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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

Date: **June 24, 2008**

SUBJECT: **Dicrotophos**

PC Code: 035201
Decision No.:356235
Petition No.: None
Risk Assessment Type: NA
TXR No.: 0053707
MRID No.: 46153203, 46153204, 46153205, 46153206, 46153207, 46153208

DP Barcode: D316123
Registration No.: 5481-447
Regulatory Action: Registration Review
Case No.: 0145
CAS No.: 141-66-2
40 CFR: NA

Ver.Apr.08

FROM: Abdallah Khasawinah, Ph.D.
Reregistration Branch 4
Health Effects Division (7509P)

THROUGH: Susan Hummel
Reregistration Branch 4
Health Effects Division (7509P)

TO: Laura Parsons
Reregistration Branch
Special Review & Reregistration Division (7508P)

I. CONCLUSIONS:

Taken together these studies are classified **Acceptable/Nonguideline** for the determination of RBC and brain cholinesterase activities following treatment with dicrotophos in adult and juvenile rats. Main deficiencies include omission of plasma measurements and lack of assessment in dams and fetuses on GD 20. The executive summary is attached.

II. ACTION REQUESTED:

Review the following studies with MRID 46153203, 46153204, 46153205, 46153206, 46153207, 46153208

JUN 26 REC'D, 2008

III. BACKGROUND

AMVAC Chemical Corporation has submitted new cholinesterase studies performed with dicrotophos. Executive Summaries of these studies are presented below:

IV. RESULTS/DISCUSSION

A series of studies was performed to investigate the effect of dicrotophos on blood and brain cholinesterse (ChE) activity and to determine the peak time of ChE inhibition (ChEI) following both acute and repeated dosing in pre-weaning, young, and older adult Alpk:AP$_f$SD rats: No plasma ChE activity was measured. The studies were conducted as follows:

- In a time-course study (MRID 46153205), a single oral dose of 0 or 5 mg dicrotophos (86.9% a.i., Lot #403001B)/kg was administered by gavage to 15-day- or 42-day-old female rats (25/group).

- In acute exposure studies, single oral doses of dicrotophos (90.4% a.i., Lot # 403001B) were administered by gavage at doses of 0.0, 0.1, 0.3, 1.0 or 5.0 mg/kg to 5 male and 5 female pre-weaning rats of 8, 15 and 22 days of age (MRID 46153205), and single oral doses of dicrotophos (87.6% a.i., Lot # 403001B) of 0.0, 0.1, 0.3, or 5.0 mg/kg were administered by gavage to groups of 15 young adult male and female rats (MRID 46153206).

- In repeat exposure studies, dicrotophos (90.4% a.i., Lot # 403001B) was administered by gavage at 0, 0.008, 0.02, 0.08, 0.4 or 1.0 mg/kg/day to pre-weaning and young adult rats (5/sex) at 12 or 42 days age, respectively, for 7 days (MRID 46153204). In another study, 5 male and 5 female adult rats received test compound (87.6% a.i., Lot # 403001B) by gavage at 0, 0.008, 0.02, or 0.4 mg/kg/day for 4 weeks (MRID 46153208). For a bridging study (MRID 46153207), adult rats (10/sex) received daily gavage doses of 0.0 or 0.4 mg dicrotophos (87.6% a.i, Lot # 403001B)/kg/day for 4 or 8 weeks.

Treatment-related mortality was observed among pups receiving a single oral dose of 5 mg/kg (one of five males in the definitive study and three of 25 females in the time-course study). At a dose of 5 mg/kg, there was inhibition of RBC and brain ChE activity in pups and adults which correlated with clinical observations of tremors (all PND 8, 15, and 22 pups and 14/15 adult males and 15/15 adult females), splayed gait, shaking, and fast/irregular breathing. Clinical signs of ChE activity inhibition were first seen in pups and young adult rats within approximately 30 minutes after dosing. In the pups the clinical effects were seen over a longer period than those in adults. No sex-related differences were evident. The peak time of effect was reached by three hours in pups and one hour in adults. At the dose of 5 mg/kg, pups had greater ChE activity inhibition than adults in both compartments. In pups, dose-dependent RBC and brain ChE activity inhibition was observed at single oral doses of ≥0.1 mg/kg (lowest dose tested), while in adult rats dose-dependent ChE activity inhibition was observed at doses of ≥0.3 mg/kg. Single doses of 0.1 mg/kg caused no significant effects in adult rats.

No mortality or clinical signs were observed in repeat-dose studies. There were no treatment-related effects on body or brain weight. Repeat oral administration for 7 days at 0.08 to PND 18 pups inhibited brain ChE activity by 15% (both sexes; NS) and RBC activity by 19 and 25% in male and female pups, respectively (both $p<0.01$). Young adults were affected at 0.4 mg/kg/day (brain: 23 and 36% ChEI in males and females, respectively; RBC: 47 and 38% ChEI in males and females, respectively). Inhibition was dose-related. Repeated 7-day exposure to ≤0.02 mg/kg/day or 0.08 mg/kg/day caused no significant ChE activity inhibition in pre-weaning and adult rats, respectively. A daily dose of 0.4 mg/kg/day for 4 weeks to adult rats caused inhibition of ChE activity in both compartments which returned to almost normal after a 4-week recovery period. No sex related differences were seen. Following repeat dosing at 0.4 mg/kg/day for 4 or 8 weeks, maximum inhibition was achieved within four weeks in adults (brain, 33-35%; RBC, 48-49%). Pre-weaning rats were not tested in this study.

Overall, the results of all six studies together provide consistent evidence of greater sensitivity in young animals with respect to ChEI following acute and repeated dicrotophos exposure. This finding is further supported by the results of an earlier developmental neurotoxicity study in rats (MRID 46153202) in which clinical signs and neuropathology were observed in pups at 0.4 mg dicrotophos/kg/day while no toxicity was evident in maternal animals indicating greater sensitivity of pups to dicrotophos treatment.

For acute exposure:

the adult LOAEL for brain ChEI is 5 mg/kg (males), 0.3 mg/kg (females)
the adult NOAEL for brain ChEI is 0.3 mg/kg (males), 0.1 mg/kg (females);

the PND 8, 15, and 22 LOAEL for brain ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for brain ChEI is not determined (<0.1 mg/kg; both sexes);

the adult LOAEL for RBC ChEI is 0.3 mg/kg (both sexes)
the adult NOAEL for RBC ChEI is 0.1 mg/kg (both sexes);

the PND 8, 15, and 22 LOAEL for RBC ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for RBC ChEI is not determined (<0.1 mg/kg; both sexes).

For acute oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition in rats is 0.3 mg/kg based on enzyme inhibition in brain and RBC; the adult NOAEL is 0.1 mg/kg.

For acute oral exposure to dicrotophos, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.1 mg/kg based on enzyme inhibition in brain and RBC; the offspring NOAEL is not determined (<0.1 mg/kg).

For repeated exposure:

the adult LOAEL for brain ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for brain ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for brain ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for brain ChEI is 0.02 mg/kg/day (both sexes);

the adult LOAEL for RBC ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for RBC ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for RBC ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for RBC ChEI is 0.02 mg/kg/day (both sexes).

For repeated oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition is 0.4 mg/kg/day based on brain and red blood cell; the NOAEL is 0.08 mg/kg/day.

For repeat oral exposure to dicrotophos, the overall offspring LOAEL is 0.08 mg/kg/day based on brain and RBC; the NOAEL is 0.02 mg/kg/day.

The cholinesterase activity measurements following acute and repeat oral doses of dicrotophos demonstrate greater susceptibility in juveniles than in adult rats. This susceptibility was observed in terms of the dose level at which an effect was observed (i.e., the LOAEL for cholinesterase inhibition was lower for juveniles than for adults in both the acute and repeat-dose studies). Although LOAELs were based on both RBC and brain ChEI, brain ChEI was generally greater than RBC ChEI for both age groups in the acute study. This difference in RBC and brain ChEI inhibition was not demonstrated in the 7-day repeat dose study. The relative susceptibility of dams and GD 20 fetuses was not addressed in this series of studies.

Taken together, these studies are classified **Acceptable/Non-Guideline** for the determination of RBC brain cholinesterase activities following treatment with dicrotophos in young adult and juvenile (pre-weaning) rats. Main deficiencies are omission of plasma ChE measurements and lack of assessment in dams and fetuses.

DATA EVALUATION RECORD

DICROTOPHOS

Study Type: SPECIAL STUDIES, CHOLINESTERASE INHIBITION [NON-GUIDELINE]

MRID 46153203 (time-course, adult and pre-weaning rats); MRID 46153205 (single dose, pre-weaning rats); MRID 46153206 (single dose, adult rats); MRID 46153204 (adult/pre-weaning comparative sensitivity, 7-day repeat dose); MRID 46153208 (28-day repeat dose, adult rats); MRID 46153207 (4- and 8-week repeat dose bridging study)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 56-2004

Primary Reviewer:
Sanjivani Diwan, M.S., Ph.D.

