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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

JAN 17 1997

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: HED Risk Assessment for Use of New Chemical Thiazopyr  
(129100) in/on the RACs, orange and grapefruit  
(Petition No. 3F4187; 707-ELN, 707-ELR). PRAT Case #  
284389 & 008262. DP Barcode D224858 & D226643.

FROM: Steve Robbins, Chemical Manager *SR 1/15/97*  
Registration Section  
Risk Characterization and Analysis Branch  
Health Effects Division (7509C)

THROUGH: Michael Metzger, Acting Chief *Michael Metzger*  
Risk Characterization and Analysis Branch  
Health Effects Division (7509C)

and

*for* Margaret J. Stasikowski, Director *Stephane K. V. 1/17/97*  
Health Effects Division (7509C)

TO: Joanne Miller, Product Manager Team 23  
Fungicide-Herbicide Branch  
Registration Division (7505C)

As requested, the Health Effects Division (HED) has completed a risk assessment for the use of the new chemical thiazopyr in/on the raw agricultural commodities (RACs) orange and grapefruit. The hazard assessment is from Pamela Hurley in Toxicology Branch I, the dietary exposure is from Jerry Stokes in Chemistry Branch I/Tolerance Support and the dietary risk evaluation is from Brian Steinwand in the Dietary Risk Evaluation Section. All the studies described below have been found acceptable except as noted.

**Executive Summary**

The Health Effects Division (HED) has reviewed toxicology and residue chemistry data submitted by the registrant in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and 40 CFR § 158 to support the use of thiazopyr in/on the RACs, orange and grapefruit. The toxicology data requirements for a food-use registration have been

satisfied. The residue chemistry data requirements remain outstanding. HED has determined that thiazopyr is a Group C (possible human carcinogen) with an RfD of 0.008 mg/kg/day. Due to the toxicity endpoints and the use pattern (orange and grapefruit) the only risk assessment required was chronic dietary. The aggregate risk, through food and water, was determined to be 4.2% of the RfD for the general U.S. population and 13.5% of the RfD for children 1 to 6 years old (the subgroup with the highest exposure). The chronic dietary risk exposure to thiazopyr appears to be minimal for this petition on oranges and grapefruits with a tolerance level of 0.05 ppm. **Therefore, HED does not consider the risk of registering thiazopyr for use on oranges and grapefruits to exceed the level of concern.**

## I. BACKGROUND

Rohm and Haas Company has submitted a request to register thiazopyr for use in/on the RACs orange and grapefruit groves. Thiazopyr will be used as a selective herbicide for the pre-emergence control of annual grasses and broadleaf weeds in orange and grapefruit. For this food use Rohm and Haas Company has requested the establishment of permanent tolerances for residues of thiazopyr (ISO common name) [3-pyridine carboxylic acid, 2-(difluoromethyl)-5-(4,5-dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-, methyl ester] and its metabolites determined as 3-pyridine carboxylic acid, 5-(aminocarbonyl)-2-(difluoromethyl)-4-(2-methylpropyl)-6-trifluoromethyl-, methyl ester and 3-pyridine carboxylic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-5-(((2-sulfoethyl)amino)carbonyl)-6-trifluoromethyl and expressed as parent equivalents in/on orange and grapefruit at 0.05 ppm.

The Health Effects Division has evaluated the data submitted to support registration of thiazopyr. A summary of the findings and an assessment of human risk resulting from the proposed use of thiazopyr are provided in this document.

## II. USE PATTERN

Thiazopyr is a herbicide active ingredient which gives pre-emergence control of annual grasses and broadleaf weeds. When thiazopyr is applied to the soil, it suppresses the development of germinating weed seeds. The mode of action of thiazopyr is disruption of spindle microtubule formation resulting in inhibition of cell division and accumulation of mitotic cells in late prometaphase. Thiazopyr will be applied by direct spraying to the bare ground to be present for action against germinating weed seeds for both orange and grapefruit.

In oranges and grapefruits, the proposed use is pre-emergence weed control under the trees and in the middles between tree rows. The product will be used either as a single application or as two or three separate sequential applications at a total maximum use rate of 2 pounds of thiazopyr per acre per year.

### III. PRODUCT CHEMISTRY

Common Name: thiazopyr

Code Number: MON 13200

Chemical Name: 3-pyridinecarboxylic acid, 2-(difluoromethyl)-5-(4,5-dihydro-2-thiazolyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-, methyl ester.

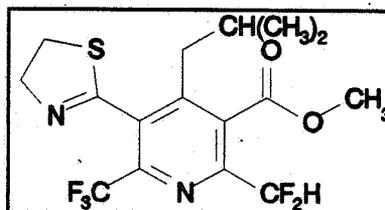
PC Code: 129100

CAS Registry Number: 117718-60-2

Chemical Family: pyridine

Molecular Formula:  $C_{16}H_{17}O_2N_2SF_5$

Chemical Structure:



Molecular Weight: 396.4

Physical State: Solid

Melting Point: 77.3 - 79.1°C

Solubility in Water: 2.3 ppm (pure active)

Octanol/Water ( $K_{ow}$ ): 7729 at room temp.

#### IV. HAZARD ASSESSMENT

##### A. Acute Toxicity

Table 1: Acute Toxicity

Acute Toxicity Studies (81-1 - 81-6)			
Guideline #	Study	MRID	Results
81-1	Acute oral (rat)	42275517	LD <sub>50</sub> : > 5 g/kg (both sexes). TOX. CAT. IV
81-2	Acute dermal (rabbit)	42275519	LD <sub>50</sub> : > 5000 mg/kg (both sexes). TOX. CAT. IV
81-3	Acute inhalation (rat)	42275521	LC <sub>50</sub> : > 1.2 mg/l (both sexes) (4-hour exposure). TOX. CAT. III
81-4	Primary eye irritation (rabbit)	42275523	Mild irritant TOX. CAT. III
81-5	Primary dermal irritation (rabbit)	42275525	Non-irritating TOX. CAT. IV
81-6	Dermal sensitization (guinea pig)	42275527	Not a sensitizer under conditions of study (Buehler).

