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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 5, 2005

MEMORANDUM

SUBJECT: EPA Id No.: 097301. Formetanate: Review of Comparative Cholinesterase Study Protocols

TXR No.: 0052987
DP Barcode No.: D31111:
Submission No.: Not provided.
PC Code: 097301

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Conclusion

ReRegistration Branch III (RRB3) has reviewed a draft protocol for the assessment of cholinesterase activity in young adult and immature rats (post natal day 11) following acute exposures to formetanate submitted by the Registrant (Gowan Company, December 10, 2004) The protocol is in general considered appropriate for the assessment of comparative cholinesterase activity data due to the carbamate formetanate. The registrant, however, should

note the comments below.

Introduction

At the request of the Agency (refer to the HIARC report for formetanate, TXR # 0050931 dated July 22, 2002), the registrant, Gowan Company, has submitted draft protocols dated October 28, 2004 and a revised protocol dated December 10, 2004 for studies that were designed to assess cholinesterase activity in adult and immature rats following acute exposures to the carbamate insecticide formetanate. The studies described in this planned series of studies are intended to satisfy the requirement for comparative cholinesterase data for the special case of the carbamate insecticide formetanate and were based on but adapted from protocols designed for organophosphate insecticide inhibitors of cholinesterase.

This study with the carbamate formetanate differs from studies with organophosphate insecticides in that only single acute doses will be assessed and no multiple dosing will be done. This is considered justified since a carbamate is a rapid acting and more easily reversible inhibitor of AChE. Whereas an organophosphate insecticide forms a stronger covalent bond with the enzyme and is considered irreversible.

The Gowan Company previously met with representatives of HED (July 20, 2004) to discuss the protocol for this special study. The draft protocol indicates that the registrant has incorporated the comments made by HED at this meeting. The following briefly describes each phase of the study and includes comments.

I. <u>Proposed study design</u>

The overall study consists of three phases as follows:

Phase I. Dose range finding study.

Four groups of 5/sex 11-day old and 6-week old adult rats will be dosed by gavage with a single doses of vehicle control, dose -1, dose -2 and dose -3 formetanate hydrochloride and at a time predetermined based on existing studies to estimate the time to peak effect for inhibition, the rats will be sacrificed and assessed for RBC and brain acetylcholinesterase (AChE) and inhibition. The dose levels selected for this aspect of the study will be based on preexisting data.

Note: This Phase will determine the dose levels to be used in the subsequent Phase II and Phase III. It is considered necessary because an existing study using acute administration did not assess for reactions and AChE inhibition in 11-day old pups.

Phase II. Determination of Time to Peak Cholinesterase Inhibition.

Four groups of "30"/sex 11-day old and 6-week old adult rats will be dosed with single

dose of vehicle and a preselected dose of formetanate hydrochloride with the dose selected based on Phase I of this study. Following dosing, groups of "five"rats/sex/group will be euthanized and RBC and brain AChE assessed at 0.25, 0.5, 1, 3, 6 and 12 hours post-dose administration.

Note #1: The notes of the July 20, 2004 meeting with the Gowan Company state that the time course aspect of the study should include an assessment of the time it takes to *recover* from the inhibition especially to determine if the pups recover more slowly than the adults. It is assumed that this aspect of the study will provide sufficient data to compare the rate of recovery from inhibition for both the neonatal and adult rats. Ideally, recovery should be to 90% of the pre-dos level for AChE. Comparison of the two age groups is considered an important aspect of the time course Phase of the study because if the pups recover more slowly than the adults, there may be an indication of potential accumulation of the inhibited enzyme with repeated exposure and since no repeated dosing will be done, it is important to establish that there is no difference in the potential for accumulation of inhibited enzyme in pups and adults.

Phase III. Comparative Cholinesterase Inhibition Phase.

10 rats per sex/group will be dosed with vehicle, dose -1, dose -2 and dose -3 of formetanate based on the doses from the preliminary studies. At the time to peak effect noted in Phase II, the rats will be euthanized and assessed for RBC and brain AChE. This aspect of the study is designed to be the definitive test for comparison of the difference (if any) between the relative sensitivity of the pups to the adults to the cholinesterase inhibitory effects of formetanate. The protocol calls for 10 rats/sex per group to increase the statistical power of the study.

II. <u>Cholinesterase measures following acute exposure to adult and immature rats</u>

The protocol indicates that cholinesterase will be assessed using a modification of the original referenced (1961) Ellman's reaction but modified by the Hunter (1997).

Few details for blood and brain sampling, preparation for assay and techniques that will be used to minimize reversal of inhibited enzyme were described. Providing the citations for the Hunter and Ellman papers is not sufficient. The final study report should clearly state how blood and brain samples were extracted from the rats, kept from the time they are obtained from the animal to the time of assay. The times between sampling and assay should be the same for all samples unless there is supporting documentation that the storage or holding conditions wont reverse the inhibition.

III. **Other comments**

A. Clinical signs and responses to treatment. The protocol does not provide sufficient detail as to what reactions to treatment will be assessed for in the adults and pups. In particular, body

temperature decrease is one response to treatment that was included in the responses to treatment in a previous study (2000, MRID No.: 45255501, Clin. Trials BioResearch, Study No.: 97555, October 19) with formetanate at the LOAEL. Thus, body temperature assessment should be included in the assessment of reactions to treatment and the time to peak effect and recovery (i.e. especially in Phase II but also in the other Phases) of this effect. The time to onset and duration of tremors, salivation and impaired gait or other signs should also be reported.

B. Comments on terminology. The protocol describes this study as a "within-litter" study and also states that the "litter and not the neonate" is the experimental unit. Neither of these terms seem to be appropriate. The litter is the experimental unit in for conventional developmental toxicity studies when the dams are dosed and the fetuses delivered and the data are expressed as number of fetuses affected/number of litters affected. However, when the rat pups are randomized for this study they lose their litter identity and the mean AChE data is based on the number of pups that come from different litters. Thus, the term "within-litter" and the reference to the litter being the experimental unit do not seem appropriate and require clarification.

Conclusion

The protocols submitted by the registrant to assess cholinesterase activity in adult and immature rats following acute or repeated exposures are generally considered adequate but have several details lacking. These include additional specific details on how the enzyme activity will be assessed for to detect the labile inhibition by the carbamate (reference to the Hunter paper is not considered sufficient). Reactions to treatment that will be assessed for (i.e. body temperature, tremors, salivation, impaired gait etc) should be assessed for. The final report should contain the additional details for preparation of AChE assessment and detailed reporting of the reactions to treatment including time to onset and duration. The terminology regarding "within-litter" and statement that the litter and not the neonate is the experimental unit should be clarified.

It should be noted that although HED has commented on the protocol as submitted it is the Registrant's responsibility to provide a study that meets acceptable standards for Quality regarding housekeeping aspects, quality data with acceptable variance and report preparation. HED review of a protocol does not guarantee acceptance of the study.

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R105431

Chemical:	**Formetanate hydrochloride**
PC Code:	**097301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**01/05/2005**
File ID:	**TX0052987**
Accession Number:	**412-05-0093**

HED Records Reference Center
03/14/2005