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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 28-January-2010

SUBJECT: **Difenoconazole** FQPA Human Health Risk Assessment to Support the Establishment of Import Tolerances on Mango and Waxapple (also known as Wax jambu)

PC Code:	128847	**DP Barcode:**	371612
Decision No.:	413927	**Registration No.:**	N/A
Petition No.:	PP#9E7573	**Regulatory Action:**	Section 3
Risk Assessment Type:	Single Chemical/Aggregate	**Case No.:**	NA
TXR No.:	NA	**CAS No.:**	119446-68-3
MRID No.:	NA	**40 CFR**	§180.475

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1.0 Executive Summary

Difenoconazole is a broad spectrum fungicide belonging to the triazole group of fungicides (Group 3). It is currently registered in the U.S. for use as a seed treatment on cereal grains, canola, and cotton and for foliar applications to pome fruits, sugar beets, fruiting vegetables, and tuberous and corm vegetables. Tolerances for difenoconazole are currently established under 40 CFR §180.475. Difenoconazole acts by blocking demethylation during sterol biosysnthesis which, in turn, disrupts membrane synthesis.

Proposed Uses

Under PP#9E7573, Syngenta Crop Protection, Inc. is proposing the establishment of import tolerances for residues of difenoconazole [1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole] in/on Mango at 0.09 ppm and on waxapple at 1.5 ppm. The end-use product (EP) for use on mangos grown in Brazil is Score®, an emulsifiable concentrate (EC) formulation containing 250 g ai/L. Up to three foliar spray applications at a target rate of 0.11 lb ai/A/application (0.125 kg ai/ha/application), with a 14-day retreatment interval (RTI), may be made to mature mango trees for a total seasonal rate of 0.33 lb ai/A (0.375 kg ai/ha) using ground equipment. The preharvest interval (PHI) is 7days. The EP for use on waxapples grown in Taiwan is a soluble concentrate (SC) formulation containing 12.5% w/v difenoconazole and 20% w/v azoxystrobin. The information for waxapple use in Taiwan is incomplete, however, the submitted field trial data will support up to eight foliar applications of a 125 g/L SC formulation of difenoconazole at a target rate of 0.34 lb ai/A/application (0.39 kg ai/ha/application), with 6- to 8-day RTI (re-entry interval), to mature waxapple trees for a total seasonal rate of 2.74 lb ai/A (3.07 kg ai/ha) and a 12-day PHI. Applications may be made using ground equipment. Consistent with the submitted field trial data, applications should be restricted to fruit protected with exclusion net bags or otherwise bagged fruit. The subject review addresses difenoconazole only.

Toxicity/Hazard

Difenoconazole possesses low acute toxicity by the oral, dermal and inhalation routes of exposure. It is not considered to be an eye or skin irritant and is not a sensitizer. Difenoconazole exhibits some evidence of neurotoxicity in the database, but the effects are transient or occur at doses exceeding the limit dose. It is not mutagenic andit is not a developmental or reproductive toxicant. Chronic effects in rats and mice are seen as cumulative decreases in body weight gains. No evidence of carcinogenicity was seen in rats. Evidence for carcinogenicity was seen in mice where liver tumors were induced at doses which were considered to be excessively high for carcinogenicity testing. Treatment-related non-neoplastic lesions were confined to the liver. Tumors were observed in mice at **46** and **58** mg/kg/day (males and females, respectively); however, based on excessive toxicity observed at the two highest doses, the absence of tumors at the lower doses and the absence of genotoxic effects, HED's Cancer Peer Review Committee (CPRC) recommended for a cancer classification of C (**possible human**

carcinogen). A margin-of-exposure (MOE) approach to risk assessment was advocated by the CPRC in July 2007. The FQPA Safety Factor (SF) is reduced to 1x.

The toxicological database for difenoconazole is sufficient to conduct this risk assessment. However, in accordance with Part 158 Toxicology Data requirements, an immunotoxicity study (870.7800) is required for difenoconazole.

Endpoints and doses for risk assessment were selected for the following scenarios: Acute dietary (general population including infants and children), chronic dietary, short-term dermal and short-term inhalation.

Dietary Exposure/Risk Assessment

HED has examined the residue chemistry database for difenoconazole and concluded that field trial data for mango are adequate and reflect the proposed use of Score® on mango in Brazil. Assuming that the use directions for waxapple in Taiwan, once fully elucidated, will comport with the submitted field trial data, HED believes that there are no residue chemistry issues that would preclude granting import tolerances for residues of difenoconazole on mango in Brazil and waxapple in Taiwan.

Acute and chronic dietary (food + water) risk assessments were conducted using the Dietary Exposure Evaluation Model - Food Consumption Intake Database (DEEM-FCID™, ver. 2.03. This model uses food consumption data from the United States Department of Agriculture's (USDA's) Continuing Surveys of Food Intakes by Individuals (CSFII; 1994-1996 and 1998).

The unrefined acute analysis assumed tolerance-level residues, 100% crop treated (CT), and default processing factors. The resulting acute food exposure estimates were less than HED's level of concern (<100% of the acute population-adjusted dose (aPAD)) at the 95th percentile of the exposure distribution for the general U.S. population (7% aPAD) and all population sub-groups; the most highly exposed population subgroup was Children1-2 years old with 16% aPAD. The somewhat refined chronic analysis assumed tolerance-level residues for some commodities, field trial residues for the majority of commodities, experimental processing factor for some crops, and 100 % CT. The resulting chronic food exposure estimates were less than HED's level of concern for the general U.S. population (17% cPAD) and all population sub-groups; the most highly exposed population subgroup was children 1-2 years old with 45% cPAD.

A cancer dietary assessment was not conducted for difenoconazole because the cancer NOAEL is higher than the chronic RfD; therefore, the chronic dietary risk estimate is more protective.

Residential Exposure/Risk Assessment

No new residential uses are being requested at this time. However, adults and adolescents may be exposed to difenoconazole from its currently registered use on ornamentals. These risks have been previously assessed. It was concluded that

residential pesticide handlers will be exposed to short-term duration (1 - 30 days) only. The dermal and inhalation (short-term) residential exposure was assessed for a homeowner mixer/loader/applicator wearing short pants and short-sleeved shirts as well as shoes plus socks using a garden hose-end sprayer, "pump-up" compressed air sprayer, or backpack sprayer. The margin of exposures (MOEs) are >100; therefore are not of concern. With respect to residential post-application exposures, current HED policy (see ExpoSAC minutes from 8/19/99 and 10/11/01) specifies that no significant post-application exposure is anticipated from ornamentals, either by residents or professional applicators; therefore, no residential post-application assessment was conducted.

Aggregate Risk Assessment

Acute and chronic aggregate exposures include food plus drinking water exposures. As stated above, acute and chronic aggregate risks are not of concern. Since a common endpoint has been identified for assessment of short-term oral, dermal, and inhalation exposures, short-term aggregate risk assessment combines chronic dietary (food and water) exposure estimates with residential exposure estimates. The proposed residential scenarios result in exposure to only adults. Aggregate MOEs are ≥180 and are not of concern.

Triazole metabolites

The requested uses of difenoconazole did not result in an increase in dietary exposure estimates for free triazole or conjugated triazoles. Therefore, the last dietary exposure analyses for the triazole metabolites (M. Negussie, 28 Oct. 2009) have not changed.

Occupational Handler and Postapplication Exposure/Risk Assessment

Not applicable since this is an import tolerance.

Recommendations for Tolerances and Registration

Pending submission of a revised Section B for waxapple (see Section 10.0 under Directions for Use) and a revised Section F (see requirements under Proposed Tolerances), there are no residue chemistry issues that would preclude granting import tolerances for residues of difenoconazole resulting from the uses on mango in Brazil and waxapple in Taiwan:

Mango .....0.07 ppm
Waxapple .....1.5 ppm

Notes to PM:

1. With regards to the use on mangos in Brazil, HED notes that Mexico and not Brazil is the largest importer of mangos into the U.S. Since only data from Brazil were submitted, the registrant should be made aware that HED can not ascertain the adequacy of the recommended tolerance to cover residues incurred in other geographical regions of South America or Mexico from the same or similar use.

2. An error was found in MRID 47760601, Section 1.3 Test Item, bottom of Page 6 of 66. The stated nominal/actual formulation content in Amistar Top 325 SC of difenoconazole and azoxystrobin are reversed. According to the label, Amistar Top 325 SC contains 125 g/L of difenoconazole and 200 g/L azoxystrobin. The MRID should be corrected.

3. The registrant should be reminded that because difenoconazole is a triazole compound, HED requires that samples from any metabolism, feeding, field trial, and/or processing studies be analyzed for the triazole metabolites triazolylalanine (TA), triazolyl acetic acid (TAA), and 1,2,4-triazole (1,2,4-T). Guidance has been issued concerning these residue chemistry data requirements for the triazole-based metabolites under DP# 327788 (4/25/06, M. Doherty) and data submitted in support of future uses of difenoconazole will be accessed accordingly.

4. The registrant should be reminded that magnitude of the residue data for papaya, as a representative crop of the tropical fruit crops group, have been deemed adequate to support the same or substantially similar use on mango (among other tropical fruits) and the establishment of a separate mango tolerance. An import tolerance is currently established under §180.475(a)(1) for residues of difenoconazole in/on papaya at 0.3 ppm.

Environmental Justice Considerations

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf).

As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas postapplication are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and

farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

Review of Human Research

This risk assessment relies in part on data from studies in which adult human subjects were intentionally exposed to a pesticide or other chemical. These studies, which comprise the Pesticide Handlers Exposure Database (PHED) and information from the Agricultural Re-Entry Task Force (ARTF) and the Outdoor Residential Exposure Task Force (ORETF), have been determined to require a review of their ethical conduct, have received that review, and were considered appropriate (or ethically conducted) for use in risk assessments.

2.0 Ingredient Profile

2.1 Summary of Proposed Uses

(HED memo of B. Cropp-Kohlligian, 01/21/10, D366507)

A list of the end-use products (EPs) relevant to this import tolerance petition is presented in Table 2.1a. A summary of the use directions for these EPs is presented in Table 2.1b.

Table 2.1a. Summary of Difenoconazole End-Use Products Proposed for Use on Mango and Waxapple.

Trade Name	Country	a.i. (% of formulation)	Formulation Type	Proposed Crops	Target Pests	Label Date
Score®	Brazil	25% (250 g ai/L)	EC	Mango	Powdery mildew and anthracnose	10/30/02
(Hao Jia Zan) [1] Azoxystrobin + Difenoconazole	Taiwan	12.5%	SC	Waxapple	Anthracnose	undated, Section B

[1] The Taiwan end-use product is a soluble concentrate formulation containing 12.5% w/v difenoconazole and 20% w/v azoxystrobin.

Table 2.1b. Summary of Proposed Directions for Use of Difenoconazole.

Applic. Timing, Type, and Equip.	Product [Formulation]	Applic. Rate (lb ai/A) [g ai/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [g ai/ha]	PHI (days)	Use Directions and Limitations
Mango						
Foliar; Ground (500-1,000 L/ha spray volume)	Score® [250 g ai/L EC]	0.022-0.11 [25-125]	3	0.33 [375]	7	Applications must start immediately after the engorgement of floral gems or before flowers bloom, using smallest dose in the first applications to control powdery mildew and a larger dose to control anthracnose; 14-day RTI.
Waxapple						
Foliar; equipment not specified (NS)	Hao Jia Zan [12.5% w/v SC]	3,000x dilution[1]	5	NS	30	Only to be applied during fluorescence; 7-day RTI.

[1] The application rate could not be determined as the Section B only reported the concentration to be applied.

2.2 Structure and Nomenclature

The nomenclature of difenoconazole is summarized in Table 2.2a, and the physicochemical properties of difenoconazole are summarized in Table 2.2b.

Table 2.2a. Difenoconazole Nomenclature.