Secondary Reviewers:
Sylvia S. Talmage, Ph.D., D.A.B.T.

Robert H. Ross, M.S., Group Leader

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: for S. Diwan
Date: 6/21/2004

Signature:
Date:

Signature:
Date:

Signature:
Date: 6/21/2004

Disclaimer

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

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:** A. Khasawinah
Re-registration Branch 4, Health Effects Division (7509P) **Date** 6-18-2008
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P V Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date** 6/18/08

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TXR#: 0053707

DATA EVALUATION RECORD

STUDY TYPE: Special Studies: Effects on Cholinesterase in Adult and Juvenile Alpk:AP$_f$SD Rats, Companion Studies to DNT Study (MRID 46153202).

PC CODE: 035201 **DP BARCODE:** D316123

TEST MATERIAL (PURITY): Dicrotophos (86.4% - 90.4% a.i.)

SYNONYMS: Bidrin; (E)-2-Dimethylcarbamoyl-1-methylvinyl dimethyl phosphate; O,O-Dimethyl O-(N,N-dimethylcarbamoyl-1-methylvinyl) phosphate

CITATIONS: Milburn G.M. (2003) Dicrotophos: Time course of cholinesterase inhibition in pre-weaning and adult rats. Central Toxicology laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/AR7311/REG/REPT. September 26, 2003. MRID 46153203. Unpublished. 175 p.

Moxon, M.E. (2003) Dicrotophos: Repeat dose cholinesterase inhibition study in pre-weaning and young adult rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/KR1491/REG/REPT. October 24, 2003. MRID 46153204. Unpublished. 69 p.

Moxon, M.E. (2003) Dicrotophos: Acute cholinesterase inhibition study in pre-weaning rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/AR7148/REG/REPT. October, 24, 2003. MRID 46153205. Unpublished. 53 p.

Brammer, A. (2002) Dicrotophos: Acute cholinesterase inhibition study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/AR7078/REG/REPT. April 4, 2002. MRID 46153206. Unpublished. 194 p.

Brammer, A. (2002) Dicrotophos: Repeat dose bridging study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/KR1455/REG/REPT. February 18, 2002. MRID 46153207. Unpublished. 107 p.

Brammer, A. (2002) Dicrotophos: Repeat dose cholinesterase inhibition study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, England. Doc. No. CTL/KR1456/REG/REPT. June 24, 2002. MRID 46153208. Unpublished. 148 p.

SPONSOR: AMVAC Chemical Corporation, Los Angeles, CA

EXECUTIVE SUMMARY: A series of studies was performed to investigate the effect of dicrotophos on blood and brain cholinesterse (ChE) activity and to determine the peak time of ChE inhibition (ChEI) following both acute and repeated dosing in pre-weaning, young, and older adult Alpk:AP$_f$SD rats. No plasma ChE activity was measured. The studies were conducted as follows:

- In a time-course study (MRID 46153205), a single oral dose of 0 or 5 mg dicrotophos (86.9% a.i., Lot #403001B)/kg was administered by gavage to 15-day- or 42-day-old female rats (25/group).

- In acute exposure studies, single oral doses of dicrotophos (90.4% a.i., Lot # 403001B) were administered by gavage at doses of 0.0, 0.1, 0.3, 1.0 or 5.0 mg/kg to 5 male and 5 female pre-weaning rats of 8, 15 and 22 days of age (MRID 46153205), and single oral doses of dicrotophos (87.6% a.i., Lot # 403001B) of 0.0, 0.1, 0.3, or 5.0 mg/kg were administered by gavage to groups of 15 young adult male and female rats (MRID 46153206).

- In repeat exposure studies, dicrotophos (90.4% a.i., Lot # 403001B) was administered by gavage at 0, 0.008, 0.02, 0.08, 0.4 or 1.0 mg/kg/day to pre-weaning and young adult rats (5/sex) at 12 or 42 days age, respectively, for 7 days (MRID 46153204). In another study, 5 male and 5 female adult rats received test compound (87.6% a.i., Lot # 403001B) by gavage at 0, 0.008, 0.02, or 0.4 mg/kg/day for 4 weeks (MRID 46153208). For a bridging study (MRID 46153207), adult rats (10/sex) received daily gavage doses of 0.0 or 0.4 mg dicrotophos (87.6% a.i, Lot # 403001B)/kg/day for 4 or 8 weeks.

Treatment-related mortality was observed among pups receiving a single oral dose of 5 mg/kg (one of five males in the definitive study and three of 25 females in the time-course study). At a dose of 5 mg/kg, there was inhibition of RBC and brain ChE activity in pups and adults which correlated with clinical observations of tremors (all PND 8, 15, and 22 pups and 14/15 adult males and 15/15 adult females), splayed gait, shaking, and fast/irregular breathing. Clinical signs of ChE activity inhibition were first seen in pups and young adult rats within approximately 30 minutes after dosing. In the pups the clinical effects were seen over a longer period than those in adults. No sex-related differences were evident. The peak time of effect was reached by three hours in pups and one hour in adults. At the dose of 5 mg/kg, pups had greater ChE activity inhibition than adults in both compartments. In pups, dose-dependent RBC and brain ChE activity inhibition was observed at single oral doses of ≥0.1 mg/kg (lowest dose tested), while in adult rats dose-dependent ChE activity inhibition was observed at doses of ≥0.3 mg/kg. Single doses of 0.1 mg/kg caused no significant effects in adult rats.

No mortality or clinical signs were observed in repeat-dose studies. There were no treatment-related effects on body or brain weight. Repeat oral administration for 7 days at 0.08 to PND 18 pups inhibited brain ChE activity by 15% (both sexes; NS) and RBC activity by 19 and 25% in male and female pups, respectively (both $p<0.01$). Young adults were affected at 0.4 mg/kg/day (brain: 23 and 36% ChEI in males and females, respectively; RBC: 47 and 38% ChEI in males and females, respectively). Inhibition was dose-related. Repeated 7-day exposure to ≤0.02 mg/kg/day or 0.08 mg/kg/day caused no significant ChE activity inhibition in pre-weaning and adult rats, respectively. A daily dose of 0.4 mg/kg/day for 4 weeks to adult rats caused inhibition of ChE activity in both compartments which returned to almost normal after a 4-week recovery period. No sex related differences were seen. Following repeat dosing at 0.4 mg/kg/day for 4 or 8 weeks, maximum inhibition was achieved within four weeks in adults (brain, 33-35%; RBC, 48-49%). Pre-weaning rats were not tested in this study.

Overall, the results of all six studies together provide consistent evidence of greater sensitivity in young animals with respect to ChEI following acute and repeated dicrotophos exposure. This finding is further supported by the results of an earlier developmental neurotoxicity study in rats (MRID 46153202) in which clinical signs and neuropathology were observed in pups at 0.4 mg dicrotophos/kg/day while no toxicity was evident in maternal animals indicating greater sensitivity of pups to dicrotophos treatment.

For acute exposure:

the adult LOAEL for brain ChEI is 5 mg/kg (males), 0.3 mg/kg (females)
the adult NOAEL for brain ChEI is 0.3 mg/kg (males), 0.1 mg/kg (females);

the PND 8, 15, and 22 LOAEL for brain ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for brain ChEI is not determined (<0.1 mg/kg; both sexes);

the adult LOAEL for RBC ChEI is 0.3 mg/kg (both sexes)
the adult NOAEL for RBC ChEI is 0.1 mg/kg (both sexes);

the PND 8, 15, and 22 LOAEL for RBC ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for RBC ChEI is not determined (<0.1 mg/kg; both sexes).

For acute oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition in rats is 0.3 mg/kg based on enzyme inhibition in brain and RBC; the adult NOAEL is 0.1 mg/kg.

For acute oral exposure to dicrotophos, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.1 mg/kg based on enzyme inhibition in brain and RBC; the offspring NOAEL is not determined (<0.1 mg/kg).

For repeated exposure:

the adult LOAEL for brain ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for brain ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for brain ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for brain ChEI is 0.02 mg/kg/day (both sexes);

the adult LOAEL for RBC ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for RBC ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for RBC ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for RBC ChEI is 0.02 mg/kg/day (both sexes).

For repeated oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition is 0.4 mg/kg/day based on brain and red blood cell; the NOAEL is 0.08 mg/kg/day.

For repeat oral exposure to dicrotophos, the overall offspring LOAEL is 0.08 mg/kg/day based on brain and RBC; the NOAEL is 0.02 mg/kg/day.

