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

OFFICE OF
PREVENTI
ON, PESTICIDES AND
TOXIC
SUBSTANCES

TXR No. 0054671

August 21, 2007

Memorandum

SUBJECT: Malathion and Malaoxon: Follow-up Analysis of Acute Dose Comparative Cholinesterase Study Protocol PC Code: 057701. Barcode: DP# 342339

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Background:

On July 19, 2007, EPA held a conference call with the malathion registrant (Cheminova represented by Paul Whatling and Teri Spanogle) and its consultant, (Judy Hauswirth) to discuss the Agency's recommendations previously outlined in a memo dated June 28, 2007 (TXR No. 0054632, DP# 340518). The EPA participants included Deborah Smegal, Elissa Reaves, John Doherty, Stephanie Padilla, Kevin Costello and Eric Meiderhoff.

HED evaluated the acetyl cholinesterase activity (AChE) data in Study TCQ00021 (2007) conducted by Charles River Laboratories to determine the time to peak effect

(TTPE) for sampling in a subsequent single dose comparative cholinesterase assay (CCA). The Agency concluded that the available data support a TTPE for inhibition of 80 minutes for both RBC and brain AChE for both malathion and malaoxon. The Agency noted the large variability in the data for both RBC and brain AChE activity and finds the appearance of a peak effect for brain inhibition in females for malathion prior to 80 minutes questionable. The Agency believes that inhibition associated with malathion results from its oxon analog, malaoxon. The phosphorylated inhibited form of the enzyme, once formed, would remain inhibited at 80 minutes, and thus, shorter times for the TTPE do not render an advantage. Based on Study TCQ00021, the variability of the data after 60 minutes (although still of high variability) appears less than at earlier times.

On July 19th, EPA also held discussions with Charles River Laboratories (Allen Hoberman and John Barnett), and made recommendations to improve the SOP for AChE to reduce the variability of the data.

Requested Action:

During the conference call, Cheminova requested that the Agency re-consider its recommendations of 80 minutes based on previous data generated by Huntingdon Life Sciences, Ltd (HLS) in 2006 that indicated a TTPE of 20 minutes for malaoxon, and the Charles River repeat dose CCA study for malathion/malaoxon (TQC00013, MRID 46822201), where malaoxon samples were collected at 30 minutes.

Comparison of Charles River and Huntingdon Life Sciences Data:

Attached is a table that summarizes and compares selected critical information from Charles River and HLS laboratories. As shown, the data from the two laboratories are different, even for the identical dose levels and time measurements. For example, in the single dose studies, HLS generally showed approximately 50% more RBC and brain ChE inhibition than Charles River data for the same dose level and sampling interval (i.e., 10 mg/kg malaoxon at 30 minutes). In addition, there were substantial differences in the AChE activity in control animals (with the control values for brain being much lower for HLS than for Charles River Laboratories), and the Standard Operating Procedure (SOP) for Cholinesterase Activity assessment.

Cursory review of the SOPs for plasma ChE and RBC and brain AChE from the Charles River and Huntingdon labs indicated that there were differences in methodology including: autoanalyzer (Hitachi 911 vs. SPECTRA max 190), solubilizer (Triton A 100 vs. tween) secondary substrate (6.6' dithiodinicotinic acid vs. 5-9'dithio-bis(2-nitrobenzoic acid), use of anticoagulant, details on centrifuging and vortexing, homogenizing (Ultra Truax vs. unspecified homogenizer and time for homogenizing) dilution of brain and secondary dilutions and acceptance criteria being discussed or not discussed.

Conclusions and Recommendations:

Thus, the Agency continues to support a TTPE of 80 minutes for both malathion and malaoxon based on the Charles River Study TCQ00021. However, the Agency acknowledges that the large variability in these data could be impacting the ability to determine AChE inhibition data with accuracy. It is, however, unknown if the TTPE results are impacted by the variability.

EPA recommends the following improvements to the Charles River SOP for AChE to reduce variability and improve the accuracy of the results:

Recommendation for brain homogenization:

> Weigh each brain, add buffer that is appropriate (given the brain weight) for the final dilution. Homogenize brain for at least 1 minute on ice using an identified homogenizer and maintain samples on ice until application to the autoanalyzer.

Recommendation for RBC preparation:

> Unless there is some need to wash red blood cells, EPA would recommend not performing the wash procedure. Alternatively, EPA recommends doing the low speed centrifugation to separate RBCs from plasma, removing plasma, and plunging a pipette into the packed RBCs and just pipetting off a sample of the RBCs and diluting them directly into the buffer, vortex well and analyze (or freeze and analyze).

The Agency recommends that Charles River considers the EPA recommendations and improve the AChE SOP prior to analyzing blood and brain samples in the CCA study for malathion/malaoxon. The dosing of the animals could be performed, and the samples could be frozen for later analysis if these SOP revisions would take significant time to adopt.

