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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

September 12, 2003
TXR # 0052088

MEMORANDUM

SUBJECT: D293004: Tetrachlorvinphos (PC Code 083701)
Protocol Review - Developmental Neurotoxicity Study

TO: Demson Fuller
Special Review and Reregistration Division (7508C)

FROM: Susan L. Makris, M.S. *Susan L Makris 9/12/03*
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THRU: Developmental Neurotoxicology Protocol Review Committee
Health Effects Division (7509C)

and

Alberto Protzel, Ph.D., Branch Senior Scientist
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Alberto Protzel 10/28/03

cc: Paul Chin, HED (7509C)
John Leahy, SRRD (7508C)

Executive Summary: The purpose of this memorandum is to provide comments on 1) a protocol for a developmental neurotoxicity (DNT) study in rats with tetrachlorvinphos by dietary exposure (MRID 46036000), and 2) dose selection rationale for the DNT study, based upon preliminary studies conducted with tetrachlorvinphos (MRID 46036001). The draft DNT protocol (Argus Research, Protocol 1608-003), dated July 18, 2003, was submitted to the Agency by SRA International, Inc. (1920 L Street, N.W., Suite 420, Washington, DC 20036) for the registrants Hartz Mountain Corporation and KMG Bernuth, Inc.

It is recommended that the DNT protocol be revised as necessary to address the detailed comments provided. Since preliminary test results suggest that dietary administration will not deliver an adequate or quantifiable dose to the offspring in the DNT study, it is recommended that tetrachlorvinphos be administered postnatally to the offspring by gavage in the DNT study, commencing as soon after birth as is feasible (i.e., between PND 1 to 4). The registrant does not

need to resubmit a revised protocol to the Agency prior to proceeding with the study.

Introduction

In addition to the draft protocol for the developmental neurotoxicity study, summaries of the results of three preliminary studies conducted with tetrachlorvinphos were submitted. These studies are described in Table 1. Selected descriptions of body weight gain data (percent reduction from control) and cholinesterase activity measures (percent difference from control) were provided, but actual data values were not included in the submission.

Table 1 Studies performed

Study No.	Study Description	Dose duration	Doses	ChE Evaluations
1608-001	Oral (gavage) maternal and fetal exposure study in rats	GD 6-21	0, 75, 150, or 300 m/k/d	Maternal and fetal plasma, RBC, brain - GD 21 - 2 hrs postdose
1608-002R	Oral (gavage) acute relative sensitivity study in neonatal and adult rats	Acute - PND 11, PND 21, PND 40	0, 75, 150, or 300 m/k/d	Plasma, RBC, brain - pre-, 1, 2, 3, 4, 8, 24 hr postdose
1608-003P	Oral (diet) perinatal and postnatal reproduction dosage-range toxicity study and preliminary pup exposure study in rats	Repeated GD 6 - LD/PND 21	0, 12.5, 94, 3750, or 5000 ppm (0, 1.0, 7.6, 283.8, and 372.4 m/k/d during gestation; 0, 2.2, 15.8, 684.8, and 881.9 m/k/d during lactation)	Pup plasma, RBC, brain - PND 4, PND 21

Dose selection rationale

Cholinesterase measures - Cholinesterase data were presented as percent change from control. The actual individual and mean values (with SDs) were not presented, making it impossible to adequately evaluate the normalcy and variation of the data. It is noted that the data contain a number of unusual findings and/or inconsistencies, which suggest poor methodologies. For example, the GD 21 results show an unexplained lack of dose response in maternal RBC cholinesterase inhibition. A lack of dose-response is also observed in the dose range finding study. The RBC ChEI for PND 4 female pups appears almost as if the low and high-dose group values were switched. Also, inexplicably, the level of apparent inhibition in the mid-high and high dose groups is sometimes nearly the same even though the dose levels are spaced widely enough to anticipate a dose response (from 3750 to 5000 ppm = 685 to 882 m/k/d during lactation) (see LD 21 maternal RBC values, PND 21 male and female pup plasma values).

Time to peak effect - Time to peak effect data (Appendix 1) are erratic. It is noted that a substantial decrease in cholinesterase activity as compared to control was observed even before dosing commenced in a number of instances (e.g., adult RBC at 75 m/k/d = -26%; PND 21 pup RBC at 150 m/k/d = -27%; PND 11 pup RBC at 300 m/k/d = -32%; PND 21 pup RBC at 300 m/k/d = -33%; adult RBC at 300 m/k/d = -26%; PND 11 and 21 pup brain ChE at 300 m/k/d = -

33%). In some cases the predosing decrease in activity was greater than any post-dosing decrease. Sometimes cholinesterase fluctuates over time (e.g., adult RBC ChEI is -40% at 4 hrs, -13% at 8 hrs, and -38% at 24 hrs). Also, in spite of the substantial increase in dose, no dose response is evident. The accuracy and reliability of these data in determining the time to peak effect is quite questionable.

Adequacy of dose to offspring - Gavage dosing of PND 11 and 21 pups with tetrachlorvinphos in the time-to-peak effect study was reported as demonstrating an apparent increase in response in pups as compared to adults in RBC ChEI at several doses. Overall, cholinesterase was inhibited for pups and adults in all 3 compartments (plasma, RBC, and brain) at all doses tested. At even greater dose levels in the dietary range-finding study (3750 ppm = 685 m/k/d; 5000 ppm = 882 m/k/d), even at PND 21, when pups should have been eating treated feed and receiving a substantial dose of test substance, the cholinesterase levels were unchanged for brain and RBC, and approximately 20% decreased from control for plasma. Additionally, the adult cholinesterase values were inhibited substantially more than pup values on LD/PND 21. These results suggest that dietary administration will not deliver an adequate or quantifiable dose to the offspring in the DNT study. It is therefore recommended that tetrachlorvinphos be administered postnatally to the offspring by gavage in the DNT study, commencing as soon after birth as is feasible (i.e., between PND 1 to 4).