##### B. Subchronic Toxicity

In a 90-day feeding study in rats (MRID#s 42275530 and 42619722), Sprague Dawley rats were fed thiazopyr in the diet for 3 months at 0, 1, 10, 100, 1000 or 3000 ppm (0, 0.07, 0.67, 6.60, 68 or 201 mg/kg/day in males and 0, 0.08, 0.79, 8.0, 79 or 227 mg/kg/day in females, respectively.) In the original Data Evaluation Record, the NOEL was set at 6.6 mg/kg/day (100 ppm), based on increased liver, thyroid and kidney weights, changes in clinical chemistry and hematological parameters and on gross and microscopic changes observed in the liver and thyroid, all observed at dose levels of 1000 ppm and higher. For the amendment, all male thyroid glands were re-examined microscopically. Based on the results of the re-examination, the NOEL and LOEL remain the same. Diffuse thyroid follicular cell hypertrophy/hyperplasia was observed in males in the 3000 ppm dose group and possibly the 1000 ppm dose group.

In a subchronic feeding study in dogs (MRID# 42275529), thiazopyr was tested at the following dose levels: 0, 10, 100, 1000 or 5000 ppm (0, 3, 6, 35 or 175 mg/kg/day for males and 0, 2, 3, 35 or 160 mg/kg/day for females, respectively). The NOEL is 2 mg/kg/day (10 ppm) and the LOEL is 3 mg/kg/day (100 ppm) based on decreased body weight gain and increased SGPT levels at 100 ppm and above; decreased total protein and albumin concentration and albumin/globulin ratio, increased AP, hepatocytic hypertrophy, oval cell proliferation and increased hepatocytic fatty content at 1000 ppm and above; and decreased calcium concentration which is thought to be related to the hypoalbuminemia, decreased cholesterol and triglyceride

concentrations, slightly increased GGT and SGPT, follicular hyperplasia of thyroid, increased colloid content in follicles and increased relative thyroid weight at 5000 ppm.

Thiazopyr was tested in a 21-day dermal study in the rat (MRID# 42619721) at 0, 100, 500 or 1000 mg/kg/day, 5 days/week for 3 weeks. The NOEL is 100 mg/kg/day. The LOEL is 500 mg/kg/day, based on increases in mean absolute and relative liver weights in high dose males and females; increases in mean absolute kidney weights in mid-dose females and in mean absolute and relative kidney weights in high dose females; and minimal multifocal or periportal hepatocyte vacuolation in mid- and high dose females.

### C. Chronic/Carcinogenicity Toxicity

In a 1-year feeding study in dogs (MRID# 42275531), thiazopyr was tested at the following dose levels: 0, 20, 200 or 2000 ppm (0, 0.8, 7.8 or 86 mg/kg/day for males and 0, 0.8, 8.8 or 78 mg/kg/day for females, respectively). The NOEL is 0.8 mg/kg/day (20 ppm) and the LOEL is 7.8 mg/kg/day (200 ppm) based upon hepatocellular hypertrophy/hyperplasia, which was observed at both 200 and 2000 ppm. In addition, an increase of approximately 10% in prothrombin time was observed at 2000 ppm with both sexes, as well as increased SGOT, SGPT, GGT and ALK and decreases in cholesterol, albumin, total protein and calcium levels. An increase in absolute and relative liver weights were also observed at 2000 ppm. Enlargement and/or discoloration in some of the high dose animals provided additional evidence of hepatotoxicity.

Thiazopyr was tested in an 18-month feeding study in mice (MRID# 42619723) at the following dose levels: 0, 1, 10, 100, 400 or 800 ppm. This was calculated to be 0, 0.17, 1.6, 16.9, 66.3 or 128.4 mg/kg/day in males and 0, 0.24, 2.6, 26.8, 108.1 or 215.9 mg/kg/day in females, respectively. The systemic NOEL is 1.6 mg/kg/day (10 ppm) and the systemic LOEL is 16.9 mg/kg/day (100 ppm.) The following effects were observed: 100 ppm - hepatocellular hypertrophy and amyloid deposition; 400 ppm - same lesions plus increase in liver weights, random and periportal hepatocellular vacuolation; 800 ppm - same lesions plus distended abdomen, slight increases in ALP, SGOT and SGPT, abnormal coloration and enlargement of the liver, decrease in absolute and relative spleen weights, increase in absolute and relative kidney weights, increase in eosinophilia in hepatocytes, kidney nephropathy and lymphocytic hyperplasia of the mesenteric lymph nodes. There were no increases in neoplastic lesions in any of the treated groups.

Thiazopyr was tested in a chronic/oncogenicity feeding study in rats (MRID# 42619724) for 24 months at the following dose levels: 0, 1, 10, 100, 1000 or 3000 ppm. This was calculated to be 0, 0.04, 0.4, 4.4, 44.2 or 136.4 mg/kg/day for males and 0, 0.06, 0.6, 5.6, 56.3 or 177.1 mg/kg/day for females,

respectively. The systemic NOEL is 4.4 mg/kg/day (100 ppm) and the LOEL is 44.2 mg/kg/day (1000 ppm) based on clinical signs of toxicity (protruding eyes in females), evidence of mild anemia in females, increases in GGT and cholesterol, increases in absolute and relative liver, kidney and thyroid weights and significant increases in microscopic lesions in the liver (hypertrophy and vacuolar change), kidney (nephropathy) and thyroid (hypertrophy and hyperplasia). These effects were all observed at 1000 ppm and greater in either males and/or females. At 3000 ppm, decreases in mean body weight and body weight gain and food consumption were observed in both sexes. In addition, at 6 months, there appeared to be a significant decrease in  $T_4$  in males and a significant increase in  $T_3$  in females at 6 months in the 3000 ppm group. These data were considered to be unreliable by the authors due to insufficient quantity of serum for analysis. Thiazopyr also induced a statistically significant increase in thyroid follicular cell adenomas/cystadenomas in males at 1000 and 3000 ppm. In addition, a non-significant increase in renal tubular adenomas were observed in high dose females. These were considered to be equivocal. No other significant increases in any tumor types were observed in either sex.