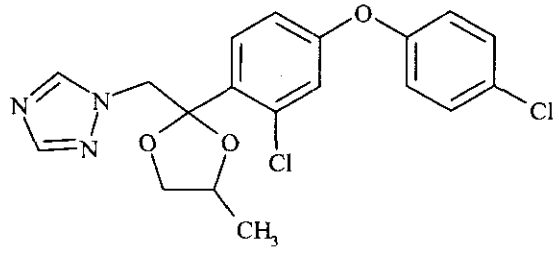
Chemical structure	
Common name	Difenoconazole
Company experimental name	CGA-169374
IUPAC name	1-({2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl}methyl)-1H-1,2,4-triazole
CAS name	1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]- 1H-1,2,4-triazole
CAS registry number	119446-68-3
End-use products (EP)	Score® - for mango grown in Brazil. 'Hao Jia Zan Azoxystrobin + Difenoconazole' - for waxapple grown in Taiwan

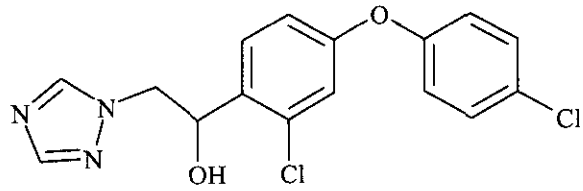
Table 2.2a. Difenoconazole Nomenclature.	
Chemical structure of CGA-205375 livestock metabolite	

Table 2.2b Physicochemical Properties of Difenoconazole.		
Parameter	Value	Reference
Melting point	78.6 °C	DP#s 172067 and 178394, 10/26/92, R. Lascola
pH	6-8 at 20 °C (saturated solution)	
Density	1.37 g/cm^3 at 20 °C	
Water solubility	3.3 ppm at 20 °C	
Solvent solubility	g/100 mL at 25 °C: n-hexane:0.5 1-octanol: 35 toluene: 77 acetone: 88 ethanol: 89	
Vapor pressure	2.5 x 10^{-10} mm Hg at 25 °C	
Dissociation constant, pK_a	Reported as <0 in MRID 42090003 which was previously reviewed in DP#s 172067 and 178394; however, questions have arisen concerning this value. Re-examination of MRID 42090003 found that the product chemistry data study to support the reported dissociation constant for the technical grade of difenoconazole was not included.	
Octanol/water partition coefficient, Log(K_{OW})	4.2 at 25 °C	DP#s 172067 and 178394, 10/26/92, R. Lascola
UV/visible absorption spectrum	λ_{max} at about 200 and 238 nm (in methanol at 26 °C)	PMRA Proposed Regulatory Decision Document on Difenoconazole, 4/14/99 (PRDD99-01)

3.0 Hazard Characterization/Assessment

(For detailed discussion, refer to HED memo of M. Sahafeyan, 11/09/07, D346591)

The toxicological database for difenoconazole is complete for the purpose of this risk assessment. However, a new data requirement for an Immunotoxicity study is required.

3.1 Hazard and Dose-Response Characterization

For a complete list of studies considered, see Appendix 1 and 2 under Section 13.0 of this document.

3.1.1 Toxicological Effects and Dose-response

Difenoconazole possesses low acute toxicity by the oral, dermal and inhalation routes of exposure. It is not considered to be an eye or skin irritant and is not a sensitizer.

In an acute neurotoxicity study in rats, reduced fore-limb grip strength was observed on day 1 in males and clinical signs of neurotoxicity in females at the limit dose of 2000 mg/kg. This effect in males is considered as transient since it was not observed at later observation points and toxicity in females was observed only at doses exceeding the limit dose. In a subchronic neurotoxicity study in rats decreased hind limb strength was observed only in males, which was considered as nonspecific in nature.

It is not a developmental or reproductive toxicant. Chronic effects in the rat study are seen as cumulative decreases in body weight gains. Similarly, chronic feeding studies in mice showed decreased body-weight gains in male and female mice at termination. In mice, treatment-related non-neoplastic lesions were confined to the liver and were supported by clinical chemistry data at a level of 300 ppm (46 and 58 mg/kg/day for males and females, respectively). No systemic toxicity was observed at the limit dose in a 28-day dermal toxicity study in rats. A dermal absorption of 15.3% was observed through rat skin using an *in vivo* method.

Difenoconazole is not mutagenic, and no evidence of carcinogenicity was seen in rats. Evidence for carcinogenicity was seen in mice, where liver tumors were induced at doses which were considered to be excessively high for carcinogenicity testing. Liver tumors were observed in mice at 300 ppm and higher; however, based on excessive toxicity observed at the two highest doses of 2500 and 4500 ppm (females terminated after two weeks due to excessive toxicity resulting in moribundity and death), the absence of tumors at two lower doses of 10 and 30 ppm and the absence of genotoxic effects, HED's Cancer Peer Review Committee (CPRC) recommended for a cancer classification of C (**possible human carcinogen**). A margin-of-exposure (MOE) approach in risk assessment was advocated by the CPRC utilizing the no-observable-adverse-effects-level (NOAEL) of **30** ppm (**4.7** and **5.6** mg/kg/day in males and females, respectively) and the lowest-observable-adverse-effects-level (LOAEL) of **300** ppm (**46** and **58** mg/kg/day in males and females, respectively) from the mouse study using only those biological endpoints which were relevant to tumor development (*i.e.*, hepatocellular hypertrophy, liver necrosis, fatty changes in the liver and bile stasis) (Memo, Jess Rowland and Esther Rinde, 27-JUL-1994; Memo, PV Shah, 1-March-2007, HED Doc. No. 005453).

The doses and toxicological endpoints selected for various exposure scenarios applicable to this risk assessment are summarized in Table 3.1.3a and Table 3.1.3b.

Table 3.1.3a. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Dietary and Non-Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, LOC for Risk Assessment	Study and Relevant Toxicological Effects
Acute Dietary (All populations)	NOAEL = 25 mg/kg	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	aRfD = aPAD = 0.25 mg/kg/day	**Acute Neurotoxicity Study in Rats** LOAEL= 200 mg/kg in males based on reduced fore-limb grip strength in males on day 1.
Chronic	NOAEL =	UF_A = 10X	cRfD = cPAD =	**Combined chronic**

Table 3.1.3a. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Dietary and Non-Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, LOC for Risk Assessment	Study and Relevant Toxicological Effects
Dietary (All populations)	0.96 mg/kg/day	UF_H = 10X UF_{FQPA} = 1X	0.01mg/kg/day	**toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Incidental Oral Short- and Intermediate-Term (1-30 days and 1-6 months)	NOAEL = 1.25 mg/kg/day	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight of F_1 males.
Dermal Short- and Intermediate-Term (1-30 days and 1-6 months)	Oral NOAEL = 1.25 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight gain of F_0 females prior to mating, gestation and lactation.
Dermal Long-Term (>6 months)	Oral NOAEL = 0.96 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Inhalation (Short- and Intermediate-term)	Oral NOAEL = 1.25 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body weight gain of F_0 females prior to mating, gestation and lactation.
Inhalation (Long- term)	Oral NOAEL = 0.96 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X UF_{FQPA} = 1X	Residential LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body weight gains.
Cancer (oral, dermal, inhalation)	Difenoconazole is classified as a Group C, possible human carcinogen with a non-linear (MOE) approach for human risk characterization (CPRC Document, 7/27/94, Memo, P. V. Shah dated March 3, 2007, HED Doc. No. 0054532).			

Abbreviations: UF = uncertainty factor, UF_A = extrapolation from animal to human (interspecies), UF_H = potential variation in sensitivity among members of the human population (intraspecies), UF_{FQPA} = FQPA

Safety Factor, NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect level, RfD = reference dose (a = acute, c = chronic), PAD = population-adjusted dose, MOE = margin of exposure, LOC = level of concern.

Table 3.1.3b. Summary of Toxicological Doses and Endpoints for Difenoconazole for Use in Occupational Human-Health Risk Assessments.

Exposure Scenario	Point of Departure	Uncertainty/FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal Short- and Intermediate-Term (1-30 days and 1-6 months)	Oral NOAEL = 1.25 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X	Occupational LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight gain of F_0 females prior to mating, gestation and lactation.
Dermal Long-Term (>6 months)	Oral NOAEL = 0.96 mg/kg/day Dermal Absorption factor=15.3%	UF_A = 10X UF_H = 10X	Occupational LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Inhalation (Short- and Intermediate-term)	Oral NOAEL = 1.25 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X	Occupational LOC for MOE<100	**Reproduction and fertility effects (rat; dietary)** Offspring LOAEL = 12.5 mg/kg/day based on reduction in body-weight gain of F_0 females prior to mating, gestation and lactation.
Inhalation (Long- term)	Oral NOAEL = 0.96 mg/kg/day 100% inhalation absorption assumed	UF_A = 10X UF_H = 10X	Occupational LOC for MOE<100	**Combined chronic toxicity/carcinogenicity (rat; dietary)** LOAEL = 24.1/32.8 mg/kg/day (M/F) based on cumulative decreases in body-weight gains.
Cancer (oral, dermal, inhalation)	Difenoconazole is classified as a Group C, possible human carcinogen with a non-linear (MOE) approach for human risk characterization (CPRC Document, 7/27/94, Memo, P. V. Shah dated March 3, 2007, HED Doc. No. 0054532).			

Abbreviations: UF = uncertainty factor, UF_A = extrapolation from animal to human (interspecies), UF_H = potential variation in sensitivity among members of the human population (intraspecies), UF_{FQPA} = FQPA Safety Factor, NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed-adverse-effect level, RfD = reference dose (a = acute, c = chronic), PAD = population-adjusted dose, MOE = margin of exposure, LOC = level of concern.

3.2 FQPA Considerations

3.2.1. Determination of Susceptibility

The Hazard Identification Assessment Review Committee (HIARC) determined that the available Agency Guideline studies indicated no increased susceptibility of rats or rabbits to in utero and/or postnatal exposure to difenoconazole. In the prenatal developmental toxicity studies in rats and rabbits and the two-generation reproduction study in rats, toxicity to the fetuses/offspring, when observed, occurred at equivalent or higher doses than in the maternal/parental animals. In the prenatal developmental toxicity study in rats, maternal toxicity was manifested as decreased body weight gain and food consumption at the LOAEL of 85 mg/kg/day; the NOAEL was 16 mg/kg/day. The developmental toxicity was manifested as alterations in fetal ossifications at 171 mg/kg/day; the developmental NOAEL was 85 mg/kg/day. In a developmental toxicity study in rabbits, maternal and developmental toxicity were seen at the same dose level (75 mg/kg/day). Maternal toxicity in rabbits were manifested as decreased in body weight gain and decreased in food consumption, while developmental toxicity was manifested as decreased fetal weight. In a 2-generation reproduction study in rats, there were decreases in maternal body weight gain and decreases in body weights of F1 males at the LOAEL of 12.5 mg/kg/day; the parental systemic and off spring toxicity NOAEL was 1.25 mg/kg/day.

3.2.2. Adequacy of Toxicity Database

There are no data gaps for the assessment of the effects of difenoconazole following in utero and/or postnatal exposure. The acute and subchronic neurotoxicity studies in rats are available. In an acute neurotoxicity study in rats, reduced fore-limb grip strength was observed on day 1 in males. This effect is considered as transient since it was not observed at later observation points. In a subchronic neurotoxicity study in rats decreased hind limb strength was observed only in males, which was considered as nonspecific in nature. Difenoconazole exhibits some evidence of neurotoxicity in the database, but the effects are transient or occur at doses exceeding the limit dose. EPA concluded that difenoconazole is not a neurotoxic compound. Based on the toxicity profile, and lack of neurotoxicity, a developmental neurotoxicity study in rats is not required.

3.2.3. Degree of Concern Analysis:

Since there is no evidence of susceptibility, there is no concern for increased susceptibility due to exposure to difenoconazole.

3.2.4. FQPA Safety Factor Recommendation

The FQPA factor for increased susceptibility to infant and children is reduced to 1x for the following considerations:

1) toxicology data base for difenoconazole is complete;

2) there is no indication of increased susceptibility of rats or rabbit fetuses to in utero and/or postnatal exposure in the developmental and reproductive toxicity data;
3) there are no concerns for neurotoxicity;
4) developmental neurotoxicity study is not required;
5) there are no residual uncertainties in the toxicology database.

3.3 Endocrine Disruption

As required under FFDCA section 408(p), EPA has developed the Endocrine Disruptor Screening Program (EDSP) to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. This list of chemicals was selected based on the potential for human exposure through pathways such as food and water, residential activity, and certain post-application agricultural scenarios. This list should not be construed as a list of known or likely endocrine disruptors.

Difenoconazole is not among the group of 58 pesticide active ingredients on the initial list to be screened under the EDSP. Under FFDCA sec. 408(p) the Agency must screen all pesticide chemicals. Accordingly, EPA anticipates issuing future EDSP test orders/data call-ins for all pesticide active ingredients.

For further information on the status of the EDSP, the policies and procedures, the list of 67 chemicals, the test guidelines and the Tier 1 screening battery, please visit our website: http://www.epa.gov/endo/.

4.0 Public Health and Pesticide Epidemiology Data

Not relevant since these are new uses.

5.0 Dietary Exposure/Risk Characterization

5.1 Pesticide Metabolism and Environmental Degradation

5.1.1 Metabolism in Primary Crops

The nature of the residue in plants is understood based on acceptable plant metabolism studies reflecting foliar applications in canola, grape, potato, tomato, and wheat and seed treatment in wheat. Based on the results of available plant metabolism studies, the petitioner has proposed that difenoconazole is metabolized in plants by the hydroxylation of the phenyl ring and/or cleavage of the dioxolane ring followed by cleavage of the carbon-carbon bridge between the phenyl and triazole rings. The metabolic pathway appears to proceed by hydrolysis of the ketal to the ketone followed by reduction of the ketone (CGA-205374) to the alkanol (CGA 205375). CGA 205375 can be conjugated with sugars or the bridge linking the phenyl and triazole moieties is cleaved. HED concluded that the residue of concern for both tolerance enforcement and risk assessment for crops included in this petition is difenoconazole *per se*.

