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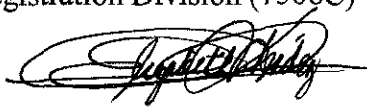
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MEMORANDUM

SUBJECT: D281185: Diazinon (PC Code 057801)
Review of Comparative Cholinesterase Study Protocols

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THRU: Developmental Neurotoxicology Protocol Review Committee
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Executive summary

Draft study descriptions for the time-to-peak effect and of cholinesterase activity assessments in adult and immature rats following acute or repeated exposures to diazinon were submitted by Makhteshim-Agan of North America Inc (MANA). In general, the study designs 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). However, several deficiencies have been identified including: 1) use of clinical signs for determination of the time-to-peak effect instead of cholinesterase inhibition data as requested by the Agency, 2) lack of

methodology description for assessing cholinesterase activity data, 3) cholinesterase activity only evaluated at termination, 4) lack of assessments of GD 20 dams and fetuses, 5) lack of correspondence in the evaluation time points between the acute and repeated dose study, and 6) use of 3 pups/sex/dose from each litter during the repeat dosing study.

Introduction

At the request of the Agency, the registrant, MANA, has submitted a general description of proposed study designs (dated February 4, 2002 and re-submitted on August 14, 2002) for studies that were designed to determine the time-to-peak effect as well as assess cholinesterase activity in adult and immature rats following acute or repeated exposures, for diazinon. The studies described in these submissions 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 | <u>Acute doses:</u> 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). |
| | <u>Repeated doses:</u> 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-to-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 study designs.

Proposed study design

The registrant proposes to conduct three independent studies to comply with the requirements specified in the Data Call-In issued on September 10, 1999. A time to peak effect study, an acute exposure comparative cholinesterase study, and a repeated-exposure comparative cholinesterase study. The study designs submitted to the Agency for review do not provide sufficient information to thoroughly evaluate the conduct of these studies. Nonetheless, based on the limited information provided, some deficiencies have been identified.

Cholinesterase measures following acute exposure to adult and immature rats

In the acute exposure comparative cholinesterase study design, pups will be acutely exposed (via gavage) to the test article at three different stages: pre-weanling (PND 11), weanling (PND 21), and 8-week old rats. In addition to daily clinical observations, pups will be subjected to in-cage, in-hand, and standardized arena assessments as well as body weight determinations, cholinesterase activity assessments, and gross necropsy evaluations. The Agency recommends that the registrant describe the age-appropriateness of the proposed neurobehavioral evaluations. In terms of cholinesterase activity determinations, the study design states that a "terminal cholinesterase" assessment will be conducted. The study design, however, does not specify the time when this evaluation will be conducted. Since the study design implies that some animals may not be sacrificed until PND 12 (Group A) or PND 22 (Group B), clarification of the timing of cholinesterase activity determination is required. The Agency reiterates that assessments of cholinesterase activity must be conducted at the time-of-peak effect (i.e. on the day of dosing). Consequently, PND 12 or 22 evaluations of animals exposed on PND 11 or 21 are not adequate.

Cholinesterase measures following repeated dose exposures to adult and immature rats

GD 20 dams and fetuses - The submitted study design does not indicate that these assessments will be conducted.

Immature rats versus young adults - Animals exposed for a total of 7 days (pre-weanlings: PND 11-17, inclusive; 8-week old rats: 7 consecutive days) will be subjected to daily clinical observations, body weight determinations (pre-weanlings: PND 1, 4, 7-17; 8-week old rats: twice/week during pre-dosing period and daily during treatment), in-hand and standardized arena assessments at the pre-determined time-to-peak effect on the last day of treatment, and gross necropsy. Cholinesterase assessments (plasma, erythrocyte, and brain), as proposed in the study design, would be conducted at the pre-determined time-to-peak effect. It is unclear, however, on what days these evaluations would be conducted. Since the study design implies that some animals may be sacrificed one day after cessation of dosing (i.e. day 18 for dams and PND 18 for pups), a clarification of the timing of cholinesterase activity determination is required. As noted previously in this review, cholinesterase activity must be assessed at the time-of peak-effect. Evaluations conducted one day after cessation of dosing are not adequate.

The Agency notes that, during the repeat dose study, the registrant intends to use 3 pups/sex/dose from each F₁ litter. This is not acceptable. The Agency recommends that a maximum of 2 pups/sex/dose be used from each litter. Clarification on pup allocation during the repeated cholinesterase study is required. Finally, the Agency notes the lack of correspondence in evaluation time points between the acute and repeated dose study. This would complicate the comparison of effects induced by acute versus repeated exposure to the test article. The Agency recommends that the evaluation time points in the repeated dose study are in concordance with those used during the acute study.

Cholinesterase measures in the main DNT study

A study design for the main DNT study, submitted by Novartis on March 23, 2000, has been 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

With the exception of a statement asserting that a “preliminary step-up, step-down acute study may be done to determine the non-lethal dose at PND11,” dose selection was not addressed in the submitted study design for any of the three age-groups that will be assessed in the studies. The registrant is requested to provide the Agency with a rationale for the doses that will be used in the comparative cholinesterase and time-to-peak effect studies for all three age groups evaluated.

The registrant has submitted a study design for a time-to-peak effect study at both PND 11 and PND 21 after a single exposure to the test article. In addition to the dose selection rationale, the registrant is requested to clarify the following aspects of the submitted protocol: 1) animal assignment to the test groups, and 2) methodology for cholinesterase activity assays. The Agency notes that the registrant intends to base their time-to-peak effect determination on clinical signs (indicative of cholinesterase inhibition) observations 6, 9, 12, 15, and 18 hours post-dosing. As previously stated in this review, the time-to-peak effect should be determined for each age group and should be based upon cholinesterase inhibition (as onset of clinical signs may not correspond to cholinesterase inhibition). While the determination of time-to-peak effect on PND 11 and PND 21 is consistent with EPA recommendations, the use of clinical signs to establish the time-to-peak effect is not acceptable and will not satisfy the Agency’s requirement.

Conclusion

In general, the study designs submitted by the registrant to assess cholinesterase activity in adult and immature rats following acute or repeated exposures 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). However, several deficiencies have been identified including: 1) use of clinical signs for determination of the time-to-peak effect instead of cholinesterase inhibition data as requested by the Agency, 2) lack of methodology description for assessing cholinesterase activity data, 3) cholinesterase activity only evaluated at termination, 4) lack of assessments of GD 20 dams and fetuses, 5) lack of correspondence in the evaluation time points between the acute and repeated dose study, and 6) use of 3 pups/sex/dose from each litter during the repeat dosing study.



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