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DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity Study in rats (EPA Guideline 81-8)

EPA ID NOs:
MRID No.: 444966-40
Pesticide Chemical Code: 129112
Toxicology Chemical Code: N/A
DP Barcode: D244009
Submission No.: S538790
CAS Reg. No.: 14517-21-7

TEST MATERIAL: CGA 279202 Technical, Trifloxystrobin, Batch P.405009, Purity 96.4%

CITATION: Classen, W. 1997. Acute Oral Neurotoxicity Study in Rats. Toxicology/Experimental Toxicology Laboratory, Novartis Crop Protection, AG, 4332 Stein, Switzerland, Study No. 973005 (Novartis Nexus Number 752-97), December 2, 1997, MRID No. 444966-40, Unpublished.

SPONSOR: Novartis Crop Protection, Inc., Greensboro, NC 27419

EXECUTIVE SUMMARY:

Trifloxystrobin (purity 96.4%, Batch No. P.405009) was administered to Tif: RAIf Sprague-Dawley rats (10/sex/dose) by gavage, at single doses of 0 or 2000 mg/kg/day. Body weights and food consumption were recorded at pretest, day 1, and twice weekly thereafter. Clinical signs were recorded daily. Neurobehavioral assessment (functional observation battery and motor activity) was performed pretest, day one at time of peak effect (approximately 6 hours post-dosing), and on days 8 and 15. At study termination, all surviving animals were sacrificed by in situ perfusion; histopathological examination of nervous system tissue was conducted on 5 animals/sex/group.

One male from the treated group was sacrificed in extremis on day 2 of the study. This death was not considered treatment-related.

No treatment-related effects were seen on body weight, weight gain, food consumption, clinical signs, functional observation battery performance or histopathological examination, or on motor-activity testing in males. For treated females, there was a slight decrease in several measured motor-activity parameters. Interpretation of

this decrease was confounded by study deficiencies: data from only 6 females/sex/group were reported for this time period, and no positive control data were submitted.

Due to study deficiencies, a LOEL and NOEL could not be determined for this study.

This study is classified as UNACCEPTABLE (GUIDELINE) and does not satisfy the requirement for a series 81-7 acute neurotoxicity study in rats. The study may be upgradable upon submission of requested information (additional procedural information for FOB and motor activity testing, analytical data presented as concentration of active ingredient, the referenced range-finding study, and further information regarding missing motor activity data for females). However, the lack of a NOEL for decreased motor activity in females may preclude upgrading the study.

COMPLIANCES: GLP Certification (p. 7), Quality Assurance Statement (p. 10), Data Confidentiality Statement (p. 2), and flagging statement (p. 4) were provided.

Review

The CALEPA review dated 6/8/98 is attached and TB-II recommends that this document be used in place of a DER prepared by HED staff or its contractors. The following additional information is being provided to bring the CALEPA review closer to HED's DER requirements and to identify several issues not addressed in the CALEPA review. Those issues and topics not addressed here were adequately described in the attached CALEPA review. The above Executive Summary, prepared by TB-II staff, should be used for regulatory purposes.

I. METHODS

A. Study Design: A range-finding study used in support of the chosen time of peak effect was briefly described in the study report, but was not submitted. This data is needed in order to verify assertions made concerning the time of peak effect used in this study.

The lack of this information was noted by the CALEPA reviewer, but the information was not requested.

B. Neurobehavioral Assessment:

1. Functional Observational Battery:

Procedural information provided regarding conduct of the FOB was incomplete. Although scoring criteria were provided, there was no information regarding whether or not the same observer was used for different test time points, or description of the procedures used for the various interventions (e.g. length of time for open field observations, manipulations used in assessing sensorimotor function). The protocol used for FOB testing should be provided.

This information was not requested by the CALEPA reviewer.

2. Locomotor activity:

Information was not provided about calibration of the motor activity equipment, time of performance of motor activity testing with respect to FOB testing (Procedure states that testing was performed between 8 A.M. and 3 P.M., after the FOB, but also states that males and females were tested on separate days [see page 24 of study report]; however, according to the report males and females were dosed on the same day [only one date, May 26, 1997, was listed for test substance administration and only one test solution was analyzed for substance concentration]). In addition, it is not clear what types of movements are included in the various types of activity counts (for example, what is the difference between vertical movements counts and rearing counts?). This information should be provided.