The cholinesterase activity measurements following acute and repeat oral doses of dicrotophos demonstrate greater susceptibility in juveniles than in adult rats. This susceptibility was observed in terms of the dose level at which an effect was observed (i.e., the LOAEL for cholinesterase inhibition was lower for juveniles than for adults in both the acute and repeat-dose studies). Although LOAELs were based on both RBC and brain ChEI, brain ChEI was generally greater than RBC ChEI for both age groups in the acute study. This difference in RBC and brain ChEI inhibition was not demonstrated in the 7-day repeat dose study. The relative susceptibility of dams and GD 20 fetuses was not addressed in this series of studies.

Taken together, these studies are classified **Acceptable/Non-Guideline** for the determination of RBC brain cholinesterase activities following treatment with dicrotophos in young adult and juvenile (pre-weaning) rats. Main deficiencies are omission of plasma ChE measurements and lack of assessment in dams and fetuses.

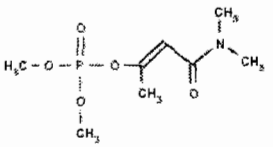
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Dicrotophos-Technical grade

Description:	Clear, brown liquid
Lot #:	403001B
Purity:	86.4 - 90.4% (E-isomer range: 86.4-86.9% in all studies)
Compound Stability:	stable under refrigeration
CAS # of TGAI:	**141-66-2**
Structure	



2. Vehicle and/or positive control: De-ionized water/no positive control was used in this study.

3. Test animals:

Species:	rat	
Strain:	Alpk:AP$_f$SD Wistar-derived	
Age and wt. at study initiation:	Time-course study: Pre-weaning female pups: 15 days, 22.1-41.7 g; young adult females: 42 days, 126-201 g Single dose studies: Pre-weaning pups: 8 days, males-13.4-20.4 g females: 11.9-20.6 g; 15 days, males-18.2-34.6 g females: 17.9-34.4 g; 22 days, males-40.2-60.3 g females: 38.9-63.1 g; young adults : 42 days, males- 143-170 g and females: 113-141 g 7-Day Repeat dose study: Pre-weaning rats: 12 days, males-19.2-26.2 g females: 16.9- 25.3g; young adults: 42 days, males: 179-221 g , females: 144-183 g 4-Week repeat dose and recovery study: Adult rats: 40 days, males; 172-202 g; females: 134-162 g 4 to 8-Week repeat dose bridging study: Adult rats: 40 days, males; 185-231 g; females: 138-174 g	
Source:	Rodent Breeding Unit., Alderly Park, Macclesfield, Cheshire, England	
Housing:	solid plastic cages with wood flake bedding; loose paper balls were used as nesting material. Young adults were housed five per cage in wire mesh cages.	
Diet:	Ctl diet supplied by Special Diet Services Ltd., Essex, UK., *ad libitum*	
Water:	tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	15/hr
	Photoperiod:	12 hrs light/dark
Acclimation period:	at least 5 days	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates:

Study Type (MRID No.)	Start	End
Time-course (MRID 46153203)	March 21, 2003	April 23, 2003
Single dose/pre-weaning rats (MRID 46153205)	March 28, 2002	May 23, 2002
Single dose/young adult rats (MRID 46153206)	November 5, 2001	January 24, 2002
7-Day repeat dose (MRID 46153204)	May 13, 2002	February 3, 2003
4-Week repeat dose/ recovery (MRID 46153208)	November 12, 2001	March 13, 2002
4-8 Weeks repeat dose bridging (MRID 46153207)	July 17, 2001	September 24, 2001

Data from p. 10, MRID 46153205; p. 12, MRID 46153207; and p. 13, MRIDs 46153203, 46153204, 46153206 and 46153208.

TABLE 1. Study design

Study Type	Dicrotophos Dose (mg/kg/day)	Number of animals/sex	Treatment
Time-course	0, 5.0	25 F/group	Single oral dose to 25 female pre-weaning (PND 15) and 25 young female adult rats (42 days of age)
Single dose	0, 0.1, 0.3, 1.0, 5.0	5/sex	Single oral dose to 5 pre-weaning rats/sex of 8, 15 or 22 days of age.
Single dose	0, 0.1, 0.3, 5.0	15/sex	Single oral dose to groups of 15 adult rats/sex
7-Day repeat dose	0.008, 0.02, 0.08, 0.4 , 1.0	5/sex	Daily oral dose starting at PND 12 (5/sex) and 42 days of age (5/sex) for 7 days
4-Week repeat dose and recovery	0, 0.008, 0.02, 0.4	10/sex	Daily oral dose to 10 male and 10 female adult rats for 28 consecutive days.
4-8 Weeks repeat dose bridging	0, 0.4	10/sex	Daily oral dose to 10 adult rats/sex for 4 or 8 weeks.

Data from pp. 15 and 16, MRID 46153203; p. 12, MRID 46153205; p. 11, MRID 46153206; pp. 12 and 15, MRID 46153204; p. 11 of MRID 46153208; and pp. 11 and 16, MRID 46153207.

3. **Animal Assignment**: Pre-weaning pups and young adult rats were allocated to group and cage positions in a sequential manner for even distribution as shown in Table 2 below.

TABLE 2. Animal assignment

Study type	Assignment
Time-course	The groups of rats were arranged in 10 replicates or randomized blocks. Each replicate had two cages, one per treatment group. Replicates 1-5 contained pre-weaning animals with their dams, replicates 6-10 contained adult animals. The rats were randomly allocated to cages as they came to hand.
Single dose	Pre-weaning rats from each litter, one/sex/litter, were allocated to each of the 5 treatment groups randomly on the day of dosing.
Single dose	Animals at either extreme of the weight distribution were discarded. A Latin Square technique was used for randomization to the control and treatment groups. Sexes were randomized separately.
7-Day repeat dose	Each pre-weaning pup was uniquely identified within the litter, upon arrival. Male pups were numbered 1-5 and female pups were numbered 6-10. Upon arrival, young adult rats, 5/sex were uniquely identified and randomly allocated, as they came to hand, to each treatment group.
4-Week repeat dose and recovery	Adult rats/were allocated to four groups (one control and three treatment), single-sex replicates randomly, each containing one cage per treatment group. Animals were randomly allocated to the cages within each replicate to an experimental group, each containing 10 rats/sex
4-8 Weeks repeat dose bridging	Following discard of weight extremes, a Latin Square technique was used for randomization to the control and treatment groups. Sexes were randomized separately. Replicates (5 /sex) 1 and 2 were dosed for 4 weeks and replicates 3 and 4 were dosed for 8 weeks.

Data from pp. 15 and 16, MRID 46153203; p. 14, MRID 46153205; pp. 16 and 17, MRID 46153206; pp.14 and 15, MRID 46153204; pp. 16 and 17, MRID 46153208; and p. 16, MRID 46153207.

4. Dose selection rationale: A single dose level for the time-course study (MRID 45153203) was chosen on the basis of data from single dose studies in pre-weaning and young adult rats. The doses for the acute ChE inhibition study in adult rats (MRID 46153206) were based on the results of previous studies in the same strain of rats carried out in the same laboratory. The dose levels for the acute ChE inhibition study in pre-weaning rats (MRID 46153205) were selected by the Sponsor from the results of an earlier study in adult rats (CTL/AR7078/Regulatory/Report). No further details were provided.

   The doses for the 7-day repeat dose ChE inhibition study in pre-weaning and young adult rats (MRID 46153204) were selected by the Sponsor based on data from acute and repeat dose ChE inhibition studies of dicrotophos in the rat (CTL/AR7078/Regulatory/Report and CTL/KR1456/ Regulatory/Report). The doses for the 4-week repeat-dose and recovery study (MRIDs 46153208 and 46153207) were selected on the basis of earlier studies (CTL/P/4692, 1995 and CTL/KR1455/Regulatory/ Report, 2002). For MRID 46153207 (bridging study), the dose level of 0.4 mg/kg, equivalent to 5 ppm in the diet, was selected as it caused slight to moderate reductions in brain, plasma, or RBC ChE activity levels, without overt toxicity in the previous 90-day dietary study (CTL/P/4692). No further details were available.

5. Dosage administration: All single or multiple doses were administered to pups, pre-weaning and young adult rats as well as older adult rats of one or both sexes as shown in Table 1 by daily oral gavage at a volume of 1 mL/100 g adjusted to body weight calculated from the most recent body weight. Dosing was performed sequentially, in group order, and repeat dosing was administered at approximately the same time each day.