5.1.2 Metabolism in Rotational Crops

The nature of the residue in rotational crops is not adequately understood because previously conducted studies did not reflect sufficiently high application rates, and/or insufficient characterization/identification of residues was achieved. An additional confined rotational crop study reflecting phenyl-ring labeling must be conducted at 1x the proposed maximum seasonal foliar application rate (0.46 lb ai/A). An acceptable limited field rotational crop study is available; these data will be reevaluated when the outstanding confined rotational crop study is received. In the meantime, this subject is not relevant since this is an import tolerance.

5.1.3 Metabolism in Livestock

The nature of the residue in livestock is understood based on acceptable goat and hen metabolism studies. The data were originally evaluated in support of seed treatment uses only, and HED concluded that the residue of concern in livestock commodities was difenoconazole *per se*. When the first foliar uses of difenoconazole on crop commodities were proposed (DP# 340379), HED re-evaluated the livestock metabolism data and concluded that the residues of concern for both tolerance setting and risk assessment for livestock commodities are difenoconazole and metabolite CGA-205375.

5.1.4 Analytical Methodology

An adequate tolerance enforcement method, method AG-575B, is available for crop commodities. The method determines residues of difenoconazole *per se* in/on crop commodities by gas chromatography with nitrogen-phosphorus detection (GC/NPD).

The method limits of quantitation (LOQs) are 0.01-0.05 ppm. A confirmatory GC method with mass-selective detection (MSD) is also available for crop commodities. Samples from the submitted the crop field trials were analyzed for residues of difenoconazole using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), Syngenta REM 147.08, or a similar method. The methods are adequate for data collection based on acceptable concurrent method recoveries. The LOQ was 0.01 ppm for difenoconazole in mango and waxapple.

5.1.5 Environmental Degradation

In soil environment, difenoconazole is persistent and slightly mobile. Difenoconazole has low potential to reach ground water, except in soils of high sand and low organic matter content. It is likely to reach surface sources of drinking water via spray drift and runoff. In the aquatic environment its main route of dissipation is partitioning into the bottom sediment, and potentially relatively fast to slow aqueous photolysis in clear water conditions.

Major degradates include CGA 205375 which was found in lab accumulation in fish at 51-64% applied dose, and in aerobic soil at 14.8%, in aerobic aquatic at 11.6% and anaerobic aquatic at 12.6% of the applied dose, and CGA 71019 (triazole) and CGA-142856 (TAA). Since triazole and TAA are common metabolites from a group of chemicals, they should be addressed separately. (See Appendix 4 for structures)

5.1.6 Comparative Metabolic Profile

Rat metabolism studies (MRID 42090028 through 31, and 42710013 through14) indicated that difenoconazole was rapidly adsorbed and mainly eliminated vis bile. Three major urinary metabolites were isolated and further identified as sulfate conjugates of CGA 205375, isomers of CGA 205375, and the hydroxyacetic metabolite of CGA 205375. Further metabolism formed free triazoles. The proposed metabolic pathway in rats is presented in Appendix 3.

Comparisons of the metabolisms indicated that the metabolic pathways in plants, livestock, rats, and the environment are very similar or identical, with the formation of CGA 205375 and then further metabolism to form free triazole metabolites.

5.1.7 Toxicity Profile of Major Metabolites and Degradates

Other than the triazole metabolites, no toxicity information is available on the CGA 205375 metabolite. Based on structural similarity, it is assumed that the CGA 205375 shares the same toxicity as the parent.

5.1.8 Pesticide Metabolites and Degradates of Concern

The most recent MARC evaluation for difenoconazole was in 1994. In 2007, HED reviewers re-evaluated the plant and livestock metabolism studies based on new data submitted in conjunction with the new uses. It was concluded that the residue of concern for both tolerance enforcement and risk assessment for currently registered crops is difenoconazole *per se*. For livestock, the residues of concern for both tolerance setting and risk assessment for livestock commodities are difenoconazole and its metabolite CGA-205375. With the subject petition, HED determined that previous conclusions on plants and livestock stand. As for drinking water assessment, HED tentatively concludes that the residues of concern are parent and CGA 205375.

5.1.9 Drinking Water Residue Profile

Although the subject petition is for import tolerances and therefore does not result in drinking water exposure, there are existing uses of difenoconazole in the U.S. The most recent drinking water assessment was conducted for parent compound only. The fate and transport database for difenoconazole were sufficient to conduct the drinking water assessment. The Tier II drinking water assessment was performed using PRZM (v3.12.2; May 12, 2005)/EXAMS (v. 2.98.04.06; April 25, 2005) modeling with index reservoir (IR) scenarios and percent cropped area (PCA) adjustment factors. The assessment was based on difenoconazole uses on citrus fruits and grapes. These are major crops, with difenoconazole maximum application rate to citrus fruits being the highest of all agricultural uses, and grapes being the second highest. An initial simulation analysis showed that grapes would produce the highest EDWCs of all proposed crop uses with the same maximum application rate of 0.46 lb ai/A hence grapes and citrus fruits were modeled.

Florida and California citrus fruit scenarios were modeled for citrus fruits, and New York and California grapes scenarios were modeled for grapes. Default PCA of 0.87 was used for surface water models. Among all the registered and proposed new uses, the highest estimated drinking water concentrations (EDWCs) from surface water sources were derived for aerial applications of difenoconazole to New York grapes at the maximum annual application rate of 0.46 lb ai/acre. The recommended peak and mean estimated drinking water concentrations (EDWCs) for the human health risk assessment are provided in Table 1. These estimates exceed the previously EDWCs from 2007 drinking water assessment (D333319).

Table 1. PCA Adjusted Difenoconazole EDWCs from Surface Water Sources.

Scenario	Application Type/Annual Fungicide Application Rate (lb ai/A))	Estimated Drinking Water Concentrations (µg/L)[a]		
		1 in 10 year annual peak	1 in 10 year annual mean	36 year annual mean
NY Grape	aerially applied 0.114 x 4 = 0.46	15.8	10.4	7.62

[a] EXAMS EECs multiplied by 0.87, a default PCA factor.

The highest SCI-GROW estimated drinking water concentration of difenoconazole from shallow ground water sources is 1.23 x 10^{-2} µg/L derived for the maximum proposed application rate to citrus fruit (0.50 lb ai/A), i.e. agricultural uses. Based on the previous drinking water assessment, this estimate is lower than an estimate for non-agricultural uses 1.28 x 10^{-2} µg/L, obtained for the maximum application rate for ornamentals (0.52 lb ai/A; D333319). These concentrations can be considered as both the acute and chronic values.

Currently, no data are available indicating whether water treatment process will increase dissipation and/or will form degradation products that may be more toxic than the parent.

5.1.10 Food Residue Profile

(HED memo of B. Cropp-Kohlligian, 01/21/10, D366507)

HED has examined the residue chemistry database for difenoconazole and no major residue deficiencies will prevent the establishment of permanent tolerances for the proposed uses. For details on data deficiencies, please see **Section 10.0**.

The nature of the residue in plants is understood based on acceptable plant metabolism studies reflecting foliar applications in canola, grape, potato, tomato, and wheat, and seed treatment in wheat. HED concludes that the residue of concern for both tolerance enforcement and risk assessment for crops included in this petition is difenoconazole *per se*. The nature of the residue in livestock is understood based on acceptable goat and hen metabolism studies. The residues of concern for both tolerance setting and risk assessment for livestock commodities are difenoconazole *per se* and its metabolite CGA-205375.

An adequate tolerance enforcement method, method AG-575B, is available for crop commodities. The method determines residues of difenoconazole *per se* in/on crop commodities by gas chromatography with nitrogen-phosphorus detection (GC/NPD). The method limits of quantitation (LOQs) are 0.01-0.05 ppm. A confirmatory GC method with mass-selective detection (MSD) is also available for crop commodities. Samples from the submitted the crop field trials were analyzed for residues of difenoconazole using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), Syngenta REM 147.08, or a similar method. The methods are adequate for data collection based on acceptable concurrent method

recoveries. The LOQ was 0.01 ppm for difenoconazole in mango and waxapple.

The submitted field trial data for mango are adequate and reflect the proposed use of Score® on mango in Brazil. The residue data from the trials conducted will support the proposed import tolerance of 0.09 ppm for mango. A Codex MRL for residues of difenoconazole *per se* has been established at 0.07 ppm for mango. To harmonization with the established Codex MRL, HED recommends a tolerance of 0.07 ppm for mango. HED is unable to assess the adequacy of the submitted field trial data for waxapple at this time because the use directions have not been fully elucidated. Syngenta is required to provide label use information as detailed under 'Directions for Use' section. Assuming that the use directions for waxapple in Taiwan, once fully elucidated, will comport with the submitted field trial data, HED tentatively recommends for the proposed import tolerance of 1.5 ppm for waxapple.

There are no feedstuffs associated with the foreign uses of difenoconazole on mango in Brazil and waxapple in Taiwan. Therefore, no livestock enforcement methods, storage stability data, or feeding studies are required to support this petition.

5.1.11 International Residue Limits

A Codex MRL for residues of difenoconazole *per se* has been established at 0.07 ppm for mango. Canadian and Mexican MRLs have been established for difenoconazole; however, no MRLs have been established for mango. No Codex, Canadian, and Mexican MRLs have been established for residues of difenoconazole in/on waxapple.

5.2 Dietary Exposure and Risk

(HED memo of T. Morton, 01/21/10, D371613)

Residue Data used for Acute and Chronic Assessments:

The unrefined acute analysis assumed tolerance-level residues, 100% crop treated (CT), and the available empirical or DEEM™ (ver. 7.81) default processing factors. The somewhat refined chronic analysis assumed tolerance-level residues for some commodities, average field trial residues for the majority of commodities, the available empirical or DEEM™ (ver. 7.81) default processing factors, and 100 % CT.

The estimated drinking water residues for 1-in-10 year annual peak (15.8µg/L) was used for the acute dietary exposure assessment, while 1-in-10 year annual mean (10.4 µg/L) was used for chronic. HED notes that degradate CGA 205375 was not included in the drinking water assessment; however, the relative amount of CGA 205375 is not significant in comparison to the parent.

DEEM-FCID™ Program and Consumption Information

Difenoconazole acute and chronic dietary exposure assessments were conducted using the

DEEM-FCID™ (ver. 2.03), which incorporates consumption data from USDA's CSFII (1994-1996 and 1998). The 1994-96, 98 data are based on the reported consumption of more than 20,000 individuals over two non-consecutive survey days. Foods "as consumed" (*e.g.*, apple pie) are linked to EPA-defined food commodities (*e.g.*, apples, peeled fruit - cooked; fresh or N/S; baked; or wheat flour - cooked; fresh or N/S, baked) using publicly available recipe translation files developed jointly by USDA/ARS and EPA. For chronic exposure assessment, consumption data are averaged for the entire U.S. population and within population subgroups, but for acute exposure assessment are retained as individual consumption events. Based on analysis of the 1994-96, 98 CSFII consumption data, which took into account dietary patterns and survey respondents, HED concluded that it is most appropriate to report risk for the following population subgroups: the general U.S. population, all infants (<1 year old), children 1-2, children 3-5, children 6-12, youth 13-19, adults 20-49, females 13-49, and adults 50+ years old.

Results and Discussion

The resulting acute food exposure estimates were less than HED's level of concern (-<100% of the acute population-adjusted dose (aPAD)) at the 95th percentile of the exposure distribution for the general U.S. population (7 % aPAD) and all population sub-groups; the most highly exposed population subgroup was children1-2 years old with 16 % aPAD. The resulting chronic food exposure estimates were less than HED's level of concern (<100% of the chronic population-adjusted dose (cPAD)) for the general U.S. population (17 % cPAD) and all population sub-groups; the most highly exposed population subgroup was children 1-2 years old with 45 % cPAD. A cancer dietary assessment was not conducted for difenoconazole because the cancer NOAEL is higher than the chronic RfD; therefore, the chronic dietary risk estimate is more protective.

Table 5.2a. Summary of Acute Dietary Exposure and Risk for Difenoconazole at the 95th Percentile.

