US EPA ARCHIVE DOCUMENT

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

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

June 2, 2006
TXR# 0053636
MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: 032501: 6(a)(2) Data on disulfoton cholinesterase activity after acute dosing in young adults and 11-day old pups at peak time [MRID# 46589701-46589704], in maternal and fetal rats [MRID# 46635901], and in young adults dose 11 days [MRID# 46637101] and 11-day old pups dosed for 11 days [MRID# 46637101].

DP Barcode: D319813 and D 321704

From: David G Anderson 6/2/06
RRB-2 HED (7509P)

To: Christina Scheltema 6/6/06
SRRD (7508P)

Thru: Alan Nielsen, BSS
RRB-2, HED (7509P)

The registrant submitted data on cholinesterase depression in the Wistar strain rather the Sprague Dawley rat used in previous studies. The use of a different strain of rat may have contributed to the decrease in dose levels at which effects were seen in cholinesterase. Increased sensitivity was seen pups over that seen in adults from acute and 11 day dosing, however dams showed cholinesterase levels generally lower than that seen in fetuses. Below are the Executive Summaries from the studies involved.

MRID# 4658901 -4658904; D319813

EXECUTIVE SUMMARY - In two independent non-guideline time of peak-effect studies (MRIDs 46589701 [young adult] and 46589702 [preweaning]), disulfoton (97.5% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered once via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/sacrifice time at doses of 0, 0.75 (females), or 1.5 (males) mg/kg. Plasma, erythrocyte, and brain cholinesterase activities were determined at 2, 4, 6, and 8 hours post-dosing in the treated animals and at 4 hours post-dosing in the controls. Similarly, disulfoton in PEG 400 was administered once via gavage (5 mL/kg) to 10 Wistar rat pups/sex/sacrifice time at doses of 0 or 0.5 mg/kg on post-natal day 11. Plasma, erythrocyte, and brain cholinesterase activities were determined at 4, 6, 8, and 24 hours post-dosing in the treated animals and at 6 hours post-dosing in the controls. Additionally, in two independent cholinesterase inhibition studies (MRIDs 46589703 [young adult] and 46589704 [preweaning]), disulfoton (same batch) in PEG 400 was administered once via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/dose at doses of 0, 0.25, 0.75, or 1.5 mg/kg (males) or 0, 0.25, 0.5, or 0.75 mg/kg (females). Plasma, erythrocyte, and brain cholinesterase activities were determined at 6 (males) and 8 (females)

JUN 22 2006

hours post-dosing (time of peak-effect) in all groups. Similarly, disulfoton in PEG 400 was administered once via gavage (5 mL/kg) to 10 Wistar rat pups/sex/sacrifice time at doses of 0, 0.125, 0.25, or 0.5 mg/kg. Plasma, erythrocyte, and brain cholinesterase activities were determined at 24 hours post-dosing (time of peak-effect) in all groups. Benchmark doses (BMD) of 10 and 20% cholinesterase inhibition were calculated for each compartment in both studies.

There were no treatment-related effects on mortality or clinical signs of toxicity in either of the time to peak effect studies. Cholinesterase activity was decreased significantly in both sexes at most post dosing time points used at 2, 4, 6, and 8 hours. Peak time points were estimated at 6 hours and 8 hours post dosing, respectively for adult males and adult females. The time to peak effect in male and female pups was estimated to be 24 hours post dosing.

There were no treatment-related effects on mortality or clinical signs of toxicity in either adults or pups of the comparative cholinesterase inhibition studies. Cholinesterase was statistically significantly inhibited in at least one cholinesterase compartment in adult males and females and in pup males and females at all dose levels. Cholinesterase activity was dose-dependently decreased ($p<=0.05$) in the adults in both sexes as follows: (i) plasma at >=0.75 mg/kg in the males (decr 35-67%) and at all doses in the females (decr 16-84%); (ii) erythrocytes at 1.5 mg/kg in the males (decr 46%) and at >=0.5 mg/kg in the females (decr 34-70%); and (iii) brain at all doses in the males (decr 4-32%) and at >=0.5 mg/kg in the females (decr 17-43%). In the pups, cholinesterase activity was dose-dependently decreased ($p<=0.05$) in both sexes as follows: (i) plasma at >=0.25 mg/kg in the males (decr 24-56%) and at all doses in the females (decr 11-53%); (ii) erythrocytes at 0.5 mg/kg (decr 52-53%); and (iii) brain at all doses (decr 5-39%).

Bench mark doses were calculated by the authors at the 10% and 20% levels.
In the adults, the following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.33 and 0.54, males; 0.09 and 0.18, females); (ii) erythrocytes (0.58 and 0.82, males; and 0.22 and 0.36, females); and (iii) brain (0.66 and 0.95, males; 0.38 and 0.54, females). Similarly in the pups, the following benchmark doses (mg/kg) were calculated: (i) plasma (0.11 and 0.20, males; 0.08 and 0.16, females); (ii) erythrocytes (0.14 and 0.24, males; and 0.11 and 0.20, females); and (iii) brain (0.15 and 0.27, males; 0.13 and 0.25, females).

An acceptable lowest effect level will be calculated from another bench mark dose level calculation within OPP.

The results of the current study not based bench mark dose levels are below:
Young adult acute LOAEL for decreased cholinesterase:
Plasma –males 0.75 mg/kg/day; -females 0.25 mg/kg/day.
Erythrocyte –males 1.5 mg/kg/day; -females 0.75 mg/kg/day.
Brain –males 0.25 mg/kg/day; -females 0.75 mg/kg/day.

Young adult acute NOAEL for decreased cholinesterase:
Plasma –males 0.25 mg/kg/day; -females none [LDT].
Erythrocyte –males 0.75 mg/kg/day; -females 0.25 mg/kg/day.
Brain –males none [LDT]; -females 0.25 mg/kg/day.

11-Day old pup acute LOAEL for decreased cholinesterase:
Plasma –males 0.25 mg/kg/day; -females 0.125 mg/kg/day.
Erythrocyte –males 0.5 mg/kg/day; -females 0.5 mg/kg/day.

Brain –males 0.125 mg/kg/day; -females 0.125 mg/kg/day.

11-Day old pup acute NOAEL for decreased cholinesterase:
Plasma –males 0.125 mg/kg/day; -females none [LDT].
Erythrocyte -males 0.25 mg/kg/day; -females 0.25 mg/kg/day.
Brain –males none [LDT]; -females none [LDT].

These studies are classified as ACCEPTABLE/NON-GUIDELINE.

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

MRID# 46635901; D321704

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46635901), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) was administered continuously in the diet to 13 pregnant Wistar rats/sex/dose at doses of 0, 0.5, 2, or 8 ppm (equivalent to 0, 0.042, 0.168, and 0.694 mg/kg/day, respectively) from gestation day (GD) 0-20. Plasma, erythrocyte, and brain cholinesterase activities were determined on GD 20 in maternal and fetal (pooled samples) rats in all groups. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no treatment-related effects on mortality, clinical signs, body weight, or feed consumption. Statistically significant dose-dependent decreases ($p<0.01$) in cholinesterase activity were observed in all compartments (plasma, blood, and brain) in the >=0.5 ppm dams, except plasma cholinesterase was not significantly depressed at 0.5 ppm. Fetal cholinesterase showed a statistically significant dose related depression in CHE as follows: plasma CHE at 0.5 ppm, erythrocyte CHE at 2 ppm and brain CHE at 8 ppm. In the dams, the following benchmark doses (mg/kg day) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.09 and 0.17); (ii) erythrocytes (0.09 and 0.17); and (iii) brain (0.09 and 0.18). In the fetuses, the following benchmark doses (mg/kg/day) were calculated: (i) plasma (0.12 and 0.23); (ii) erythrocytes (0.10 and 0.19); and (iii) brain (0.38 and 0.59). In this study, the fetuses were less sensitive than their dams.

Maternal LOAEL for decreased cholinesterase:
Plasma **-2 ppm [0.168 mg/kg/day]**;
Erythrocytes **-0.5 ppm [LDT] [0.042 mg/kg/day]**; and
Brain **-0.5 ppm [LDT][0.042 mg/kg/day]**.

Maternal NOAEL for decreased cholinesterase:
Plasma **0.5 ppm [0.042 mg/kg/day]**,
Erythrocytes, **-none [LDT]**; and
Brain, **-none [LDT]**.

Litter LOAEL for decreased cholinesterase:
Plasma **-0.5 ppm [LDT][0.042 mg/kg/day]**;
Erythrocyte **-2 ppm**;
Brain **-8 ppm [0.694 mg/kg/day]**.

Litter NOAEL for decreased cholinesterase:
Plasma, -none [LDT];
Erthrocytes -0.5 ppm [0.042 mg/kg/day]:
Brain -2 ppm [0.168 mg/kg/day].

This study is classified as ACCEPTABLE/NON-GUIDELINE.

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

MRID# 46637101; D321704

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46637101), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered once daily for 11 consecutive days via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/dose at doses of 0, 0.25, 0.5, or 1.0 mg/kg in males and 0, 0.125, 0.25, or 0.5 mg/kg in females. Plasma, erythrocyte, and brain cholinesterase activities were determined at 1 hour after final dosing (time of peak-effect) in all groups. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no mortalities or treatment-related clinical signs of toxicity. Cholinesterase activity was dose-dependently decreased (p<=0.05) as follows: (i) plasma in the >=0.5 mg/kg males (decr 32-41%.) and in the >=0.125 mg/kg females (decr 30-73%); (ii) erythrocytes in the >=0.5 mg/kg males (decr 38-72%) and in the >=0.125 mg/kg females (decr 15-63%); and (iii) brain in the >=0.25 mg/kg males (decr 15-70%) and in the >=0.125 females (decr 11-70%). The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.20 and 0.40, males; 0.05 and 0.11, females); (ii) erythrocytes (0.12 and 0.23, males; and 0.08 and 0.15, females); and (iii) brain (0.12 and 0.24, males; 0.07 and 0.14, females).
Young adult LOAEL for decreased cholinesterase:
Plasma –males 0.5 mg/kg/day; -females 0.125 mg/kg/day
Erythrocyte –males 0.5 mg/kg/day; -females 0.125 mg/kg/day
Brain –males 0.25 mg/kg/day; -females 0.125 mg/kg/day.

