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

OFFICE
OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

TXR# 0052748
Date: July 25, 2006

MEMORANDUM

SUBJECT: PC 036501: Coumaphos: Comparative cholinesterase study of adult male and females and 11-day old pups [MRID# 46258301].

DP Barcode: D305691
PC Code: 036501

FROM: David G Anderson, PhD 7/25/06
Reregistration Branch II
HED (7509P)

THRU: Alan Nielsen, BSS 7/25/06
Reregistrant Branch II
HED (7509P)

TO: Robert McNally/Wilhelamena Livingston
SRRD (7508P)

The study [MRID# 46258301] has been reviewed as requested and the conclusions are presented below. This study was submitted as a supplement to a DNT study with coumaphos [MRID# 45912101][TXR# 0051861]. This request was part of urgent request to review several DNT studies with OP's to evaluate DNT studies submitted to date.

MRID# 46258301: Comparative Cholinesterase in adult and 11-day old Wister rats with coumaphos

Conclusions:

For young-adult males/females the NOAEL/LOAEL = 1.0/2.0 mg/kg based on increased plasma (male/females=33%/38%) and erythrocyte (males/females=34%30%) cholinesaterase inhibition. Brain cholinesterase was not inhibited at any dose level in males

or females.

For males/female 11 day old pups the NOAEL/LOAEL = 0.25/0.5 mg/kg based on plasma (males/females 19%/22%) and erythrocyte (males /females 20%/19%) and brain (8%/7%) cholinesterase inhibition.

The study is ACCEPTABLE/NON-GUIDELINE for a cholinesterase data supplemental to the developmental neurotoxicity study in rats with coumaphos.

COMPLIANCE: A statement of no-confidentiality of data and compliance with GLPs was signed.

EPA Reviewer: David G Anderson, Ph.D.
Reregistration Branch 2, Health Effects Division (7509C)
EPA Secondary Reviewer: Elissa Reaves, Ph.D.
Reregistration Branch 2, Health Effects Division (7509C)

Signature: David G Anderson
Date 7/25/06
Signature: Elissa R
Date 7/25/06

TXR#: 0052748

DATA EVALUATION RECORD
Supplement to MRID# 45912101

STUDY TYPE: Cholinesterase Inhibition in Young Adults and 11 Day Old Pups - Rat [Supplement to OPPTS 870.6300 (§83-6)] OECD 426

PC CODE: 036501 **DP BARCODE**: D305691

TEST MATERIAL (PURITY): Technical Grade Coumaphos (93.4-94.7%)

SYNONYMS: O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl)O,O-diethyl phosphorothioate

CITATION: Sheets, L. P. (2004) Cholinesterase inhibition in young adult and neonatal (11-day-old) Wistar rats treated by gavage with a single dose of technical grade coumaphos. Bayer Crop Science LP, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 201016 (other #s 03-P12-QD, 03-N12-QG, 03-P12-SF, 03-D12-TK); April 16, 2004. MRID 46258301. Unpublished

SPONSOR: Bayer Health Care, LLC, Animal Health Division, 12707 Shawnee Mission Parkway, Shawnee, KS 66216-1846.

EXECUTIVE SUMMARY: The relative sensitivities to cholinesterase inhibition at peak inhibition by coumaphos were measured in young-adult and neonatal rats (MRID 46258301). In these studies on young-adult and neonatal Wistar (Hanover Crl:WI[GIxBRL/Han]GS BR) rats, coumaphos (93.4-94.7% a.i., batch # 795-010-112) was administered in a single dose by gavage to 58 to 63 day old young-adults (6/sex/group)(4 hours after dosing) or 11-day-old neonatal rats (8 hours after dosing) at peak cholinesterase inhibition. Peak cholinesterase inhibition was measured, 4 hours and 8 hours, respectively in young-adults and neonatal rats administered coumaphos at 0, 1.0, 2.0 or 4.0 mg/kg/day and 0, 0.25, 0.50, or 1.0 mg/kg/day, respectively.

These data suggest that coumaphos treatment of 11 day old male and female pups by a single dose by gavage results in cholinesterase inhibition at a lower dose than a similar treatment of 58-63 day old male and female young-adults. In addition, brain cholinesterase was inhibited at the same LOAEL as plasma and erythrocyte cholinesterase in male and female pups, whereas young-adults showed no brain cholinesterase inhibition at any dose level in males or females.

The report did not mention any observations of possible cholinergic effects in the adult or pups or weight decrement after treatment.

For young-adult males/females the NOAEL/LOAEL = 1.0/2.0 mg/kg based on increased plasma (male/females=33%/38%) and erythrocyte (males/females=34%30%) cholinesaterase inhibition. Brain cholinesterase was not inhibited at any dose level in males or females.

For males/female 11 day old pups the NOAEL/LOAEL = 0.25/0.5 mg/kg based on plasma (males/females 19%/22%) and erythrocyte (males /females 20%/19%) and brain (8%/7%) cholinesterase inhibition.

The study is **ACCEPTABLE/NON-GUIDELINE** for a cholinesterase data supplemental to the developmental neurotoxicity study in rats with coumaphos.