6. Dosage preparation and analysis: For the single dose study in pre-weaning rats, dose preparations were made separately for each dosing day. The highest concentration (0.5 mg/mL) was prepared by adding an appropriate amount of de-ionized water to a weighed amount of test substance without making any adjustments for purity. The lower concentrations were prepared by serial dilution of the highest concentration. The dose preparations were stored in the refrigerator. For adult rats, dosing was prepared in the same manner; however, adjustment for purity was made to the weighed amount of test substance. For the time-course study, an appropriate amount of vehicle was added to a weighed amount of test substance to provide one preparation (w/v) of the required concentration. The dosing formulations were stored at room temperature. For a 7-day repeat dose study, the dose preparations were made as two batches for each phase. Each batch was split into aliquots for daily dosing. Dosing preparations were made and stored in the same manner but without any adjustment for purity and with the exception that the dose preparations for phase 2 were stored under nitrogen. For the 4-week repeat dose/recovery and bridging studies, each dosing preparation made in de-ionized water was throughly mixed before subdividing into daily aliquots that were stored at 2 to 10°C. Dosing solutions were brought to room temperature at least one hour prior to dosing. For phase 2 of the repeat dose study in pre-weaning and young adult rats, dose preparations were stored under nitrogen. Formulations were prepared weekly with the exception of acute studies where dose-preparations were made daily.

   For single dose studies, the stability analysis of the chemical in samples at each dose preparation was determined over 7 days. For the time-course study, the stability of the dosing preparation under the condition of storage was demonstrated in a previous study (KR 1491).

Prior to the start of the repeat dose study, stability of the test substance in samples of the 0.4 and 0.0008 mg/mL concentrations stored at 4°C was analyzed over 12 days; the stability of the 0.1 mg/mL concentration was determined over 13 days at room temperature. For the bridging study, stability of dicrotophos in de-ionized water, stored at 4°C, was determined. Representative samples of dosing preparations used in various studies were analyzed to verify the achieved concentrations of dicrotophos in de-ionized water.

Results:

Homogeneity analysis: Assessment was not made.

Stability analysis: In all studies the stability of dicrotophos in de-ionized water was found to be satisfactory when stored at 4°C. The results of analysis for each study are provided in Table 3 below.

TABLE 3. Stability analysis

Study type	Stability analysis
Time-course	Stability was demonstrated in a previous study (KR1491).
Single dose/pre-weaning rats	Demonstrated in study # KR 1491; stable at concentrations of 0.0008 and 0.04 mg/mL over 12 days; in addition, as reported in study # LR05871, the stability was demonstrated at concentration of 1 mg/mL over 11 days.
Single dose/young adult rats	Stable at concentration of 0.5 mg/mL for 7 days; stability at concentration of 0.0008 mg/mL for 7 days was demonstrated in study # KR1456.
7-Day repeat dose and recovery	Upon re-analysis, dicrotophos at concentrations of 0.0008 and 0.04 mg/mL was stable (93-100% and 88.5-100%, respectively) for at least 12 days. The stability at concentration of 0.01 mg/mL was determined for 13 days at room temperature in CTL Study # RR0883.
4-Week repeat dose and recovery	Stable at concentration of 0.0008 mg/mL for at least 7 days; the 0.5 mg/mL concentration was analyzed in a separate study (AR 7078).
4-8 Weeks repeat dose bridging	Stable (100-113.5%) at concentration of 0.04 mg/mL for at least 7 days. The rise in concentration was attributed to analytical variability.

Data from Table 1, p. 24 MRID 46153205; p. 19 and Table 2 and p. 25, MRID 46153206; p. 14, MRID 46153203; Table 2, p. 24, MRID 46153204; Table 2, p. 29, MRID 46153208; and Table 2, p. 30, MRID 46153207.

Concentration analysis: All doses were within ±14% of nominal with the exception of the 4-week repeat dose and recovery study where the re-analysis for the 0.02 mg/mL level showed a mean concentration 135% of nominal. The results of analysis for each study are provided in Table 4 as follows:

TABLE 4. Concentration analysis	
Study type	**Concentration analysis**
Time-course	The mean achieved concentration for the 0.04 mg/mL level was within 3% of nominal concentration.
Single dose/pre-weaning rats	The mean concentrations for the 0.01 and 0.5 mg/mL levels were within ±14% of the nominal concentration
Single dose/young adult rats	The mean achieved concentration for each dose preparation was within 10% of nominal concentration.
7-Day repeat dose	The mean achieved concentration for each dose preparation was within 12% of nominal concentration.
4-Week repeat dose and recovery	The mean concentration for the 0.04 mg/mL dose was 102-105% of nominal; for 0.02 and 0.008 mg/mL the mean concentrations were 108 to 160% of nominal. The initial method was revised and validated before dose preparation analysis for a second time (mean concentrations: 130-135% of nominal).
4-8 Weeks repeat dose bridging	The mean concentration for the 0.04 mg/mL level was 92.5-100% (within 8%) of nominal.

Data from Table 1, p. 25, MRID 46153203; Table 1, pp. 23 and 24, MRID 46153205; Table 1, p. 24, MRID 46153206; Table 1, pp. 22 and 23, MRID 46153204; Table 1, p. 28, MRID 46153208; and Table 1, p. 29, MRID 46153207.

Overall, the analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

Clinical observations/Body weight: Clinical observations and body weight were recorded as shown in Table 5 below.

TABLE 5. Clinical observations and body weight	
Study type	**Observations**
Time-course	All pups and adult females were checked on the day of dosing and prior to termination. All pups and adult females were weighed on day 1 prior to dosing and prior to study termination.
Single dose/pre-weaning rats	Gross observations of all pups were made prior to start of the study. The detailed clinical observations for pups were recorded immediately prior to dosing and between 1.5 and 2 hours post dosing, i.e. at the estimated time of peak effect. The body weights were recorded immediately prior to dosing, for the calculation of dose volume.
Single dose/young adult rats	Gross observations of all adult rats were made prior to start of the study. The detailed clinical observations were recorded prior to dosing on day 1, at the time of expected peak effect on day 1 (2-3 hours post dosing), and then on days 8 and 15 as appropriate. For group 4, clinical observations in replicates 1 and 2 at approximately 1-1.5 hours post dosing on day 1 were conducted, as critical signs were evident at this time. Cage-side observations were made at least once daily throughout the study. Body weights were recorded on day -1, prior to dosing on day 1, and on days 8 and 15, where appropriate.
7-Day repeat dose	Gross observations of pups and young adults were made prior to dosing period and daily thereafter. On dosing days, detailed clinical observations were recorded immediately prior to dosing and between 1.5 and 2 hours post dosing (estimated time of peak effect). Body weight was recorded daily, immediately prior to dosing.
4-Week repeat dose and recovery	Gross observations of all rats were made prior to treatment. Detailed clinical observations were made daily and at 3-4 hours after dosing at week 1 and 2. At week 2, cage-side observations were also made soon after dosing and towards the end of the working day. Any rat found dead was subjected to postmortem examination. Body weights were recorded daily, immediately prior to dosing, (for dose calculation) and at weekly intervals during the recovery period and/or prior to termination on day 29 or 57.
4-8 Weeks repeat dose bridging	Detailed clinical observations were recorded prior to dosing and daily, thereafter, at the same time when body weights were recorded. Cage-side observations were made soon after dosing, and at 2-3 hours after dosing. Body weights were recorded daily, immediately prior to dosing, (for dose calculation) and at weekly intervals during the recovery period and/or prior to termination of day 29 or 57.

Data from p. 15, MRID 46153205; p. 18, MRID 46153206; pp. 16 and 17, MRID 46153203; p. 16, MRID 46153204; p. 18, MRID 46153208; and pp. 16 and 17, MRID 46153207.

2. **Termination Schedule and sample collection**: Adults and/or pups were sacrificed according to the schedule shown in Table 6.

All pups and adults were sacrificed by over exposure to haloethane Ph. Eur vapor followed by exsanguination. Blood was collected by cardiac puncture and placed in tubes containing lithium heparin as the anticoagulant and submitted to the clinical pathology unit (CTL) for analysis of cholinesterse activity. Plasma samples were stored at -70°C or -80°C. The whole brain was removed, kept on ice, and weighed before or after submitting to CTL for analysis of brain cholinesterase activity. Brain weight was taken but not reported for single dose studies. With the exception of the 7-day repeat dose study, brain weight was included in the report for other repeat dose studies.

TABLE 6. Termination schedule

Study Type	Day	Samples	Treatment
Time-course	PND 16 and 17 or Day 43 and 44	Blood/brain	Five rats/group were sacrificed at 1, 3, 8, 24 or 72 hours after dosing. At each time point one pup or one adult was sacrificed.
Single dose to pups	PND 8, 15 or 22	Blood/brain	Five pre-weaning rats/sex were sacrificed at the estimated time of peak effect, approximately 2 hours post-dosing.
Single dose to adults	Day 1, 8 or 15	Blood/brain	Five rats/group were sacrificed on day 1 (2-3 hours after dosing), day 8 and day 15.
7-Day repeat dose	PND 18 or 48	Blood/brain	Pre-weaning (5/sex) and young adult (5/sex) rats were sacrificed approximately 2 hours after the 7th dose (i.e. 18 and 48 days of age, respectively).
4-Week repeat dose/recovery	Day 29 or day 57	Blood/brain	Five/sex were sacrificed on day 29 and 5/sex were retained for further 28 days.
4-8 Week repeat dose/ bridging	Day 29 or 57	Blood/brain	Five/sex were sacrificed on day 29 (at least 69 days old); remaining were sacrificed after recovery period of 8 weeks (on day 57 or at least 97 days old).

