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MEMORANDUM

SUBJECT: D281187: Acephate (PC Code 103301)
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 acephate were submitted by Valent U.S.A. Corporation. 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). It is recommended that an evaluation of cholinesterase inhibition in GD 20 dams and fetuses (following maternal dosing from GD 6-20) be conducted.

Introduction

At the request of the Agency, the registrant, Valent U.S.A Corporation, has submitted four draft

protocols (dated February 19, 2002) for studies that were designed to assess cholinesterase activity in adult and immature rats following acute or repeated exposures, for acephate. These include the following:

1. Oral (gavage) dosage range study of acephate technical in neonatal rats (Argus Research, Horsham, PA; protocol 222-003)
2. Oral (gavage) dosage range study of acephate technical in adult rats (Argus Research, Horsham, PA; protocol 222-004)
3. Oral (gavage) acute relative sensitivity study of acephate technical in neonatal and adult rats (Argus Research, Horsham, PA; protocol 222-005)
4. Oral (gavage) repeated dose relative sensitivity study of acephate technical in neonatal and adult rats (Argus Research, Horsham, PA; protocol 222-006)

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 designs

Brief descriptions of the study designs follow:

1. Oral (gavage) dosage range study of acephate technical in neonatal rats

   This study will be conducted in three segments. In Part A, neonatal pups will be administered a single gavage dose of acephate on PND 11 or 21 and observed for 24 hours. In Part B, acephate will be administered to pups by daily gavage on PND 11 through 21; pups will be observed for 24 hours after the last dose. In Part C, the time of peak cholinesterase inhibition will be determined for pups following a single dose on PND 11 or 21. Two dose levels will be selected from the range of doses tested in Part A

2. Oral (gavage) dosage range study of acephate technical in adult rats

   Adult rats will be administered single or repeated doses of acephate by gavage; toxicity will be evaluated. Time of peak effects will also be determined.

3. Oral (gavage) acute relative sensitivity study of acephate technical in neonatal and adult rats

   Adult and neonatal rats will be administered acute gavage doses of acephate. Cholinesterase activity will be measured at the time of peak effect.

4. Oral (gavage) repeated dose relative sensitivity study of acephate technical in neonatal and adult rats

   Eleven repeated doses of acephate will be administered to adult and neonatal rats; pups will be dosed on PND 11 through 21. Cholinesterase activity will be measured at the acute time of peak effect following the last treatment.

Cholinesterase measures following acute exposure to adult and immature rats

In general, the protocols as described adequately address the collection of data on cholinesterase measures following acute exposure to adult and immature rats. It is noted, however, that Protocol 222-005 (page 10) specifies a sample size of 5 pups/sex/dose for cholinesterase measures on PND 11 and on PND 21, although sample sizes in adult rats are 10/sex/dose. Due to the variability in cholinesterase data in immature pups, this sample size may not be sufficient to adequately characterize the response to treatment. It is recommended that the number of pups/sex at each postnatal day of assessment be increased.

Cholinesterase measures following repeated dose exposures to adult and immature rats

GD 20 dams and fetuses - The protocols do not address cholinesterase measures in GD 20 dams and fetuses following maternal treatment from GD6-20.

Immature rats versus young adults - The protocols as described adequately address the collection of data on cholinesterase measures following 11 repeated exposures to adult and immature rats.

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

The determination of time of peak effect is not described. As discussed in the guidance, the endpoint that should be used to determine the time of peak effect is cholinesterase inhibition, and the time of peak effect should be determined for each age group.

It is important that doses be selected in such a manner to allow for characterization of the dose effect curves for all 3 compartments (i.e., plasma, erythrocyte, and brain).

Specific comments on the Argus Laboratories Standard Operating Procedures (SOPs) for cholinesterase measures have been previously provided to Valent U.S.A. Corporation in the Agency review of the dose-range-finding and main developmental neurotoxicity protocols for acephate (TXR 0050295, dated January 10, 2002). These comments are reiterated as follows:

> Items 5.1.5 and 5.1.6: The procedures indicate the use of 0.1% Tween 80 as a detergent, although generally Triton X-100 is used. The laboratory needs to provide evidence that Tween 80 does not affect the enzyme activity.
> Item 5.2: Insufficient information is provided regarding the source of the AChE and BChE standards.
> Items 5.3.1 and 5.3.2: It is stated that adult and pup brains will be rinsed or soaked in approximately 100 mL of saline for a minimum of 1 hour. This procedure is excessive and may compromise the results; a brief rinse with saline is sufficient.
> Item 5.3.2: The specified 1:50 (approximately) brain homogenate dilution may be adequate for samples from adults or PND 21 pups, but will be too dilute for samples from GD 20 fetuses and PND 4 pups. A 1:10 solution is recommended for GD 20 fetuses, and approximately 1:20 solution is recommended for PND 4 pups.
> Item 5.3.3: The methodology states that collection tubes may contain *either* heparin or EDTA as an anticoagulant. While generically this is true, it is very important that only one of the two anticoagulants be selected for consistent use throughout this entire study.
> Item 5.4: For brain and plasma analysis, incubation times of 10 minutes are specified. However, the substrate may be used up in 10 minutes, and reactions will not be optimal. Therefore, it is recommended that brain and plasma be incubated for 5 minutes.
> Item 5.4 (also see 8.4 and 8.5): The methods specify adding a total of 360 mcL of

materials to the 96-well microtiter plate; however, generally such plates have a maximum volume of 300 mcL. Is this an error, or is a larger plate being used?
Item 8: A microtiter plate assay is indicated for red blood cells. This methodology is not appropriate for red blood cells; instead, a radiometric assay should be used. It is anticipated that little activity will be observed with the microtiter plate method.
Items 8.4 and 8.5: A 60-minute incubation period is indicated; 15-minutes is recommended for red blood cells.

Proposed schedules are provided for the dose range-finding studies. These schedules appear to be reasonable. The schedules for the acute and repeated dose comparative sensitivity studies have yet to be determined. The Agency expects that the cholinesterase study data will be available at the time of submission of the developmental neurotoxicity study for acephate.

Study	Proposed in-life start date	Proposed in-life completion date	Proposed final report date
Dose RF study in neonates	April 11, 2002 (Part A)	June 18, 2002 (Part C)	July 31, 2002
Dose RF study in adults	May 7, 2002 (Part A)	May 24, 2002 (Part B)	July 30, 2002

Conclusion

The protocols submitted by the registrant to assess cholinesterase activity in adult and immature rats following acute or repeated exposures are considered partially adequate for the evaluation 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). It is recommended that an evaluation of cholinesterase inhibition in GD 20 dams and fetuses (following maternal dosing from GD 6-20) be conducted.

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