Population Subgroup	aPAD (mg/kg/day)	Exposure (mg/kg/day)	%aPAD
General U.S. Population	0.25	0.017747	7
All Infants (< 1 year old)		0.0255139	10
Children 1-2 years old		**0.039565**	**16**
Children 3-5 years old		0.031818	13
Children 6-12 years old		0.017949	7
Youth 13-19 years old		0.008828	4
Adults 20-49 years old		0.013083	5
Adults 50+ years old		0.017255	7
Females 13-49 years old		0.013281	5

The bolded %aPAD is the highest.

Table 5.2b. Summary of Chronic Dietary Exposure and Risk for Difenoconazole.

Population Subgroup	cPAD (mg/kg/day)	Exposure (mg/kg/day)	%cPAD

Table 5.2b. Summary of Chronic Dietary Exposure and Risk for Difenoconazole.			
Population Subgroup	**cPAD (mg/kg/day)**	**Exposure (mg/kg/day)**	**%cPAD**
General U.S. Population	0.01	0.001709	17
All Infants (< 1 year old)		0.002445	25
Children 1-2 years old		**0.004484**	**45**
Children 3-5 years old		0.003624	36
Children 6-12 years old		0.002061	21
Youth 13-19 years old		0.001372	14
Adults 20-49 years old		0.001376	14
Adults 50+ years old		0.001548	16
Females 13-49 years old		0.001402	14

The bolded %cPAD is the highest.

The requested uses of difenoconazole did not result in an increase in dietary exposure estimates for free triazole or conjugated triazoles. Therefore, the last dietary exposure analyses for the triazole metabolites (M. Negussie, 28 Oct. 2009) have not changed.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

(HED memo of M. Sahafeyan, 11/09/07, D346591)

6.1 Residential Handler Exposure and Risk Characterization

No new residential uses are being requested at this time. However, adults and adolescents may be exposed to difenoconazole from its currently registered use on ornamentals. These risks have been previously assessed. Below are the results from HED's previous assessment:

HED believes residential pesticide handlers will be exposed to short-term duration (1 - 30 days) only. The dermal and inhalation (short-term) residential exposure was assessed for "homeowners" mixer/loader/applicator wearing short pants and short-sleeved shirts as well as shoes plus socks using garden hose-end sprayer, "pump-up" compressed air sprayer, and backpack sprayer. A MOE of 100 is adequate to protect residential pesticide handlers from exposures to difenoconazole. MOEs are >100; therefore are not of concern. A summary of these exposures and risks is presented in Table 6.1.

Table 6.1 Summary of Exposure & Risk for Homeowners Applying Difenoconazole.				
Unit Exposure[1] mg ai/lb handled	Applic. Rate[2] lb ai/unit	Units Treated[3]	Avg. Daily Exposure[4] mg ai/kg bw/day	Short-term MOE[5]
Mixer/Loader/Applicator Using Garden Hose-end Sprayer				
Dermal:	0.13 lb ai/A		Dermal:	

SS&SP 11 Inhal. 0.017		0.5 A/day	shrtsl&pants 0.00156 Inhal. 0.0000158	790
Mixer/Loader/Applicator Using "Pump-Up" Compressed Air Sprayer				
Dermal: SS&SP 38 Inhal 0.0027	0.13 lb ai/A	0.5 A/day	Dermal: shrtsl&pants 0.00539 Inhal. 0.0000025	230
Mixer/Loader/Applicator Using Backpack Sprayer				
Dermal: SS&SP 5.1 Inhal. 0.03	0.13 lb ai/A	0.5 A/day	Dermal: shrtslv&pants 0.000725 Inhal. 0.000028	1,700

1. Unit Exposures are taken from "PHED SURROGATE EXPOSURE GUIDE", Estimates of Worker Exposure from The Pesticide Handler Exposure Database Version 1.1, August 1998. Inhal. = Inhalation. Units = mg a.i./pound of active ingredient handled. Unit exposures are also taken from ORETF studies OMA 004,OMA006 and from the Draft Residential SOPs, DECEMBER 1997. SS & SP = short sleeved shirt and short pants. LS & LP = long sleeved shirt and long pants.
2. Applic. Rate. = Taken from the draft Inspire® label.
3. Units Treated are taken the residential SOPs.
4. Average Daily Dose (ADD) = Unit Exposure * Applic. Rate * Units Treated * absorption factor (15.3 % for dermal) ÷ Body Weight (70 kg).
5. NOAEL = No Observable Adverse Effect Level (1.25 mg a.i./kg bw/day for short-term and intermediate-term dermal and inhalation).
6. MOE = Margin of Exposure = No Observable Adverse Effect Level (NOAEL) ÷ ADD. ADD = dermal + inhalation.

With respect to residential post-application exposures, current HED policy (see ExpoSAC minutes from 8/19/99 and 10/11/01) specifies that no significant post-application exposure is anticipated from ornamentals, either by residents or professional applicators; therefore, no residential post-application assessment was conducted.

7.0 Aggregate Risk Assessments and Risk Characterization

In accordance with the FQPA, HED must consider and aggregate (add) pesticide exposures and risks from three major sources: food, drinking water, and residential exposures. In an aggregate assessment, exposures from relevant sources are added together and compared to quantitative estimates of hazard (e.g., a NOAEL or PAD), or the risks themselves can be aggregated. When aggregating exposures and risks from various sources, HED considers both the route and duration of exposure.

7.1 Acute & Chronic Aggregate Risk

Acute and chronic aggregate exposures include food plus drinking water exposures. As demonstrated under Section 5.2. Acute and chronic aggregate risks are not of concern.

7.2 Short- and Intermediate-Term Aggregate Risk

Short-Term Aggregate Risk Assessment: Since a common endpoint has been identified for assessment of short-term oral, dermal, and inhalation exposures (changes in body weights and body-weight gains) the short-term aggregate risk assessment considered

exposure from food, water, and residential sources. Since the doses corresponding to the identified oral, dermal, and inhalation endpoints were different but the level of concern for all three routes of exposure are identical, the short-term aggregate exposures were calculated using the 1÷MOE approach. HED combines chronic dietary (food and water) exposure estimates with residential exposure estimates when conducting short-term aggregate risk assessments. Short-term exposure has been defined as from 1- 30 days and HED has concluded that chronic dietary exposure estimates will more accurately reflect actual dietary exposure over these time periods than will high-end acute-dietary exposures. The proposed residential scenarios result in exposure to only adults. Therefore, short-term aggregate assessments were not conducted for infants and children. Table 7.2 is a summary of the short-term aggregate exposures and risk estimates. Since the aggregate MOEs are ≥180, short-term aggregate exposure to difenoconazole is not of concern.

Table 7.2. Short-Term Aggregate Risk Calculations for Handlers

Population	Target Aggregate MOE[1]	dietary MOE[2]	dermal + inhalation MOE[3]	agg. MOE (dietary and residential)[4]
Youth 13-19 years old	100	910	230	180
Adults 20-49 years old		910		180
Adults 50+ years old		810		180
Females 13-49 years old		890		180

[1] total uncertainty factor for all routes of exposure is 100x; therefore, the target MOE is 100.
[2] dietary MOE = short-term incidental oral NOAEL ÷ chronic dietary exposure.
[3] dermal MOE = short-term dermal NOAEL ÷(dermal + inhalation residential exposure) (see text).
[4] aggregate MOE (dietary and residential) = $1 \div ((1 \div MOE_{dietary}) + (1 \div MOE_{dermal}) + (1 \div MOE_{inhalation}))$.

8.0 Cumulative Risk Characterization/Assessment

Difenoconazole is a member of the triazole-containing class of pesticides. Although conazoles act similarly in plants (fungi) by inhibiting ergosterol biosynthesis, there is not necessarily a relationship between their pesticidal activity and their mechanism of toxicity in mammals. Structural similarities do not constitute a common mechanism of toxicity. Evidence is needed to establish that the chemicals operate by the same, or essentially the same, sequence of major biochemical events (EPA, 2002). In conazoles, however, a variable pattern of toxicological responses is found. Some are hepatotoxic and hepatocarcinogenic in mice. Some induce thyroid tumors in rats. Some induce developmental, reproductive, and neurological effects in rodents. Furthermore, the conazoles produce a diverse range of biochemical events including altered cholesterol levels, stress responses, and altered DNA methylation. It is not clearly understood whether these biochemical events are directly connected to their toxicological outcomes. Thus, there is currently no evidence to indicate that conazoles share common mechanisms of toxicity and EPA is not following a cumulative risk approach based on a common mechanism of toxicity for the conazoles. For information regarding EPA's procedures for cumulating effects from substances found to have a common mechanism of toxicity,

see EPA's website at http://www.epa.gov/pesticides/cumulative.

Difenoconazole is a triazole-derived pesticide. This class of compounds can form the common metabolite 1,2,4-triazole and two triazole conjugates (triazolylalanine and triazolylacetic acid). To support existing tolerances and to establish new tolerances for triazole-derivative pesticides, including difenoconazole, U.S. EPA conducted a human health risk assessment for exposure to 1,2,4-triazole, triazolylalanine, and triazolylacetic acid resulting from the use of all current and pending uses of any triazole-derived fungicide. The risk assessment is a highly conservative, screening-level evaluation in terms of hazards associated with common metabolites (e.g., use of a maximum combination of uncertainty factors) and potential dietary and non-dietary exposures (i.e., high end estimates of both dietary and non-dietary exposures). In addition, the Agency retained the additional 10X FQPA safety factor for the protection of infants and children. The assessment includes evaluations of risks for various subgroups, including those comprised of infants and children. The Agency's complete risk assessment is found in the propiconazole reregistration docket at http://www.regulations.gov, Docket Identification (ID) Number EPA-HQ-OPP-2005-0497.

9.0 Occupational Exposure/Risk Pathway

Not applicable since this is a import tolerance.

10.0 Data Needs and Label Recommendations

Residue Chemistry

<u>860.1200 Directions for Use - Waxapple</u>

- Syngenta must submit a complete description of the use of difenoconazole on waxapple grown in Taiwan (a translated label or complete Section B). As required by the NAFTA Guidance Document on Data Requirements for Tolerances on Imported Commodities in the United States and Canada (December 2005), it is necessary to submit copies of registered/approved label(s) translated to English. The information must include, but is not limited to, the maximum single application rate, the maximum annual application rate, application timing (as it relates to plant growth stage), retreatment interval, application tank-mix preparation, volume of spray mix per unit area, application equipment, and the preharvest interval. The application rates should be expressed in units of pounds of active ingredient per acre (or kilograms per hectare).

- Syngenta must submit a complete description of the use of difenoconazole on waxapple grown in Taiwan consistent with the use profile of the submitted field trial data (MRID 47760601). The submitted field trial data will support eight foliar applications of a 125 g/L suspension concentrate (SC) formulation of

difenoconazole at a target rate of 0.39 kg ai/ha/application (0.34 lb ai/A/application), with 6- to 8-day retreatment intervals, for a total rate of 3.07 kg ai/ha (2.74 lb ai/A) and a 12-day PHI. Applications may be made using ground equipment in 2,000 L/ha spray volumes (214 gal/A which is considered a dilute spray volume), without an adjuvant.

- Consistent with the submitted field trial data, applications should be restricted to fruit protected with exclusion net bags or otherwise bagged fruit.

860.1550 Proposed Tolerances

- The proposed tolerances should be revised to reflect the recommended tolerance levels and correct commodity definitions as specified in Table 12.0.

Toxicity

- Immunotoxicity study (870.7800)

In accordance with Part 158 Toxicology Data requirements, an immunotoxicity study (870.7800) is required for difenoconazole. In the absence of specific immunotoxicity studies, EPA has evaluated the available difenoconazole toxicity data to determine whether an additional database uncertainty factor is needed to account for potential immunotoxicity. There are no indications in the available studies that organs associated with immune function, such as the thymus and spleen, are affected by difenoconazole, and difenoconazole does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. Therefore, EPA does not believe that conducting immunotoxicity testing will result in a point of departure lower than those already selected for difenoconazole risk assessment, and an additional database uncertainty factor is not needed to account for the lack of this study.

ORE

- none

11.0 References:

1. HED memo of B. Cropp-Kohlligian, 01/28/10, "Difenoconazole. Import Tolerance Request on Mango and Waxapple (also known as Wax jambu). Summary of Analytical Chemistry and Residue Data. D371612.

2. HED memo of T. Morton, 01/21/10, "**Difenoconazole.** Acute and Chronic Aggregate Dietary Exposure and Risk Assessments for the Section 3 Registration Request for Mango and Wax Apple. D371613.

3. EFED memo of I. Maher, 05/28/09, Difenoconazole (Parent Only) Drinking Water Assessment in Support of New Use Registration Action for Bulb Vegetables, Brassica (Cole) Leafy Vegetables, Cucurbit Vegetables, Citrus Fruit, Grapes, and Tree Nuts".

12.0 Tolerance Summary

The proposed tolerances should be revised to reflect the recommended tolerance levels and correct commodity definitions as specified in Table 12.0.

