EPA Reviewer: Irving Mauer, Ph.D. Date
Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: Ching-Hung Hsu, Ph.D. Date
Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Cytotoxicity in rat hepatocyte cultures, and effects on mitochondrial Function (non-guideline studies).

DP BARCODE:D243979 SUBMISSION CODE:S538757
P.C. CODE:129112 TOX. CHEM. NO.:N/A
MRID NO: 44496720

TEST MATERIAL (PURITY): Trifloxystrobin (99%), and CGA-321113 (carboxylic acid metabolite, 99%)

SYNONYMS: CGA-279202; (none for CGA-321113)

CITATION: Bouis, P. (1997). Cytotoxicity in Primary Cultured Rat Hepatocytes and Effects on Mitochondrial Function of Rat Liver, conducted at Novartis, Basel (Switzerland), Laboratory Report. CB 97/59 (Novartis Nexus No. 754-97) dated December 17, 1997. MRID 44496720. Unpublished.

SPONSOR: Novartis Crop Protection, Inc. (Formerly CIBA-GEIGY Ltd.), Greensboro, NC

EXECUTIVE SUMMARY: Cytotoxicities of triflxystrobin (CGA-279202, 99.9%) and its carboxylic acid metabolite, CGA-321113 (99%) were compared by exposing rat hepatocytes isolated from a young adult male Crl:(WI)Br(Wistar) rat (BW = 183g) to test articles dissolved in dimethylsulfoxide, and (1) monitoring the morphology of hepatocyte monolayer cultures after 1, 4 and 24 hours exposure, as well as (2) determining the release of lactic dehydrogenase (LDH, a biochemical measure of toxicity) spectrophotometrically after 4 and 24 hour exposures. A second young adult male (Tif:RAIF(SPF) rat (BW = 220g) was starved overnight (to deplete hepatic glycogen and fatty acids), and the mitochondrial fraction of its liver and oxygen consumption (both ADP stimulated state-3 and resting state-4 respiration) measured before and after exposure to test articles; the corresponding respiratory control ratio (RCR) and P/0 ratio were calculated as well as IC_{50} values. The protein content of the mitochondrial fractions was measured by the conventional BCA assay method adopted for microliter plates.

Trifloxystrobin was visually toxic after 1 hour exposure, and lethal (no live cells) after 4 and 24 hour exposure to 30 and 100 uM, whereas the acid metabolite, CGA-321113 could survive a concentration 20 times higher, 600 uM (with moderate effects) with no visible cellular effects below that. LDH leakage paralleled the morphological findings, with elevations at concentrations producing moderate to severe visual alterations or cell death.

In the presence of a suitable metabolic substrate (succinate), the addition of ADP is expected to increase oxygen consumption in mitochondrial preparations (termed "state-3 respiration") at least two-fold above the resting state (called basal, or "state-4 respiration"), when the added ADP is converted to ATP. Addition of trifloxystrobin at concentrations ranging from 10 to 600 nM to succinate-supplemented mitochondrial fractions resulted in a dose-related inhibition of state-4 and -3 respiration (from 14-16% to over 90%), and calculated IC_{50} values of 154 and 68 nM respectively. In contrast, the acid metabolite, CGA-321113, did not inhibit mitochondrial respiration at concentrations up to 30,000 nM. Therefore, inhibition of mitochondrial respiration may be considered a potentially significant mechanism of trifloxystrobin toxicity.

This study is classified as Acceptable-Non-Guideline.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided, but no Quality Assurance or Flagging statements.

NB: This EPA-generation summary agrees in major respects with the attached CALEPA 6/4/98 conclusions.

TRIFLO.IM/LCA

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TRIFLOXYSTROBIN

CYTOTOXICITY

SignOff Date:	8/3/99
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HED DOC Number:	013599
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