Young adult NOAEL for decreased cholinesterase:
Plasma –males 0.25 mg/kg/day; -females none [LDT]
Erythrocyte –males 0.25 mg/kg/day; -females none [LDT]
Brain –males none [LDT]; -females none [LDT].

This study is classified as ACCEPTABLE/NON-GUIDELINE.

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

MRID# 46637102; D321704

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46637102), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered daily via gavage (5 mL/kg) to 10 Wistar rat pups/sex/dose at doses of 0, 0.06, 0.125, or 0.250 mg/kg for 11 consecutive days beginning on postnatal day 11. Plasma, erythrocyte, and brain cholinesterase activities were determined at 1 hour (time of peak effect) following the final dosing. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no treatment-related effects on clinical signs of toxicity. Four pups of different dose groups, but originating from the same litter (female no. 9) were found dead or cannibalized between PND 12-18. Cholinesterase activity was dose-dependently (unless otherwise stated) decreased ($p<=0.05$) as follows: (i) plasma in the >=0.125 mg/kg males (decr 22-38%,) and in the 0.25 mg/kg females (decr 36%); (ii) erythrocytes at >=0.125 mg/kg in the males (decr 19-42%) and at >=0.06 mg/kg in the females (decr 29-52%); and (iii) brain at >=0.06 mg/kg in both sexes (decr 7-44%). The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.059 and 0.117, males: 0.09 and 0.148, females); (ii) erythrocytes (0.086 and 0.136, males; and 0.042 and 0.085, females); and (iii) brain (0.053 and 0.107, males; 0.051 and 0.102, females).

Pup LOAELs for cholinesterase were as follows:
Plasma- males 0.125 mg/kg/day –females 0.250 mg/kg/day;
Erythrocyte- males 0.125 mg/kg/day –females 0.06 mg/kg/day [LDT];
Brain –males 0.06 mg/kg/day [LDT]; -females 0.06 mg/kg/day [LDT].

Pup NOAELs for cholinesterase were as follows:
Plasma –males 0.06 mg/kg/day; -females 0.125 mg/kg/day;
Erythrocyte –males 0.06 mg/kg/day; -females none [LDT];
Brain –males none [LDT]; -females none [LDT].

This study is classified as **ACCEPTABLE/NON-GUIDELINE**.

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

DATA EVALUATION RECORD

DISULFOTON

Study Type: Non-guideline; Time of Peak Cholinesterase Inhibition and Comparative Cholinesterase Studies in Preweaning and Young-adult Rats

Work Assignment No. 3-01-80; formerly 2-01-80 (MRIDs 46589701 through 46589704)

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

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer:
David McEwen, B.S.

Signature: David A. McEwen
Date: 9/29/05

Secondary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 9/30/05

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 9/30/05

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 9/30/05

Disclaimer

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

EPA Reviewer: David G Anderson, PhD Signature:
Reregistration Branch-2, Health Effects Division (7509C) Date
Work Assignment Manager: PV Shah, Ph.D. Signature:
Registration Action Branch 1, Health Effects Division (7509C) Date

Template version 11/01

TXR# 0053636

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Time of Peak Cholinesterase Inhibition and Comparative Cholinesterase Studies in Preweaning and Young-adult Rats.

PC CODE: 032501 **DP BARCODE: DP319813**

TEST MATERIAL (PURITY): Disulfoton (97.5% a.i.)

SYNONYMS: O,O-diethyl S-[2-(ethylthio)ethyl] phosphorodithioate

CITATION Langewische, F.W. (2005) Disulfoton: Study to establish the time of peak cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade disulfoton. Bayer HealthCare AG, PH-R&D-PD Toxicology International, Wuppertal, Germany. Laboratory Report No.: AT02018, Study No.: T6073934, March 31, 2005. MRID 46589701. Unpublished.

Langewische, F.W. (2005) Disulfoton: Study to determine the time of peak cholinesterase inhibition in preweaning Wistar rats treated by gavage with an acute dose of technical grade disulfoton. Bayer HealthCare AG, PH-R&D-PD Toxicology International, Wuppertal, Germany. Laboratory Report No.: AT02019, Study No.: T0073938, April 14, 2005. MRID 46589702. Unpublished.

Langewische, F.W. (2005) Disulfoton: Cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade disulfoton. Bayer HealthCare AG, PH-R&D-PD Toxicology International, Wuppertal, Germany. Laboratory Report No.: AT02065, Study No.: T9073937, May 30, 2005. MRID 46589703. Unpublished.

Langewische, F.W. (2005) Disulfoton: Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage with an acute dose of technical grade disulfoton. Bayer HealthCare AG, PH-R&D-PD Toxicology International, Wuppertal, Germany. Laboratory Report No.: AT02066, Study No.: T1073939, May 30, 2005. MRID 46589704. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY - In two independent non-guideline time of peak-effect studies (MRIDs 46589701 [young adult] and 46589702 [preweaning]), disulfoton (97.5% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered once via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/sacrifice time at doses of 0, 0.75 (females), or 1.5 (males) mg/kg. Plasma, erythrocyte, and brain cholinesterase activities were determined at 2, 4, 6, and 8 hours post-dosing in the treated animals and at 4 hours post-dosing in the controls. Similarly, disulfoton in PEG 400 was administered once via gavage (5 mL/kg) to 10 Wistar rat pups/sex/sacrifice time at doses of 0 or 0.5 mg/kg on post-natal day 11. Plasma, erythrocyte, and brain cholinesterase activities were determined at 4, 6, 8, and 24 hours post-dosing in the treated animals and at 6 hours post-dosing in the controls. Additionally, in two independent cholinesterase inhibition studies (MRIDs 46589703 [young adult] and 46589704 [preweaning]), disulfoton (same batch) in PEG 400 was administered once via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/dose at doses of 0, 0.25, 0.75, or 1.5 mg/kg (males) or 0, 0.25, 0.5, or 0.75 mg/kg (females). Plasma, erythrocyte, and brain cholinesterase activities were determined at 6 (males) and 8 (females) hours post-dosing (time of peak-effect) in all groups. Similarly, disulfoton in PEG 400 was administered once via gavage (5 mL/kg) to 10 Wistar rat pups/sex/sacrifice time at doses of 0, 0.125, 0.25, or 0.5 mg/kg. Plasma, erythrocyte, and brain cholinesterase activities were determined at 24 hours post-dosing (time of peak-effect) in all groups. Benchmark doses (BMD) of 10 and 20% cholinesterase inhibition were calculated for each compartment in both studies.

There were no treatment-related effects on mortality or clinical signs of toxicity in either of the time to peak effect studies. Cholinesterase activity was decreased significantly in both sexes at most post dosing time points used at 2, 4, 6, and 8 hours. Peak time points were estimated at 6 hours and 8 hours post dosing, respectively for adult males and adult females. The time to peak effect in male and female pups was estimated to be 24 hours post dosing.

There were no treatment-related effects on mortality or clinical signs of toxicity in either adults or pups of the comparative cholinesterase inhibition studies. Cholinesterase was statistically significantly inhibited in at least one cholinesterase compartment in adult males and females and in pup males and females at all dose levels. Cholinesterase activity was dose-dependently decreased ($p<=0.05$) in the adults in both sexes as follows: (i) plasma at >=0.75 mg/kg in the males (decr 35-67%) and at all doses in the females (decr 16-84%); (ii) erythrocytes at 1.5 mg/kg in the males (decr 46%) and at >=0.5 mg/kg in the females (decr 34-70%); and (iii) brain at all doses in the males (decr 4-32%) and at >=0.5 mg/kg in the females (decr 17-43%). In the pups, cholinesterase activity was dose-dependently decreased ($p<=0.05$) in both sexes as follows: (i) plasma at >=0.25 mg/kg in the males (decr 24-56%) and at all doses in the females (decr 11-53%); (ii) erythrocytes at 0.5 mg/kg (decr 52-53%); and (iii) brain at all doses (decr 5-39%).

Bench mark doses were calculated by the authors at the 10% and 20% levels.
In the adults, the following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.33 and 0.54, males; 0.09 and 0.18, females); (ii) erythrocytes (0.58 and 0.82, males; and 0.22 and 0.36, females); and (iii) brain (0.66 and 0.95, males; 0.38 and 0.54, females). Similarly in the pups, the following benchmark doses (mg/kg) were calculated: (i) plasma (0.11 and 0.20, males; 0.08 and 0.16, females); (ii) erythrocytes (0.14 and 0.24, males; and 0.11 and 0.20, females); and (iii) brain (0.15 and 0.27, males; 0.13 and 0.25, females).

An acceptable lowest effect level will be calculated from another bench mark dose level calculation within OPP.

The results of the current study not based bench mark dose levels are below:
Young adult acute LOAEL for decreased cholinesterase:
Plasma –males 0.75 mg/kg/day; -females 0.25 mg/kg/day.
Erythrocyte –males 1.5 mg/kg/day; -females 0.75 mg/kg/day.
Brain –males 0.25 mg/kg/day; -females 0.75 mg/kg/day.