#### D. Developmental Toxicity

In a developmental toxicity study in rat (MRID# 42275532), thiazopyr was tested at the following dose levels: 0, 10, 100 or 250 mg/kg/day. The maternal NOEL is 100 mg/kg/day and the maternal LOEL is 250 mg/kg/day based on increased liver weights, salivation, decreased body weight gains and food consumption. The developmental NOEL was 100 mg/kg/day and the developmental LOEL was 250 mg/kg/day based on increased incidences of unossified sternebra(e) and 7th cervical rib variations.

In a developmental toxicity study in rabbits (MRID# 42275533), thiazopyr was tested at the following dose levels: 0, 10, 75 or 175 mg/kg/day. The maternal NOEL is 75 mg/kg/day and the maternal LOEL is 175 mg/kg/day based on reduced body weight gain and food consumption. The developmental NOEL is 175 mg/kg/day (HDT). No effects were observed.

#### E. Reproductive Toxicity

Thiazopyr was tested in a reproduction study in rats (MRID# 42275534) at the following dose levels: 0, 10, 100 or 1000 ppm (0, 0.5, 5 or 50 mg/kg/day). The parental/systemic NOEL was 0.5 mg/kg/day (10 ppm). The LOEL was 5 mg/kg/day (100 ppm) based on the following effects: at 100 ppm and above: centrilobular hepatocellular hypertrophy ( $\delta$ ); and at 1000 ppm: centrilobular hepatocellular hypertrophy ( $\text{♀}$ ), centrilobular hepatocellular vacuolation ( $\delta$ ), periportal hepatocellular vacuolation ( $\text{♀}$ ), hepatocellular degeneration/necrosis ( $\text{♀}$ ); increased absolute and relative liver weights ( $\delta+\text{♀}$ ) and discoloration and enlarged

livers (♂). The thyroid was not examined. The reproductive NOEL was 50 mg/kg/day (1000 ppm, the highest dose tested). There were no reproductive effects.

#### F. Mutagenicity

##### Gene Mutation

- Mutagenicity of Thiazopyr in *Salmonella typhimurium* (MRID# 42275535):

Thiazopyr was tested for potential to induce reverse mutations in the *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 or TA 1537; both with and without metabolic activation (S9 mix) at dose levels up to 10,000 µg/plate. The results were negative. The study was considered to be acceptable for regulatory purposes.

- Mutagenicity of Thiazopyr in Chinese Hamster Ovary Cells (CHO) at the HGPRT Locus Both With and Without Metabolic Activation (MRID# 42275536):

Thiazopyr was tested for potential to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells, either in the presence of or in the absence of metabolic activation at dose levels up to 1000 µg/ml. The results were negative. The study was originally considered to be acceptable for regulatory purposes, however, the Cancer Peer Review Committee (CPRC) considers this study to be unacceptable because the chemical was not tested at a high enough concentration (no signs of cytotoxicity or solubility problems).

##### Structural Chromosomal Aberration

- Induction of Micronuclei In Vivo in Bone Marrow Cells of Mice Treated With Thiazopyr (MRID# 42275537):

Thiazopyr was tested for potential to induce chromosomal aberrations in a mouse bone marrow assay. The mice were administered single i.p. doses of the test material at 80, 400 or 800 mg/kg (7%, 35%, 70% of the LD<sub>50</sub>). They were sacrificed 24, 48 and 72 hours later and the femoral bone marrow was prepared by conventional smear technique on microscope slides, air-dried overnight, and stained using the Wright-Giemsa Stain Pak. The slides were first coded, then scored for micronuclei in 1000 polychromatic erythrocytes (PCE) per experimental point. The positive control was cyclophosphamide. The results were negative under the conditions of the study. The study was considered to be acceptable for regulatory purposes.

## Other Genotoxic Effects

- Unscheduled DNA Synthesis in Rat Hepatocytes That Have Been Treated In Vitro With Thiazopyr (MRID# 42275538):

Thiazopyr was tested for induction of unscheduled DNA synthesis in rat hepatocytes treated in vitro up to the solubility limit dose of 3000  $\mu\text{g/ml}$ . Primary hepatocyte cultures from male F-344 rats were simultaneously exposed for 19 hours for both tritiated thymidine and either solvent (1% acetone) or graded concentrations of the test material (5 - 3000  $\mu\text{g/ml}$ ). The slides were prepared from the cells, dipped in photographic emulsion and stored in light-tight boxes at  $-20^{\circ}\text{C}$  for 7 days. They were then developed and stained with 1% methyl green-pyronin. 2-Acetylaminofluorene was used as a positive control. Thiazopyr induced neither cytotoxicity nor increased nuclear grain counts over the control values. The positive control induced a strong positive response. The study was considered to be acceptable for regulatory purposes.

## G. Metabolism

A metabolism study was conducted in rats (MRID#s 422755-39 and 433194-01) in which thiazopyr was administered as: 1) a single low oral dose (1 mg/kg), 2) a single high dose (oral) (100 mg/kg), 3) a single low i.v. dose (1 mg/kg) or 4) multiple low dose (oral) (14 daily unlabeled 1 mg/kg doses, followed by 1 radiolabeled (oral) dose at 1 mg/kg.) Thiazopyr with  $^{14}\text{C}$  at the 4 position of the pyridine ring and  $^{14}\text{C}$  at the 4' and 5' positions of the thiazole ring was tested. The apparent rate of absorption was less for the thiazoline labeled MON and the apparent plasma half-life of the thiazoline labeled material was 5-fold that of the pyridine labeled molecule. The absorption of an orally administered dose was  $\geq 90\%$  for both test materials. The overall radiolabel recovery for all study groups was  $88.9 \pm 0.6\%$ . No significant sex-related differences were observed in the total percent recovery. However, the distribution of recovery was sex-related. There was little radiolabel detected in the tissues at study termination. Preferential sites for localization of the radiolabel included liver, adipose tissue, muscle and bone. Ten metabolites of thiazopyr were found in the excreta, each constituting greater than or equal to 5% of the dose for either sex. The metabolic pathway is essentially an oxidative pathway. Vulnerable sites of the molecule are the thiazoline ring, the isobutyric side chain and the pyridine rings. The test material appears to be rapidly and extensively eliminated with low amounts of residues remaining in the tissues and carcass. However, the percentage of radiolabel remaining in the carcasses following thiazoline labeled thiazopyr was between 6.9 - 10.8%.

