.8	11.3 ± 3.4	10.1 ± 3.8	6.6 ± 2.7*
Number of litters weaned	18	16	17	9
Live birth index	99.4	97.3	99.2	98.5
Lactation index (d 1-22)[a]	92.7	91.1	84.0	75.4*

Data taken from Tables 25, 26, 28 and 30, pp. 126, 127-128, 131, and 136, respectively, MRID 44296101.
[a]Excluding whole litter losses.
Significantly different from control: *p<0.05; **p<0.01.

TABLE 14: Viability of F_2 litters during lactation				
Observation/study time	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Number of litters	20	23	21	23
Whole litter losses	0	1	2	6*
Proportion of litters with all pups surviving[a]	12/20	16/22	8/19	2/17**
Number of pups born alive	253	252	220	257
Mean # live pups/litter[a]				
Day 1	12.7 ± 2.4	11.0 ± 3.0*	10.9 ± 2.5*	11.6 ± 3.3
Day 5	12.3 ± 2.4	10.3 ± 3.1*	9.2 ± 2.8**	8.9 ± 4.0**
Day 15	12.3 ± 2.4	10.2 ± 3.1*	9.1 ± 2.8**	8.6 ± 3.8**
Day 29	12.3 ± 2.4	10.2 ± 3.1*	9.1 ± 2.8**	8.6 ± 3.8**
Number of litters weaned	20	22	19	17
Live birth index	97.9	98.3	98.8	97.9
Lactation index (d 1-22)[a]	96.8	94.1	83.8*	75.6**

Data taken from Tables 25, 26, 29, and 30, pp. 126, 127-128, 133 and 137, respectively, MRID 44296101.
[a]Excluding whole litter losses.
Significantly different from control: *p<0.05; **p<0.01.

2. Body weight

Selected body weights of the F_{1a}, F_{1b}, and F_2 pups during lactation are given in Tables 15, 16, and 17, respectively. No statistically significant differences were observed between the treated F_{1a} or F_2 male or female pups and their respective control groups. During lactation of the F_{1b} pups, male and female body weights from all treated groups were occasionally statistically significantly less than the control group with treated male pups showing a slight dose-related decrease on lactation day 29.

TABLE 15: Mean pup body weights of F_{1a} litter during lactation (g)				
Day of lactation	0 ppm	0.5 ppm	5.0 ppm	25/10 ppm
Males				
Day 1	5.7	5.9	5.6	5.5
Day 5	8.3	8.3	7.9	8.4
Day 11	17.7	17.3	17.1	16.3
Day 29	76.2	72.5	72.3	73.9
Females				
Day 1	5.4	5.5	5.3	5.1
Day 5	8.0	7.9	7.5	7.7
Day 11	17.2	16.4	16.3	16.3
Day 29	71.5	67.9	67.2	71.0

Data taken from Table 34, pp. 145-146, MRID 44296101.

TABLE 16: Mean pup body weights of F_{1b} litter during lactation (g)				
Day of lactation	0 ppm	0.5 ppm	5.0 ppm	15 ppm
Males				
Day 1	6.2	6.0	5.8*	5.9
Day 5	9.1	9.2	8.5	8.6
Day 11	19.6	18.0	17.2*	17.3
Day 29	84.7	77.0*	76.1*	74.5*
Females				
Day 1	5.7	5.6	5.4	5.6
Day 5	9.0	8.6	7.8**	8.2
Day 11	18.6	17.0	16.8	16.8
Day 29	77.5	71.1*	72.3	71.0

Data taken from Table 35, pp. 147-148, MRID 44296101.
Significantly different from control: *p<0.05; **p<0.01.

TABLE 17: Mean pup body weights of F_2 litter during lactation (g)				
Day of lactation	0 ppm	0.5 ppm	5.0 ppm	10 ppm
Males				
Day 1	5.9	6.2	6.2	5.9
Day 5	9.4	9.7	9.3	8.8
Day 11	18.5	19.5	19.2	18.8
Day 29	78.3	82.4	80.1	78.8
Females				
Day 1	5.5	5.8	5.8	5.5
Day 5	9.1	9.2	8.9	8.5
Day 11	18.0	18.6	18.3	18.0
Day 29	73.4	76.6	74.9	74.5

Data taken from Table 36, pp. 149-150, MRID 44296101.