Young adult acute NOAEL for decreased cholinestyerase:
Plasma –males 0.25 mg/kg/day; -females none [LDT].
Erythrocyte –males 0.75 mg/kg/day; -females 0.25 mg/kg/day.
Brain –males none [LDT]; -females 0.25 mg/kg/day.

11-Day old pup acute LOAEL for decreased cholinesterase:
Plasma –males 0.25 mg/kg/day; -females 0.125 mg/kg/day.
Erythrocyte –males 0.5 mg/kg/day; -females 0.5 mg/kg/day.
Brain –males 0.125 mg/kg/day; -females 0.125 mg/kg/day.

11-Day old pup acute NOAEL for decreased cholinesterase:
Plasma –males 0.125 mg/kg/day; -females none [LDT].
Erythrocyte –males 0.25 mg/kg/day; -females 0.25 mg/kg/day.
Brain –males none [LDT]; -females none [LDT].

These studies are classified as ACCEPTABLE/NON-GUIDELINE.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description:	Light brown liquid
Batch #:	3-08-5013JC158
Purity (w/w):	97.5% a.i.
Stability of compound:	The test material was shown to be stable in the vehicle for up to 8 days at room temperature.
CAS #:	298-04-4
Structure:	(CH3-CH2-O)2P=O | S-(CH2)2-S-CH2-CH3

2. **Vehicle** - PEG 400

3. Test animals

Species:	Rat
Strain:	Wistar Crl:GLxBrl Han: WI
Adult age/weight at study initiation:	9-10 weeks old/ 224-274 g males and 165-193 g females (for both studies)
Pup Age/weight at dosing:	11 days old/ weight not reported (both studies)
Source	Charles River Wiga (Deutschland, Sulzfeld, Germany)
Housing:	Individually in Type IIIh Makrolon® cages
Diet:	Mouse and Rat Maintenance Diet No. 3883.0.15 (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water, ad libitum
Environmental conditions:	
Temperature:	20±2°C
Humidity:	Approximately 50%
Air changes:	At least 10/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 7 days

B. STUDY DESIGN

1. Study purpose - The purpose of these studies was to determine the time of peak cholinesterase inhibition in young-adult and preweaning rats treated once via gavage with disulfoton, and to determine cholinesterase inhibition in young-adult and preweaning rats at the time of peak effect. These data were used to calculate the benchmark dose (BMD10 and BMD20) response for each compartment (i.e. plasma, erythrocytes, and brain), and to provide a comparison of cholinesterase activity effects in adult versus neonatal animals. These studies were designed to support a separate Development Neurotoxicity Study (OPPTS 870.6300) performed in August, 1998.

2. In-life dates - Start: 12/21/04 End: 12/22/04 (MRID 46589701)

Start: 01/17/05 End: 01/26/05 (MRID 46589702)
Start: 01/06/05 End: 01/07/05 (MRID 46589703)
Start: 02/01/05 End: 02/04/05 (MRID 46589704)

3. **Mating procedure for preweaning studies** - Females were paired 2:1 with males of the same strain and source overnight. Each female was examined following the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. If sperm or a copulatory plug were found, that day was designated gestation day (GD) 0, and each female was placed individually in a Type IIIh Makrolon® cage.

4. **Animal assignment and treatment** - For the studies using young adult rats, the animals were randomly assigned to the test groups noted in Tables 1a and 1b. It was stated that the randomization was performed taking body weight into consideration (no further details were provided). Each young adult animal received a single gavage dose at a volume of 5 mL/kg. For the studies using pups, litters were standardized to 8 pups/litter (preferably 4 pups of each sex) on post-natal day (PND) 4. If the number of males or females was less than 4, a partial adjustment was made (e.g. 3 of one sex and 5 of the other). Pups were culled randomly; pups not chosen for the study and litters that had an insufficient number of pups were killed and discarded without further examination. The pups chosen for the study were consecutively allocated (10/sex/dose group, or as nearly possible) to the groups noted in Tables 1a and 1b. Each pup received a single gavage dose at a volume of 5 mL/kg on PND 11.

Table 1a. Study design for time of peak effect studies. [a]

Group	# of Animals (M/F)	Dose (mg/kg)		Sample Time (Hrs post-dosing)
		Males	Females	
Young adult				
1 (control)	6/6	0	0	4
2	6/6	1.5	0.75	2
3	6/6	1.5	0.75	4
4	6/6	1.5	0.75	6
5	6/6	1.5	0.75	8
Preweaning				
1 (control)	9/10	0	0	6
2	11/9	0.5	0.5	4
3	10/10	0.5	0.5	6
4	10/9	0.5	0.5	8
5	10/10	0.5	0.5	24

a The data were obtained from Table 5-1 on pages 19 and 20 in MRIDs 46589701 and 46589702, respectively.

Table 1b. Study design for comparative cholinesterase inhibition studies. [a]

Group	# of Animals (M/F)	Dose (mg/kg)		Sample Time (Hrs post-dosing)
		Males	Females	
Young adult				
1 (control)	6/6	0	0	6 [M] and 8 [F]
2	6/6	0.25	0.25	6 [M] and 8 [F]
3	6/6	0.75	0.5	6 [M] and 8 [F]
4	6/6	1.5	0.75	6 [M] and 8 [F]
Preweaning				
1 (control)	10/10	0	0	24
2	10/10	0.125	0.125	24
3	10/10	0.25	0.25	24
4	9/10	0.5	0.5	24

a The data were obtained from Table 5-1 on pages 20 and 21 in MRIDs 46589703 and 46589704, respectively.

5. Dose selection rationale - It was stated that the dose levels used in these studies were requested by the Sponsor.

6. Test substance preparation and analysis - Dose formulations were prepared once prior to dosing by mixing the appropriate amounts of Disulfoton with PEG 400, and stored at room temperature until dosing the following day. The doses for each animal were determined using individual body weights. Stability data were provided from a previous study (# F701 1320) in which concentrations of 0.02 and 0.40 mg/mL were evaluated for stability for up to 8 days at room temperature. These concentrations bracketed the concentrations used in the current study. Actual concentrations of the dosing formulations were determined at 1 to 3 days prior to compound administration in each study except for MRID 46589701. Homogeneity was not determined.

Results

Stability analysis (Range as % of initial): 84-99%

Concentration analysis:

MRID 46589702		MRID 46589703		MRID 46589704	
Dose (mg/kg)	% of Nominal	Dose (mg/kg)	% of Nominal	Dose (mg/kg)	% of Nominal
0.5	88	0.25	94	0.125	91
		0.5	97	0.25	89
		0.75	107	0.5	88

		1.5	80		

The analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

7. **Statistics** - The cholinesterase data were analyzed using an adjusted Welch test. Significance was indicated at $p \leq 0.05$ and 0.01 in the study tables.

C. METHODS

1. Observations

a. Young adults - All animals were observed once for mortality and clinical signs of toxicity.

b. Maternal animals - Dams were observed daily for mortality, moribundity, and clinical signs of toxicity. Body weight gain and food and water consumption were not evaluated.

c. Offspring - The number of live and stillborn pups was recorded for each litter, but was not reported. Pups were observed daily for mortality, moribundity, and clinical signs of toxicity from birth until sacrifice. The sex and weight (not reported) of each pup was determined as soon as possible following parturition (PND 0). Pup body weights were also recorded (not reported) on PNDs 4 and 11.

2. Body weight - All young adults and pups were weighed prior to treatment to determine individual doses.

3. Cholinesterase activity determination

a. Young adults - At each sacrifice time, blood was collected via the retroorbital venous plexus under ether anesthesia for plasma and erythrocyte cholinesterase activity determinations. Following blood collection, the animals were sacrificed by cervical dislocation under CO_2 anesthesia, and the whole brain was removed from the skull and frozen (≤-18°C) until analysis. Cholinesterase activity determination was performed using a modification of the Ellman method. The modification consisted of using 6,6'-dithiodinicotinic acid as the coupling agent and measuring the change in absorbance at 340 nm. A gross necropsy was not performed. It was stated that brain weights were recorded after dissection (not reported).

b. Pups - At each sacrifice time, blood was collected from the pups via decapitation for plasma and erythrocyte cholinesterase activity determinations. Following blood collection, the whole brain was removed from the skull and frozen (≤-18°C) until analysis. Cholinesterase activity determination was performed using the modification of the Ellman method described above. The dams were killed following sacrifice of the pups. A gross necropsy of the dams or pups was not performed. It was stated that brain weights of the pups were recorded after dissection (not reported).

4. Benchmark dose response - It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2)

versus analytically confirmed doses. Benchmark dose responses of 10 and 20% cholinesterase inhibition were calculated for each compartment.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - It was stated that no clinical signs of toxicity were observed in any animal in all studies; however, individual data were not provided.

2. Mortality - All animals survived to scheduled sacrifice in all studies.

B. CHOLINESTERASE ACTIVITY - The cholinesterase data for all compartments and time points are summarized and presented in Tables 2a and 2b (time of peak effect studies) and 3a and 3b (comparative cholinesterase inhibition studies) below.

1. Plasma cholinesterase - In the time to peak effect studies, plasma cholinesterase activity was decreased ($p \leq 0.05$) throughout the study periods by 56-72% in the adult males; 35-85% in the adult females, 32-54% in the male pups; and 29-49% in the female pups. The maximum peak inhibition (decreased activity) in the plasma of the adults was at 6 hours post-dosing in both sexes, and in the pups it was 8 hours post-dosing in the males and 8-24 hours post-dosing in the females.