Comparison of Malathion/Malaoxon Studies

Parameter		Charles River						HLS Single Dose PND 11 (Jan 2006)				
Study		Repeat Study PND 11-21 TQC00013 (2006) (n=12/sex)				Single Dose PND 11 (June 2007) (TCQ00021) (n=8/sex)		CHV 112/050147 (n=8/sex)	CHV 110/050069 (n=5-6/sex) (3-phase study)			
Compound		Malathion		Malaoxon		Malathion	Malaoxon	Malaoxon	Malaoxon			
Dose (mg/kg)		50	150	2.5	4	150	10	7 (7.75)	1	3	5	10
Time to peak effect (minutes)	RBC	Sampled at 2 hr		Sampled at 30 min		120 (M); 60 (F)	120 (M); 120 (F)	20 (M); 20 (F) (n=3)	Sampled at 30 min			
	Brain					80 (M); 60 (F)	60 (M); 120 (F)	20 (M); 20-40 (F)				
Maximum RBC inhibition (ug/L)	Males	34%	54%	46%	51%	47% --120	64%--120 39%--20 54%--40 (looks low— 51% at 4 mg/kg at 30 min)	48%	5%	45% (II) 44% (III)	68%	80% (39-54% CR)
	Females	30%	52%	35%	45%	54%--60 31%--120	65%--120 37%--20 52%--40 (looks low— 45% at 4 mg/kg at 30 min)	49% (n=3) 5 clotted	41% (II) 49% (III)	62%	72%	68% (37-52% CR)

Comparison of Malathion/Malaoxon Studies

Parameter		Charles River						IHLS Single Dose PND 11 (Jan 2006)				
Study		Repeat Study PND 11-21 TQC00013 (2006) (n=12/sex)				Single Dose PND 11 (June 2007) (TCQ00021) (n=8/sex)		CHV 112/050147 (n=8/sex)	CHV 110/050069 (n=5-6/sex) (3-phase study)			
Compound		Malathion		Malaoxon		Malathion	Malaoxon	Malaoxon	Malaoxon			
Dose (mg/kg)		50	150	2.5	4	150	10	7 (7.75)	1	3	5	10
Maximum Brain inhibition (ug/K)	Males	0.1%	15% (33% in single dose) looks low	1%?? (46% RBC)	None?? (51% RBC)	36% --80 33%--120	31%--60 30%--120 22%--20 25%--40	46% (more than CR)	--	6% (II) 12% (III)	25%	43% (22-25% CR)
	Females	none	16.8% (40% in single dose) looks low	2%?? (35% RBC)	None?? (45% RBC)	56%--60 40%--120	33%--120 6%-20 18%--40	46% (more than CR)	9% (II+ III)	17%	19%	60% 6-18% CR
Administration volume and vehicle		5 mL/kg; Corn oil				5 mL/kg; Corn oil		5 mL/kg; Corn oil	5 mL/kg; Corn oil			
Concentration mg/mL						30	2	1.4 malaoxon	0.2	0.6	1	2
Strain		Crl:CD(SD)				Crl:CD(SD)		Crl:CD(SD) IGS BR	Crl:CD(SD) IGS BR			
purity		96%; 9010501		97.7%; Batch 849-Bse-42C		96%; 9010501	97.7%; Batch 849-Bse-42C	97.7%; Batch 849-Bse-42C	97.7%; Batch 849-Bse-42C			

Comparison of Malathion/Malaoxon Studies												
Parameter		Charles River						HLS Single Dose PND 11 (Jan 2006)				
Study		Repeat Study PND 11-21 TQC00013 (2006) (n=12/sex)				Single Dose PND 11 (June 2007) (TCQ00021) (n=8/sex)		CHV 112/050147 (n=8/sex)	CHV 110/050069 (n=5-6/sex) (3-phase study)			
Compound		Malathion		Malaoxon		Malathion	Malaoxon	Malaoxon	Malaoxon			
Dose (mg/kg)		50	150	2.5	4	150	10	7 (7.75)	1	3	5	10
Control values	males	2316 RBC 12104 brain		1930 RBC 11867 brain		2024-2396 RBC 6946-7083 brain	1955-2176 RBC 7079-7166 brain	1647 RBC 4678 brain	2290 RBC (II+III) 2080 (II) 4500 (III) brain			1600 RBC 6970 brain
	females	2119 RBC 11880 brain		1788 RBC 12311 brain		1868-2076 RBC 6978-7259 brain	1796-2087 RBC 6718-7313 brain	1627 RBC 4463 brain	3180 (II) 2645 (III) RBC 2080 (II) 4200 (III) brain			1145 RBC 5840 brain

II=phase 2
III=phase 3

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Chemical:	**Malathion** **Malaoxon (metabolite of malathion)**
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