Data from p. 17, MRID 46153203; p. 16, MRID 46153205; p. 18, MRID 46153206; p. 16, MRID 46153204; p. 18, MRID 46153208; and pp. 11 and 17, MRID 46153207.

3. **Cholinesterase determination:** ChE assays were done on all red blood cell (RBC) and brain samples. ChE activity determination was based on the Ellman method and modified as recommended by EPA taking in to account concerns by Wilson *et al*, 1996 (Factors in standardizing automated cholinesterase assays. J. Toxicol. Environ. Hlth. 48: 187-195) and the UK Animal Clinical Chemistry Association concerns in their Spring 1997 Newsletter. All ChE assays were done on a Konelab 60i automated analyzer. RBC ChE activity was measured following the hydrolysis of acetylthiocholine to thiocholine and its subsequent reaction with 6,6'-dithiodinicotinic acid (DTNA) in phosphate buffer, pH 8.0 at 37°C. Absorption was measured at 405 nm. Analyses were performed in accordance with Clinical Pathology unit SOP CT91-059. Although not stated in the report, it is assumed that for the single dose, 4-week repeat dose and recovery, and bridging studies in adult rats, the same methodology was followed for the measurement of ChE activity.

4. **Necropsy procedures:** No gross or microscopic examination was performed on brain and nerve tissue samples.

D. DATA ANALYSIS: Data were analyzed using SAS (1999). Body weight, food consumption and ChE measurements were analyzed by ANOVA. Brain weight to body weight ratios were analyzed by ANCOVA. Differences in body weight from control were analyzed using a two-sided Student's t-test. For all statistical analyses, the level of significance was at 5% and 1%.

II. RESULTS:

A. Mortality and clinical observations: All adult rats survived to individual group termination with the exception of an incidental death of one male from the 0.3 mg/kg dose group that died prior to dosing on day 1. The single dose of 5.0 mg/kg resulted in mortality among three pups (one male in the single-dose study and two females in the time-course study) within 1.5

to 5.5 hours after dosing while one female pup (in the time-course study) was sacrificed six hours after dosing due to severe tremors. The single dose of 5.0 mg/kg to pups and adult rats caused clinical signs of toxicity within 30 minutes of dosing. Clinical effects in the pups including splayed gait, tremors, shaking and fast/irregular breathing were seen over an 8-hour period in contrast to effects in adults which lasted for one hour.

In repeat dosing studies, one control male died of gavage error on day 16 during the 4-week study. No signs of toxicity were evident in these studies. Mortality and clinical observations data are shown in Tables 7a, 7b, 7c, and 7d.

TABLE 7a. Mortality and clinical signs of toxicity

Study type/ Dose	Mortality	Clinical signs
Time-course/pre-weaning and young adult	Two female pups died approximately 2 and 5.5 hours after dosing and a third female pup was sacrificed due to severe clinical signs approximately 6 hours after dosing.	At 5 mg/kg, all animals had clinical signs of toxicity beginning 30 minutes after dosing and lasting up to 7-8 hours after dosing. The majority of animals showed decreased activity (18/25 pups and 24/25 young adults), and ataxia was noted in 19/25 pups. Effects of ChEI correlated with clinical observations for splayed gait, shaking, tremors, and fast/irregular breathing. Clinical effects in the pups were seen over a longer period than those in the adult. In all animals, clinical signs were normal after 24 hours.
Single dose/pre-weaning and young adult	One PND 8 male pup at 5 mg/kg was found dead approximately 1.5 hours after dosing. All other pups survived to scheduled termination.	Tremors were seen in all pups given 5 mg/kg dicrotophos. Pups of 15 days of age were more affected than those of 8 and 22 days of age (Table 7b). In addition to the tremors observed, clinical signs included signs of salivation, eye discharge, thickened eyelids, blinking, decreased activity (slight - moderate), splayed gait and reduced hind limb function. No clinical signs were seen at lower dose levels with the exception of one 8 day old male pup given 1 mg/kg that had slight tremors.
Single dose/adult	At 0.3 mg/kg, one male died prior to dosing on day 1.	Clinical signs seen in one or both sexes at 5 mg/kg within 1.5-2 hours after dosing included, tremors, decreased activity, tiptoe gait, splayed gait and/or reduced stability, irregular breathing and piloerection (Table 7c), but all animals recovered from day 2 onward. In addition, pinched-in-sides and upward curvature of the spine were seen in all animals only on day 1. There were no significant clinical observations in animals treated with 0.1 or 0.3 mg/kg dicrotophos.
7-Day repeat dose	All rats survived to scheduled termination	No treatment related clinical condition was reported.
4-Week repeat dose/recovery	One control male died on day 16 prior to dosing due to gavage error.	Study report states that cage-side observations were made 2-3 hours after dosing but no treatment related findings were observed.
Repeat dose/ bridging	All rats survived to scheduled termination	No treatment related clinical signs were observed.

Data from pp. 18 and 26, MRID 46153203; Appendix C, p. 32, MRID 46153205; Table 3, pp. 27 and 28, MRID 46153206; p. 18, MRID 46153204; p. 20, MRID 46153208; and Table 3, pp. 32 and 33, MRID 46153207.

TABLE 7b. Clinical findings in pre-weaning rats receiving a single dose of 5 mg dicrotophos						
Observation	**PND 8**		**PND 15**		**PND 22**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Tremors (slight or moderate)	3/3[a]	5/5	5/5	5/5	5/5	5/5
Excessive yawning	3/3	5/5	2/5	–[b]	–	–
Blinking Eye discharge Eyelids thickened	–	–	3/5 2/5 2/5	3/5 1/5 2/5	1/5 1/5	1/5 1/5
Salivation	3/3	–	–	2/5	–	–
splayed gait	–	–	–	1/5	–	–
Reduced hind-limb function decreased activity	–	–	5/5	5/5	–	–
Diarrhoea	–	–	–	–	1/5	–

Data from p. 31-36, Appendix C, MRID 46153205.
[a] One male PND 8 pup died by 1.5 hours postdosing another was missing.
[b] = No findings.

TABLE 7c. Clinical findings in young adult rats receiving a single dose of 5 mg dicrotophos/kg		
Observations	**No. of rats with findings/Total number of rats in the group**	
	Males	**Females**
Tremors (slight or moderate)	14/15	15/15
Sides pinched in	15/15	15/15
Upward curvature of spine	15/15	15/15
Decreased activity	9/15	9/15
Splayed gait	7/15	6/15
Reduced stability	8/15	9/15
Tip toe gait	1/15	4/15
Piloerection	6/15	3/15
Breathing-irregular	5/15	6/15
Chromodacryorrhea	5/15	5/15
Reduced response to sound	2/15	—[a]
Diarrhoea	1/15	—
Salivation	1/15	—

Data from Table 3, pp. 27 and 28, MRID 46153206.
[a]= No findings.

TABLE 7d. Intergroup comparison of clinical observation in the time-course of cholinesterase inhibition in pre-weaning and adult female rats

Time/Observation	Pre-weaning (PND 15)		Adults (PND 42)	
	Dose (mg/kg)			
	0	5	0	5
1 HOUR POST-DOSING				
Splayed gait	0/5	5/5 (100%)[a]	0/5	4/5 (80%)
Shaking	0/5	1/5 (20%)	0/5	0/5
Tremors	0/5	4/5 (80%)	0/5	1/5 (20%)
Fast/irregular breathing	0/5	0/5	0/5	5/5 (100%)
3 HOURS POST-DOSING				
Splayed gait	0/5	5/5 (100%)	0/5	0/5
Tremors	0/5	3/5 (60%)	0/5	0/5
Labored breathing	0/5	2/5 (40%)	0/5	0/5
Piloerection	0/5	2/5 (40%)	0/5	0/5
8 HOURS POST-DOSING				
Splayed gait	0/5	4/5 (80%)	0/5	0/5
Tremors	0/5	3/5 (60%)	0/5	0/5
Labored breathing	0/5	1/5 (20%)	0/5	0/5
Shaking	0/5	1/5 (20%)	0/5	0/5

Data from Table 2, p. 26, MRID 46153203.
n =5 per group, except PND 15 treated at 8 hrs post-dosing, n=4.
Data reported as incidence/number of animals.
[a] Results in parenthesis are percent occurrence relative to control.
*p≤ 0.05, **p ≤ 0.01.
Clinical signs of ChE inhibition were first seen approximately 30 minutes after dosing and were still evident in animals 7-8 hours after dosing. The majority of pre-weaning and adult animals had decreased activity. All pre-weaning animals continued to shake during the study and most also had ataxia. Note: All adult animals had tremors on day 1, although a low incidence was observed during the hourly observations immediately post-dosing. All animals (except one pup with slightly decreased activity) were normal 24 hours after dosing.

