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MEMORANDUM

SUBJECT: D283831: Azinphos-methyl (PC Code 058001)
Comparative Cholinesterase Study Protocols

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Special Review and Reregistration Division (7508C)

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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 azinphos-methyl (supplemental information to a developmental neurotoxicity study in rats with azinphos-methyl) were submitted by Bayer Corporation. These protocols are considered 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).

Introduction

At the request of the Agency, the registrant, Bayer Corporation, has submitted four draft

protocols (electronic cover letter dated June 10, 2002) for studies that were designed to assess cholinesterase activity in adult and immature rats following acute or repeated exposures to azinphos-methyl (AZM). These include the following:

1. Pilot study to establish the time of peak brain, erythrocyte and plasma cholinesterase inhibition in young adult Wistar rats treated by gavage with an acute dose of technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-P12-[incomplete ID no.])
2. Pilot study to establish the time of peak brain, erythrocyte and plasma cholinesterase inhibition in preweaning (postnatal days 11 and 21) Wistar rats treated by gavage with an acute dose of technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-P12-[incomplete ID no.])
3. Brain, erythrocyte and plasma cholinesterase inhibition in young adult Wistar rats treated by gavage with an acute dose of technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-N12-[incomplete ID no.])
4. Brain, erythrocyte and plasma cholinesterase inhibition in preweaning (postnatal days 11 and 21) Wistar rats treated by gavage with an acute dose of technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-N12-[incomplete ID no.])
5. Brain, erythrocyte and plasma cholinesterase inhibition in young adult Wistar rats treated daily by gavage for eleven days with technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-N12-[incomplete ID no.])
6. Brain, erythrocyte and plasma cholinesterase inhibition in preweaning Wistar rats treated daily by gavage for eleven days (PND 11-21) with technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-N12-[incomplete ID no.])
7. Determination of maternal and fetal brain, erythrocyte and plasma cholinesterase activities in Wistar rats following gestational exposure via the diet to technical grade Azinphos-Methyl (GUTHION®) (Bayer Corporation, Agricultural Division, Study number 02-D72-[incomplete ID no.])

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. Pilot study - time of peak ChEI in young adult rats - acute gavage dose of AZM

   This study will be conducted to establish the time following oral gavage administration when inhibition of brain, RBC, and plasma ChE activities reach maximum levels in young adult rats. Doses selected (6 mg/kg for males and 3 mg/kg for females) were based upon the findings of an acute neurotoxicity study in rats. Six males and females will be assigned to separate groups that will be sacrificed at 1.5, 3, 8, or 24 hours after treatment, at which times tissues will be collected for measurement of brain, RBC, and plasma cholinesterase activity.

2. Pilot study - time of peak ChEI in PND 11 and 21 rats - acute gavage dose of AZM

   This study will be conducted to establish the time following oral gavage administration when inhibition of brain, RBC, and plasma ChE activities reach maximum levels in PND 11 and 21 rat pups. Pups will originate from 10 litters per dose level and per age tested; each litter will provide 1 pup/sex per each of four time-points examined (within-litter study design). The protocol specifies that the doses and time points selected for evaluation will be based upon the findings of an acute neurotoxicity study in rats and the time-course study conducted in young adult rats. When pups from each litter are sacrificed at various time-points after treatment, tissues will be collected for measurement of brain, RBC, and plasma cholinesterase activity.

3. ChEI in young adult rats - acute gavage dose of AZM

   Young adult rats (6/sex/dose) will be administered an acute gavage dose of azinphos-

methyl. Plasma, RBC, and brain cholinesterase activity will be measured at the time of peak cholinesterase effect, as established in the pilot study.

4. ChEI in PND 11 and 21 rats - acute gavage dose of AZM

Groups of preweaning rats will be administered acute gavage doses of azinphos-methyl on PND 11 or PND 21. Pups will originate from 10 litters per age; each litter will provide 1 pup/sex/dose (within-litter study design). Plasma, RBC, and brain cholinesterase activity will be measured at the time of peak cholinesterase effect, as established in the pilot study.

5. ChEI in young adult rats - eleven days of repeated gavage dosing with AZM

Eleven repeated daily gavage doses of azinphos-methyl will be administered to young adult rats (6/sex/dose). Cholinesterase activity will be measured at the acute time of peak cholinesterase effect following the last treatment.

6. ChEI in preweaning rats - eleven days (PND 11-21) of repeated gavage dosing with AZM

Eleven repeated doses of azinphos-methyl will be administered by gavage to preweaning rats (PND 11 through 21). Pups will originate from 10 litters per age; each litter will provide 1 pup/sex/dose (within-litter study design). Cholinesterase activity will be measured at the acute time of peak cholinesterase effect following the last treatment.

7. ChEI in maternal and fetal rats - dietary administration of AZM during gestation

Azinphos-methyl will be administered in the diet to pregnant female rats (15/dose) from gestation days 0 through 20. The dose levels will be the same as those used in the developmental neurotoxicity study in rats with azinphos-methyl (nominal concentrations of 3, 10, or 15 ppm). Dams will be sacrificed on gestation day 20, and fetuses will be removed from the uterus. Plasma, RBC, and brain cholinesterase activity will be measured for dams with litters and for fetuses (samples from fetuses in each litter will be pooled).

Cholinesterase measures following acute exposure to adult and immature rats

The protocols as described adequately address the collection of data on cholinesterase measures following acute exposure to adult and immature rats.

Cholinesterase measures following repeated dose exposures to adult and immature rats

GD 20 dams and fetuses - The protocols address cholinesterase measures in GD 20 dams and fetuses following maternal treatment from GD 0-20. This is considered to be an appropriate dosing period, since it is the same as the gestation dosing period used in the developmental neurotoxicity study (MRID 45711201).

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 information provided with each supplementary study protocol indicates that the developmental neurotoxicity study with azinphos-methyl had already been conducted, and that cholinesterase activity was measured for pups on PND 4 and PND 21 and for dams on lactation day 21. Such procedures are consistent with the current Agency guidance (10/29/01) which recommends the measurement of cholinesterase activity during the course of the DNT study, as a tool in assessing the adequacy of postnatal dosing. The adequacy of the cholinesterase activity data will be assessed when the study is evaluated by the Agency.

Conclusion

The protocols submitted by the registrant to assess cholinesterase activity in adult and immature rats following acute or repeated exposures of azinphos-methyl are considered 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).

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