Table 12.0. Tolerance Summary for Difenoconazole.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments; *Correct Commodity Definition*
Mango, fruit	0.09	0.07	*Mango*
Waxapple, fruit	1.5	1.5	*Wax jambu*

13.0 Appendices

Appendix 1: Acute Toxicity Data on Difenoconazole Technical

Table 13.0a Acute Toxicity Profile - Difenoconazole

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral rat	42090006	LD_{50} = 1450 mg/kg	III
870.1200	Acute dermal rat	42090007	LD_{50} > 2010 mg/kg	III
870.1300	Acute inhalation rat	42090008	LC_{50} > 3.3 mg/L	III
870.2400	Acute eye irritation rabbit	42090009	Mild ocular irritation reversible in 7 days	III
870.2500	Acute dermal irritation rabbit	42090010	Slight irritation	IV
870.2600	Skin sensitization mouse	42090011, 42710004	Negative	N/A

Appendix 2: Subchronic, Chronic and Other Toxicity Profile

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100	90-Day oral toxicity (rat)	42090022 (1987) Acceptable/guideline 0, 20, 200, 750, 1500 or 3000 ppm 0, 1, 10, 37.5, 75 and 150 mg/kg/d	NOAEL = 20 ppm (1 mg/kg/day) LOAEL = 200 ppm (10 mg/kg/day) based on the 10% decrease in body weight in the 200 ppm females (as well as a negative trend in feed consumption) and Increases in absolute liver weights in both sexes
870.3100	90-Day oral toxicity (mouse)	42090021 (1987) Minimum/guideline 0, 20, 200, 2500, 7500 or 15,000 ppm M: 0, 2.9, 30.8, 383.6, 1125 and 2250 mg/kg/d F: 0, 4.1, 41.5, 558.9, 1125 and 2250 mg/kg/d	NOAEL = 20 ppm (2.9 mg/kg/day) LOAEL = 200 ppm (30.8 mg/kg/day) based on body weight changes & liver histopathology.
870.3150	26-Week oral toxicity	42090012 (1987) Minimum/ guideline 0, 100, 1000, 3000 or 6000 ppm M: 0, 3.6, 31.3, 96.6 and 157.8 mg/kg/d F: 0, 3.4, 34.8, 110.6 and 203.7 mg/kg/d	NOAEL = 3000 ppm (31.3 mg/kg/day in males/34.8 mg/kg/day in females) LOAEL = 6000 ppm (96.6 mg/kg/day in males/110.6 mg/kg/day in females), based primarily on microscopic examination of CGA 169374-related lenticular cataracts.
870.3200	21/28-Day dermal toxicity (rat)	42090013 (1987) Minimum/ guideline 0, 10, 100 and 1000 mg/kg/d	NOAEL = 10 mg/kg/day LOAEL = 100 mg/kg/day based on statistically significant decrements in body weight, body weight gain, and food consumption.
870.3200	21/28-Day dermal toxicity (rat)	46950310 (2000) Acceptable/ guideline 0, 10, 100 and 1000 mg/kg/d	NOAEL (systemic) = 1000 mg/kg/day LOAEL (systemic) was not determined. NOAEL (dermal) = 100 mg/kg/day LOAEL (dermal) = 1000 mg/kg/day based on hyperkeratosis at the skin application site.

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3700a	Prenatal developmental in (rat)	42090016, 42710007 (1987) Minimum/ guideline 0, 2, 20, 100 or 200 mg/kg/d from GD 6-15 (nominal doses differed widely from theoretical, this required altering NOAEL/LOAEL values)	**Maternal** NOAEL = 16 mg/kg/day LOAEL = 85 mg/kg/day based on decreased body weight gain and food consumption. **Developmental** NOAEL = 85 mg/kg/day LOAEL = 171 mg/kg/day based on alterations in fetal ossification.
870.3700b	Prenatal developmental in (rabbit)	42090017, 42710008 (1987) Minimum/ guideline 0, 1, 25 or 75 mg/kg/d from GD 7-19	**Maternal** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on decreased body weight gain and food consumption. **Developmental** NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day based on nonsignificant increases in postimplantation loss and resorptions/doe and a significant decrease in fetal weight.
870.3800	Reproduction and fertility effects (rat)	42090018 (1988) Minimum/ guideline 0, 25, 250 or 2500 ppm 0, 1.25, 12.5 and 125 mg/kg/d	**Parental/Systemic** NOAEL = 25 ppm (1.25 mg/kg/day) LOAEL = 250 ppm (12.5 mg/kg/day) based on reductions (statistically nonsignificant) in body weight gain which appear to be part of a dose-related trend days 70-77 prior to mating, days 0-7 of gestation, and days 7-14 of lactation **Reproductive** NOAEL = 25 ppm (1.25 mg/kg/day) LOAEL = 250 ppm (12.5 mg/kg/day) based on a significant reduction in the body weight of F1 male pups at day 21 in the 250 ppm group.
870.4100b	Chronic toxicity (dog)	42090012, 42710005 (1988) Minimum/ guideline 0, 20, 100, 500 or 1500 ppm M: 0, 0.71, 3.4, 16.4 and 51.2 mg/kg/d F: 0, 0.63, 3.7, 19.4 and 44.3 mg/kg/d	NOAEL = 100 ppm (3.4 mg/kg/day in males/3.7 mg/kg/day in females) LOAEL = 500 ppm (16.4 mg/kg/day in males/19.4 mg/kg/day in females), based on significant inhibition of body weight gain in females.

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.4200	Carcinogenicity (rat)	42090019, 42710010 (1989) Minimum/ guideline 0, 10, 20, 500 or 2500 ppm M: o, 0.48, 0.96, 24.12 and 123.7 mg/kg/d F: 0, 0.64, 1.27, 32.79 and 169.6 mg/kg/d	NOAEL = 20 ppm (0.96 mg/kg/day in males/1.27 mg/kg/day in females) LOAEL = 500 ppm (24.1 mg/kg/day in males/ 32.8 mg/kg/day in females) based on reductions in cumulative body weight gains in the 500 and 2500 ppm groups. **No evidence of carcinogenicity**
870.4300	Carcinogenicity (mouse)	42090015, 42710006 (1989) Minimum/ guideline 0, 10, 30, 300, 2500 or 3000 ppm M: 0, 1.51, 4.65, 46.29, 423.1 and 818.9 mg/kg/d F: 0, 1.9, 5.63, 57.79 and 512.6 mg/kg/d	NOAEL = 30 ppm (4.7 mg/kg/day in males/5.6 mg/kg/day in females) LOAEL = 300 ppm (46.3 mg/kg/day in males/57.8 mg/kg/day in females) based on reductions in the cumulative body weight gains in the 300, 2500 & 4500 ppm groups. **Evidence of carcinogenicity (liver adenoma/carcinoma in both sexes)**
870.5100	*In vitro* bacterial gene mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	42090019, 42710010 (1989) Minimum/ guideline 340 - 5447 µg/plate; 85 - 1362 µg/plate (repeat assay with TA1537 and TA98)	There were sufficient and valid data to conclude that CGA 169374 technical was negative in the microbial gene mutation assay.
870.5300	*in vitro* mammalian cell gene mutation assay in mouse lymphoma cells	42090024 (1986) Unacceptable/ guideline	No conclusion can be reached from the three nonactivated and two S9 activated mouse lymphoma forward mutation assays conducted with difenoconazole technical. The study was seriously compromised.
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells	46950319 (2001) Acceptable/ guideline 0, 21.99, 27.49, or 34.36 µg/mL (-S9) 0, 34.36, 53.69 or 67.11 µg/mL (+S9)	There was evidence of a weak induction of structural chromosomal aberrations over background in the presence of S9-mix.

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells	46950321 (2001) Acceptable/ guideline 0, 26.3, 39.5 or 59.3 µg/mL (-S9) 0, 11.7 or 17.6 µg/mL (+S9)	There was evidence of a weak induction of structural chromosomal aberrations over background.
870.5375	*In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in human lymphocytes	46950323 (2001) Acceptable/ guideline 0, 5, 30 or 75 µg/mL (-S9) 0, 5, 30 or 62 µg/mL (+S9)	There was no evidence of structural chromosomal aberrations induced over background.
870.5385	*In vivo* mammalian chromosomal aberration test Assay in Mice	42090023 (1986) Unacceptable/guideline 250, 500 or 1000 mg/kg	There was no evidence of a cytotoxic effect on the target organ or significant increase in the frequency of nuclear anomalies (micronuclei). However, the study was compromised.
870.5395	*In vivo* mammalian cytogenetics - erythrocyte micronucleus assay in mice	41710011 (1992) Acceptable/guideline Doses up to 1600 mg/kg	Mice bone marrow - No increase in micronucleated polychromatic erythrocytes occurred with CGA-1 69374 (91.2% a.i).
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	4210012 (1992) Acceptable/ guideline Doses up to 50 µg/mL	CGA-i69374 tech. (92.2% a.i.) was considered to be negative in the unscheduled DNA synthesis assay in rat primary hepatocytes as measured by an autoradiographic method at concentrations up to 50.0 µg/mL.
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	42090027 (1985) Unacceptable/ guideline 0.25-31.25 µg/mL	No conclusion can be reached from the unscheduled DNA synthesis (UDS) primary rat hepatocyte assay conducted with difenoconazole technical at concentrations ranging from 0.25 to 31.25 µg /mL. The sensitivity of the study was severely compromised.
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture	42090026 (1985) Unacceptable/ guideline 0.08-10 µg/mL	No conclusion can be reached from the unscheduled DNA synthesis (UDS) human fibroblast assay conducted with difenoconazole tech. at conc. ranging from 0.08 to 10 µg /mL.

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.6200a	Acute neurotoxicity screening battery	46950327 (2006) Acceptable/ guideline 0, 25, 200 or 2000 mg/kg/d	NOAEL (M) = 25 mg/kg/day LOAEL (M) = 200 mg/kg/day based on reduced fore-limb grip strength in males on day 1 and increased motor activity on Day 1. NOAEL (F) = 200 mg/kg/day LOAEL (F) = 2000 mg/kg/day based on decreased body weight, the following clinical signs: upward curvature of the spine, tip-toe gait, decreased activity, piloerection and sides pinched in and decreased motor activity.
870.6200b	Subchronic neurotoxicity screening battery	46950329 (2006) Acceptable/ guideline 0, 40, 250, or 1500 ppm M; 0, 2.8, 17.3 or 107.0 mg/kg/d F: 0, 3.2, 19.5, or 120.2 mg/kg/d	NOAEL (M) = 40 ppm (2.8 mg/kg/day) LOAEL (M) = 250 ppm (17.3 mg/kg/day) based on decreased hind limb strength. NOAEL (F) = 250 ppm (19.5 mg/kg/day) LOAEL (F) = 1500 (120.2 mg/kg/day) based on decreased body weight, body weight gain and food efficiency.
870.7485	Metabolism and pharmacokinetics (rat)	42090028 (1990) Acceptable/ guideline 14 daily doses of 0.5 or 300 mg/kg	The absorption, distribution, metabolism, and excretion of CGA 169374 were studied in groups of male and female Sprague-Dawley rats. Animals were administered a single oral gavage dose of 0.5 or 300 mg/kg [^{14}C]CGA- 169374, or 0.5 mg/kg unlabeled GGA-169374 by gavage for 14 days followed by a single gavage dose of 0.5 mg/kg [^{14}C)CGA-169374 on day 15. The test compound was labeled with C^{14} at either the phenyl or triazole ring.
870.7485	Metabolism and pharmacokinetics (rat)	42090031 (1988) Acceptable/ guideline 0.5 or 300 mg/kg	These studies indicate that distribution, metabolism, and elimination of CGA-169374 were not sex related. There was a slight dose difference in the metabolism and elimination of CGA-169374. In phenyl and triazole labeling studies, fecal excretion of radioactivity was higher in the high dose animals compared to the low dose animals, and an additional metabolite was found in the feces of the high dose animals compared to the low dose animals. There was no major difference in the distribution and excretion of radioactivity with labeling at the phenyl and triazole ring positions, however, there were some different metabolites identified. The studies also showed that administration of 0.5 and 300 mg/kg CGA- 169314 did not induce any treatment related clinical effects.

