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MEMORANDUM

SUBJECT: D281332: Terbufos (PC Code 105001)
Review of Comparative Cholinesterase Study Protocols

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THRU: Developmental Neurotoxicology Protocol Review Committee
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Executive summary

Draft protocols for the assessment of cholinesterase activity in adult and immature rats following acute or repeated exposures to Terbufos (BAS 316I, AC 92100) were submitted by BASF Corporation (BASF Agro Research, PO Box 400, Princeton, NJ 08543-0400). These protocols are considered 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 address cholinesterase evaluation in GD 20 dams and pups. EPA prefers that young adults be approximately 60 days old, instead of 35-42 days as proposed in the protocol. The intervals proposed for dose volume adjustment (weekly) should be more frequent (see below). Comments are also provided regarding the described cholinesterase assay, as well as dose suspension analyses.

Introduction

At the request of the Agency, the registrant, BASF, has submitted draft protocols (dated 2/1/02) for studies that were designed to assess cholinesterase activity in adult and immature rats following acute or repeated exposures, for BAS 316I (AC 92100, Terbufos). 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. The EPA position regarding the optimal schedule for measurement of cholinesterase activity is summarized 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.

Proposed study design

Phorate (doses to be determined) will be administered to pups (8/sex/dose group) via oral gavage (in corn oil), either a single dose on PND 11 or 21, or repeated dosing from PND11-21. In addition, adult rats (8/sex/dose group) will be administered a single acute dose or 11 repeated daily doses. Proposed dosing levels were not included in the protocol, but will be selected based on the results of a range-finding study (not described). Either three or four doses will be used, depending on the range-finding study results.

In addition to cholinesterase evaluations, physical examinations and standard arena observations will be performed in pups on days 11, 16, and 21.

Cholinesterase measures following acute exposure to adult and immature rats

The protocol states that cholinesterase inhibition (plasma, RBC, and brain) will be determined following a single dose of phorate, on PNDs 11 and 21, at the time of peak effect. The time of peak effect will be determined in a range-finding study (not described). As described in the above-referenced guidance, 1) the time of peak effect should be determined for each age group and should be based upon cholinesterase inhibition and 2) it is important that doses be selected in a manner that allows characterization of the dose effect curves for all 3 compartments (i.e., plasma, erythrocyte, and brain). Cholinesterase inhibition will also be evaluated following a single dose in adults (male and female), with sacrifice at time of peak effect (stated in the protocol to be 4-5 h post-dosing, based on clinical signs in the acute neurotoxicity study).

Cholinesterase measures following repeated dose exposures to adult and immature rats

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 pups on GD 20, in pups on PND 4, and in dams and pups on PND 21. The previously reviewed protocol did not include GD 20 measures for dams or pups, nor do the current protocols include these measurements.

Immature rats versus young adults: As for acute evaluations, pups will be sacrificed at the time of peak effect following the last dose (PND21) for evaluation of cholinesterase (plasma, RBC, and brain). Time of sacrifice for adult rats was not specified, but should be performed at the time of peak effect for cholinesterase inhibition.

Cholinesterase measures in the main DNT study

The protocol for the main DNT study has been previously reviewed by the Agency, and is not under consideration at this time. However, the registrant is reminded that the current Agency guidance (10/29/01) recommends the measurement of cholinesterase activity during the course of the DNT study, as a tool in assessing the adequacy of postnatal dosing. Animals should be available for these cholinesterase assessments at PND 4 (culled pups) and at PND 21 (dams and extra weanlings).

Other comments

We note that the age listed for adult rats is 35-42 days at receipt. The time between receipt and testing is not stated, but we recommend that adult animals be at least 60 days old at the time of testing.

We note that the protocol states on p. 4 (Sec. 11.1, test substance preparation) that daily dose

volumes will be adjusted weekly to compensate for body weight changes. In pups, dose volumes should be adjusted daily, based on current body weight (we note in Sec. 13.1.3 that pups will be weighed daily from day 11-21). For young adults, we recommend that dosage volumes be adjusted twice per week.

The protocol states that cholinesterase activity will be expressed on a "per mL" basis in addition to percent of control activity, but the referent is not stated (e.g. per mL of what?). This measurement should be standardized in some way, e.g. as "nm substrate hydrolyzed per ml blood" or "per mg brain tissue."

The protocol states that "suspensions of all dose levels will be analyzed weekly for the first eight weeks and monthly thereafter ..." (Sec. 11.3). We assume that this is an error. Frequency of analysis for concentration confirmation should be included in the study report, along with results of the analyses.

Although the protocol referred to the results of a range-finding study, which would be used to determine peak effect time as well as doses for the current study, no further information about the range-finding study was provided. The registrants should make sure issues regarding dose-response and time of peak effect (discussed above) are addressed by the range-finding study, and the results of that study should be included in the study report.

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