COMPLIANCE: A statement of no-confidentiality of data and compliance with GLPs was signed.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:**	Technical grade Coumaphos
Description:	Cream-colored, slightly yellowish to light grey powder or lumps
Lot/Batch #:	795-010-112
Purity:	93.4-94.7 % a.i.
Compound Stability:	Confirmed for 32 days, frozen
CAS # of TGAI:	56-72-4

---

2. **Vehicle control:** 0.5% (w/v) methyl cellulose Tween 80 in a dose volume of 10 ml/kg.

3. **Test animals (P):**	For suppling neonatal pups for dosing and time to peak activity.	
Species:	Rat	
Strain:	Wistar Hannover Crl:WI(Glx/BRL/Han) IGS BR	
Age at study initiation:	males & females: at least 12 wks when bred	
Wt. at study initiation:	164.0-229.0 g	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually or with litter in stainless steel grid or plastic cages	
Diet:	Purina Mills Rodent Lab Chow 5002, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	10-15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	About 7 days	

Doses were analyzed and found within acceptable variation of nominal.

Cholinesterase activity was measured by a modified Ellman method, using 6,6'-diothiodinicotinic acid as coupling reagent and measuring the changes in absorbance at 340 nm.

Litter size reduction at day 4 by random selection was erroneously referred to as culling. Culling pups is inappropriate in toxicity studies submitted to EPA.

B. RESULTS:

1. Observations: Observations were stated to have been made, but no data was presented. Weight data was stated to have been collected, but no data was presented.

2. Cholinesterase Inhibition: Dose related cholinesterase inhibition was stated to have been collected for young-adult males and females at 4 hours after dosing and for male and female 11 day old pups at 8 hours after dosing, the respective peak cholinesterase inhibition (Tables 1 and 3). However, notations in the individual animal data suggested that different time periods of collection may have been reported (Tables 2 & 4). It is assumed in the current review that the blood collection times after dosing (4 hours for young adults and 8 hours for 11 day old pups) were as stated elsewhere in the report and the letter notations referred to in Table 2 and 4 were in error.

Blood collection times after dosing in various tables in the report were Males, A=4 hr, B-2 hr, C=4 hr, D=8 hr, E=24 hr; and Females E= ? hr, F=4 hr, G=2, H=4 hr, I=8 hr, J=24 hr. In other places in the tables in the report, blood collection times after dosing were: Males, A=4 hr, B=2 hr, C=4 hr, D=8 hr; Females, E=4 hr, F=2 hr, G=4 hr, H=8 hr. The registrant should clarify the letters used in the Table of individual animal data.

3. **Young-adult rats** (time to peak activity): Young adult rats about 58 to 63 days old were used to determine the time between dosing and peak cholinesterase inhibition activity. Male and female rats were dosed at about 10mg/kg and blood taken at 2, 4, 8 and 24 hours after dosing (Table 1). Time to peak cholinesterase inhibition was found at 4 hours after dosing.

4. **Young-adult rats** (dose related cholinestease inhibition at peak activity): Male and female rats were dosed by gavage in a single dose at 1.0, 2.0 or 4.0 mg/kg (Table 2). Blood was taken at 4 hours after dosing.

Statistically significant cholinesterase inhibition was seen at 2.0 mg/kg and greater in plasma and erythrocyte. No increased cholinesterase inhibition was seen in brain. A statistically significant erythrocyte cholesterase inhibition was seen at 1.0 mg/kg in males (19%) only. The report author discounted this value as being below 20% inhibition and females showed no depression at this dose. In addition, no difference in cholinesterase inhibition between males and females has been shown in these studies with coumaphos and the standard deviation in control values for erythrocyte cholinesterase was large.

5. **Neonatal 11-day old rats** (time to peak activity): Litters from approximately 10 undosed dams were allocated to the study after litter size reduction to 4 pups/sex/litter. Pups were chosen 1 male and 1 female from each litter and allocated to four groups. Neonatal pups at 11 days of age were dose at 3.0 mg/kg in a single dose and decapitated for blood 2, 4 or 8 hours after dosing (Table 3). Peak cholinesterase inhibition was found to be 8 hours after dosing.

6. **Neonatal 11-day old rats** (dose related cholinesterase inhibition at peak activity): Litters from approximately 10 undosed dams were allocated to the study after litter size reduction to 4 pups/sex/litter. A within litter treatment design was used, with four groups represented by one males and one female in each litter, to the extent pups were available. Within each litter, pups were designated to receive a single dose on day 11 of lactation. No more than one male or one female in a litter were assigned to one dose level. Neonatal pups 11 days old were dosed by gavage in a single dose at 0, 0.25, 0.5 or 1.0 mg/kg and blood taken by decapitation at 8 hours after dosing (peak cholinesterase inhibition)(Table 4).

Statistically significant cholinesterase inhibition was seen at 0.50 mg/kg and greater in plasma , erythrocyte and brain.