## H. Neurotoxicity

In an acute mammalian neurotoxicity study (MRID# 43855508) thiazopyr (97.8% a.i.) was administered to groups of 10 Sprague-Dawley CD rats/sex/dose by gavage in a volume of 10 ml/kg body weight. A single dose was administered at the following levels: 0, 500, 1000 or 2000 mg/kg. All animals were observed in behavioral tests (functional observational battery [FOB] and motor activity) prior to dosing, at the time of peak effect within 8 hours of dosing, and 7 and 14 or 15 days after dosing.

The results from this study are considered to be inconclusive for neurotoxicity. There was no set pattern for the FOB and motor activity observations and the effects were transient. The effects (lacrimation, salivation and decreases in alertness, gait score, rearing, excretion, righting reflex, microscopic lesions included dilatation of the lateral ventricle, degeneration of the sciatic, sural, tibial and thoracic spinal cord nerve fibers) were observed at dose levels known to be lethal in other studies with younger rats. Particularly because of the high dose levels used, in this case it is difficult to distinguish between neurotoxicity and general signs of toxicity. The microscopic examination suggests a possible effect, but the lesions were minimal. The controls also had minimal lesions, albeit fewer. The LOEL is 1000 mg/kg, based on differences in the FOB and motor activity at 1000 and 2000 mg/kg when compared to the control groups at 7 hours but not at 7 days or greater. The NOEL is 500 mg/kg.

## I. Special Toxicity Studies

### **Special Mechanistic Studies for Thyroid Pathology**

#### Effect of Dietary Exposure of Thiazopyr on Serum Levels of T<sub>3</sub>, T<sub>4</sub>, and TSH, Hepatic Drug Metabolizing Activity and Thyroid Pathology in Male Sprague Dawley Rats (MRID# 42619725):

Thiazopyr was tested in a special thyroid function study to investigate the subchronic effects on hormone levels and other biochemical endpoints. The chemical was administered through the diet at either 0 or 3000 ppm and the animals were sacrificed at 7, 14, 28, 56 or 90 days. Statistically significant decreases in body weight gain were observed at 90 days (89% of controls.) Starting fairly early on, increases in TSH ( $p < 0.05$ ) and decreases in T<sub>4</sub> ( $p < 0.05$ ) were observed. In addition, there were increases in absolute and relative liver weights ( $p < 0.05$ ), and absolute and relative thyroid weights ( $p < 0.05$ ), and increases in thyroid follicular cell hypertrophy/hyperplasia. Reverse T<sub>3</sub> was increased at 28 days (only timepoint measured,  $p < 0.05$ ). T<sub>3</sub> was either not affected or increased.

There were indications of increases in hepatic UDPGT activity ( $p < 0.05$ ), at 7, 56 and 90 days; and significant

increases in  $T_4$  UDPGT activity ( $p < 0.05$ ) at all times except 90 days. Hepatic 5'-monodeiodinase activity was either not affected or decreased. **The effects observed in this study are supportive of the theory that thiazopyr may induce thyroid tumors through a disruption in the thyroid-pituitary hormonal feedback mechanisms.**

A Dose Response and Reversibility Study of Effects of Thiazopyr on Biochemical Mechanisms of Thyroid Toxicity in Sprague Dawley Rats (MRID# 43072801):

In a second special thyroid function study, groups of twenty 12-week-old male Sprague Dawley Crl:CD SD(BR) strain rats were given diets containing 0, 10, 30, 100, 300, 1000 or 3000 ppm (3 groups per dose level) thiazopyr for 56 days. The dose levels are equivalent to 0, 0.5, 1.5, 5, 15, 50 or 150 mg/kg/day, respectively. Two experiments were conducted with these animals. The first was designed to investigate dose response effects of the test substance on body, liver and thyroid weight,  $T_4$  uridine diphosphate glucuronyl transferase (UDPGT) activity in liver tissue, serum concentrations of thyroxine ( $T_4$ ), triiodothyroxines ( $T_3$ ), reverse triiodothyroxine ( $rT_3$ ) and thyroid stimulating hormone (TSH), and thyroid histology. In the second experiment, two groups receiving the 3000 ppm diet (150 mg/kg/day) were maintained on a control diet following the 56 day treatment period for 56 or 112 days before they were sacrificed along with a group of control animals and observed for reversibility of effects on the parameters observed in the first experiment.

In the dose-response study, liver weights were significantly increased at 300, 1000 and 3000 ppm. Thyroid weights were significantly increased for the 1000 and 3000 ppm dose groups. There were no significant effects of the test substance on body weight or body weight gain during the study. The  $T_4$  UDPGT levels were increased in the 1000 and 3000 ppm dose levels. The highest dose level (3000 ppm) was associated with increased  $T_3$ , TSH, and  $rT_3$  serum concentrations ( $p < 0.05$ ), an increased incidence of follicular cell hypertrophy/hyperplasia, and significantly decreased  $T_4$ . Based on the liver weight increases, the NOEL and LOEL in this study are 15 and 50 mg/kg/day (100 and 300 ppm) respectively. Thyroid weight was the only one of the above mentioned parameters that did not return to values comparable to controls.

Effect of Dietary Exposure of Thiazopyr on Biochemical Mechanisms Involved in the Disposition of  $T_4$  (MRID# 43072802):

In a third special thyroid function study, groups of 5-10 twelve-week-old male Sprague Dawley Crl:CD SD(BR) strain rats were given diets containing 0 or 3000 ppm (4 groups per dose level) Thiazopyr for 56 days. The dose level is equivalent to 150 mg/kg/day. A series of four experiments were conducted to evaluate the test substance's effect on blood concentration half-life of  $T_4$ , biliary excretion rate of  $T_4$ , thyroid gland iodine

uptake, and hepatic enzyme activity including T<sub>4</sub> UDPGT, deiodinase, aryl hydrocarbon dehydrase, ethoxy O-dehydrase, and cytochrome P-450.

