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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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

July 17, 2002

MEMORANDUM

SUBJECT: **Dicrotophos:** Review of Acute and Repeated Exposure Comparative Cholinesterase Study Protocols

TO: Laura Parsons
Reregistration Branch
Special Review and Reregistration Division (7508C)

FROM: William F. Sette, Ph.D. F Sette 7-17-02
Toxicology Branch
Health Effects Division (7509C)

THRU: Developmental Neurotoxicology Protocol Review Committee
Health Effects Division (7509C)

and
Alberto Protzel, Ph.D., Branch Senior Scientist 7/19/02
Toxicology Branch
Health Effects Division (7509C)

cc: Michael Nieves, SRRD (7508C)
Bill Greear, HED (7509C)
David Hrdy, HED (7509C)

Chemical: Dicrotophos
PC Code: 035201
DP Barcode: D281500
TXR #: 0050545

ACTION: The purpose of this memo is to review draft protocols submitted by AMVAC for studies to be conducted at Central Toxicology Laboratories for the assessment of cholinesterase activity in adult and immature rats following acute or repeated exposures to Dicrotophos.

CONCLUSION: These protocols are considered only partially adequate for the assessment of comparative cholinesterase activity data as specified in the EPA Data-Call-In (DCI) for adult and developmental neurotoxicity (DNT) studies on the organophosphate (OP) pesticides (issued September 10, 1999). The protocols fail to provide for the contemporaneous assessment of plasma cholinesterases; fails to assess dams and fetuses on GD 20; in the pup repeated exposure study confounds litter and dose; does not describe the rationale or data supporting one hour as the time of peak effect; fails to identify a source for acute data on young adults; does not describe the adequacy of proposed doses for characterizing the dose effect curves for all 3 compartments; and contains a lack of correspondence between the acute testing time point and the repeated exposure time point in pre-weanling rats.

Introduction

At the request of the Agency, the registrant, AMVAC, has submitted draft protocols for studies that were designed to assess cholinesterase activity in immature rats following acute or repeated exposures to Dicrotophos. The studies described in this submission are intended to satisfy the requirement for comparative cholinesterase data as specified in the EPA Data-Call-In (DCI) for adult and developmental neurotoxicity (DNT) studies on the organophosphate (OP) pesticides (issued September 10, 1999). Additional instructions provided to the registrant in a document entitled *Guidance on Cholinesterase Measures in DNT and Related Studies (10/29/01)* form the basis for the review of the comparative cholinesterase protocols. A summary of the EPA guidance regarding the subjects and times for measurement of cholinesterase activity is given in the following table:

Summary of EPA Guidance on Required Cholinesterase Measures	
Study	Populations
Main DNT study	1. PND 4 (pups) 2. PND 21 (pups and dams)
Maternal GD 6-20 study	1. GD 20 dams 2. GD 20 fetuses
Sensitivity study	Acute doses: 1. Pre-weaning pups (both sexes); a) Early-Mid lactation [no later than PND11]; b) Late lactation [7-10 days after first time point, no later than PND 21]; 2. Young adults (both sexes).
	Repeated doses: 1. Pre-weaning pups -- exposure beginning during early lactation, with a duration of 7-10 days (starting no later than PND 11, e.g., PND 11-21), with ChE evaluations after dosing on last day of exposure; 2. Young adults (both sexes) -- repeated dose exposure using duration and doses as for pre-weaning.

The following discussion presents the Agency response to the draft protocols.

Cholinesterase measures in the main DNT study and in GD 20 dams and fetuses

The protocol for the main DNT study has been previously reviewed by the Agency. The Agency guidance (10/29/01) recommends the measurement of cholinesterase activity during the course of the DNT study in dams and fetuses on GD 20, in pups on PND 4, and in dams and pups on PND 21. Neither the DNT protocol nor these protocols propose measurements of ChEs in dams and fetuses at GD20.

Generic Issues

This protocol proposes measures of erythrocyte and brain ChE in pups one hour following acute or repeated exposure. Plasma would be stored at -70° C and not analyzed.

EPA finds it unacceptable not to analyze plasma cholinesterase (ChE) at the same time as analyses of RBC and brain ChEs, as data from all 3 compartments are considered essential to these evaluations.

The protocol proposes a single time of testing, i.e., one hour after exposure, after acute or subacute exposures for each age group. There is no discussion or data shown here to support one hour as the time of peak effect.

Cholinesterase measures following acute exposure to adult and immature rats

The acute protocol proposes assessment of 5 pups/sex/dose on PND 8, 15, and 22. This is consistent with the guidance document, and is laudable in using an intermediate lactation time point, i.e., PND 15. Five pups/sex/dose should generally be acceptable if the sensitivity of the cholinesterase assay is adequate, e.g., CVs of roughly 20% or less. However, there is no discussion of any data on young adults, that would serve as a basis for comparison with the data on the pups. The source of young adult data, and the details to describe their comparability is an essential element of defining comparative sensitivity.

The acute protocol proposes four doses and a control group: 0, 0.1, 0.3, 1 and 5 mg/kg. No discussion of the adequacy of this dose range is proffered, which is an essential component of the study and must be provided in the study report.

Cholinesterase measures following repeated dose exposures to adult and immature rats

The protocol will have 8 litters and dams and proposes to allocate 2 litters to each treatment group, from which a total of 5 male and 5 female pups will be drawn. This scheme is undesirable since it will confound litter source with dose; that is, differences seen between dose groups may be a function of effects due to being from the same litter, rather than from the dose

itself. It is preferable, as is proposed in the acute protocol, to allocate one pup/sex/litter to each of the four different dose groups (3 doses and controls), so that animals from all litters are in each dose group.

The protocol proposes dosing of groups of 5 pups/sex/dose on PND 12 for 7 days with assessment of ChE on PND 18, and dosing groups of 5 young adults/sex/dose on PND 42 for 7 days with assessment of ChE on PND 48. Proposed dose levels are 0, 0.008, 0.02, 0.08, and 0.4 mg/kg/day. Detailed clinical observations will be made to dosed animals prior to dosing and one hour after exposure on each dosing day [See above regarding this time point]. Body weights will be recorded prior to dosing.

This is generally consistent with EPA recommendations, except that the assessment on PND 18, the latest day of repeated pre-weaning testing, does not correspond with any day of acute testing (which are on PND 15, and PND 22). This is an important comparison that might indicate whether age alone or age and duration of exposure cause different results. Second, 42 day old rats may not be fully mature with respect to enzyme systems relevant to metabolizing OPs. Day 60 or later would be preferable.

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Chemical:	**Dimethyl phosphate ester with 3-hydroxy-**
PC Code:	**035201**
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/17/2002**
File ID:	**TX050545**
Accession Number:	**412-03-0016**

HED Records Reference Center
09/30/2002