Table 13.0b Subchronic, Chronic and Other Toxicity Profile of Difenoconazole

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.7485	Metabolism and pharmacokinetics (rat)	420710013, 42710014 (1990) Acceptable/ guideline 0.5 or 300 mg/kg	These two studies described the absorption, distribution, and excretion as the pharmacokinetics and isolated and identified urinary metabolites. Issues raised in the previous supplementary studies were answered. In conjunction with these studies, the previous studies are upgraded.
870.7485	Metabolism and pharmacokinetics (rat)	42090029 (1987) Acceptable/ guideline	[14C]CGA-169374 was rapidly and extensively distributed. metabolized, and excreted in rats for all dosing regimens. The extent of absorption is undetermined pending determination of the extent of biliary excretion. The 4-day recoveries were 97.4-107.75% of the administered dose for all dosing groups. The elimination of radioactivity in the feces (78.06- 94.61% of administered dose) and urine (8.48-21.86%) were almost comparable for all oral dose groups, with slightly higher radioactivity found in the feces of the high dose group than the low dose groups. This was probably due to biliary excretion, poor absorption or saturation of the metabolic pathway. The radioactivity In the blood peaked at about 24-48 hours for all dosing groups. Half-lives of elimination appear to be approximately 20 hours for the low dose groups and 33 - 48 hours for the high dose group. The study results also indicate that CGA-1 69374 and/or its metabolites do not bioaccumulate to an appreciable extent following oral exposure since all the tissues contained negligible levels (<1%) of radioactivity 7 days postexposure.
870.7485	Metabolism and pharmacokinetics (rat)	42090030 (1987) Acceptable/ guideline	The metabolism of CGA-169374 appears to be extensive because the metabolites accounted for most of the recovered radioactivity in the excreta. Three major metabolites were identified in the feces (i.e., A, B, and C). Two of the metabolites were separated into isomers (i.e., A1, A2, B1, and B2). Metabolite C was detected only In the high dose groups, indicating that metabolism of CGA-169374 is dose related and involves saturation of the metabolic pathway. Free triazole metabolite was dete6ted in the urine of triazole labeled groups and its byproduct was detected In the liver of phenyl labeled groups only. Other urinary metabolites were not characterized.

Table A.1.3. Subchronic, Chronic and Other Toxicity Profile of Difenoconazole Metabolites

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	46950314 (1991) Unacceptable/ guideline 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate in strains TA100 and TA1537 (-S9) 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate in all strains (+S9) 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate in strains TA1535, WP2 *uvrA* and TA98 (-S9) 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate in strains WP2 *uvrA* (+S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. There was no evidence of induced mutant colonies over background. Tested: CGA-189138 (metabolite of difenoconazole)
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	46950315 (1991) Unacceptable/ guideline 0, 156, 313, 625, 1250, 2500 or 5000 µg/plate (±S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. There was no evidence of induced mutant colonies over background. Tested: CGA205374 (metabolite of difenoconazole)
870.5100	*In vitro* Bacterial Gene Mutation *(Salmonella typhimurium/ E. coli)/* mammalian activation gene mutation assay	46950317 (1991) Unacceptable/ guideline 0, 2.50, 5.00, 10.0, 20.0, 40.0 or 80.0 µg/plate in all strains (-S9) 0, 5.00, 10.0, 20.0, 40.0, 80.0 or 160 µg/plate in strains TA100 and TA1535 (+S9) 0, 10.0, 20.0, 40.0, 80.0, 160 or 320 µg/plate in strains WP2 *uvrA* and TA1537 (-S9) 0, 2.50, 5.00, 10.0, 20.0, 40.0, or 80.0 µg/plate in strain TA98 (+S9)	The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.** Tested: CGA205375 (metabolite of difenoconazole)

EXECUTIVE SUMMARIES OF SOME STUDIES:

STUDY TYPE: 28-Day Dermal Toxicity – Rat
(OPPTS 870.3200 [§82-2] (rodent); OECD 410).

CITATION: Gerspach, R. Difenoconazole: 28-Day Repeated Dose Dermal Toxicity Study in Rats. Novartis Crop protection AG Toxicology (Switzerland). Novartis Report Number: 993072; Syngenta Report Number: T002728-06. July 11, 2000, MRID 46950310 and MRID 46950311. Unpublished.

EXECUTIVE SUMMARY: In a 28-day dermal toxicity study (MRID 46950310) CGA 169374 Technical (91.8% a.i., Batch No. P807002) was applied to the shaved skin of ten male and ten female rats at dose levels of 0, 10, 100 and 1000 mg/kg bw/day. There were no treatment-related effects on body weight or food consumption. Non clinical signs of toxicity were noted including specific indicators of neurotoxicity. The dose level of 1000 mg/kg bw/day caused hyperkeratosis at the skin application site. A high incidence of follicular cell hypertrophy of the thyroid was observed in males and females of control and all treatment groups and variations with dose are not considered treatment-related. Minimal inconsequential changes were noted on clinical chemistry parameters in high dose males that were not relevant toxicologically. The incidence and severity was increased in animals in the highest dose group. There was an increase in the absolute (12%) and relative (16%) weight of the liver in males in the high dose group accompanied by an increased incidence of slight hepatocellular hypertrophy (7/10) compared to controls (2/10). Females in the high dose group also had an increase in the relative weight of the liver (10%) with an increased incidence of slight hepatocellular hypertrophy (7/10) compared to controls (1/10). These effects are consistent with adaptive responses of the liver.

A systemic LOAEL for male and female rats was not established. The NOAEL for male and female rats is 1000 mg/kg bw/day.

A dermal irritation LOAEL for male and female rats is 1000 mg/kg bw/day based on hyperkeratosis at the skin application site. The dermal NOAEL for male and female rats is 100 mg/kg bw/day.

This 28-day dermal toxicity study in the Fischer 344 rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in the rat.

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

CITATION: Pinto, P.J. (2006) Difenoconazole Technical (CGA169374): Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory (Cheshire,

UK). Laboratory report number AR7517-REG-R1, July 28, 2006. MRID 46950327. Unpublished.

Pinto, P.J. (2006) Difenoconazole Technical (CGA169374): Preliminary Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory (Cheshire, UK). Laboratory report number AR7518-REG, June 16, 2006. MRID 46950325. Unpublished.

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 46950327), groups of fasted Alpk:AP_fSD Wistar-derived rats (10/sex/dose), at least 42 days old, were given a single oral dose of difenoconazole technical (CGA169374) (94.3% w/w, batch/lot # WM806228) in 1% w/v aqueous carboxymethylcellulose (CMC) at doses of 0, 25, 200, or 2000 mg/kg bw and observed for 14 days. Dose levels selected for this study were based on the results of a preliminary acute neurotoxicity study (MRID 46950325). Neurobehavioral assessment (functional observational battery and motor activity testing) was performed on 10 animals/sex/group on days -7, 1, 8, and 15. Body weight and food consumption were measured weekly throughout the study. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination; brain weight was recorded from these animals. Of the perfused animals, 5 animals/sex from the control and high dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no unscheduled deaths at any dose level. Weight change on the day of dosing by the control, low-, mid-, and high-dose groups was -2.1, -1.0, -7.8, and -18.3 g, respectively, for males and 0.0, 2.1, -3.8, and -13.0 g, respectively, for females. Body weight for females had recovered to control levels by day 8. Food consumption for males given 2000 mg/kg was approximately 20% less than control during week 1 only ($p<0.01$). Food consumption for these animals recovered to control levels during week 2. There were no differences from control for females at any dose level or for males at the lower dose levels. These effects on body weight and food consumption were not toxicologically significant.

At 2000 mg/kg, a number of adverse clinical signs were observed on day 1 (at the time of peak effect), including: upward curvature of the spine (8 males, 9 females); tip-toe gait (3, 8); decreased activity (6, 7); piloerection (3, 5); sides pinched in (3, 7); and subdued (1, 0). Females were affected more than males. All treatment-related clinical signs observed on day 1 showed complete recovery by day 5 (males) or day 7 (females).

Significant decreases in fore-limb grip strength were seen in mid- (↓23%) and high-dose (↓26%) males on day 1. Females dosed with 2000 mg/kg had lower motor activities on day 1 (37%), at the time of peak effect, and on day 8 (31%). Males dosed with 200 or 2000 mg/kg had higher motor activities than the controls on day 1, 50% and 55%, respectively, at the time of peak effect.

There were no effects on brain weight at any dose level. Neuropathological

examination of the central and peripheral nervous system showed no effects of treatment at doses of 2000 mg/kg in both sexes.

The LOAEL for acute neurotoxicity of difenoconazole technical (CGA169374) in male rats is 200 mg/kg bw based on reduced fore-limb grip strength in males on day 1 and increased motor activity on Day 1. The NOAEL is 25 mg/kg bw.

The LOAEL for acute neurotoxicity of difenoconazole technical (CGA169374) in female rats is 2000 mg/kg bw based on decreased body weight, the following clinical signs: upward curvature of the spine, tip-toe gait, decreased activity, piloerection and sides pinched in and decreased motor activity. The NOAEL is 200 mg/kg bw.

This acute neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). Positive control data have been submitted for review and were considered acceptable.

STUDY TYPE: Subchronic Neurotoxicity, OPPTS 870.6200b [§82-7] feeding - rat; (OECD 424).

CITATION: Pinto, P J. (2006). Difenoconazole technical (CGA 169374) subchronic neurotoxicity study in rats, final report. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Report number PR1330-REG-R1. July 28, 2006. MRID 46950329. Unpublished.

Pinto, P.J. (2006). Difenoconazole technical (CGA 169374) 28-day dietary rangefinding study in rats, final report. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK 10 4TJ. Report number KR1606-REG. June 13, 2006. MRID 46950326. Unpublished.

Alexander, O. (2006) Difenoconazole technical (CGA 169374) subchronic neurotoxicity study in rats – study profile. Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419-8300. Report number PR1330-REG-R1. September 19, 2006. MRID 46950330. Unpublished.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 46950329) difenoconazole technical (94.5% w/w, batch no. WM806228) was administered to groups of 12 male and 12 female Alpk:AP$_f$SD (Wistar-derived) rats at concentrations of 0, 40, 250, or 1500 ppm in the diet for 90 days. Respective dose levels corresponded to 0, 2.8, 17.3 or 107.0 mg/kg bw/day for males and 0, 3.2, 19.5, or 120.2 mg/kg bw/day for females. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group pretest and during weeks 2, 5, 9, and 14. Cholinesterase activity was not determined. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 5/sex from the control group and 5/sex from the

1500 ppm group were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

Treatment with difenoconazole at concentrations up to 1500 ppm in the diet had no effect on mortality or clinical signs. Relative to respective control weight, final body weight of males and females in the 1500 ppm group was reduced by 9% and 7%. Body weight gain was reduced by 22% in males and 23% in females. Food consumption was reduced in this group (statistically significant only in females [7%]), and food efficiency was significantly reduced in males by 21% ($p \leq 0.05$) and in females by 21% (ns). Lower dose groups were unaffected. Absolute liver weight in males and females in the 1500 ppm group was increased over respective control weight by 38% and 45%. Liver was not weighed in lower dose groups. The increase in liver weight was considered a normal response to chemical treatment.

During weeks 2, 9 and 14, hind-limb grip strength in males in the 1500 ppm group was reduced by 18 to 27% relative to the control values. At week 14, hind-limb grip strength in males in the 250 ppm group was significantly ($p \leq 0.05$) reduced by 20% relative to the control values. FOB observations in females were unaffected by treatment. Motor activity was unaffected in both sexes at all observation times. Brain weight was unaffected by treatment and there were no treatment-related neuropathological lesions.

The LOAEL in male rats is 250 ppm in the diet (17.3 mg/kg bw/day), based on decreased hind limb strength. The NOAEL is 40 ppm (2.8 mg/kg bw/day).

The LOAEL in female rats is 1500 ppm in the diet (120.2 mg/kg bw/day), based on decreased body weight, body weight gain and food efficiency. The NOAEL is 250 ppm (19.5 mg/kg bw/day).

The study is classified as **Acceptable/Guideline** and does satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b). Positive control data have been submitted for review and were considered acceptable.

STUDY TYPE: Rodent *In Vivo* Dermal Penetration Study – Rat
OPPTS 870.7600 [§85-2]; OECD none.