The 3000 ppm dose level of thiazopyr increased the clearance rate from the blood (p < 0.05) and rate of accumulation in the bile (p < 0.05). The test substance was also associated with increases in T<sub>4</sub> UDPGT activity (p < 0.05) and total deiodinase activity in the liver (p < 0.05 for  $\mu\text{g T}_3/\text{mg protein}/30$  minutes). A two-fold increase in mixed function oxidase enzyme activity was also associated with administration of 3000 ppm Thiazopyr in the diet of rats for 56 days (p  $\leq$  0.01 for all parameters).

These results, along with those of the previously summarized studies suggest that increased glucuronidation, deiodination of T<sub>4</sub> to T<sub>3</sub>, and increased rate of clearance of T<sub>4</sub> from the blood and excretion of the hormone and its metabolites in the bile could significantly reduce the level of circulating T<sub>4</sub> in the male rat.

## V. DOSE RESPONSE ASSESSMENT

### A. Reference Dose

The HED RfD/Peer Review Committee met on October 28, 1993 to discuss and evaluate the toxicology data submitted in support of registration of thiazopyr and assess the Reference Dose (RfD) for this chemical. At this meeting the Committee recommended that an RfD for this chemical be established on the basis of a chronic toxicity study in dogs with a NOEL of 0.8 mg/kg/day (MRID# 42275531). Hepatocellular hypertrophy and hyperplasia were observed at the next higher dose level (7.8 mg/kg/day in males and 8.8 mg/kg/day in females) or higher. An uncertainty factor (UF) of 100 was used to account for the interspecies extrapolation and intra-species variability. On this basis the RfD was calculated to be 0.008 mg/kg/day.

The Committee also found that there was no evidence, based on the available information, that thiazopyr was associated with significant reproductive or developmental effects. Therefore, HED has no concern regarding special sensitivities for infants and children.

### B. Carcinogenicity Classification

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on March 9, 1994 to discuss and evaluate the weight-of-the-evidence on thiazopyr with particular reference to its carcinogenic potential. The Peer Review Committee classified thiazopyr as a Group C - possible human carcinogen and recommended that for the purpose of risk characterization a Margin of Exposure (MOE) approach should be used for evaluation of the consequences of human exposure.

The classification was based on a 2-year dietary feeding study (MRID# 42619724) coupled with several special studies conducted with thiazopyr in the rat. In the chronic feeding study, thiazopyr induced a statistically significant increase in thyroid follicular cell tumors in males. In addition, there were numerical increases in renal tubular adenomas (a rare tumor type) in both sexes; however, there was a statistically significant positive trend in females only. The Committee agreed that due to the rarity of the renal tumors in males, the adenomas observed in this sex were also biologically significant, even though the appearance of tumors was not dose-related.

The MOE approach was selected because the non-neoplastic toxicological evidence (thyroid growth, thyroid follicular cell hypertrophy/hyperplasia, thyroid and pituitary hormonal changes and data on the site of action [hepatocellular hyperplasia/hypertrophy, enhanced liver metabolism and excretion of T<sub>4</sub>]) indicated that Thiazopyr was inducing a disruption in the thyroid-pituitary hormonal status. In addition, there was no evidence of genotoxicity. Therefore, a threshold consideration was to be applied in estimating human health risks.

#### C. Other Toxicity Endpoints

##### Acute Dietary Endpoint (One Day)

An acute dietary risk assessment is not required because no endpoints indicating potential for adverse effects were identified by the Toxicology Endpoint Selection Committee (TESC).

##### Short (1-7 days), Intermediate (7 days to several months) and Chronic Term Occupational or Residential Exposure

A short- or intermediate-term risk assessment is not required because no endpoints, indicating potential for adverse effects, were identified by the TESC.

#### VI. DIETARY EXPOSURE AND RISK CHARACTERIZATION

##### A. Dietary Exposure From Food Sources

###### i. Nature of the Residue in Plants and Animals

##### PLANTS:

Lemon trees grown in 5 gallon containers in a sandy loam soil were treated with thiazopyr labelled at the C-4 position of the pyridine moiety at the maximum proposed label rate of 2 lb a.i./A and at twice the proposed maximum label rate using 4 lb a.i./A. Foliage and immature fruit were collected at 133 and 124 days, respectively, after treatment, and mature fruits were harvested 236 days after treatment. Only the mature fruit were

analyzed and fractionated for determination of residues.

The low  $C^{14}$  thiazopyr activity in this metabolism study suggests that 1) thiazopyr metabolites containing the intact pyridine ring undergo negligible translocation from the soil to fruit, 2) under actual field conditions at the maximum proposed label rate of 2.0 lb a.i./A, residues of thiazopyr and its metabolites would be undetectable ( $<0.05$  ppm). Most of the metabolites in the lemon tissues were partitioned into the aqueous layer. Thiazopyr is relatively nonpolar and is water-insoluble. Several metabolites were partially distributed in the organic and the aqueous layers. The levels of organic-soluble residues (thiazopyr, and metabolites defined as #3, #5, #6, and #8) totaled  $<4\%$  of the total radioactive residue (TRR).

Although cottonseed has been removed from the initial petition the metabolism in cotton will be discussed to provide additional support to the proposed metabolic pathway in plants.

Cotton plants grown in sandy loam soil in individual greenhouse pots were divided into 4 treatment groups: untreated, treated with soil incorporation with unlabeled thiazopyr, soil incorporation treated with thiazopyr labeled in the pyridine or thiazoline rings, surface treatment with thiazopyr labeled in the pyridine or thiazoline rings. The pesticide was applied to each pot in an amount equivalent to 0.125 lbs a.i./A. The proposed maximum seasonal label rate is 0.375 lb a.i./A (0.5 lb a.i./A in AZ and CA). Cotton foliage was harvested at 56 days; cottonseed and cotton plant hay at 249 days after treatment. Residue levels were determined in plant matrices by combustion and the captured radioactivity was determined by a liquid scintillation counter (LSC.) Uptake of  $C^{14}$  thiazopyr was approximately 0.5% of the applied radioactivity. Residue levels for both ring labels were similar for both application methods.

