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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

PC 113961

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 4, 2003
TXR No. 0051604

MEMORANDUM

SUBJECT: D287620: Prothioconazole (JAU 6476/Metabolite SXX 0665) (PC Code Not Assigned [presubmission chemical])
Developmental Neurotoxicity Study Protocol

TO: Terri Stowe
Registration Division

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THRU: Stephen Dapson Stephen C. Dapson 03/5/2003
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cc: Paula Deschamp, Branch Chief, RAB3

ACTION REQUESTED: Review submitted developmental neurotoxicity study protocol for SXX 0665 (plant metabolite of prothioconazole [JAU 6476]) .

Introduction

BayerCropScience, Research Triangle Park, NC, has submitted a developmental neurotoxicity study protocol, dated December 18, 2002, for: SXX 0665 (plant metabolite of JAU 6476 /Prothioconazole), PC Code unavailable.

Bayer is proposing to perform a DNT study using the major plant metabolite of JAU 6476, SXX 0665, based on the following rationale:

> The parent compound, JAU 6476 (prothioconazole) rapidly converts to the primary metabolite, desthio-JAU 6476, otherwise known as SXX 0665. The extensive toxicology database on SXX 0665 shows it to produce lower toxicity endpoints, i.e. lower NOELs,

than the parent. Therefore, the rationale for utilizing SXX 0665 as the test agent in the DNT study is two-fold: (1) it better represents the practical dietary exposure, and (2) it will represent a more conservative (worse-case) exposure scenario.

The following discussion presents the Agency response to the contents of the protocol.

Adequacy of the main study design

Dosing period

The study design includes dietary administration of SXX 0665, from gestation day (GD) 0 through lactation/postnatal day (PND) 21.

This is considered to be an acceptable dosing duration that meets the current recommendations of the Health Effects Division (HED).

Adequacy of postnatal dosing

As noted above, the protocol specifies administration of SXX 0665 to the dams via the diet. In a developmental neurotoxicity study, pup exposure to test substance during the lactation period may occur via three pathways: 1) maternal transfer via milk; 2) consumption of treated diet by pups; and/or 3) direct dosing of pups.

The most reliable means to define postnatal exposure is to directly gavage the pup for some or all of early life (Chapin et al., 1997; Beyrouty et al., 2001; Moser et al., 2001). Direct gavage dosing of pups can be initiated as early as PND 1 or as late as PND 11; initiation between PND 4 and 7 is preferred. Initiation of gavage dosing in older pups may result in a relatively long period of minimal exposure for the first week or more of lactation. Without direct dosing, there is concern about the extent to which continuing to dose the dams during lactation provides exposure to the offspring during this dynamic phase of neurological development.

In studies where the dosage to the dam is by gavage, and during dietary studies prior to the onset of diet consumption by pups, postnatal exposure to pups depends exclusively on exposure through the milk. Available data indicate that exposure via milk is variable, both among compounds and across time for a given compound (Dorman et al., 2001).

In dietary studies, postnatal exposure to pups may include some ingestion of test substance by pups during late lactation, but limited data available to EPA suggest that this may only happen between days 18-21 (Gerrish et al., 1998; Hanley & Watanabe, 1985). Further, the levels of enzymes that may activate (e.g., Basu et al., 1971; Atterberry et al., 1997; Moser et al., 1998) and metabolize pesticides change during the lactation period. The changing nature of pup exposure and metabolism during this critical period means that adequacy of dosing for pups and the impact of that dosing, e.g., measurement of milk content of test substance, measurement of a biomarker (such as ChE inhibition for organophosphates), and clinical signs (such as decreased body weight) must be assessed repeatedly during the pre-weaning period. If assessment of a

biomarker is used to support adequacy of dosing, at least two, and preferably three adequately spaced measures during lactation are recommended.

Preliminary studies to evaluate the adequacy of postnatal dosing (e.g., milk concentrations of the test substance, along with milk consumption and food consumption data in pups) may be used as a means to estimate the extent of exposure during lactation. Evaluation of traditional toxicity measures in the offspring (e.g., survival, body weight, and clinical signs) may also be used in preliminary studies, or alternatively in the main DNT study to define the adequacy of postnatal dosing. In the absence of signs of toxicity in the pups during one or more critical phases of neurological development, the adequacy of the developmental neurotoxicity study may be called into question.

Number of offspring examined for neuropathology

The protocol states that 10 animals/sex/dose will be allocated for neuropathological assessment, following sacrifice on PNDs 21 and 50-60. The number and approximate evaluation dates of animals are consistent with current HED recommendations. However, collection of adult tissue samples over a period of 10 days may lead to increased variability in the data. It is recommended that adult tissues be collected within a 5 day period, and that day of sacrifice be balanced with respect to dose group.

Sample collection and processing

For the purpose of the current study, the collection of nervous tissue on PND 11 *or* on PND 22, and at approximately PND 60 will be acceptable. Some general issues have been raised regarding the methods used to evaluate developmental processes as they are taking place; these issues have not yet been resolved. The current EPA guideline calls for collection of nervous tissue on PND 11, based, in part on the desire to assess toxicant impact on early developmental processes, and in part, on dosing that ended on PND 10. Since this protocol includes dosing through postnatal day 21, data collected on PND 21 will assess the full impact of this extended dosing on a longer period of neurological development, although at a later stage. EPA would be very interested in seeing data that addressed both PND 11 and PND 22 in order to assess the relative sensitivity at these two time points.