B. **Body weight and food consumption:** No adverse effect on body weight was noted in pre-weaning and adult rats with the exception of the bridging study in which after 5 weeks, a decrease in body weight up to 10% during the last three weeks of the recovery period compared to control was noted in males in the 0.4 mg/kg/day dose group. This decrease was not considered treatment-related since this finding was not seen during the treatment period. No treatment-related effects were found on food consumption of adult male or female rats.

C. **Brain weight:** With the exception of the 4-week and 4- to 8- week repeat dose studies, brain weight was not included in the study reports. In the 4-week repeat dose and recovery study, the mean brain weight of males in the 0.4 mg/kg/day dose group sacrificed at week 5 was slightly but statistically significantly (6%) higher than controls. This finding was possibly attributed to the greater weight of one treated male and slightly smaller weight of 2 control males. In the 4- to 8-week repeat dose bridging study, brain weight was 6% heavier than controls in treated males on day 29, but not at later time points and not in females. Therefore, these changes in brain weight are not considered treatment-related.

D. Cholinesterase activity: The treatment-related RBC and brain ChEI in young adult, and pre-weaning male and female rats following the time course, acute, 7-day repeat, 4-week, and bridging study are reported in Tables 8, 9, 10a and 10b, 11, and 12, respectively. The studies are also discussed below.

1. Time course: ChE inhibition of RBCs and brain in young adult rats dosed with 5 mg/kg was observed on day 1 within 1-3 hours post-dosing (Table 8). The peak time effect was reached by 3 hours in pups (both RBC and brain) and 1 hour for adults. Pups had greater RBC ChE inhibition than adults. There was partial recovery in both pups and adults by 24 and 72 hours post-dosing.

2. Acute exposure: In young adult male and female rats, ChE activity inhibition was observed at ≥0.3 mg/kg dicrotophos (Table 9). RBC inhibition was statistically significant in males and females at 0.3 mg/kg (15% and 10%, respectively) and 5.0 mg/kg (47 and 39%, respectively). Brain ChEI occurred at 0.3 and 5.0 mg/kg in females (22% and 76%, respectively) and at 5.0 mg/kg in males (74%).

   In several pup groups, ChE activity inhibition was observed at ≥0.1 mg/kg dicrotophos. RBC ChEI occurred at 0.1 mg/kg in PND 15 males and females and PND 22 females (20-25%). For PND 8 males and females and PND 22 males, RBC inhibition occurred at 0.3 mg/kg (19-26%). Inhibition of brain ChE was observed at 0.1 mg/kg in PND 8 males, PND 15 males and females, and PND 22 females (19-32%). PND 8 females and PND 22 males were affected at 0.3 mg/kg. Recovery from ChEI was usually complete in adults by days 8 and 15 (data not shown).

TABLE 8. RBC and brain ChE activity in pre-weaning and adult rats treated with a single dose of dicrotophos - time course

Age group/ Time post-treatment	RBC (U/L)		Brain (IU/g)	
	Dose (mg/kg)			
	0	5	0	5
PND 15 Female				
1 hour post-dosing	4381 ± 726	1758** ± 160 (60)	4.61 ± 0.38	0.86** ± 0.06 (81)
3 hours post-dosing	6481 ± 1256	**1930** ± 259 (70)**	4.59 ± 0.54	**0.78** ± 0.05 (83)**
8 hours post dosing	5658 ± 1100	1856* ± 239 (67)	5.88 ± 0.93	1.09** ± 0.21 (82)
24 hours post dosing	4852 ± 536	3030** ± 436 (38)	5.98 ± 0.94	3.77* ± 0.24 (37)
72 hours post dosing	4317 ± 584	2850* ± 339 (34)	7.26 ± 1.25	5.48 ± 0.81 (24)
PND 42 Female				
1 hour post-dosing	3232 ± 136	**1379** ± 60 (57)**	6.40 ± 0.39	**1.46** ± 0.08 (77)**
3 hours post-dosing	3043 ± 208	1706** ± 69 (44)	8.78 ± 3.05	2.56** ± 0.48 (71)
8 hours post dosing	3372 ± 203	1881** ± 129 (44)	6.03 ± 0.44	2.49** ± 0.32 (59)
24 hours post dosing	2970 ± 152	2205** ± 176 (26)	7.17 ± 1.21	4.09* ± 0.65 (43)
72 hours post dosing	3171 ± 220	2445** ± 103 (23)	5.94 ± 1.14	4.18* ± 0.60 (30)

Data from Table 4, pp. 30-33, MRID 46153203.
n =5 per group, except PND 15 treated at 8, 24, & 72 hrs post-dosing, n=4.
[a] Results in parenthesis are percent inhibition relative to control, numbers in bold are peak effect.
* p≤ 0.05, **p ≤ 0.01.

TABLE 9. RBC and brain ChE activity in adult and pre-weaning rats treated with dicrotophos – acute exposure

Age group/Tissue	Dose (mg/kg)				
	0	0.1	0.3		5.0
PND 42 Adult Males					
RBC (U/L)	2642 ± 102	2771 ± 145	2237** ± 75 (15)	–	1409** ± 153 (47)
Brain (IU/g)	4.77 ± 0.27	4.99 ± 0.32	4.57 ± 0.60 (4)	–	1.24** ± 0.09 (74)
PND 42 Adult Females					
RBC (U/L)	2449 ± 110	2399 ± 138 (2)	2214** ± 101 (10)	–	1498** ± 95 (39)
Brain (IU/g)	5.22 ± 0.63	4.76 ± 0.19 (9)	4.07** ± 0.24 (22)	–	1.26** ± 0.06 (76)
	0	**0.1**	**0.3**	**1.0**	**5.0**
PND 8 Males					
RBC (U/L)	2911 ± 547	3081 ± 551	2146** ± 435 (26)	1624** ± 608 (44)	1465** ± 98 (50)
Brain (IU/g)	3.39 ± 0.28	2.83** ± 0.23 (16)	1.95** ± 0.17 (42)	1.14** ± 0.20 (66)	0.64** ± 0.07 (81)
PND 8 Females					
RBC (U/L)	3153 ± 486	3337 ± 531	2466 ± 722 (22)	1771** ± 495 (44)	1580** ± 654 (50)
Brain (IU/g)	3.19 ± 0.26	2.99 ± 0.09 (6)	2.09** ± 0.26 (34)	1.17** ± 0.18 (63)	0.66** ± 0.05 (79)
PND 15 Males					
RBC (U/L)	3150 ± 285	2361** ± 304 (25)	2006** ± 527 (36)	1716** ± 143 (46)	1171** ± 184 (63)
Brain (IU/g)	4.69 ± 0.38	3.73** ± 0.60 (21)	2.95** ± 0.18 (37)	1.48** ± 0.25 (68)	0.77** ± 0.04 (84)
PND 15 Females					
RBC (U/L)	2959 ± 143	2318** ± 505 (22)	2203** ± 205 (26)	1519** ± 246 (49)	1320** ± 194 (55)
Brain (IU/g)	4.90 ± 0.30	3.31** ± 0.45 (32)	2.69** ± 0.10 (45)	1.40** ± 0.17 (71)	0.77** ± 0.04 (84)
PND 22 Males					
RBC (U/L)	2849 ± 384	2584 ± 227 (9)	2312** ± 231 (19)	1583** ± 113 (44)	1360** ± 152 (52)
Brain (IU/g)	5.18 ± 0.31	4.79 ± 0.69 (8)	4.38** ± 0.48 (15)	2.28** ± 0.11 (56)	1.16** ± 0.18 (78)
PND 22 Females					
RBC (U/L)	3028 ± 319	2438** ± 302 (20)	2280** ± 176 (25)	1700** ± 203 (44)	1321** ± 147 (56)
Brain (IU/g)	5.43 ± 0.44	4.41** ± 0.26 (19)	4.02** ± 0.31 (26)	2.51** ± 0.35 (54)	1.33** ± 0.19 (76)

Data from Tables 2-3, pp. 25-26, MRID 46153205 (pups, n=5 for all groups).
Data from Tables 7-8, pp. 36-37, MRID 46153206 (adults, n=5 for all groups).
[a] Results in parenthesis are percent inhibition relative to control. Five adult rats per group were killed on day 1 (at time of peak effect, 2-3 hours after dosing), day 8, and day 15 (only day 1 post-dosing data are shown in table, because they are most relevant time point in pup data).
*p≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

3. Repeated exposure: ChEI data following 7-day repeated dosing with dicrotophos in adult and pre-weaning rats are summarized in Tables 10a and 10b (data for the 1.0 mg/kg/day groups were reported separately, i.e., the study was conducted in two phases). In adult males and females, both RBC and brain ChEI were observed at 0.4 and 1.0 mg/kg/day (the 10% RBC ChE activity inhibition at 0.08 mg/kg/day in males was not considered biologically significant). The results were generally dose-related. RBC ChEI in PND 18 males and females was observed at a lower dose, 0.08 mg/kg/day (19 and 25%, respectively). Statistical significance for brain ChEI was attained at 0.4 mg/kg/day; however, the 15% inhibition in

brain ChE activity inhibition for both sexes at 0.08 mg/kg/day is biologically significant. At the 0.4 and 1.0 mg/kg/day repeat doses, the magnitude of ChEI was greater in pups than in adult rats (both compartments). No gender differences were apparent for either age group.