The very low level of  $C^{14}$  radioactivity in cottonseed prohibited extraction and isolation of metabolites. The metabolism of thiazopyr in cotton was extensive, although some parent remained in crop tissues. In a representative experiment, 93.1% of the total radioactivity of the leaves was extractable. The organic extract had 42% of the initial radioactivity, and of this, 13.2% was resolved into 11 discrete peaks. The aqueous phase had 57.8% of the initial radioactivity. High performance liquid chromatography (HPLC) resolved 29% of the initial extract radioactivity into 26 discernable peaks. In all, of over 40 peaks present, nine significant metabolites were identified and characterized. Recoveries of the identified peaks ranged from 0.1% to 9.4% of the TRR.

In summary, thiazopyr undergoes extensive and rapid degradation in plants to a large number of polar metabolites, all found at low levels ( $<10\%$  of the TRR). Major routes of metabolism include sulfur oxidation, thiazoline ring opening and

methyl ester hydrolysis, and transformation of the isobutyl side chain. About 30 metabolites have been positively or tentatively identified. The complete breakdown of thiazopyr to many low-level polar metabolites closely resembles the metabolic pathways observed in other crops and soil metabolism. For example in cotton, 8 metabolites are found, 13 in peanuts, and 12 in lemons. The submitted metabolic data for plants are adequate to support the proposed uses with oranges and grapefruits. No additional data are needed.

#### Animals:

##### Lactating Goat:

Two lactating goats were dosed orally for 4 consecutive days with 19.3 mg of C<sup>14</sup>/C<sup>13</sup> thiazopyr (labeled with C<sup>13</sup> and C<sup>14</sup> in the C-4 position of the pyridine ring; specific activity, 16.1 mCi/mmmole). One goat dosed with a placebo served as the control. The dosage administered to the animals was equivalent to 12 (goat #1) and 21 ppm (goat #2) in the diet, based on actual feed consumption. After four days the animals were sacrificed, aliquots of tissues, feces, milk, and urine were combusted and counted for radioactivity by LSC. Other aliquots were extracted with acetonitrile/water, partitioned, and after sample cleanup were analyzed by HPLC with radiometric detection to identify metabolites. The total percentage recoveries of administered radioactivity in goat #1 and goat #2 in blood, feces, milk, tissues, and urine were approximately 89 and 90, respectively. Radiolabeled residues plateaued in milk by day 3.

Thiazopyr in a lactating ruminant and plants undergoes metabolic degradation to a large number of polar metabolites, all found at low levels. Milk contained 3 identifiable metabolites, Liver contained 7 metabolites including the parent, kidney had 2 metabolites, renal and omental fat had 5 metabolites, and muscle had 5 metabolites. In all, parent plus 11 individual metabolites were identified in goat tissues and milk. Of these, 6 were identical to plant metabolites and 2 others were very similar. The major residues in ruminant muscle and milk are thiazopyr and its unsaturated nitrile acid. The major residues in fat are thiazopyr and its sulfone ester metabolite.

##### Poultry:

Laying hens were dosed orally for 4 days with either 1.3 mg (Group #'s 6 and 7) or 10.4 mg (Group #4) of labeled thiazopyr (C<sup>14</sup>/C<sup>13</sup> in the C-4 position of the pyridine ring). The administered dosages based on actual feed consumption, were equivalent to 12 and 78 ppm in the diet in the low and high dose diet, respectively. After four days the animals were sacrificed, aliquots of tissues, excreta, and eggs were combusted and counted for radioactivity by LSC. Other aliquots were extracted with acetonitrile/water, partitioned, and analyzed after sample

cleanup by HPLC with radiometric detection to identify metabolites.

Residues in eggs in the low dose groups plateaued by day 4. In the high dose birds the residues had not plateaued by day 4. Since normal egg formation requires 7 days, the low dose may not really be a plateau, but a variation of residues from day to day.

Tissues of group 3 hens were extracted and the extracts subjected to HPLC. Total accountabilities ranged from 92 to 114% (ave.: 104%).

Thiazopyr in laying hens and plants undergoes metabolic degradation to a large number of polar metabolites, all found at low levels. Liver had 5 metabolites including the parent (4 of which were found as plant metabolites), while kidney had 3 (2 of which are plant metabolites). Egg yolk had 5 (3 of which are plant metabolites) and muscle had 6 (4 of which are plant metabolites). In all, 8 individual metabolites were identified in hen tissue, eggs, and excreta. Of these, 4 were identical to plant metabolites. The major terminal residues in poultry are thiazopyr and its nitrile acid ester metabolite.

HED considers the metabolism of thiazopyr in plants and animal as adequately understood. The petitioner initially proposed that tolerances be established for thiazopyr and those metabolites that can be converted to two common entities, referred to as the sulfonic diacid (SAA) and the amide acid (AA). However, the petitioner has now proposed that tolerances be established for thiazopyr and its metabolites convertible to one common chemophore and has submitted an analytical enforcement methodology for thiazopyr and its metabolites using this approach. The submitted field trial residue data in support of the proposed use on orange and grapefruit used methodology involving two common entities. The HED Metabolism Committee, in a meeting held on April 11, 1995, determined that with respect to enforcement, the appropriate tolerance expression will be based on the methodology available and its ease of use. The Agency has validated the proposed methodology using the one chemophore for the RACs orange and grapefruit. However, acceptance is still dependent upon the petitioner making the recommended correction/changes as suggested by the Agency. **HED will only consider the proposed enforcement methodology adequate if the petitioner provides the requested rewrite.** HED will also consider the submitted residue field trial and processing data adequate even though it is supported by analyses using the two chemophore methodology.

In summary, thiazopyr undergoes extensive and rapid degradation in plants and animals to a large number of polar metabolites, each present at low levels (<10% of TRR). Major routes of metabolism include sulfur oxidation, thiazoline ring opening and methyl ester hydrolysis, and transformation of the isobutyl side chain. More than 30 metabolites have been

positively or tentatively identified.