In the comparative cholinesterase inhibition studies, plasma cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) in the adult males at ≥0.75 mg/kg (↓35-67%) and in the adult females at all doses (↓16-84%). Similarly in the pups, plasma cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) in the males at ≥0.25 mg/kg (↓24-56%) and in the females at all doses (↓11-53%).

2. Erythrocyte cholinesterase - In the time to peak effect studies, erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) throughout the study periods by 40-51% in the adult males; 25-65% in the adult females; 23-50% in the male pups; and 30-56% in the female pups. The maximum peak inhibition in the erythrocytes of the adults was at 6 hours post-dosing in the males and 8 hours post-dosing in the females, and in the pups it was 24 hours post-dosing in both sexes.

In the comparative cholinesterase inhibition studies, erythrocyte cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) in the adult males at 1.5 mg/kg (↓46%) and in the adult females at ≥0.5 mg/kg (↓34-70%). Similarly in the pups, erythrocyte cholinesterase activity was decreased ($p \leq 0.05$) in the 0.5 mg/kg males (↓53%) and females (↓52%).

3. Brain cholinesterase - In the time to peak effect studies, brain cholinesterase activity was decreased ($p \leq 0.05$) throughout the study periods by 27-42% in the adult males; 15-38% in the adult females; 16-34% in the male pups; and 12-30% in the female pups. The maximum peak inhibition in the brains of the adults was at 6 hours post-dosing in the males and 8 hours post-dosing in the females, and in the pups it was 8 hours post-dosing in both sexes.

In the comparative cholinesterase inhibition studies, brain cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) in the adult males at all doses (↓4-32%) and in the adult females at ≥0.5 mg/kg (↓17-43%). Similarly in the pups, brain cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at all doses in the males (↓8-39%) and females (↓5-36%).

Table 2a. Mean (±SD) cholinesterase activity in young-adult rats treated once via gavage with Disulfoton. [a]

Dose (mg/kg)	0	1.5	1.5	1.5	1.5
Time Post-dosing (Hrs)	4	2	4	6	8
Males					
Plasma (kU/L)	0.36±0.10	0.27±0.07 (25)	0.16±0.05** (56)	0.10±0.04** (72)	0.16±0.09** (56)
Erythrocyte (kU/L)	1.19±0.10	1.04±0.15 (13)	0.67±0.14** (44)	0.58±0.14** (51)	0.71±0.27* (40)
Brain (U/g)	11.03±0.78	10.16±0.62 (8)	8.06±0.98** (27)	6.40±0.68** (42)	7.68±1.84** (30)
Females					
Plasma (kU/L)	1.67±0.40	1.09±0.14* (35)	0.54±0.27** (68)	0.25±0.07** (85)	0.27±0.04** (84)
Erythrocyte (kU/L)	1.69±0.23	1.26±0.05** (25)	1.16±0.33** (31)	0.69±0.07** (59)	0.59±0.13** (65)
Brain (U/g)	11.24±0.47	11.21±0.23	9.50±0.97* (15)	7.17±0.60** (36)	6.97±1.37** (38)

a Data were obtained from Table 6-2 on page 26 and pages 31 and 32 of MRID 46589701; n=6. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Table 2b. Mean (±SD) cholinesterase activity in rat pups treated once via gavage with Disulfoton on PND 11. [a]

Dose (mg/kg)	0	0.5	0.5	0.5	0.5
Time Post-dosing (Hrs)	6	4	6	8	24
Males					
Plasma (kU/L)	0.72±0.06	0.49±0.11** (32)	0.39±0.08** (46)	0.33±0.10** (54)	0.37±0.10** (49)
Erythrocyte (kU/L)	2.20±0.48	1.70±0.37* (23)	1.70±0.37* (23)	1.34±0.43** (39)	1.10±0.57** (50)
Brain (U/g)	5.51±0.24	4.63±0.75** (16)	4.20±0.64** (24)	3.63±0.60** (34)	4.10±0.78** (26)
Females					
Plasma (kU/L)	0.72±0.09	0.51±0.07** (29)	0.45±0.11** (38)	0.37±0.10** (49)	0.37±0.12** (49)
Erythrocyte (kU/L)	2.20±0.41	1.88±0.23 (15)	1.53±0.23** (30)	1.27±0.50** (42)	0.97±0.47** (56)
Brain (U/g)	5.61±0.54	4.95±0.56* (12)	4.44±0.69** (21)	3.94±0.84** (30)	4.13±0.73** (26)

a Data were obtained from Table 6-2 on page 28 and pages 33 and 34 of MRID 46589702; n=9-11. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at p≤0.05 ** Significantly different from controls at p≤0.01

Table 3a. Mean (±SD) cholinesterase activity at the estimated time of peak effect in young-adult rats treated once via gavage with Disulfoton. [a]

Males				
Compartment	Dose (mg/kg)			
	0	0.25	0.75	1.5
Plasma (kU/L)	0.43±0.08	0.39±0.04 (9)	0.28±0.05** (35)	0.14±0.04** (67)
Erythrocyte (kU/L)	1.69±0.21	1.53±0.10 (9)	1.40±0.20 (17)	0.92±0.14** (46)
Brain (U/g)	11.54±0.30	11.08±0.32* (4)	9.92±0.29** (14)	7.81±0.93** (32)
Females				
Compartment	Dose (mg/kg)			
	0	0.25	0.5	0.75
Plasma (kU/L)	1.56±0.17	1.31±0.20* (16)	0.57±0.12** (63)	0.25±0.10** (84)
Erythrocyte (kU/L)	1.51±0.24	1.41±0.21 (7)	1.00±0.13** (34)	0.45±0.10** (70)
Brain (U/g)	10.74±0.42	10.71±0.50	8.87±0.71** (17)	6.10±0.64** (43)

a Data were obtained from Table 6-2 on page 26 and pages 32 and 33 of MRID 46589703; n=6. The estimated time of peak effect was 6 (males) and 8 (females) hours post-dosing. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at p≤0.05 ** Significantly different from controls at p≤0.01

Table 3b. Mean (±SD) cholinesterase activity at 24 hours post-dosing in rat pups treated once via gavage with Disulfoton on PND 11. [a]

Compartment	Dose (mg/kg)			
	0	0.125	0.25	0.5
Males				
Plasma (kU/L)	0.71±0.08	0.66±0.08 (7)	0.54±0.08**(24)	0.31±0.13**(56)
Erythrocyte (kU/L)	2.01±0.56	1.62±0.49 (19)	1.72±0.27 (14)	0.95±0.44**(53)
Brain (U/g)	6.49±0.42	5.90±0.37** (8)	5.38±0.54**(16)	3.88±0.86**(39)
Females				
Plasma (kU/L)	0.73±0.06	0.65±0.06** (11)	0.49±0.08** (33)	0.34±0.13** (53)
Erythrocyte (kU/L)	2.18±0.58	1.90±0.48 (13)	1.70±0.14 (22)	1.05±0.51** (52)
Brain (U/g)	6.47±0.39	6.13±0.28* (5)	5.23±0.51** (19)	4.12±0.73** (36)

a Data were obtained from Table 6-2 on page 27, and pages 32 and 33 of MRID 46589704; n=9-10. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at p≤0.05 ** Significantly different from controls at p≤0.01

C. **BENCHMARK DOSE RESPONSE** - In the adults, the following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.33 and 0.54, males; 0.09 and 0.18, females); (ii) erythrocytes (0.58 and 0.82, males; and 0.22 and 0.36, females); and (iii) brain (0.66 and 0.95, males; 0.38 and 0.54, females; Table 4a). Similarly in the pups, the following benchmark doses (mg/kg) were calculated: (i) plasma (0.11 and 0.20, males; 0.08 and 0.16, females); (ii) erythrocytes (0.14 and 0.24, males; and 0.11 and 0.20, females); and (iii) brain (0.15 and 0.27, males; 0.13 and 0.25, females; Table 4b).

Table 4a. Benchmark dose estimates (mg/kg) for cholinesterase inhibition in young-adult rats treated once via gavage with Disulfoton. [a]

Compartment	Males		Females	
	BMD10	BMD20	BMD10	BMD20
Plasma	0.33	0.54	0.09	0.18
Erythrocytes	0.58	0.82	0.22	0.36
Brain	0.66	0.95	0.38	0.54

a Data were obtained from Table 7-1 on page 29 of MRID 46589703. It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. BMD10 = 10% inhibition BMD20 = 20% inhibition

Table 4b. Benchmark dose estimates (mg/kg) for cholinesterase inhibition in rat pups treated once via gavage with Disulfoton on PND 11. [a]

Compartment	Males		Females	
	BMD10	BMD20	BMD10	BMD20
Plasma	0.11	0.20	0.08	0.16
Erythrocytes	0.14	0.24	0.11	0.20
Brain	0.15	0.27	0.13	0.25

a Data were obtained from Table 7-1 on page 29 of MRID 46589704. It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. BMD10 = 10% inhibition BMD20 = 20% inhibition

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS** - The investigators concluded that based on the time to peak-effect in all compartments, time points of 6 (adult males), 8 (adult females), and 24 (all pups) hours were recommended as the time of sample collection for subsequent studies investigating a possible age-related relative sensitivity for ChE-inhibition by treatment with Disulfoton. It was also concluded that cholinesterase activity was dose-dependently decreased in the young adult animals at doses up to and including 1.5 (males) and 0.75 mg/kg (females); and in the pups at up to and including 0.5 mg/kg.

B. **REVIEWER COMMENTS** - There were no treatment-related effects on mortality or clinical signs of toxicity in any study.

In the time of peak effect studies, cholinesterase inhibition was observed in all compartments (plasma, blood, and brain) in both sexes throughout the study observation periods. Based on the levels of cholinesterase inhibition, 6 and 8 hours post-dosing were determined to be the estimated time of peak-effect in adult males and females, respectively, and 24 hours post-dosing was determined to be the estimated time of peak-effect in pups of both sexes. These times should be used for sampling in subsequent studies.