TABLE 10a. RBC and brain ChE activity in adult and pre-weaning rats treated with dicrotophos – 7-day repeat dose (Phase 1)

Treatment group/ Tissue	Dose (mg/kg/day)				
	0	0.008	0.02	0.08	0.4
Adult males (48 days)					
RBC (U/L)	2892 ± 120	2637* ± 69 (9)[a]	2706 ± 170 (6)	2587* ± 189 (10)	1544** ± 318 (47)
Brain (IU/g)	4.57 ± 0.54	4.87 ± 0.97	5.24 ± 0.13	4.75 ± 0.48	3.35** ± 0.34 (27)
Adult females (48 days)					
RBC (U/L)	2733 ± 67	2724 ± 129	2645 ± 139 (3)	2556 ± 123 (6)	1703** ± 307 (38)
Brain (IU/g)	5.01 ± 0.45	4.70** ± 0.53 (6)	5.70* ± 0.65	5.01 ± 0.30	3.19** ± 0.27 (36)
PND 18 males					
RBC (U/L)	3161 ± 315	3148 ± 441	3046 ± 206 (4)	2565** ± 355 (19)	1245** ± 86 (61)
Brain (IU/g)	4.84 ± 1.34	4.06 ± 0.38 (16)	4.85 ± 1.11	4.09 ± 0.95 (15)	2.41** ± 0.18 (50)
PND 18 females					
RBC (U/L)	3066 ± 307	3264 ± 326	2906 ± 429 (5)	2297** ± 214 (25)	1250** ± 133 (59)
Brain (IU/g)	4.46 ± 0.41	4.14 ± 0.46 (7)	4.41 ± 1.07 (1)	3.79 ± 0.29 (15)	1.89** ± 0.20 (58)

Data from Table 3, pp. 25 and 27, MRID 46153204.
pups and adults: n=5 for all groups.
[a] Results in parenthesis are percent inhibition relative to control.
*p≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

TABLE 10b. RBC and brain ChE activity in adult and pre-weaning rats treated with dicrotophos - 7-day repeat exposure (Phase 2)

Treatment group/Tissue	Dose (mg/kg/day)	
	0	1.0
Adult males (48 days)		
RBC (U/L)	2271 ± 134	1254** ± 161 (45)
Brain (IU/g)	5.76 ± 0.36	1.99** ± 0.19 (65)
Adult females (48 days)		
RBC (U/L)	2241 ± 80	1178** ±244 (47)
Brain (IU/g)	5.74 ± 0.74	2.09** ± 0.38 (64)
PND 18 males		
RBC (U/L)	3380 ± 360	1154** ± 98 (66)
Brain (IU/g)	5.39 ± 0.50	1.17** ± 0.15 (78)
PND 18 females		
RBC (U/L)	2982 ± 354	1143** ± 111 (62)
Brain (IU/g)	5.92 ± 1.62	1.24** ± 0.15 (79)

Data from Table 37, pp. 26 and 28, MRID 46153204 (adults, n=5 for all groups).
[a] Results in parenthesis are percent inhibition relative to control.
*p≤ 0.05, **p ≤ 0.01.

4. 4-Week repeat dose with recovery: In adult male and female rats dosed orally for 28 consecutive days, dicrotophos inhibited ChE activity at 0.4 mg/kg/day but not at 0.02 or 0.008 mg/kg/day (Table 11). Inhibition of ChE activity for those animals treated with 0.4 mg/kg/day dicrotophos persisted into the recovery period (RBC for males and RBC and brain for females).

TABLE 11. RBC and brain ChE activity in adult rats treated with dicrotophos – 4-week repeated exposure with recovery

Group/Tissue	Dose (mg/kg/day)			
	0	0.008	0.02	0.4
4-Week repeat dosing				
Adult males				
RBC (U/L)	2168 ± 135	2160 ± 56	2005 ± 201 (8)	1507** ± 143 (30)
Brain (IU/g)	4.72 ± 0.87	5.08 ± 0.30	4.86 ± 1.26	2.47** ± 0.32 (48)
Adult females				
RBC (U/L)	2274 ± 321	2282 ± 158	2402 ± 143	1817** ± 133 (20)
Brain (IU/g)	5.00 ± 0.95	4.49 ± 0.65 (10)	4.33 ± 0.28 (13)	2.71** ± 0.52 (46)
Recovery groups				
Adult males				
RBC (U/L)	2549 ± 97	2286 ± 113 (10)	2452 ± 228 (4)	2145** ± 172 (16)
Brain (IU/g)	3.64 ± 1.04	3.83 ± 0.69	3.53 ± 0.69 (3)	3.49 ± 0.35 (4)
Adult females				
RBC (U/L)	2344 ± 85	2358 ± 141	2514 ± 151	2122* ± 121 (10)
Brain (IU/g)	4.43 ± 0.38	3.81 ± 0.74 (14)	3.48 ± 0.61 (21)	3.30** ± 0.50 (25)

Data from Table 7, pp. 42-43, MRID 46153208.
n =5 per group.
[a] Results in parenthesis are percent inhibition relative to control.
* $p \leq 0.05$, ** $p \leq 0.01$.
Repeated dose treated, 68 days of age. Recovery treated, additional 28 non-dosing days, 96 days of age.

5. Bridging study (4 or 8 week repeat-dose study): Among rats dosed orally for 4 or 8 weeks with the single dose of 0.4 mg/kg/day, RBC and brain ChE activity were inhibited (Table 12). Compared with the 4-week data, after 8 weeks of administration, there was no significant further inhibition of ChE activity, indicating that maximal inhibition was achieved within 4 weeks. No gender difference was apparent. As noted previously, brain weight was 6% heavier in treated males than controls (1.97±0.6 vs.1.86±0.06 g, $p<0.05$) after 4 weeks (day 29), but not at the later time point and not in females.

TABLE 12. Four or eight-week repeat-dose study		
Treatment group/Tissue	Dose (mg/kg/day)	
	0	0.4
4-Week repeat dose		
Adult males		
RBC (U/L)	2165 ± 52	1442** ± 207 (33)
Brain (IU/g)	5.64 ± 0.36	2.90** ± 0.19 (49)
Adult females		
RBC (U/L)	2284 ± 33	1482** ± 89 (35)
Brain (IU/g)	5.47 ± 0.73	2.84** ± 0.37 (48)
8-Week repeat dose		
Adult males		
RBC (U/L)	2111 ± 206	1349** ± 67 (36)
Brain (IU/g)	5.07 ± 1.24	2.21** ± 0.15 (56)
Adult females		
RBC (U/L)	2384 ± 97	1427** ± 87 (40)
Brain (IU/g)	4.59 ± 0.21	2.13** ± 0.09 (54)

Data from Table 7, pp. 43, MRID 46153207 (adults, n=5 for all groups).
[a] Results in parenthesis are percent inhibition relative to control.
*p≤ 0.05, **p ≤ 0.01.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that administration of a single dose of 5.0 mg dicrotophos/kg did not affect body weight or body weight gain. However, an increased incidence of clinical signs was seen in both pups and adult rats. Treatment with dicrotophos was associated with a dose-dependent increase in inhibition of RBC and brain ChE activity at 0.1 mg/kg and above in pups and at 0.3 mg/kg and above in adult rats. No sex related differences were seen. At 5 mg/kg, the decrease in ChE activity was most marked from 1 to 8 hours post dosing. There was no difference in the extent or time of the response seen in pups and adult rats. Recovery in RBC and brain ChEI activity occurred after 24 and 72 hours. In single dose studies, the authors established a NOAEL of 0.1 mg/kg in adult rats based on lack of inhibition in ChE activity. For pups, no NOAEL was established since the lowest dose of 0.1 mg/kg caused up to 32 and 25% inhibition of brain and RBC ChE activity, respectively, in male and female pups.

