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MEMORANDUM

SUBJECT: D281364: Ethoprop (PC Code 041101)
Comparative Cholinesterase Study Protocol

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Executive summary

A draft protocol for the assessment of cholinesterase activity in adult and immature rats following an acute exposure to ethoprop (supplemental information to a developmental neurotoxicity study in rats with ethoprop) was submitted by Aventis CropScience. This protocol is 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 following major deficiencies were noted: 1) The protocol did not include measurement of plasma cholinesterase activity following acute doses of ethoprop to adult and immature rats. 2) Time-to-peak cholinesterase effect in immature animals was not provided. 3) The time of sample collection in relationship to time of dosing was not addressed. 4) Cholinesterase activity in adult

and immature rats and in pregnant dams and fetuses following repeated exposures to ethoprop are not addressed. 5) The use of only 4 animals per sex per dose group may not be sufficient to establish a dose response and ED50 in each compartment.

Introduction

At the request of the Agency, the registrant, Aventis CropScience, has submitted a draft protocol (dated January 28, 2002) for a study designed to assess cholinesterase activity in adult and immature rats following acute exposure to ethoprop. The study described in this submission is 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 protocol. 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.

In addition, as described in the 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).

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

Proposed study design

In the proposed study design, a single dose of ethoprop will be administered by oral gavage to pregnant (gestation day 20), immature (postnatal day 11), or adult (postnatal day 80) rats. Three treated groups plus control will be used at each age; dose levels were not provided. Brain and red blood cell cholinesterase will be measured for 4 fetuses/sex (from 4 dams/group), 4 PND 11 pups/sex/group, and 4 adults/sex/group.

Cholinesterase measures following acute exposure to adult and immature rats

The protocol as described addresses the collection of data on cholinesterase measures following acute exposure to adult and immature rats with partial adequacy. However, the following problems are noted:

1. Measurement of plasma cholinesterase is not included in the protocol; plasma cholinesterase must be measured for both adult and immature rats.
2. The time to peak effect in immature animals is not provided. The draft protocol cites a previous study (MRID 43442402) as a source of information regarding time of peak effect (claimed as 2 hours post dose) in rats following oral administration of ethoprop at dose levels of 30 and 60 mg/kg. It is noted that this study was performed in young adult rats (approximately 7-8 weeks of age). Cholinesterase activity was measured on Day 1 at 2 hours post dose, and on Days 3, 8, and 15. Maximum cholinesterase inhibition occurred on Day 1, and recovery was noted at all later time points. At 60 mg/kg (the HDT) in males, the percent cholinesterase inhibition was 93% for plasma, 53% for RBC, and 93% for caudate putamen; at 40 mg/kg (the HDT) in females, the percent cholinesterase inhibition was 94% for plasma, 49% for RBC, and 92% for caudate putamen. Although the precise time of peak effect was not identified, since only one time point was evaluated on the day of dosing, the magnitude of the response suggests that 2-hours post-dose may be at or near the time of peak cholinesterase response for adult rats. However, the time of peak response in immature rats could be quite different from adults and must be evaluated.
3. The time of sample collection in relation to time of dosing is not specified, although it is suggested that 2 hours post dose will be utilized, based on the cited time to peak effect data for adult rats.
4. The use of only 4 animals per sex per dose group may not be sufficient to establish a dose response and ED50 in each compartment.

Additionally, the following issues are noted:

1. Acute cholinesterase measures in dams and fetuses on gestation day 20 are included in the protocol but have not been requested by the Agency. However, the Agency does require measures at GD 20 following repeated exposures (see below).
2. The male weight range specified in paragraph 9.4.2 (225-300 grams) is low for the intended age range (75-80 days). According to Charles River, Sprague-Dawley males at 72-77 days of age fall within an average weight range of 325-350 grams (www.CRiver.com). To achieve a lower weight range, younger adult animals (e.g., 60 days of age) could be used for the cholinesterase studies (obviously, females would also

be smaller).

3. The number of pregnant dams and pups specified for placement on study (paragraph 9.4.1, page 6) does not match the description of animal assignment in paragraph 7.2 on page 4.
4. In general, detailed procedures for cholinesterase measures were not provided in the study protocol. The method for separation of RBCs from whole blood is not specified. This information should be provided in the final protocol.

Cholinesterase measures following repeated dose exposures to adult and immature rats

GD 20 dams and fetuses - The protocol does not address cholinesterase measures in GD 20 dams and fetuses following maternal treatment from GD 6-20.

Immature rats versus young adults - The protocol does not include the collection of data on cholinesterase measures following 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).

Conclusion

The protocol submitted by the registrant to assess cholinesterase activity in adult and immature rats following acute exposure of ethoprop is considered only 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). The following major deficiencies were noted: 1) The protocol did not include measurement of plasma cholinesterase activity following acute doses of ethoprop to adult and immature rats. 2) Time-to-peak cholinesterase effect in immature animals was not addressed. 3) The time of sample collection in relationship to time of dosing was not specified. 4) Cholinesterase activity in adult and immature rats and in pregnant dams and fetuses following repeated exposures to ethoprop are not addressed. 5) The use of only 4 animals per sex per dose group may not be sufficient to establish a dose response and ED50 in each compartment.

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