Table 1: Time to peak cholinesterase inhibition in young-adult rats administered a single dose by gavage at 10 mg/kg with coumaphos technical. [a]

Time	Males			Females		
Samples collected	Plasma	Erythrocyte	Brain	Plasma	Erythrocyte	Brain
Control in IU/mL ±SD	0.37±0.05	1.05±0.13	12.2±0.7	1.65±0.68	0.97±0.09	12.3±0.3
2 hr	57%*	53%*	2%	83%*	67%*	10%
4 hr	70%*	72%*	3%	78%*	72%*	3%
8 hr	62%*	71%*	5%	58%*	73%*	8%
24 hr	30%*	58%*	2%	47%*	54%*	2%

[a] % inhibition relative to a single set of controls. Samples collected from separate groups [6/sex/group/time point] 2, 4, 8 and 24 hours after treatment using one control group. * Significantly different from control value at p >=0.05.

Table 2: Dose related cholinesterase inhibition at peak values (4 hours) [a] in young-adult Wistar rats administered a single dose by gavage at 0, 1.0, 2.0 or 4.0 mg/kg. [b] Blood taken by orbital plexus at peak cholinesterase inhibition [4 hours].

Dose in mg/kg	Males				Females			
	Time period blood collected	Plasma	Erythrocyte	Brain	Time period blood collected	Plasma	Erythrocyte	Brain
0	A	0.40±0.09 IU/mL	0.94±0.18 IU /mL	12.1±0.4 UI/mL	E	2.10±0.58 IU/mL	1.16±0.19 IU/mL	11.8±0.7 IU/mL
1.0	B	10%	19%* (0.76±0.06 IU/mL)	[3%]	F	17%	16%	2%
2.0	C	33%	34%* (0.62±0.05 IU/mL)	[2%]	G	38%	30%*	[1%]
4.0	D	50%	38%*(0.58± 0.07 IU/mL)	1%	H	59%	52%*	3%

[a] Blood stated to be taken at peak cholinesterase inhibition. The letters A, B, C, D, E, F, G, H in columns 2 & 6 are unexplained and confusing. The letters A, B, C, D, E, F, G, H are found in individual animal data, page 50-59 of the report may suggest collection from a time period differed from 4 hours. The registrant should explain these letters in the table in the report. It is assumed in the current review that the blood collection times were as stated (4 hr) elsewhere in the report.

[b] Based on results for males and females [n=6/sex/group]. Results indicated % inhibition of ChE activity for controls compared with treated groups. Values in brackets show % greater than control values. Values in parentheses are actual mean cholinesterase values in IU/mL±SD. * Statistically significant at p<=0.05. ** Statistically significant at p<=0.01.

Table 3: Time to peak cholinesterase inhibition in 11 day old rat pups administered a single dose by gavage at 3.0 mg/kg coumaphos. [a]

Time after treatment	Males			Females		
Samples collected	Plasma	Erythrocyte	Brain	Plasma	Erythrocyte	Brain
Control in IU/mL±SD	0.68±0.07	1.83±0.23	5.7±0.3	0.71±0.08	1.82±0.18	5.9±0.4
2 hr	72%*	62%*	33%*	76%*	65%*	39%*
4 hr	88%*	87%*	63%*	87%*	87%*	61%*
8 hr	88%*	91%*	67%*	93%*	95%*	71%*

[a] % inhibition compared with a single set of controls. Samples collected from separate groups [8-10/sex/group/time point], 2, 4, and 8 hours after treatment with coumaphos using one control group. * Statistically significant at p<=0.05. ** Statistically significant at p<=0.01.

Table 4: Cholinesterase inhibition at peak time (8 hours) [a] in 11-day old Wistar rat pups after treatment by gavage with coumaphos. [b]

Dose in mg/kg	Males				Females			
	Time period blood collected	Plasma	Erythrocyte	Brain	Time period blood collected	Plasma	Erythrocyte	Brain
0	A	0.67±0.07 IU/mL	1.25±0.11 IU/mL	6.1±0.3 IU/mL	A	0.73±0.08 IU/mL	1.15±0.17 IU/mL	6.0±0.3 IU/mL
0.25	B	3%	[6%]	3%	B	7%	[4%]	2%
0.50	C	19%*	20%*	8%*	C	22%*	19%	7%*
1.00	D	60%*	64%*	38%*	D	60%*	68%*	37%*

[a] Blood stated to be taken at peak cholinesterase inhibition. The letters A, B, C, D in column 2 & 6 of this Table are unexplained and confu
sing.. These letters A, B, C, D are found in the individual animal data, page 61-65 of the report. It is assumed for the current review that the blood collection times were as stated elsewhere in the report (8 hr after dosing).

[b] Based on results for male and female litter mates combined [n=8-10 pups/sex/group]. Results indicate ChE activity for controls and % inhibition compared with control. Values in brackets show % greater than control values. * Statistically significant at p<=0.05. ** Statistically significant at p<=0.01.

13544

R132422

Chemical: Coumaphos

PC Code: 036501
HED File Code: 13000 Tox Reviews
Memo Date: 7/25/2006
File ID: TX0052748
Accession #: 000-00-9999

HED Records Reference Center
9/20/2006