CITATION: Hassler, S. (2003) Difenoconazole 250 EC (A7402G): Dermal absorption of [Triazole-U-^{14}C] CGA 169374 formulated as Score® 250 EC (A-7402G) in the rat (*in vivo*). Syngenta Crop Protection AG, Health Assessment/Animal Metabolism CH-4002 Basel, Switzerland. Syngenta Number T002729-06, May 6, 2003. MRID 46950333. Unpublished.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 46950333), [Triazole–U-^{14}C] CGA 169374 (radiolabled: batch # 50.2-1 and 50.2-2 contained 98 and 99.3% a.i., respectively; nonradiolabeled: batch # AMS 255/3 contained 99.3% a.i.) formulated as Score® 250 EC (A-7402G) was administered to 16 male HanBrl: WIST

(SPF) rats/dose to a skin area of 10 cm^2 at nominal dose levels of 0, 0.005, 0.0125, and 2.5 mg/cm^2 skin. The 2.5 mg/cm^2 dose was repeated because of a high variability in the results of the washing procedure. Measured dose levels were 0.005, 0.0130, 2.4, and 2.6 mg/cm^2 for the low, mid, and high dose and high dose repeat groups, respectively. Exposure duration was 6 hours and animals were monitored for 6, 24, 48, or 72 hours. The remaining discussion of dermal penetration at the high dose will include only the "high-dose repeat" data (i.e., the results of the first high-dose exposure will not be discussed). Recovery of the applied dose was acceptable with group means ranging from 95.44 to 103.67%. Results were not adjusted for incomplete recovery of the applied dose. The majority of the applied dose was recovered in the skin wash, accounting for 49-69%, 73-78%, and 76-86% of the low, mid, and high dose, respectively. The amount of the applied dose retained at the application site was 8-12%, 3-5%, and 2-5% of the low, mid, and high dose, respectively. At the low and mid dose, the major part of the radioactivity remaining in the skin was associated with the *stratum corneum* (7-11% and 2-5%, respectively), while only 1-2% of the high dose was recovered in the upper skin layer. Dermal absorption (sum of blood, carcass, urine, feces, skin test site, gastrointestinal tract, untreated skin, and cagewash) accounted for 15-38%, 7-15%, and 3-11% of the low, mid, and high doses, respectively. Of the test substance systemically absorbed, excretion into the feces was generally the primary route of elimination, accounting for up to 18%, 8%, and 2% of the low, mid, and high doses, respectively. Of the radioactivity remaining in the animal 72 hours after application, the gastrointestinal tract contained 3.0%, 1.4%, and 0.3% of the low, mid, and high doses, respectively, and the carcass contained 1.5%, 0.7%, and 1.1%, respectively. Blood concentrations during and after the exposure period were at or below the limits of determination. Based on the limited blood concentration data available, maximum blood concentrations were measured between 6-8 hours after dose application.

Based on the amount of radioactivity entering the systemic circulation within 6 hours of exposure, the calculated penetration rates at the low, mid, and high doses were 0.013, 0.162, and 30.4 $\mu g\ cm^{-2}\ h^{-1}$, respectively. The penetration rates increased somewhat proportionally with the increase of the test substance concentration at the three dose levels (1:26:5100 for the concentration ratio of the dose levels versus 1:12:2300 for the ratio of the penetration values).

This study in the rat is **unacceptable/guideline** and does not satisfy the guideline requirement for a dermal penetration study (870.7600) in rats. Major deficiencies include uncertainty in the ability of the laboratory to perform the experiment, and only one exposure duration was tested (6 hours), despite minimum Guideline recommendations for durations of 1, 10, and 24 hours. See "Study Deficiencies" for listing of numerous minor deficiencies.

STUDY TYPE: ***In Vitro* Dermal Penetration Study – Rat and Human**
OPPTS 870.7600 [§85-2]; OECD none.

CITATION: Hassler, S. (2003) Difenoconazole 250 EC (A7402G): The percutaneous

penetration of [Triazole-U-^{14}C] CGA 169374 formulated as Score® 250 EC (A-7402G) through rat and human split-thickness skin membranes (*in vitro*). Syngenta Crop Protection AG, Health Assessment/Animal Metabolism CH-402 Basel, Switzerland. Syngenta Number T002730-06, April 9, 2003. MRID 46950332. Unpublished.

EXECUTIVE SUMMARY: In an *in vitro* percutaneous penetration study (MRID 46950332), [Triazole–U-^{14}C] CGA 169374 (98% a.i., batch number 50.2-1) mixed with nonradiolabeled CGA 169374 (batch number AMS 255/3 containing 99.3% a.i.) formulated as SCORE 250® (A-7402) was applied to skin membranes prepared from rat [male HanBrl: WIST (SPF)] and human (cadaver) abdominal skin. Percutaneous absorption at low, mid, and high doses of 0.5, 12.5, or 2500 µg/cm^2 (actual applied doses of 0.5, 12, or 2345 µg/cm^2) was assessed over 24 hours.

Results clearly indicate that transfer of [Triazole–U-^{14}C] CGA 169374 across skin membrane was notably greater for the rat skin membrane than for human skin membrane as shown by flux values that were 10-, 12-, and 32-fold greater for the low, mid, and high concentrations, respectively. A concentration-dependent absorption was also indicated by greater flux values with increasing concentration: flux values at the low, mid, and high doses for the rat skin membranes were 0.020, 0.455, and 26.2 ug/cm^2, respectively, and for human skin membranes were 0.002, 0.037, and 0.822 ug/cm^2, respectively. The increasing flux values resulted in greater absolute amounts of test article being transferred across the skin membranes with increasing concentration: values at the low, mid, and high doses for the rat skin membranes were 0.35, 7.7, and 539.2 ug/cm^2, respectively, and for human skin membranes were 0.04, 0.84, and 15.6 ug/cm^2, respectively. However, the percutaneous absorption was decreased, indicating saturated kinetics (absorption values at the low, mid, and high doses expressed as percent of applied dose for the rat skin membranes were 71%, 64%, and 23%, respectively, and for human skin membranes were 8%, 7%, and 0.7%, respectively).

This *in vitro* percutaneous absorption study in the rat is **acceptable/nonguideline**, but does not satisfy the guideline requirement for a dermal penetration study (870.7600) in rats. The study is a specialty study and was designed to provide only supplemental information to the OPPTS 870.7600 requirement. Results of this study provide information on the differences in dermal absorption between rat and human skin membranes.

STUDY TYPE: ***In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells; OPPTS 870.5375 [§84-2]; OECD 473**

CITATION: Lloyd, M. (2001) Difenoconazole Technical: Induction of chromosome aberrations in cultured Chinese hamster ovary (CHO)cells. Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY,

England. Laboratory Project ID: Covance Number 252/293, Syngenta Number T002874-06, December 11, 2001. MRID 46950319. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950319), Chinese hamster CHO cells in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 21.99, 27.49, or 34.36 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 34.36, 53.69 or 67.11 µg/mL with S9-mix. Cells were harvested 17 hours following the end of exposure. Cells were exposed in a second confirmatory study for three hours at concentrations of 0, 21.99, 27.49 or 34.36 µg/mL without S9-mix and for three hours at concentrations of 0, 34.36, 53.69, 67.11 or 83.89 µg/mL with S9-mix. Cells were harvested 17 hours following exposure. Cells were evaluated for the presence of structural chromosomal aberrations and for numerical aberrations (polyploidy, endoreduplication and hyperploidy). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. There was a statistically significant increase in the percentage of cells with structural chromosomal aberrations at a CGA 169374 Technical concentration of 34.36 µg/mL without S9-mix in the first study. The slides were rescored to determine if aberrations at the fragile X site were present. Aberrations at the fragile X site are not likely relevant to clastogenicity. Aberrations at the fragile X site were not found but the values obtained on rescoring were within the historical solvent control range. There was no clear reason given why the percent of aberrant cells was lower when the slides were rescored. Possibly the distribution of cells on the slides was uneven. The increase at this dose without S9-mix was not seen in the confirmatory assay and thus the increase was not considered biologically significant. A statistically significant increase in the percent of aberrant cells was seen in the first study at 67.11 µg/mL with S9-mix. The statistical significance remained upon rescoring and all values exceeded the historical solvent control range. No statistically significant increase was seen at this or a higher concentration in the confirmatory study with S9-mix. The failure to see a significant increase in the percent of aberrant cells in the confirmatory study makes the results equivocal. The solvent and positive controls (4-Nitroquinoline 1-oxide without S9-mix and cyclophosphamide with S9-mix) induced the appropriate responses. **There was evidence of a weak induction of structural chromosomal aberrations over background in the presence of S9-mix.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

STUDY TYPE: ***In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in Chinese hamster CHO cells; OPPTS 870.5375 [§84-2]; OECD 473**

CITATION: Ogorek, B. (2001) Difenoconazole Technical: Cytogenetic test on Chinese

hamster cells *in vitro*. Syngenta Crop Protection AG, Health Assessment 2 Stein/Genetic Toxicology, CH-4332 Stein, Switzerland. Laboratory Project ID: Syngenta AG Test Number 20013013, Syngenta Number T002875-06, December 3, 2001. MRID 46950321. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950321), Chinese hamster CHO cells in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 26.3, 39.5 or 59.3 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 11.7 or 17.6 µg/mL with S9-mix. Cells were harvested 18 hours following the end of exposure. Cells were exposed in a second confirmatory study for 21 hours at concentrations of 0, 2.3, 5.2 or 11.7 µg/mL without S9-mix and for three hours at concentrations of 0, 7.8, 11.7 or 17.6 µg/mL with S9-mix. Cells were harvested immediately following the 21-hour exposure and 18 hours after the three-hour exposure. Cells were evaluated for the presence of structural chromosomal aberrations and for polyploidy. The S9-fraction was obtained from Aroclor 1254 induced male HanIbm:WIST(SPF) rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. There was a statistically significant increase in the percentage of CHO cells with structural chromosomal aberrations at a CGA 169374 Technical concentration of 59.3 µg/mL without S9-mix in the original study when aberrations at the fragile X site were included but not when they were excluded. Aberrations at the fragile X site are not likely relevant to clastogenicity. No statistically significant increase in the percent of aberrant cells was seen in the original study with S9-mix. An increase in the percent of aberrant cells was seen in the confirmatory study at 17.6 µg/mL with S9-mix and the increase was statistically significant ($p \leq 0.001$) when aberrations at the fragile X site were excluded. The value of 6.5% aberrant cells exceeded the value of >6% set as a criterion for a positive effect in the testing laboratory. The failure to see a significant increase in the percent of aberrant cells in the original study makes the results equivocal. No statistically significant increase in the percentage of aberrant cells was seen at any of the three test material concentrations without S9-mix in the confirmatory study. The solvent and positive controls (Mitomycin C without S9-mix and Cyclophosphamide with S9-mix) induced the appropriate responses. **There was evidence of a weak induction of structural chromosomal aberrations over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

STUDY TYPE: ***In vitro* Mammalian Cytogenetics (chromosomal aberrations) assay in human lymphocytes; OPPTS 870.5375 [§84-2]; OECD 473**

CITATION: Fox, V. (2001) Difenoconazole Technical: *In* vitro cytogenetic assay in human lymphocytes. Central Toxicology Laboratory, Alderley

Park/Macclesfield, Cheshire, UK SK10 4TJ. Laboratory Project ID: CTL Number SV1090, Syngenta Number T002876-06, August 29, 2001. MRID 46950323. Unpublished

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (Chromosomal aberrations) (MRID 46950323), human lymphocytes in culture were exposed to CGA 169374 Technical (94.3% w/w, Lot No. WM806228) in DMSO for three hours at concentrations of 0, 5, 30 or 75 µg/mL without metabolic activation (S9-mix) and at concentrations of 0, 5, 30 or 62 µg/mL with S9-mix. Cells were harvested 17 hours following the end of exposure. Cells were exposed in a second experiment for 20 hours at concentrations of 0, 1, 5 or 10 µg/mL without S9-mix and for three hours at concentrations of 0, 5, 30 or 50 µg/mL with S9-mix. Cells were harvested immediately following the 20-hour exposure and 17 hours after the three-hour exposure. Cells were evaluated for the presence of structural chromosomal aberrations. The S9-fraction was obtained from Phenobarbital + β-naphthoflavone induced male Sprague-Dawley rat liver.

CGA 169374 Technical was tested up to cytotoxic concentrations as evidenced by a dose-related reduction in mitotic activity seen with and without S9-mix. No statistically significant increases in the percentage of cells with structural aberrations, excluding gaps, over the solvent control values were seen at any test material concentration with or without S9-mix in the first experiment or without S9-mix in the second experiment. A statistically significant increase over the solvent control value was seen at 5µg/mL with S9-mix in the second experiment; however, the increase was not considered biologically significant because the value (4.00%) was within the historical solvent control range, the values at the two higher concentrations were not significantly increased and no increase was seen in the first experiment. The solvent and positive controls (Mitomycin C without S9-mix and Cyclophosphamide with S9-mix) induced the appropriate responses. **There was no evidence of structural chromosomal aberrations induced over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *OPPTS 870.5375; OECD 473* for *in vitro* cytogenetic mutagenicity data.