5. Offspring postmortem results

a. Organ weights

Testes and epididymes weights of the male pups were not affected by treatment.

b. Pathology

1) **Macroscopic pathology** - Empty stomach was a common finding in high-dose F_{1a} and F_2 pups that died intercurrently.

2) **Microscopic pathology** - Microscopic examinations of tissues from the pups were not conducted.

III. DISCUSSION

Male and female Sprague-Dawley rats were fed up to 25 ppm dicrotophos in the diet for two generations. Two litters were produced by the F_0 generation and one litter was produced by the F_1 generation.

A. INVESTIGATOR'S CONCLUSIONS

The study author concluded that administration of dicrotophos to Alpk:AP$_f$SD rats over two generations resulted in toxicity. Decreased body weights during the premating period were observed in the F_0 adults receiving ≥5 ppm. During gestation of litter A, body weights of the F_0 females given 25 ppm were reduced. Treatment-related reductions in body weights and food consumption occurred during lactation for F_0 females receiving ≥10 ppm and F_1 females receiving ≥5 ppm. Dietary levels of 5 ppm and greater were associated with decreased pup survival of both generations.

The author stated that the NOEL in this study was 0.5 ppm. Separate systemic and reproductive NOELs were not specified.

B. REVIEWER'S DISCUSSION

1. SYSTEMIC TOXICITY

No deaths of the adult animals of either generation were attributable to treatment with dicrotophos. Clinical signs of toxicity were manifested in high-dose F_0 males and females as involuntary shaking of the limbs during the first half of the premating period. Reduced body weights of the mid- and high-dose F_0 males and high-dose F_0 females during the premating period correlated with reduced food consumption and/or food utilization. These effects were most pronounced during the first few weeks of the premating period and probably result from the diets not being palatable to the animals. Once the animals adjusted to the taste of the test article, recovery was apparent in these treated groups with final body weights and overall weight gains similar to the control group levels.

The reason for the lower body weights of mid- and high-dose F_1 males and all treated F_1 females during week 1 of premating is also probably due to a lack of palatability of the diets to the animals. Lower body weights of these animals on lactation day 29 correlated with a pronounced reduction in food consumption during weeks 3 and 4 of lactation when the pups should have started eating the test diets. Corresponding with the

lower body weights was a significant reduction in food utilization by the mid- and high-dose males and a slight reduction by the high-dose females during weeks 1-4 of premating.

The Systemic Toxicity NOEL is 0.5 ppm and the Systemic Toxicity LOEL is 5.0 ppm based on lower body weights in the F_0 and F_1 males and females and reduced food utilization in F_0 males and females and F_1 males.

2. **REPRODUCTIVE TOXICITY**

F_0 maternal body weights during gestation of litter A were reduced at 25 ppm despite an increase in food consumption. No effects on gestational body weights were observed in either generation at lower dietary concentrations. F_0 and F_1 maternal body weights were also reduced at the mid- and high-dose during lactation and correlated with lower food consumption by these groups. Although the body weights for the low-dose F_1 dams were statistically significant, they were 97% of the control group level and, therefore, is not considered toxicologically significant.

The main toxicological effect of dicrotophos was on pup survival. Whole litter losses were increased in animals of both generations given ≥10 ppm. The number of pups/litter was significantly decreased at the high dose for the F_{1a} and F_{1b} litters and at the mid- and high-doses for the F_2 litters. Despite the effect on pup survival, there were no treatment-related effects on pup body weights during lactation.

The Reproductive Toxicity NOEL is 0.5 ppm and the Reproductive Toxicity LOEL is 5.0 ppm based on a reduced number of F_2 pups/litter during lactation.

C. **STUDY DEFICIENCIES**

No deficiencies were identified in the conduct of this study.

D. **CLASSIFICATION**

This study is classified as Acceptable-Guideline and satisfies the guideline requirement for a reproduction study (§83-4) in rats.