Seven-day repeat dosing of 0.4 or 1.0 mg dicrotophos/kg/day inhibited both RBC and brain ChE activity in both pups and young adult rats. At 0.08 mg/kg/day, only RBC ChEI was observed in pups. ChEI was not observed at 0.008 and 0.02 mg/kg/day in pups or young adult rats. Daily oral administration of 0.4 mg dicrotophos/kg/day to adult male and female rats for 4 weeks resulted in inhibition of RBC and ChE activity which returned almost to normal after a 4-week recovery period. The NOAEL was 0.02 mg/kg/day. After 8 weeks of administration of the single dose, there was no further significant decrease in cholinesterase activity indicating that the maximum inhibition was achieved within 4 weeks. No clear sex related differences were evident in repeat dosing studies.

B. DISCUSSION AND REVIEWER COMMENTS: These studies were conducted to determine the time to peak effect and effects of dicrotophos on RBC and brain ChE activity in male and female adult and juvenile SD rats following single or repeated oral administration. Plasma ChE activity was not measured.

The time of peak effect after single oral dosing with dicrotophos was reached by three hours in 15-day-old female pups compared to one hour in 42-day-old females (but still statistically significant at three hours in adults). By 24 and 72 hours postdosing, the ChE activity in pups and adults had returned to normal levels. The collection of blood and brain from both pups and adults for ChEI measurement at 2 hours post-dosing is reasonable.

In adult rats, single oral doses of dicrotophos up to 5.0 mg/kg did not adversely affect body weight or body weight gain at necropsy. However, treatment-related mortality was observed among pre-weaning rats that received a single oral dose of 5.0 mg/kg. The observed mortality at 5 mg/kg in pups correlated with the 76-84% brain ChEI in pups observed in the acute study. The effects on RBC and brain ChE activity in juvenile and adult rats correlated with clinical signs of toxicity. Although the clinical signs of ChE inhibition were first seen in pups and young adult rats within approximately 30 minutes after dosing, the clinical effects were seen over a longer period (8 hours) in the pups than those in adult rats (1 hour). Acute exposure to dicrotophos resulted in greater inhibition of RBC and brain ChE activities in 15 day old pups vs. 42 day old adult rats and occurred in pups at a lower dose than in adults. Brain weight was not reported in single dose studies.

The data set provided a number of statistically significant findings at levels of ChEI between 10-84%. Statistically significant and dose-dependent RBC and brain ChE inhibition were observed at a single oral dose of 0.1 mg/kg (lowest dose tested) and above in pups and at a dose of 0.3 mg/kg and above in young adult rats. Acute doses of 0.1 mg/kg caused no significant effects on ChE activity in adults. Thus, the available data indicate that pups were more sensitive than adults to the effects of dicrotophos with regard to survival, incidence and duration of clinical signs, extent of ChEI and the dose at which these effects were observed. Based on the magnitude of ChEI, the brain appears to be more a sensitive compartment than the RBC, with a variety of statistically significant effects seen at ≥0.1 mg/kg in pups and at ≥0.3 mg/kg in adult rats confirming these as LOAELs. The NOAEL was <0.1 mg/kg for pups and 0.1 mg/kg for young adult rats.

No treatment-related deaths were reported in the repeat-dose studies. Seven-day repeat exposure at doses ranging from 0.08 to 1.0 mg/kg/day induced dose-related statistically significant decreases in RBC and brain ChE activity in pups and adult rats of both sexes. Repeated 7-day dosing with dicrotophos in pre-weaning rats of 18 days of age resulted in higher brain and RBC ChE inhibition and at lower doses than in adult rats of 48 days of age. In adult rats, RBC and brain ChE inhibition response to repeated dosing with dicrotophos was still evident even after dosing had ceased for 28 days; however, the amount of inhibition was reduced. After 8 weeks of treatment there was no further significant decrease in ChE levels indicating that the maximal inhibition was achieved within 4 weeks.

When comparison was made between acute versus repeat dosing with regard to ChEI at 1.0 mg/kg dose, a slight cumulative effect was evident in pups (both sexes) following repeat

dosing for 7 days. In the acute study, blood and brain ChEI ranged from 44-49% and 54-66%, respectively; whereas in the repeat-dose study, the respective ranges were 62-66% and 78-79%. Adults were not tested at 1.0 mg/kg in the acute study. Following 4 weeks of dosing at 0.4 mg/kg/day, the brain (46-49% ChEI) appears to be a slightly more sensitive compartment than the RBC (20-35% ChEI). Following 7-day repeat exposures, statistically significant effects on brain and/or RBC ChEI were observed in PND 18 pups at 0.08 mg/kg/day and at 0.4 mg/kg/day in PND 48 adults. Although the 15% brain ChEI in both sexes of PND 18 pups at 0.08 mg/kg/day was not statistically significant, the dose dependence for the pups and adult rats suggests that the best point of departure is where the increasing slope of the dose effect curve begins. For both compartments, then, the weight of the evidence shows consistent significant and dose dependent effects of ≥15% in both sexes and at most time points, leading to the conclusion that 0.08 and 0.4 mg/kg/day are the LOAEL for ChEI in pups and adults, respectively. The NOAELs for ChEI are 0.02 mg/kg/day and 0.08 mg/kg/day for pups and adult rats, respectively.

The evidence of systemic toxicity observed in these studies suggests that dosing was adequate. Overall, based on these data, it is concluded that there was consistent evidence of greater sensitivity in young animals to ChEI following acute or repeated dicrotophos exposure. This finding is further supported by the results of an earlier developmental neurotoxicity study in rats (MRID 46153202). In this study, neuropathology was observed in pups at 0.4 mg/kg/day which caused no toxicity in maternal animals indicating greater sensitivity of pups to dicrotophos treatment.

For acute exposure:

the adult LOAEL for brain ChEI is 5 mg/kg (males), 0.3 mg/kg (females)
the adult NOAEL for brain ChEI is 0.3 mg/kg (males), 0.1 mg/kg (females);

the PND 8, 15, and 22 LOAEL for brain ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for brain ChEI is not determined (<0.1 mg/kg; both sexes);

the adult LOAEL for RBC ChEI is 0.3 mg/kg (both sexes)
the adult NOAEL for RBC ChEI is 0.1 mg/kg (both sexes);

the PND 8, 15, and 22 LOAEL for RBC ChEI is 0.1 mg/kg (both sexes)
the PND 8, 15, and 22 NOAEL for RBC ChEI is not determined (<0.1 mg/kg; both sexes).

For acute oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition in rats is 0.3 mg/kg based on enzyme inhibition in brain and RBC; the adult NOAEL is 0.1 mg/kg.

For acute oral exposure to dicrotophos, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.1 mg/kg based on enzyme inhibition in brain and RBC; the offspring NOAEL is not determined (<0.1 mg/kg).

For repeated exposure:

the adult LOAEL for brain ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for brain ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for brain ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for brain ChEI is 0.02 mg/kg/day (both sexes);

the adult LOAEL for RBC ChEI is 0.4 mg/kg/day (both sexes)
the adult NOAEL for RBC ChEI is 0.08 mg/kg (both sexes);

the offspring LOAEL for RBC ChEI is 0.08 mg/kg/day (both sexes)
the offspring NOAEL for RBC ChEI is 0.02 mg/kg/day (both sexes).

For repeated oral exposure to dicrotophos, the overall adult LOAEL for cholinesterase inhibition is 0.4 mg/kg/day based on brain and red blood cell; the NOAEL is 0.08 mg/kg/day.

For repeat oral exposure to dicrotophos, the overall offspring LOAEL is 0.08 mg/kg/day based on brain and RBC; the NOAEL is 0.02 mg/kg/day.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted.

1. Cholinesterase activity was not measured in dams and fetuses on GD 20 as recommended by the protocol review included with the study package (memo dated July 17, 2002).

2. Plasma ChE activity should have been analyzed at the same time as brain and RBC ChE were measured. Since WOE analysis is based on data from all three compartments the pattern of effect between the compartments affects the WOE.

3. The rationale supporting the selection of the times of sample collections should have been provided. There was lack of correspondence between the acute exposure testing time and repeated exposure testing time. The time point (i.e. PND) assessed at the end of repeated exposure should correspond to one of the time points assessed following acute exposure. For example, PND 18 in the repeat exposure study does not correspond to any day in acute testing time (i.e. PND 8, 15, or 22). As recommended in EPA's guidance (10/29/01), for acute exposure testing time, the first PND time point should be no later than PND 11, the second lactation time point should be 7-10 days later. Also, as noted in the protocol review, rats at 42 days of age are not fully mature with respect to enzyme systems. Therefore, Day 60 or a later day would be preferable.

4. With the exception of the 4-week repeat dose and recovery and 4 to 8-week repeat dose bridging studies, brain weight was not included in the reports. Morphometric analysis of brain would have been useful in correlating clinical signs and ChE inhibition with neuropathology.

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