Using the initial methodology (two chemophore, SAA and AA) the major residues in ruminant muscle and milk are thiazopyr and its unsaturated nitrile acid. The major residues in fat are thiazopyr and its sulfone ester metabolite. The major terminal residues in poultry are thiazopyr and its nitrile acid ester metabolite. In the April 1995 meeting, the HED Metabolism Committee concluded that there is no special toxicological concern with any one metabolite. The metabolites would be considered to have comparable or lower toxicity than the parent. In addition, the present reference dose for thiazopyr is low (0.008 mg/kg/day). Therefore, for risk assessment purposes the metabolites convertible to the sulfonic triacid as measured by the proposed analytical method.

#### ii. Analytical Method

The parent and most of the identified metabolites share a common moiety, 2-difluoromethyl-4-(2-methylpropyl)-6-trifluoromethyl pyridine carboxylate. The residues of thiazopyr and its metabolites can be converted to a sulfonic triacid followed by derivatization to a trimethyl ester (i.e., 3,4,5-pyridine tricarboxylic acid, 2-(difluoromethyl)-6-(trifluoromethyl) trimethyl ester). Residues are quantitated by GC/MS with all residues being calculated as thiazopyr equivalents.

The Agency validation has determined a limit of detection (LOD) at 0.003 ppm and a limit of quantitation (LOQ) at 0.015 ppm (see comments on page 15 of this document in regards to the Agency's acceptance of the methodology.) No methods have been submitted for livestock meat, meat by-products, and fat, and milk and eggs, because the petitioner expected amounts of identifiable residues from the proposed use would not be finite (<0.01).

#### iii. Storage Stability

Data submitted by Monsanto (MRID #42619703) regarding the storage stability indicates that thiazopyr is stable for 6 months at ambient conditions packaged in double-lined low density polyethylene bags; 1-year study still in progress (TGAI).

#### iv. Magnitude of the Residue

Residues of thiazopyr and its metabolites convertible to AA and SAA in/on orange and grapefruit following a single soil broadcast application at 2 lb a.i./A or two sequential soil broadcast applications each at 1 lb a.i./A/application of the 2 lb a.i./gal EC formulation (MRID #42641401) are presented in Table 2. (Residues were not corrected for method recoveries.)

Table 2: Thiazopyr Residues in Orange and Grapefruit

Total Rate (lb a.i./A)	Number of Appl.	PHI, in days	Test Sites (no. of samples)	Residues in ppm		
				AA <sup>a</sup>	SAA <sup>a</sup>	Combined Residues
Orange						
2	1	90-98	FL (12)	<0.003-0.0042	<0.013	<0.016-<0.0172
2	2	90-98	FL (12)	<0.003-0.0052	<0.013	<0.016-<0.0182
2	1	32-92	CA (8)	<0.003-0.0039	<0.013	<0.016-<0.0169
2	2	32-92	CA (8)	<0.003	<0.013	<0.016
2	1	65	AZ (2)	<0.003	<0.013	<0.016
2	2	65	AZ (2)	<0.003	<0.013	<0.016
Control	--	--	FL, CA, AZ (22)	<0.003	<0.013	<0.016
Grapefruit						
2	1	90-98	FL (6)	<0.003-0.0051	<0.013	<0.016-0.0181
2	2	90-98	FL (6)	<0.003-0.006	<0.013	<0.016-0.019
2	1	89, 91	CA (4)	<0.003	<0.013	<0.016
2	2	89, 91	CA (4)	<0.003	<0.013	<0.016
2	1	91	AZ (2)	<0.003	<0.013	<0.016
2	2	91	AZ (2)	<0.003	<0.013	<0.016
Control	--	--	FL, CA, AZ (12)	<0.003	<0.013	<0.016

<sup>a</sup> The limits of detection for AA and SAA are 0.003 ppm and 0.013 ppm, respectively.

The available data indicate that residues of Thiazopyr and its metabolites convertible to AA and SAA are not likely to exceed the proposed tolerance of 0.05 ppm in/on citrus fruits harvested 32-98 days following a single soil broadcast application of the 2 lb a.i./gal EC formulation at 2 lb a.i./A or two sequential soil broadcast applications at 1 lb a.i./A/application for a maximum seasonal rate of 2 lb a.i./A. Apparent residues of AA and SAA metabolites in/on 40 untreated samples of the citrus fruits were nondetectable (<0.003 ppm and <0.013 ppm, respectively). Using the proposed triacid methodology would give similar results.

#### v. Processing Study

Monsanto Company submitted data (MRID #42619705) depicting the potential for concentration of residues of thiazopyr and its metabolites convertible to AA and SAA in orange and grapefruit processed commodities. In five tests conducted in CA(1) and FL(4), orange and grapefruit were harvested 89-92 days following a single soil broadcast application of the 2 lb a.i./gal EC formulation at 10 lb a.i./A/application (5x the proposed maximum seasonal rate) using ground equipment.

Treated and control samples from the FL and CA sites were harvested and delivered to the University of Florida, Citrus Research Center and Education Center (Lake Alfred, FL) or to California State Polytechnic University (Pomona, CA), respectively, for processing. Samples were cooled (4-7 C) prior

to processing. Citrus fruits were processed and untreated fruits, washed fruits, finisher pulp, wet peel, dry peel, juice, molasses, and cold-pressed oil were collected using a simulated commercial procedure. The processed fractions were shipped frozen (temperature unspecified) by freezer trucks to the analytical laboratory (Monsanto Company, St. Louis, MO). Samples were stored frozen at -24 to -23 C for up to 428 days for oranges, 369 days for grapefruit, 511 days for orange juice, and 414 days for grapefruit juice prior to analysis. Untreated control and treated samples were analyzed for residues of AA and SAA using Method RES-017-91, which has a lower limit of method validation of 0.025 ppm for both AA and SAA.

The data indicate that residues of thiazopyr and its metabolites convertible to AA and SAA are not likely to concentrate in the pulp (finisher), peel (wet and dry), and juice that had been processed from orange and grapefruit treated with a single broadcast application of the thiazopyr EC formulation at 5x the proposed maximum seasonal rate. Although based on residue data from the 5x trial, it appears that thiazopyr residue concentration in citrus oil, when these residues are adjusted to reflect the maximum proposed rate, thiazopyr residues in the citrus oil are not expected to exceed the proposed 0.05 ppm RAC tolerance for orange and grapefruit. Therefore, a 408 tolerance would not be needed for citrus oil.