In the comparative cholinesterase inhibition studies, dose-dependent decreases in cholinesterase activity were observed in all compartments (plasma, blood, and brain) in the adults at up to 1.5 (males) and 0.75 mg/kg (females), and in the pups at up to 0.5 mg/kg. These data were used to calculate benchmark doses for 10 and 20% cholinesterase inhibition in the adults and pups of both sexes.

This study is classified as acceptable/non-guideline.

C. <u>STUDY DEFICIENCIES</u> - None

DATA EVALUATION RECORD

DISULFOTON

Study Type: Non-guideline; Cholinesterase Inhibition in Rats

Work Assignment No. 3-01-96 B (MRID 46637101)

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

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer:
David A. McEwen, B.S. Signature: _____
Date: _____

Secondary Reviewer:
John W. Allran, M.S. Signature: _____
Date: _____

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T. Signature: _____
Date: _____

Quality Assurance:
Mary L. Menetrez, Ph.D. Signature: _____
Date: _____

Disclaimer

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

EPA Reviewer: David G Anderson, PhD Signature:
Reregistration Branch 2, Health Effects Division (7509C) Date
Work Assignment Manager: PV Shah, Ph.D. Signature:
Registration Action Branch 1, Health Effects Division (7509C) Date

Template version 11/01

DATA EVALUATION

STUDY TYPE: Non-guideline; supplement to Guideline OPPTS 870.6300; Cholinesterase Inhibition in Rats

PC CODE: 032501 **DP BARCODE: DP321704**
TXR#: 0053636

TEST MATERIAL (PURITY): Disulfoton (97.3% a.i.)

SYNONYMS: O,O-diethyl S-[2-(ethylthio)ethyl] phosphorodithioate

CITATION: Klaus, A.M. (2005) Cholinesterase inhibition in young-adult Wistar rats treated daily by gavage for eleven days with technical grade Disulfoton. Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany. Laboratory Report No.: AT02202, Study No.: T1073353, July 25, 2005. MRID 46637101. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46637101), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered once daily for 11 consecutive days via gavage (5 mL/kg) to 6 young-adult Wistar rats/sex/dose at doses of 0, 0.25, 0.5, or 1.0 mg/kg in males and 0, 0.125, 0.25, or 0.5 mg/kg in females. Plasma, erythrocyte, and brain cholinesterase activities were determined at 1 hour after final dosing (time of peak-effect) in all groups. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no mortalities or treatment-related clinical signs of toxicity. Cholinesterase activity was dose-dependently decreased ($p<=0.05$) as follows: (i) plasma in the >=0.5 mg/kg males (decr 32-41%,) and in the >=0.125 mg/kg females (decr 30-73%); (ii) erythrocytes in the >=0.5 mg/kg males (decr 38-72%) and in the >=0.125 mg/kg females (decr 15-63%); and (iii) brain in the >=0.25 mg/kg males (decr 15-70%) and in the >=0.125 females (decr 11-70%). The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.20 and 0.40, males; 0.05 and 0.11, females); (ii) erythrocytes (0.12 and 0.23, males; and 0.08 and 0.15, females); and (iii) brain (0.12 and 0.24, males; 0.07 and 0.14, females).
Young adult LOAEL for decreased cholinesterase:

Plasma –males 0.5 mg/kg/day; -females 0.125 mg/kg/day
Erythrocyte –males 0.5 mg/kg/day; -females 0.125 mg/kg/day
Brain –males 0.25 mg/kg/day; -females 0.125 mg/kg/day.

Young adult NOAEL for decreased cholinesterase:
Plasma –males 0.25 mg/kg/day; -females none [LDT]
Erythrocyte –males 0.25 mg/kg/day; -females none [LDT]
Brain –males none [LDT]; -females none [LDT].

This study is classified as **ACCEPTABLE/NON-GUIDELINE.**

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Disulfoton

Description:	Light brown liquid
Batch #:	3-08-5013JC158
Purity (w/w):	97.3% a.i.
Stability of compound:	The test material was shown to be stable in the vehicle for up to 8 days at room temperature.
CAS #:	298-04-4
Structure:	[CH3CH2O]2P=O | SCH2CH2SCH2CH3

2. Vehicle - The test material was dissolved in polyethylene glycol (PEG) 400.

3. Test animals

Species:	Rat
Strain:	Wistar CRL: GlxBrl Han:WI
Age/weight at study initiation:	9 weeks old/ 237-267 g males and 158-176 g females
Source:	Charles River Wiga (Deutschland, Sulzfeld, Germany)
Housing:	Individually in Type IIIh Makrolon® cages
Diet:	Mouse and Rat Maintenance Diet No. 3883.0.15 (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water, ad libitum
Environmental conditions:	
Temperature:	20±2°C

Humidity:	Approximately 50%
Air changes:	At least 10/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 7 days

B. STUDY DESIGN

1. Study purpose - The purpose of this study was to determine cholinesterase inhibition in young-adult rats treated daily via gavage with Disulfoton for 11 consecutive days. The benchmark dose (10% and 20% cholinesterase inhibition) responses for each compartment (i.e. plasma, erythrocytes, and brain) were calculated to provide a comparison of cholinesterase activity effects in adult animals. This study was designed to support a separate Development Neurotoxicity Study (OPPTS 870.6300).

2. In-life dates - Start: 5/08/05 End: 5/18/05

3. Animal assignment and treatment - Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. It was stated that the randomization was performed taking body weight into consideration (no further details were provided). Each animal received daily gavage doses (5 mL/kg) for 11 consecutive days.

Table 1. Study design [a]

Group	Dose (mg/kg)		# of Animals (M/F)
	Males	Females	
1 (control)	0	0	6/6
2	0.25	0.125	6/6
3	0.5	0.25	6/6
4	1.0	0.5	6/6

a Data were obtained from page 20 of the study report.

4. Dose selection rationale - It was stated that the doses used in this study were requested by the Sponsor, with the expectation of inhibiting cholinesterase activity without causing overt toxicity.

5. Test substance preparation and analysis - Dose formulations were prepared prior to dosing by mixing the appropriate amounts of Disulfoton with PEG 400. The formulations were stored (for a maximum of 8 days) at room temperature. The doses for each animal were determined using individual body weights. Stability data were provided from a previous study (# F7011320) in which concentrations of 0.02 and 0.4 mg/mL were evaluated for up to 8 days at room temperature. These concentrations bracketed the concentrations used in the current study.

Actual concentrations of all dose formulations (0.025, 0.05, 0.1, and 0.2 mg/mL) were determined once during the administration period. Homogeneity was not determined.

Results

Stability analysis (% of Day 0 after 8 days at room temperature): 84-87%
Concentration analysis (Range as % of nominal): 82-85%

The analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

6. **Statistics** - The body weight data were analyzed using ANOVA followed by Dunnett's test, if necessary. The cholinesterase data were analyzed using an adjusted Welch test. Significance was indicated at $p \leq 0.05$ and 0.01 in the study tables.

C. METHODS

1. **Observations** - Animals were observed once daily for mortality and clinical signs of toxicity.

2. **Body weight** - Animals were weighed daily prior to dosing to determine individual doses.

3. **Cholinesterase activity determination** - At 1 hour after the final dosing, blood was collected via the retroorbital venous plexus under ether anesthesia for plasma and erythrocyte cholinesterase activity determinations. Immediately following blood collection, the animals were sacrificed by cervical dislocation under CO_2 anesthesia, and the whole brain was removed and frozen (≤-18 °C) until analysis. Cholinesterase activity determination was performed using a modification of the Ellman method. The modification consisted of using 6,6'-dithiodinicotinic acid as the coupling reagent and measuring the change in absorbance at 340 nm.

4. **Benchmark dose response** - It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. Benchmark dose responses of 10 and 20% cholinesterase inhibition were calculated for each compartment.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - No treatment-related signs of toxicity were observed.

2. **Mortality** - All animals survived to scheduled sacrifice.

3. **Body weight** - No treatment-related effect on body weight was observed at any dose in either sex.

B. CHOLINESTERASE ACTIVITY - The cholinesterase data for all compartments are summarized and presented in Tables 2a and 2b below.

1. **Plasma cholinesterase** - Plasma cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at ≥0.5 mg/kg in the males (↓32-41%) and at all doses the females (↓30-73%).
2. **Erythrocyte cholinesterase** - Erythrocyte cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at ≥0.5 mg/kg in the males (↓38-72%) and at all doses the females (↓15-63%).
3. **Brain cholinesterase** - Brain cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at all doses in the males (↓15-70%) and the females (↓11-70%).