Motor activity was not recorded for 8 females (4 control and 4 treated) during peak effect testing on day 1. The report states that data for these animals was not recorded due to a `technical defect' (not otherwise described). Since these represent almost half of the animals in these groups, it is difficult to interpret the motor activity findings for this group (see below).

These issues were not raised by the CALEPA reviewer.

C. Positive Controls:

No positive control data were provided. This must be submitted before the study can be accepted, especially in view of the complete lack of findings in the FOB in the current study. This information was also requested by the CALEPA reviewer.

II. RESULTS

A. Analytical Chemistry: Homogeneity of the test solution was assumed (not tested), due to continuous stirring. Thus, there is no documentation that the solution was in fact homogeneous. In addition, complete analytical data were not submitted; results were submitted only as percent of target, instead of actual concentration of test substance in vehicle. Complete analytical data should be submitted.

This issue was not raised by the CALEPA reviewer.

B. Neurobehavioral results

1. FOB Findings:

All animals received a score of 0 for all measures at all time points for these evaluations, raising questions about the sensitivity of the method used. This issue cannot be resolved in the absence of positive control data.

This issue was not raised by the CALEPA reviewer.

2. Motor activity:

There were decreases in motor activity on day 1, for females only, in several of the motor activity parameters measured (depending on the statistical test used, total distance, movement time, vertical time, and center time, $p<.05$ [unadjusted]). These effects were seen in spite of the missing values for almost half the animals in these groups (see above). The missing data complicates the interpretation of these differences, since the presence of additional animals with similar tendencies (toward decreased activity on these measures) could have led to increased levels of significance for these measures. For treated males on day 1, there was an apparent increase in variance of motor activity (for most parameters, standard deviations were almost as large as the mean

for treated males, whereas they were close to 50% of the mean for controls).

Table 1. Motor activity in females, day 1 (time of peak effect).

Measured Parameter	Control	Trifloxystrobin	Percent Decrease
Total distance	2841±805	1728±890	39%
Movement time	219±52	135±73	38%
Vertical time	296±159	188±122	36%
Center time	139±95	34±31	75%

n=6; at pretest, percent difference (treated vs. control) was -7% for total distance, -13% for movement time, +19% for vertical time, and -13% for center time.

In addition, the graphs depicting motor activity for females include incorrect values for control animals (e.g., see graphs on p. 40, vs. mean values on p. 94, 95 of report).

In view of the missing data and significant decreases in activity seen in females on several motor activity parameters, we consider decreased motor activity in females to be a treatment-related effect. This conclusion is supported by the reduced activity seen at a higher dose (3500 mg/kg; reduction seen for up to 24 hours post-dosing) in the range-finding study [see summary of the range-finding study results, p. 18].

These problems were not noted in the CALEPA review.

III. DISCUSSION and CONCLUSIONS:

The study author asserts that no adverse effects associated with test substance administration were noted in this study. Although significant decreases were seen in motor activity of females at the time of peak effect, there was no comparable finding in males, and the study authors felt the decrease in females was not compound-related. We believe that the absence of data from 40% of the female subjects for this time point makes this finding difficult to interpret, and we consider this finding to be a treatment-related effect, in females only.

No other test-substance associated effects were found in this study. However, in the absence of positive control data, we are unable to evaluate the sensitivity of the test method (as noted above).

We agree with the CALEPA reviewer that the current study is unacceptable, and that positive control data needs to be submitted. In addition, the following information should be submitted:

1) Additional procedural information for the FOB and motor activity

testing (see above for details).

2) Analytical data presented as concentration of active ingredient, instead of percent of target concentration.

3) Further explanation of the missing data on female motor activity at the time of peak effect (day 1).

4) The referenced range-finding study (Acute Oral Rangefinding Neurotoxicity Study in Rats; test no. 973004 [Study report p. 33]).

Upon receipt of the requested information, the study classification will be re-evaluated; however, the study may not be upgradable, due to lack of a NOEL in females for decreased motor activity.

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