Developmental Neurotoxicity Study Protocol (Argus Research Protocol 1608-003)

Contrary to a claim included in the submission, a DNT protocol for tetrachlorvinphos has not been “previously submitted to and approved by the EPA.”

This protocol specifies that the study will be conducted in the following manner: 25 pregnant female Sprague-Dawley rats will be assigned to each of three treatment groups and a concurrent control group. Dams will be treated with tetrachlorvinphos in the diet from gestation day 6 through lactation day 22 (with the day of delivery designated as lactation day 1). The proposed dietary dose levels will be 0, 12.5, 3750, or 5000 ppm.

Maternal animals will be examined daily for viability; observations on maternal behavior will be recorded on days 1, 5, 8, 14, and 22 postpartum; and a battery of functional observations will be conducted daily (outside of the home cage and by an observer that is unaware of treatment group) beginning on gestation day 6. Maternal body weights and food consumption will be recorded daily from gestation day 0; however, food consumption will not be tabulated after postpartum day 14. The dams will be allowed to deliver; the duration of gestation and any adverse clinical signs during parturition will be recorded. Dams will be sacrificed on lactation day 22, and a gross necropsy will be performed.

Litter size, pup viability, and clinical observations will be recorded daily throughout lactation. Pup body weights will be recorded on postnatal days 1, 5, 8, 12, 18, and 22. A battery of functional observations will be conducted (outside of the home cage and by an observer that is unaware of treatment group) for 1 pup/sex/litter on postnatal days 5, 12, 22, 36, 46, and 61. On lactation day 5, litters will be standardized to 8 pups/litter. Post-weaning offspring clinical observations, body weight, and food consumption data will be recorded weekly. The age of

preputial separation or vaginal patency will be recorded.

Motor activity testing (on PND 14, 18, 22, and 61±2) and auditory startle habituation (on PND 23 and 61±2) will be examined for 1 pup/sex/litter. Learning and memory testing will be conducted with a separate set of offspring (1 pup/sex/litter); passive avoidance testing will be performed on PND 24±1 and 31±2, while performance in a water maze will be evaluated on PND 61±2 and 68±2.

At PND 22 and at study termination (approximately PND 70), cohorts of 10 pups/sex/group will be killed by *in situ* perfusion under anesthesia, and necropsies will be performed. The fixed brains of these offspring will be weighed, sectioned, stained, and examined microscopically without knowledge of treatment group. Linear morphometric measurements will be taken on each brain (nine measures for PND 22 brains and eight for PND 70 brains). Peripheral nervous system tissues will be examined microscopically for the adult offspring. All histopathology will be performed on control and high-dose specimens first; if a treatment-related adverse effect is observed, then similar evaluations will be performed for the low- and intermediate-dose groups.

Agency comments on Protocol 1608-003

Since preliminary test results suggest that dietary administration will not deliver an adequate or quantifiable dose to the offspring in the DNT study, it is recommended that tetrachlorvinphos be administered postnatally to the offspring by gavage in the DNT study, commencing as soon after birth as is feasible (i.e., between PND 1 to 4).

The description of pup assignment to various evaluations on protocol pages 11-12 does not indicate selection of pups for a functional observation battery. Instead, clinical observations are described in the protocol on pages 13-14 for dams and pages 15-16 for pups. As described, these observations are not as extensive as those required by the guideline (OPPTS 870.6300) and should be revised to adhere to guideline specifications. The protocol does not specify that trained observers or standardized procedures (including ranking of response) will be used, and not all endpoints included in the guideline are listed in the protocol (e.g., pupillary function is not included).

Food consumption values should be tabulated following postpartum day 14 (page 14 of the protocol states that they will be recorded daily throughout the lactation period). Information on litter food consumption in late lactation, even though imprecise due to consumption of varying amounts of feed by pups and due to spillage as pups become more active, can sometimes be useful in the interpretation of toxicity information. This is particularly important in a study that uses the diet as the route of test substance administration.

Appropriate, adequate positive control data from the laboratories that perform the DNT studies should be provided to the Agency at the time of study submission. These positive control data should demonstrate the sensitivity of the procedures used, including the ability to detect both increases and decreases in measured parameters, as appropriate. In the submitted protocol (page 17), positive control data are mentioned only with respect to motor activity testing, where data relating to increases, but not decreases, in activity are promised; data on decreases should also be

obtained. Positive control data are not mentioned for any other tests that will be performed (e.g., FOB, auditory startle, passive avoidance, water maze, neuropathology), nor does the description of the final report (page 27) include mention of positive control data. While the positive control studies do not need to be performed using prenatal exposures, the laboratory must demonstrate competence in the evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for all critical endpoints, and for appropriate age groups. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies.

Auditory startle testing should include measurements of both amplitude and latency. The description of testing on page 17 suggests that latency will not be recorded or reported.

The *latency (seconds) for trial 1 on the first day of testing* should be added to the list of dependent measures that will be compared between control and treated groups for water maze testing (p 19). Individual data from all of the trials should be included in the study report for both latency and errors.

Conclusions and recommendations

It is recommended that the DNT protocol be revised as necessary to address the detailed comments provided above. Since preliminary test results suggest that dietary administration will not deliver an adequate or quantifiable dose to the offspring in the DNT study, it is recommended that tetrachlorvinphos be administered postnatally to the offspring by gavage in the DNT study, commencing as soon after birth as is feasible (i.e., between PND 1 to 4). The registrant does not need to resubmit a revised protocol to the Agency prior to proceeding with the study.



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