Table 2a. Mean (±SD) cholinesterase activity at 1 hour after final dosing in young-adult male rats treated daily via gavage with Disulfoton for eleven consecutive days. [a]

Compartment	Dose (mg/kg)			
	0	0.25	0.5	1.0
Plasma (kU/L)	0.37±0.06	0.33±0.08	0.25±0.03** (↓32)	0.22±0.03** (↓41)
Erythrocyte (kU/L)	1.91±0.23	1.53±0.35	1.18±0.16** (↓38)	0.54±0.16** (↓72)
Brain (U/g)	11.45±1.27	9.72±0.80* (↓15)	6.98±0.76** (↓39)	3.39±0.23** (↓70)

a Data were obtained from Table 6-2 on page 28, and page 46 of the study report; n=6. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Table 2b. Mean (±SD) cholinesterase activity at 1 hour after final dosing in young-adult female rats treated daily via gavage with Disulfoton for eleven consecutive days. [a]

Compartment	Dose (mg/kg)			
	0	0.125	0.25	0.5
Plasma (kU/L)	1.53±0.36	1.07±0.19* (↓30)	0.77±0.20** (↓50)	0.41±0.07** (↓73)
Erythrocyte (kU/L)	1.83±0.16	1.55±0.16* (↓15)	1.32±0.15** (↓28)	0.67±0.05** (↓63)
Brain (U/g)	11.58±0.91	10.31±0.65** (↓11)	7.74±0.68** (↓33)	3.43±0.21** (↓70)

a Data were obtained from Table 6-2 on page 28, and page 47 of the study report; n=6. Percent inhibition (decreased activity) is included in parentheses. * Significantly different from controls at $p \leq 0.05$.
** Significantly different from controls at $p \leq 0.01$

C. BENCHMARK DOSE RESPONSE - The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.20 and 0.40, males; 0.05 and 0.11, females); (ii) erythrocytes (0.12 and 0.23, males; and 0.08 and 0.15, females); and (iii) brain (0.12 and 0.24, males; 0.07 and 0.14, females; Table 3).

Table 3. Benchmark dose estimates (mg/kg) for cholinesterase inhibition in young-adult rats treated daily via gavage with Disulfoton for eleven consecutive days. [a]

Compartment	Males		Females	
	BMD10	BMD20	BMD10	BMD20
Plasma	0.20	0.40	0.05	0.11
Erythrocytes	0.12	0.23	0.08	0.15
Brain	0.12	0.24	0.07	0.14

a Data were obtained from Table 7-1 on page 31 of the study report. It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. BMD10 = 10% inhibition BMD20 = 20% inhibition

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that cholinesterase activity was dose-dependently decreased in all compartments in the males at ≥0.5 mg/kg and in the females at all dose levels.

B. REVIEWER COMMENTS - There were no treatment-related effects on mortality, body weight, or clinical signs.

Dose-dependent decreases in cholinesterase activity were observed in all compartments (plasma, blood, and brain) at ≥0.125 mg/kg/day in the females and at ≥0.5 mg/kg/day in the males. Additionally, brain cholinesterase activity was decreased (↓15%; $p \leq 0.05$) in the 0.25 mg/kg/day males. These data were used to calculate benchmark doses for 10 and 20% cholinesterase inhibition in both sexes.

This study is classified as **ACCEPTABLE/NON-GUIDELINE.**

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not change the conclusions of this DER:

- Homogeneity was not determined.

DATA EVALUATION RECORD

DISULFOTON

Study Type: Non-guideline; Cholinesterase Inhibition in Rat Pups

Work Assignment No. 3-01-96 C (MRID 46637102)

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

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer:
David A. McEwen, B.S.
Signature: _____
Date: _____

Secondary Reviewer:
John W. Allran, M.S.
Signature: _____
Date: _____

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: _____
Date: _____

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: _____
Date: _____

Disclaimer

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

EPA Reviewer: David G Anderson, PhD
Toxicology Branch , Health Effects Division (7509C)
Work Assignment Manager: PV Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature:
Date
Signature:
Date

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; supplement to Guideline OPPTS 870.6300; Cholinesterase Inhibition in Rat Pups

PC CODE: 032501 **DP BARCODE: DP321704**
TXR#: 0053636

TEST MATERIAL (PURITY): Disulfoton (97.3% a.i.)

SYNONYMS: *O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate

CITATION: Klaus, A.M. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage for eleven days with technical grade Disulfoton. Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany. Laboratory Report No.: AT02282, Study No.: T6073358, August 17, 2005. MRID 46637102. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46637102), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered daily via gavage (5 mL/kg) to 10 Wistar rat pups/sex/dose at doses of 0, 0.06, 0.125, or 0.250 mg/kg for 11 consecutive days beginning on postnatal day 11. Plasma, erythrocyte, and brain cholinesterase activities were determined at 1 hour (time of peak effect) following the final dosing. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no treatment-related effects on clinical signs of toxicity. Four pups of different dose groups, but originating from the same litter (female no. 9) were found dead or cannibalized between PND 12-18. Cholinesterase activity was dose-dependently (unless otherwise stated) decreased ($p<=0.05$) as follows: (i) plasma in the >=0.125 mg/kg males (decr 22-38%,) and in the 0.25 mg/kg females (decr 36%); (ii) erythrocytes at >=0.125 mg/kg in the males (decr 19-42%) and at >=0.06 mg/kg in the females (decr 29-52%); and (iii) brain at >=0.06 mg/kg in both sexes (decr 7-44%). The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.059 and 0.117, males; 0.09 and 0.148, females); (ii) erythrocytes (0.086 and 0.136, males; and 0.042 and 0.085, females); and (iii) brain (0.053 and 0.107, males; 0.051 and 0.102, females).

Pup LOAELs for cholinesterase were as follows:
Plasma- males 0.125 mg/kg/day –females 0.250 mg/kg/day;
Erythrocyte- males 0.125 mg/kg/day –females 0.06 mg/kg/day [LDT];
Brain –males 0.06 mg/kg/day [LDT]; -females 0.06 mg/kg/day [LDT].

Pup NOAELs for cholinesterase were as follows:
Plasma –males 0.06 mg/kg/day; -females 0.125 mg/kg/day;
Erythrocyte –males 0.06 mg/kg/day; -females none [LDT];
Brain –males none [LDT]; -females none [LDT].

This study is classified as **ACCEPTABLE/NON-GUIDELINE.**

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

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Disulfoton

Description:	Light brown liquid
Batch #:	3-08-5013JC158
Purity (w/w):	97.3% a.i.
Stability of compound:	The test material was shown to be stable in the vehicle for 1 day (0.012 mg/mL) or up to 8 days (0.025 and 0.05 mg/mL) at room temperature.
CAS #:	298-04-4
Structure:	(CH3CH2O)2P=O | SCH2CH2SCH2CH3

2. **Vehicle** - The test material was dissolved in polyethylene glycol (PEG) 400.

3. **Test animals**

Species:	Rat
Strain:	Wistar CRL: GlxBrl Han:WI
Pup age/mean weight at dosing:	11 days old/ weight not reported
Source:	Charles River Wiga (Deutschland, Sulzfeld, Germany)
Housing:	Type IIIh Makrolon® cages were used throughout the study. During mating, one male was housed overnight with two females; dams with their litters were housed together until sacrifice.
Diet:	Mouse and Rat Maintenance Diet No. 3883.0.15 (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	20±2°C

EPA Reviewer: David G Anderson, PhD **Signature:**
Toxicology Branch , Health Effects Division (7509C) **Date**
Work Assignment Manager: PV Shah, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date**

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; supplement to Guideline OPPTS 870.6300; Cholinesterase Inhibition in Rat Pups

PC CODE: 032501 **DP BARCODE: DP321704**
TXR#: 0053636

TEST MATERIAL (PURITY): Disulfoton (97.3% a.i.)

SYNONYMS: *O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate

CITATION: Klaus, A.M. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage for eleven days with technical grade Disulfoton. Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany. Laboratory Report No.: AT02282, Study No.: T6073358, August 17, 2005. MRID 46637102. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46637102), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) in PEG 400 was administered daily via gavage (5 mL/kg) to 10 Wistar rat pups/sex/dose at doses of 0, 0.06, 0.125, or 0.250 mg/kg for 11 consecutive days beginning on postnatal day 11. Plasma, erythrocyte, and brain cholinesterase activities were determined at 1 hour (time of peak effect) following the final dosing. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no treatment-related effects on clinical signs of toxicity. Four pups of different dose groups, but originating from the same litter (female no. 9) were found dead or cannibalized between PND 12-18. Cholinesterase activity was dose-dependently (unless otherwise stated) decreased ($p<=0.05$) as follows: (i) plasma in the >=0.125 mg/kg males (decr 22-38%,) and in the 0.25 mg/kg females (decr 36%); (ii) erythrocytes at >=0.125 mg/kg in the males (decr 19-42%) and at >=0.06 mg/kg in the females (decr 29-52%); and (iii) brain at >=0.06 mg/kg in both sexes (decr 7-44%). The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.059 and 0.117, males; 0.09 and 0.148, females); (ii) erythrocytes (0.086 and 0.136, males; and 0.042 and 0.085, females); and (iii) brain (0.053 and 0.107, males; 0.051 and 0.102, females).

Dose formulations were stored at room temperature for 1 day (0.012 mg/mL) or up to 8 days (0.025 and 0.05 mg/mL). The doses for each animal were determined using individual body weights. Stability data were provided from previous studies (# F7011320 and #F0011413) in which concentrations of 0.01, 0.02, and 0.4 mg/mL were evaluated for up to 8 days at room temperature. These concentrations bracketed the concentrations used in the current study. Actual concentrations of all dose formulations (0.012, 0.025, and 0.05 mg/mL) were determined once during the administration period. Homogeneity was not determined.

Results

Stability analysis (% of Day 0):

Low dose (0.01 mg/mL) after 1 day at room temperature: 97.9%

Higher doses (0.02 and 0.4 mg/mL) after 8 days at room temperature: 83.9-87 5%

Concentration analysis (Range as % of nominal): 90-91%

The analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

7. **Statistics** - The cholinesterase data were analyzed using an adjusted Welch test. Significance was indicated at $p \leq 0.05$ and 0.01 in the study tables.

C. METHODS

1. Observations

a. Maternal animals - Dams were observed daily for mortality, moribundity, and clinical signs of toxicity. Body weight gain and food consumption were not evaluated.

b. Offspring - The number of live pups at parturition and stillborn pups was recorded for each litter, but was not reported. Pups were observed daily for mortality, moribundity, and clinical signs of toxicity from birth until sacrifice. The sex and weight (not reported) of each pup was determined as soon as possible following PND 0. Pup body weights were also recorded (not reported) on PND 4 and on the days of treatment prior to dosing.