#### vi. Meat, Milk, Poultry, and Eggs

In the citrus field trials, no residues of thiazopyr or its metabolites convertible to either **SAA** or **AA** were found to be greater than the lower limit of the method validation (0.025 ppm.) Similar results would be expected using the triacid methodology. Based upon the available data for the proposed uses, tolerances are not required for livestock tissues, milk, or eggs.

#### B. Dietary Exposure From Ground Water

A ground water exposure estimate for thiazopyr is based on findings from an ongoing prospective ground water monitoring study in Florida. Study results up to 14 months after application show that no Thiazopyr residues leached to ground water. Thiazopyr was detected sporadically in soil-pore water at concentrations up to 1.3 ppb. The monoacid degradate (**AA**) was detected in soil-pore water at concentrations up to 60.6 ppb and in ground water at concentrations up to 7.6 ppb. Since the metabolites were to be included in estimating dietary exposure, the following calculation is used:

Adult Exposure = (chemical concentration in  $\mu\text{g/L}$  in consumed water) ( $10^{-3}$  mg/ $\mu\text{g}$ )  $\div$  (70 kg body weight) (2 L/day)

Adult Exposure = (7.6 ppb) ( $10^{-3}$ ) / (70 kg) (2 L/day)

Adult Exposure =  $2.17 \times 10^{-4}$  mg/kg/day

Kids Exposure = (chemical concentration in  $\mu\text{g/L}$  in consumed water) ( $10^{-3}$  mg/ $\mu\text{g}$ )  $\div$  (10 kg body weight) (1 L/day)

Kids Exposure = (7.6 ppb) ( $10^{-3}$ ) / (10 kg) (1 L/day)

Kids Exposure =  $7.6 \times 10^{-4}$  mg/kg/day

Therefore, the %RfD taken up by ground water exposure is calculated as follows:

%RfD = (Exposure mg/kg/day)  $\div$  (RfD mg/kg/day)  $\times$  100

%RfD Adults =  $2.17 \times 10^{-4}$  mg/kg/d  $\div$  0.008 mg/kg/d  $\times$  100

%RfD for Adults = 2.7

%RfD Kids (1-6 yrs) =  $7.6 \times 10^{-4}$  mg/kg/d  $\div$  0.008 mg/kg/d  $\times$  100

%RfD for Kids (1-6 yrs) = 9.5

### C. Dietary Risk Characterization

#### i. Chronic Dietary Risk

A DRES chronic exposure analysis was performed using tolerance level residues and 100 percent crop treated information to estimate the Theoretical Maximum Residue Contribution (TMRC) for the general population and 22 subgroups. As a new chemical, tolerances for thiazopyr have yet to be published in the CFR. No anticipated residue (AR) information was used. No tolerances now exist for residues of thiazopyr in animal commodities and no food/feed additive tolerances have been established.

The chronic analysis showed that exposure from the proposed new tolerances on oranges and grapefruit for children 1 to 6 years old (the subgroup with the highest exposure) would be 4.0% of the RfD. While the exposure for the general U.S. population would be 1.5% of the RfD.

This chronic analysis for Thiazopyr is a worst case estimate of dietary exposure with all residues at tolerance level and 100 percent of the commodities assumed to be treated with Thiazopyr. Even without refinements, the chronic dietary risk exposure to Thiazopyr appears to be minimal for this petition on oranges and grapefruit at 0.05 ppm and does not exceed the RfD for any of the DRES subgroups.

ii. Aggregate Dietary/Drinking Water Risk

The dietary and drinking water combined exposure to Thiazopyr would be 13.5% (9.5 + 4.0) of the RfD for children 1 to 6 years old. While the exposure for the general U.S. population would be 4.2% (2.7 + 1.5) of the RfD. HED does not consider the combined risk to exceed the level of concern.

iii. Acute Dietary Risk

An acute dietary risk assessment is not required because no toxicity endpoints were identified indicating a potential for adverse effects.

VII. Determination of Safety for Infants and Children

The toxicological database for evaluating pre- and postnatal toxicity for thiazopyr is mostly complete. Available data indicate that no developmental toxicity was observed in the rabbit study at the highest dose tested (175 mg/kg/day). Maternal toxicity was observed in the rabbit in the 175 mg/kg/day dose group which consisted of reductions in body weight gain and food consumption. In the rat developmental study, a reduction in maternal body weight gain and body weight was observed at the highest dose tested (250 mg/kg/day). Developmental toxicity was observed in the high dose (250 mg/kg/day) as increased incidences of unossified sternebra and 7th cervical rib variations.

The NOEL for systemic (parental) toxicity is 0.5 mg/kg/day. The NOEL for reproductive toxicity is 50 mg/kg/day (highest dose tested). There were no reproductive effects noted in the study. These data taken together suggest minimal concern for developmental or reproductive toxicity and do not indicate any increased pre- or postnatal sensitivity in the offspring; no additional uncertainty factor for increased sensitivity in infants and children is appropriate.

VIII. Other Considerations

A. Cumulative Effects

Thiazopyr is structurally similar to other chemicals in the pyridine chemical family. Further, other pesticides may have common toxicity endpoints with thiazopyr.

However the Agency has not made a determination whether thiazopyr and any other pesticide have a common mode of toxicity and require cumulative risk assessment. For the purposes of this tolerance and registration application, the Agency has considered only risks from thiazopyr. If required, cumulative risks will be assessed as part of Reregistration and tolerance reassessment, and when methodologies for determining common mode of toxicity and for performing cumulative risk assessment are finalized.

## IX. Occupational/Residential Exposure and Risk Characterization

For short term and intermediate term exposures there are no identified toxicological endpoints (see section V, c, Other Toxicity Endpoints). In light of the use patterns of this compound, as a preemergence herbicide for annual grasses and broadleaf weeds, HED does not consider this a chronic exposure situation.

## X. References

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