It is expected that all histopathological procedures be validated for those days chosen. Nervous tissue collected on PND 11 can be immersion fixed; however nervous tissue from PND 21 or PND 60 rats should be perfusion fixed. The submitted protocol specifies perfusion fixation at both time points.

Histopathological evaluation

The submitted protocol includes processing of tissues from all animals for histopathological evaluation, through slide preparation.

Like other guidelines, the developmental neurotoxicity guideline allows a sequential histopathological analysis of tissues (first high dose and control groups, then sequential

examination of lower dose groups if a treatment related effect is observed at the high dose, until a no-effect-level is found). In the submitted protocol, tissues from high dose and control animals will be evaluated qualitatively and morphometric measurements will be made for those groups. Agency experience suggests that it may be preferable to evaluate all treated groups at the same time, to allow for interpretation of neuropathology data in the context of the contemporaneous analysis of all the data from the other groups. It also avoids delays in study reviews that could result if questions arise about the need for lower dose evaluation.

The number of brain regions examined morphometrically need not be restricted to those specified in the guideline as a minimum set, i.e., neocortex, hippocampus, and cerebellum. The submitted protocol includes morphometric evaluations of frontal cortex, parietal cortex, caudate putamen and globus pallidus, corpus callosum, hippocampal gyrus, and cerebellum. Behavioral findings or qualitative histopathological findings may also guide the selection of additional brain regions for morphometric assessment. For parietal cortex and caudate putamen and globus pallidus, it is unclear whether unilateral or bilateral measurements will be taken; for hippocampus, the protocol states that bilateral measurements will be made, and the mean will be recorded. We recommend that bilateral measurements be made for each of these tissues, and that both measures be reported separately.

Assignment of pups to testing

Animal allocation is described in the submitted protocol on pp. 8-9. The proposed allocation plan appears acceptable.

It is important to ensure that the method of animal assignment minimize potential problems related to litter effects, i.e., by using one pup/sex/litter (or one measure/litter, e.g., mean body weight for each litter) and that the statistical analyses consider the impact of multiple measures and repeated measures, as well as litter effects. Additional information is provided by the following references: Cox, 1994; Holson and Pearce, 1992; and Tamura and Buelke-Sam, 1992.

When allocating animals to FOB and motor activity testing, the same individual animals should be evaluated at all scheduled time points. The submitted protocol will accomplish this by assigning the same animals to motor activity testing for all four time points (set A); a different set of animals (Set C) is assigned to detailed observational battery testing for all time points.

When selecting animals and testing paradigms for cognitive (learning and memory) assessment, it is important to select tasks and/or allocate animals so that comparable assessments of learning may be made at both times, i.e., shortly after PND 21 and around PND 60. In general, the use of separate animals at the two time points is preferred, because for many tasks initial learning may confound later (PND 60) assessment of learning. In the submitted protocol, the same set of animals (set C) is used for both time points, but different procedures are used (passive avoidance on PND 22/29, water maze on PND 60/67±2). This is acceptable.

Behavioral evaluations

Auditory startle reflex testing should include measurements of both amplitude and latency and

should demonstrate habituation. The submitted protocol states that both response amplitude and latency to peak will be recorded, but only that response amplitude will be reported. Both measures should be included in the study report.

Additional comments

BayerCropScience has proposed conducting the DNT study using SXX 0665, the major plant metabolite of JAU 6476 (prothioconazole). Based on the available information (mostly registrant-generated summaries of toxicity and chemistry studies), we find it reasonable to conduct a DNT study using the major plant metabolite of JAU 6476, SXX 0665. This conclusion must be caveated, as follows:

1) It is based entirely on presentations provided by registrants, since no data have been submitted. It is possible we might interpret the data differently once it is submitted. This applies to both the toxicity data and the chemistry (residue) data.

2) If residue profiles indicate high exposure to parent, additional toxicology data on JAU 6476 itself may be needed.

3) Available toxicity data for JAU 6476 were obtained using gavage studies; SXX 0665 studies used dietary administration. Thus, results may not be strictly comparable. In addition, no neurotoxicity studies were available for SXX 0665, thus those comparisons could not be made. BayerCropScience noted deficiencies in SXX 0665 studies; it is unknown whether these deficiencies might affect our evaluation of study results.

Positive control data

Appropriate, adequate positive control data from the laboratory that performs the DNT study 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. 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.

Dose levels

The doses selected for use in the study (0, 40, 160, and 400 ppm) appear reasonable based on submitted summaries of findings in the reproduction study. However, we note that the reproduction study (on which the dose selection is based) was conducted with Sprague-Dawley rats, while the DNT will be conducted with Wistar rats. It is possible that there may be some differences in response across strains, making dose selection less reliable.

The final doses selected, and the rationale upon which these selections were based, should be included in the study report.

Electronic submission of reports and data

As part of a voluntary pilot program intended to develop greater efficiency in study submission and review processes, Registrants are invited to submit developmental neurotoxicity study reports to the Agency in electronic format. **Attachment 1** contains information on electronic submission of reports and data.

References

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R091100

Chemical:	3H-1,2,4-Triazole-3-thione, 2-[2-(1-chlo
PC Code:	**113961**
HED File Code	**13000 Tox Reviews**
Memo Date:	03/04/2003
File ID:	TX005160
Accession Number:	412-04-0139

HED Records Reference Center
02/23/2004