2. Cholinesterase activity determination - Approximately 1 hour after the final dosing, blood was collected from the pups via decapitation for determination of plasma and erythrocyte cholinesterase activity. Immediately following blood collection, the whole brain was removed from the skull and frozen (≤-18°C) until analysis. Cholinesterase activity determination was performed using a modification of the Ellman method. The modification consisted of using 6,6'-dithiodinicotinic acid as the coupling reagent and measuring the change in absorbance at 340 nm. The dams were killed following sacrifice of the pups. A gross necropsy of the dams or pups was not performed. It was stated that brain weights of the pups were recorded after dissection (not reported).

3. Benchmark dose response - It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2)

versus analytically confirmed doses. Benchmark dose responses of 10 and 20% cholinesterase inhibition were calculated for each compartment.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - No treatment-related clinical signs of toxicity were observed at any dose.

2. Mortality - Four pups of different dose groups, but originating from the same litter (female no. 9) were found dead or cannibalized between PND 12-18. All other pups survived to scheduled sacrifice.

B. CHOLINESTERASE ACTIVITY - The cholinesterase data for all compartments are summarized and presented in Table 2 below.

1. Plasma cholinesterase - Plasma cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at ≥0.125 mg/kg in the males (↓22-38%) and at 0.25 mg/kg in the females (↓36%).

2. Erythrocyte cholinesterase - Erythrocyte cholinesterase activity was dose-dependently decreased ($p \leq 0.05$) at ≥0.125 mg/kg in the males (↓19-42%) and at ≥0.06 mg/kg in the females (↓29-52%).

3. Brain cholinesterase - Brain cholinesterase activity was dose-dependently decreased ($p \leq 0.01$) at ≥0.06 mg/kg in the males (↓7-41%) and the females (↓9-44%). However, cholinesterase inhibition was less than 10% in the 0.06 mg/kg males and females.

Table 2. Mean (±SD) cholinesterase activity at 1 hour after final dosing in rat pups treated daily via gavage with Disulfoton for 11 consecutive days. [a]

Compartment	Dose (mg/kg)			
	0	0.06	0.125	0.25
Males				
Plasma (kU/L)	0.58±0.11	0.52±0.07	0.45±0.08* (↓22)	0.36±0.05** (↓38)
Erythrocyte (kU/L)	1.99±0.36	2.14±0.20	1.61±0.30* (↓19)	1.15±0.25** (↓42)
Brain (U/g)	9.47±0.34	8.81±0.41** (↓7)	7.29±0.46** (↓23)	5.58±0.20** (↓41)
Females				
Plasma (kU/L)	0.56±0.10	0.57±0.11	0.47±0.10	0.36±0.04** (↓36)
Erythrocyte (kU/L)	2.02±0.19	1.44±0.29** (↓29)	1.22±0.29** (↓40)	0.96±0.21** (↓52)
Brain (U/g)	9.50±0.37	8.64±0.24** (↓9)	7.22±0.33** (↓24)	5.36±0.27** (↓44)

a Data were obtained from Table 6-2 on page 30, and pages 37 and 38 of the study report; n=9-10. Percent inhibition (decreased activity) is included in parentheses.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

C. BENCHMARK DOSE RESPONSE - The following benchmark doses (mg/kg) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.059 and 0.117, males: 0.09 and 0.148, females); (ii) erythrocytes (0.086 and 0.136, males; and 0.042 and 0.085, females); and (iii) brain (0.053 and 0.107, males; 0.051 and 0.102, females; Table 3).

Table 3. Benchmark dose estimates (mg/kg) for cholinesterase inhibition in rat pups treated daily via gavage with Disulfoton for 11 consecutive days. [a]

Compartment	Males		Females	
	BMD10	BMD20	BMD10	BMD20
Plasma	0.059	0.117	0.090	0.148
Erythrocyte	0.086	0.136	0.042	0.085
Brain	0.053	0.107	0.051	0.102

a Data were obtained from Table 7-1 on page 33 of the study report. It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses.
BMD10 = 10% inhibition
BMD20 = 20% inhibition

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that cholinesterase activity was clearly decreased ($p \leq 0.01$) in both sexes at 0.25 mg/kg. The toxicological relevance of the findings at 0.06 and 0.125 mg/kg was questionable.

B. REVIEWER COMMENTS - There were no treatment-related effects on clinical signs of toxicity. Four pups of different dose groups, but originating from the same litter (female no. 9) were found dead or cannibalized between PND 12-18.

Dose-dependent decreases in cholinesterase activity were generally observed in all compartments (plasma, blood, and brain) in both sexes at doses ≥0.125 mg/kg. These data were used to calculate benchmark doses for 10 and 20% cholinesterase inhibition in both sexes.

This study is classified as **acceptable/non-guideline.**

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not change the conclusions of this DER: Homogeneity was not determined.

DATA EVALUATION RECORD

DISULFOTON

Study Type: Non-guideline: Cholinesterase Inhibition in Maternal and Fetal Rats

Work Assignment No. 3-01-96 A (MRID 46635901)

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

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer:
David McEwen, B.S.

Signature: David A. McEwen
Date: 1/30/06

Secondary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 1/30/06

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E. Viana
Date: 1/30/06

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 1/30/06

Disclaimer

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

EPA Reviewer: David G Anderson, PhD Signature:
Reregistretion Branch-2, Health Effects Division (7509C) Date
Work Assignment Manager: PV Shah, Ph.D. Signature:
Registration Action Branch 1, Health Effects Division (7509C) Date

Template version 11/01

TXR# 0053636

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; supplement to Guideline OPPTS 870.6300; Cholinesterase Inhibition in Maternal and Fetal Rats

PC CODE: 032501 **DP BARCODE**: D321704

TEST MATERIAL (PURITY): Disulfoton (97.3% a.i.)

SYNONYMS: O,O-diethyl S-[2-(ethylthio)ethyl] phosphorodithioate

CITATION: Klaus, A.M. (2005) Cholinesterase inhibition in maternal and fetal Wistar rats following gestational exposure via the diet with technical grade Disulfoton. Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany. Laboratory Report No.: AT02212, Study No.: T5073348. July 28, 2005. MRID 46635901. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY - In a non-guideline cholinesterase inhibition study (MRID 46635901), Disulfoton (97.3% a.i.; Batch #: 3-08-5013JC158) was administered continuously in the diet to 13 pregnant Wistar rats/sex/dose at doses of 0, 0.5, 2, or 8 ppm (equivalent to 0, 0.042, 0.168, and 0.694 mg/kg/day, respectively) from gestation day (GD) 0-20. Plasma, erythrocyte, and brain cholinesterase activities were determined on GD 20 in maternal and fetal (pooled samples) rats in all groups. Benchmark dose (BMD) responses of 10% and 20% cholinesterase inhibition were calculated for each compartment.

There were no treatment-related effects on mortality, clinical signs, body weight, or feed consumption. Statistically significant dose-dependent decreases ($p<0.01$) in cholinesterase activity were observed in all compartments (plasma, blood, and brain) in the >=0.5 ppm dams, except plasma cholinesterase was not significantly depressed at 0.5 ppm. Fetal cholinesterase showed a statistically significant dose related depression in CHE as follows: plasma CHE at 0.5 ppm, erythrocyte CHE at 2 ppm and brain CHE at 8 ppm. In the dams, the following benchmark doses (mg/kg/day) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.09 and 0.17); (ii) erythrocytes (0.09 and 0.17); and (iii) brain (0.09 and 0.18). In the fetuses, the following benchmark doses (mg/kg/day) were calculated: (i) plasma (0.12 and 0.23); (ii) erythrocytes (0.10 and 0.19); and (iii) brain (0.38 and 0.59). In this study, the fetuses were less sensitive than their dams.

Maternal LOAEL for decreased cholinesterase:
Plasma **-2 ppm [0.168 mg/kg/day]**;
Erythrocytes **-0.5 ppm [LDT] [0.042 mg/kg/day]**; and
Brain **-0.5 ppm [LDT][0.042 mg/kg/day]**.

Maternal NOAEL for decreased cholinesterase:
Plasma **0.5 ppm [0.042 mg/kg/day],**
Erythrocytes, **-none [LDT]**; and
Brain, **-none [LDT]**.

Litter LOAEL for decreased cholinesterase:
Plasma **-0.5 ppm [LDT][0.042 mg/kg/day]**;
Erythrocyte **-2 ppm**;
Brain **-8 ppm [0.694 mg/kg/day]**.

Litter NOAEL for decreased cholinesterase:
Plasma, **-none [LDT]**;
Erthrocytes **-0.5 ppm [0.042 mg/kg/day]**;
Brain **-2 ppm [0.168 mg/kg/day]**.

This study is classified as **ACCEPTABLE/NON-GUIDELINE.**

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Disulfoton

Description:	Light brown liquid
Batch #:	3-08-5013JC158
Purity (w/w):	97.3% a.i.
Stability of compound:	The test material was shown to be stable in the diet for up to 8 days frozen (-18°C) and for 1 day at room temperature.
CAS #:	298-04-4
Structure:	(CH3CH2O)2P=O | SCH2CH2SCH2CH3

2. **Vehicle** - Diet

3. Test animals

Species:	Rat
Strain:	Wistar CRL: GlxBrl Han:WI
Age/weight at study initiation:	9-22 weeks old/ 170-249 g females only
Source:	Charles River Wiga (Deutschland, Sulzfeld, Germany)
Housing:	Individually in Type IIIh Makrolon® cages
Diet:	Mouse and Rat Maintenance Diet No. 3883.9.25 (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water, ad libitum
Environmental conditions:	
Temperature:	20±2°C
Humidity:	Approximately 50%
Air changes:	At least 10/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 7 days

B. STUDY DESIGN

1. Study purpose - The purpose of this study was to determine cholinesterase inhibition in pregnant and fetal rats after continuous exposure in the diet with Disulfoton from gestation day (GD) 0-20. The benchmark dose (10% and 20% cholinesterase inhibition) responses for each compartment (i.e. plasma, erythrocytes, and brain) were calculated to provide a comparison of cholinesterase activity effects in maternal versus fetal animals. This study was designed to support a separate Development Neurotoxicity Study (OPPTS 870.6300).

2. In-life dates - Start: 5/03/05 End: 6/01/05

3. Mating procedure - Females were paired 1 or 2:1 with males of the same strain and source overnight. Each female was examined following the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. If sperm or a copulatory plug were found, that day was designated gestation day (GD) 0, and each female was placed individually in a Type IIIh Makrolon® cage.

4. Animal assignment and treatment - Animals were assigned to the test groups noted in Table 1 in the order that they were determined to be inseminated (the first inseminated female was assigned to the control group, the 2nd inseminated female was assigned to the 0.5 ppm group and so on). Each animal received the test material continuously in the diet for 21 days (GD 0-20).

Table 1. Study design [a]

Group	# of Animals	Nominal Dose (ppm)	Intake (mg/kg/day)[b]
1 (control)	13	0	0
2	13	0.5	0.042
3	13	2	0.168
4	13	8	0.694

a Data were obtained from pages 20-21 of the study report.
b Mean intake from GD 0-20

5. Dose selection rationale - It was stated that the doses used in this study were identical to those used in the preceding developmental neurotoxicity study.

6. Test substance preparation and analysis - Dietary formulations were prepared weekly by mixing the appropriate amounts of Disulfoton with the diet. The dietary batches were divided into 7 portions; one portion was used on the day of preparation, and the remaining portions were stored frozen (-18°C) until use. Homogeneity, stability, and content were provided from a previous study (# F2011361) in which concentrations of 0.5, 2, and 8 ppm were evaluated. Additional concentration analyses of all dietary formulations (0.5, 2, and 8 ppm) were performed at the beginning and end of the treatment period.

Results

Homogeneity analysis (Range as % RSD): 1.69-8.18

Stability analysis (Range as % of initial):
After 1 day at room temperature: 91-94%
After 8 days frozen (-18°C): 92-109%
Concentration analysis (Range as % of nominal):

Dose (ppm)	% of Nominal
0.5	103-106
2	105-112
8	114-116

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

7. **Statistics** - The body weight and feed intake data were analyzed using ANOVA followed by Dunnett's test, if necessary. The cholinesterase data were analyzed using an adjusted Welch test. Significance was indicated at $p \leq 0.05$ and 0.01 in the study tables.

C. METHODS

1. **Observations** - Dams were observed once daily for mortality and clinical signs of toxicity from GD 0-20.

2. **Body weight** - Dams were weighed daily from GD 0-20.

3. **Feed consumption** - Feed consumption was measured daily on GD 0-20.

4. **Cholinesterase activity determination**

a. **Maternal animals** - On GD 20, blood was collected via the retroorbital venous plexus under ether anesthesia for plasma and erythrocyte cholinesterase activity determinations. Immediately following blood collection, the animals were sacrificed by cervical dislocation under CO_2 anesthesia, the whole brain was removed from the skull and frozen (≤-18°C) until analysis, and a cesarean section was performed. Cholinesterase activity determination was performed using a modification of the Ellman method. The modification consisted of using 6,6'-dithiodinicotinic acid as the coupling reagent and measuring the change in absorbance at 340 nm.

b. **Fetal animals** - Following blood collection from the dams on GD 20, the fetuses were removed from the uterus and sacrificed by decapitation for collection of blood samples. The blood samples were pooled by litter. Immediately thereafter, the whole brains were removed from the fetuses, pooled litterwise, and frozen (≤-18°C) until analysis. Cholinesterase activity determination was performed using the same modification of the Ellman method reported above.

5. **Benchmark dose response** - It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. Benchmark dose responses of 10 and 20% cholinesterase inhibition were calculated for each compartment.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - No clinical signs of toxicity were observed throughout the study.

2. **Mortality** - All animals survived to scheduled sacrifice.

3. **Body weight** - No treatment-related effect on body weight was observed.

4. Feed consumption - No treatment-related effect on feed consumption was observed. The decreased feed consumption noted at 8 ppm on GD 20 (↓22%, p≤0.01) was not considered to be toxicologically relevant.

B. CHOLINESTERASE ACTIVITY - The cholinesterase data for all compartments are summarized and presented in Table 2 below.

1. Plasma cholinesterase - Plasma cholinesterase activity was dose-dependently decreased in the dams at ≥2 ppm (p=<0.01, ↓37-91%) and in the fetuses at all doses (p=<0.01,↓12-69%). However, the inhibition in the 0.5 and 2 ppm fetuses was less than 20%.

2. Erythrocyte cholinesterase - Erythrocyte cholinesterase activity was dose-dependently decreased in the dams at all doses (p=<0.05↓18-90%) and in the fetuses at ≥2 ppm (p=<0.05, ↓20-83%). However, the inhibition in the 0.5 ppm dams was less than 20%.

3. Brain cholinesterase - Brain cholinesterase activity was dose-dependently decreased in the dams at all doses (p=<0.01,↓5-85%) and in the fetuses at 8 ppm (p=<0.01, ↓35%). However, the inhibition in the 0.5 dams was less than 10%.

Table 2. Mean (±SD) cholinesterase activity in maternal and fetal rats treated with Disulfoton in the diet from GD 0-20. [a]

Compartment	Dose (mg/kg/day)/(ppm)			
	0/0	0.042/0.5	0.168/2	0.694/8
Dams				
Plasma (kU/L)	2.10±0.29	1.89±0.23	1.33±0.16** (↓37)	0.19±0.06** (↓91)
Erythrocyte (kU/L)	2.02±0.34	1.66±0.31* (↓18)	1.13±0.37** (↓44)	0.20±0.13** (↓90)
Brain (U/g)	11.97±0.53	11.35±0.50** (5)	8.12±0.44** (↓32)	1.76±0.19** (↓85)
Fetuses [b]				
Plasma (kU/L)	0.26±0.01	0.23±0.02** (↓12)	0.21±0.02** (↓19)	0.08±0.01** (↓69)
Erythrocyte (kU/L)	1.27±0.16	1.21±0.20	1.02±0.19** (↓20)	0.22±0.11** (↓83)
Brain (U/g)	1.81±0.30	1.75±0.28	1.74±0.26	1.18±0.21** (↓35)

a Data were obtained from Table 6-3 on page 31, and pages 84 and 85 of the study report; n=11-13. Percent inhibition (decreased activity) is included in parentheses. bFetal data were pooled by litter. * Significantly different from controls at p≤0.05 ** Significantly different from controls at p≤0.01

C. BENCHMARK DOSE RESPONSE - In the dams, the following benchmark doses (mg/kg/day) were calculated for levels of 10 and 20% cholinesterase inhibition, respectively: (i) plasma (0.09 and 0.17); (ii) erythrocytes (0.09 and 0.17); and (iii) brain (0.09 and 0.18; Table 3). In the fetuses, the following benchmark doses (mg/kg/day) were calculated: (i) plasma (0.12 and 0.23); (ii) erythrocytes (0.10 and 0.19); and (iii) brain (0.38 and 0.59).

Table 3. Benchmark dose estimates (mg/kg/day) for cholinesterase inhibition in maternal and fetal rats treated with Disulfoton in the diet from GD 0-20. [a]

Compartment	Dams		Fetuses	
	BMD10	BMD20	BMD10	BMD20
Plasma	0.09	0.17	0.12	0.23
Erythrocytes	0.09	0.17	0.10	0.19
Brain	0.09	0.18	0.38	0.59

a Data were obtained from Table 7-1 on page 34 of the study report. It was stated that the BMD was calculated using the non-positive quadratic polynomial model as provided in the US EPA BMDS software (version 1.3.2) versus analytically confirmed doses. BMD10 = 10% inhibition BMD20 = 20% inhibition

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u> - The investigators concluded that cholinesterase activity was dose-dependently decreased in the dams in all compartments at all doses; however, inhibition was <20% in all compartments at 0.5 ppm. In the fetuses, cholinesterase activity was significantly decreased and exceeded 20% inhibition at 8 ppm in all compartments.

B. <u>REVIEWER COMMENTS</u> - There were no treatment-related effects on mortality, clinical signs, body weight, or feed consumption.

Significant dose-dependent decreases ($p \leq 0.01$) in cholinesterase activity were observed in all compartments (plasma, nominal decrease), blood, and brain) in the ≥0.5 ppm dams and in the 0.5 [plasma], 2 [erythrocytes], and 8 ppm [brain] fetuses. These data were used to calculate benchmark doses for 10 and 20% cholinesterase inhibition in both maternal animals and the fetuses. In this study, the fetuses were less sensitive than their dams.

This study is classified as **ACCEPTABLE/NON-GUIDELINE.**

C. <u>STUDY DEFICIENCIES</u> - None

13544

R128714

Chemical: Disulfoton

PC Code:
032501
HED File Code: 13000 Tox Reviews
Memo Date: 6/2/2004
File ID: DPD319813
DPD321704
Accession #: 412-06-0192

HED Records Reference Center
7/12/2006