DIFENOCONAZOLE METABOLITES:

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA189138 (metabolite of difenoconazole): reverse mutation assay of CGA189138. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1809, October 21, 1991. MRID 46950314. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950314), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-189138, a metabolite of difenoconazole, (97.8% a.i., lot number 910806) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 31.3, 62.5, 125, 250, 500 or 1000 µg/plate were tested in the absence of S9-mix in strains TA100 and TA1537 and in the presence of S9-mix in all *Salmonella* strains; concentrations of 0, 62.5, 125, 250, 500, 1000 or 2000 µg/plate were tested in the absence of S9-mix in strains TA1535, WP2 *uvrA* and TA98 and in the presence of S9-mix in strain WP2 *uvrA*. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-189138 was tested at concentrations up to the limit concentration for the assay in the pilot assay, and many of the higher concentrations tested in both assays showed cytotoxicity and sometimes also insolubility. In the absence of S9-mix in the pilot assay, the test material was cytotoxic, as judged by stereomicroscopic examination of the bacterial lawns, at concentrations of 800 µg/plate and higher in strains TA100 and TA1537 and at concentrations of 2,000 µg/plate and higher in strains TA1535, WP2 *uvrA* and TA98. In the presence of S9-mix in the pilot assay, the test material was cytotoxic at concentrations of 800 µg/plate and higher in all four *Salmonella* strains and at concentrations of 2,000 µg/plate and higher in strain WP2 *uvrA*. In the absence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 500 µg/plate and higher in strain TA100, at concentrations of 1,000 µg/plate and higher in strains TA1535 and TA98, and at the maximum concentrations tested in strains WP2 *uvrA* and TA1537. In the presence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 500 µg/plate and higher in strains TA100, TA1535 and TA1537 and at the maximum concentrations tested in strains WP2 *uvrA* and TA98. At cytotoxic concentrations there was often also a marked decrease in the number of revertant colonies found. In the pilot assay, precipitation of the white powdery test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 5,000 µg/plate in the absence of S9-mix and at concentrations of 2,000 µg/plate and above in the presence of S9-mix. In the main assay, such precipitation was observed only in the presence of S9-mix and at the highest concentration tested in strain WP2 *uvrA*. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA205374 (metabolite of difenoconazole): reverse mutation assay of CGA205374. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1746, August 14, 1991. MRID 46950315. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950315), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-205374, a metabolite of difenoconazole, (99.3% a.i., lot number 9106054) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 156, 313, 625, 1250, 2500 or 5000 µg/plate were tested with and without S9-mix. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-205374 was tested at concentrations up to the limit concentration for the assay, but the effective concentrations tested were limited by insolubility. Evidence of cytotoxicity, which was collected by stereomicroscopic examination of the bacterial lawns, was seen only in strain TA1537 at the maximum concentration tested, and then only in the main assay in the presence of S9-mix. The test material was quite insoluble, with cloudiness of the preincubation mixture being observed even at 128 µg/plate. The white powdery precipitate of the test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 320 µg/plate and higher in the pilot assay in the absence of S9-mix and at concentrations of 800 µg/plate and higher in the presence of S9-mix. In the main assay, this precipitate was noted at concentrations of 313 µg/plate and higher both in the presence and absence of S9-mix. This precipitate became heavy enough to make it difficult to observe the bacterial lawn at concentrations of 1250 µg/plate or higher in the absence of S9-mix and at the concentration of 5000 µg/plate in the presence of S9-mix. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity

(bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

STUDY TYPE: *In vitro* Bacterial Gene Mutation (Bacterial system, *Salmonella typhimurium* and *Escherichia coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

CITATION: Nakajima, M. (1991) CGA205375 (metabolite of difenoconazole): reverse mutation assay of CGA205375. Biosafety Research Center; Foods, Drugs and Pesticides (An-Pyo Center); 582-2, Arahama Shioshinden; Fukude-Cho Iwata-Gun.; Shizuoka 437-12; Japan. Laboratory Project ID: BRC Number 1747, August 14, 1991. MRID 46950317. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 46950317), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2 *uvrA of E. coli* were exposed to CGA-205375, a metabolite of difenoconazole, (99.8% a.i., lot number 9106055) dissolved in DMSO in two independent assays using a 20-minute preincubation procedure and duplicate plating. In the first mutagenicity assay, which was alternatively called the pilot assay and the dose-finding assay, concentrations of 0, 51.2, 128, 320, 800, 2000 or 5000 µg/plate were tested with and without S9-mix. In the second assay, which was called the main assay, concentrations of 0, 2.50, 5.00, 10.0, 20.0, 40.0 or 80.0 µg/plate were tested in the absence of S9-mix in all strains; concentrations of 0, 5.00, 10.0, 20.0, 40.0, 80.0 or 160 µg/plate were tested in the presence of S9-mix in strains TA100 and TA1535; concentrations of 0, 10.0, 20.0, 40.0, 80.0, 160 or 320 µg/plate were tested in the presence of S9-mix in strains WP2 *uvrA* and TA1537; and concentrations of 0, 2.50, 5.00, 10.0, 20.0, 40.0, or 80.0 µg/plate were tested in the presence of S9-mix in strain TA98. The S9 fraction was obtained from phenobarbital and 5,6-benzoflavone-induced male Sprague-Dawley rat liver.

CGA-205375 was tested at concentrations up to the limit concentration for the assay in the pilot assay. Most of the concentrations in that assay showed cytotoxicity and some of the higher ones also showed insolubility. Some of the higher concentrations in the main assay, which used much lower concentrations, also showed cytotoxicity. In the absence of S9-mix in the pilot assay, the test material was cytotoxic, as judged by stereomicroscopic examination of the bacterial lawns, at all concentrations in strains TA100 and TA1537 and at concentrations of 128 µg/plate and higher in strains TA1535, WP2 *uvrA* and TA98. In the presence of S9-mix in the pilot assay, the test material was cytotoxic at all tested concentrations in strain TA98, at concentrations of 128 µg/plate and higher in strains TA100 and TA1535, and at concentrations of 320 µg/plate and higher in strains WP2 *uvrA* and TA1537. In the absence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 40.0 µg/plate and higher in strain TA100 and at the highest tested concentration in the other strains. In the presence of S9-mix in the main assay, the test material was cytotoxic at concentrations of 40.0 µg/plate and higher in strain TA98, at concentrations of 80.0 µg/plate and higher in strain TA100, at concentrations of 160

μg/plate and higher in strain TA1537 and at the maximum concentrations tested in strains TA1535 and WP2 *uvrA*. At cytotoxic concentrations there was often also a marked decrease in the number of revertant colonies found. Because cytotoxicity was excessive at most concentrations, the pilot assay provided only slight useful information on mutagenesis in most strains. In the pilot assay, the needle crystalline precipitate of the test material was observed on the surface of the agar plates at the time of colony counting at concentrations of 800 μg/plate and above in the absence of S9-mix and at concentrations of 2,000 μg/plate and above in the presence of S9-mix. No precipitation was observed at any of the much lower concentrations tested in the main assay. The number of revertants per plate was not increased over the concurrent solvent control value at any test material concentration, with or without S9-mix, in any tester strain. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Unacceptable/Guideline** and does not satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. Five strains of *S. typhimurium* were not used in the assay. The study can not be upgraded.

Appendix 3. Proposed Metabolic Pathway for difenoconazole in Rats

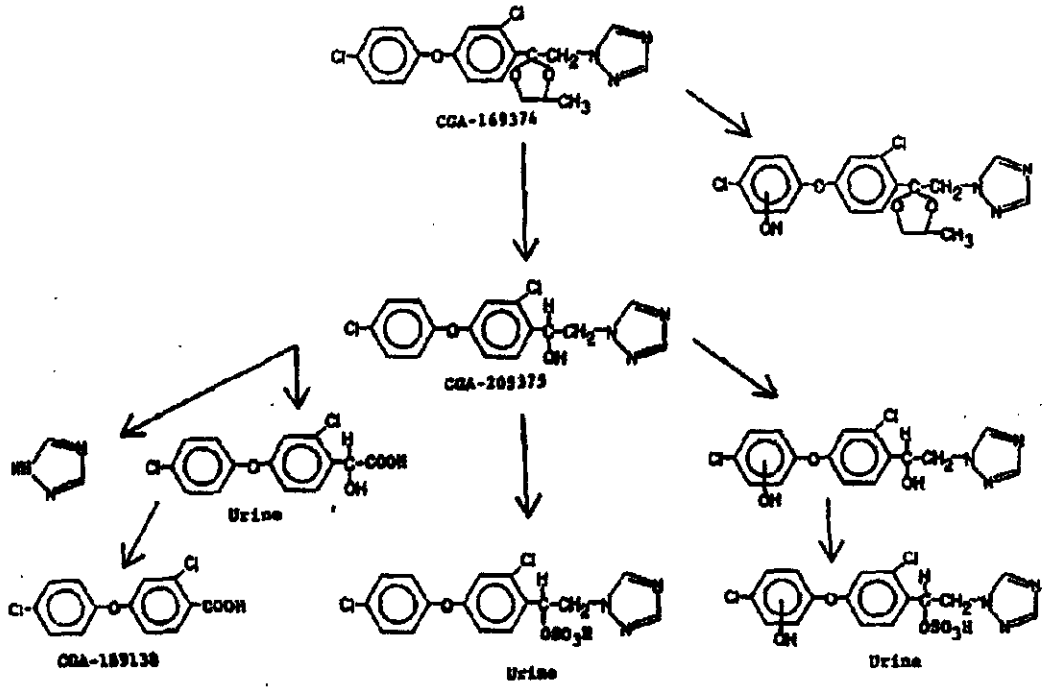


Figure 1. Proposed Metabolic Pathway for CGA 169374 in Rats

SOURCE: CBI, page 33

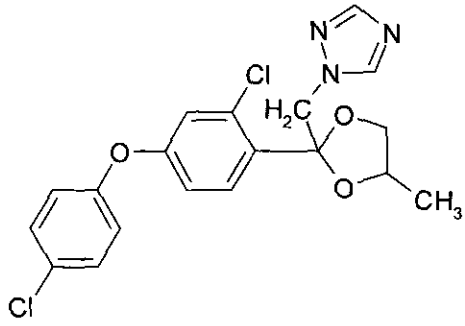
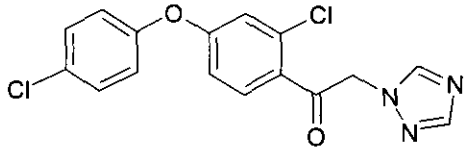
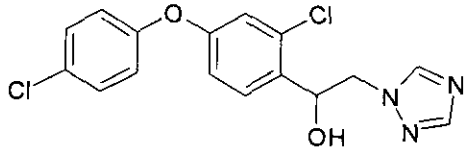
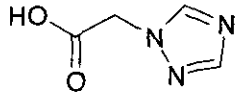
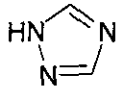
Appendix 4 Environmental Fate Degradates

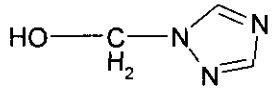
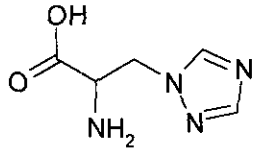
Table 13.0c. Summary of Difenoconazole Major Degradates and Maximum Percent Formation Observed in the Laboratory and Field Studies.

	Max Degradate Concentration (% of applied) and Time (days) to Max Concentration					*Analyzed Degradates*	
Degradate [1]	***Lab Accumulation in Fish***	***Aqueous Photolysis*** [2,3,4]	***Aerobic Soil***	***Anaerobic Aquatic***	***Aerobic Aquatic***	***TFD*** [5]	***Ground Water***
CGA 205375	51-64%	3.8% (4)	14.8% (360)*	12.6% (175)	11.6% (90)	4.5% (121)[A] 5.3% (364)[B] 3.5% (123)[C] 6.9% (182)[D]	No study
CGA 205374		1.1% (14)	2.1 % (272)	0.8% (247)			
CGA 71019			20.6% (190)	35.9% (350)*			
CGA-142856		41.8% (30)[4]*					
CGA-107069/ CGA-71019		12.27% (30)[4]* 12.9% (9)[4]					

[1] Refer to Table I-2 for name and structure; [2] Difenoconazole was stable under hydrolysis; [3] No meaningful amount of degradates were formed in soil photolysis study (≤ 0.2% and only single replicates); [4] In sterile natural water (MRID 46950105 and MRID 42245128); [5] % of the total applied difenoconazole, based on four applications; [A] under bare soil conditions in GA (MRID 46950126); [B] under potato production condition in ND (MRID 46950129); [C] under a bare plot of loam soil in CA (MRID 46950129); [D] in CA bare loamy sand soil (MRID 42245140); and * The max concentration was observed in the last sampling interval.

Table 13.0d. Chemical Structures of Difenoconazole and Degradation Products Detected in Submitted Environmental Fate Studies.

Name(s)	Structure	Known Chemical and Fate Parameters
CGA-169374 Difenoconazole 1-[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole. 1-[[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole. 1-(2-[4-(4-Chlorophenyoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl)-methyl])-1H-1,2,4-triazole. CAS #: 119446-68-3		
CGA-205374 [CGA-176459] 1-[2-Chloro-4-(4-chlorophenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanone 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1H-1,2,4-triazol-1-yl)-ethanone CAS #: 136815-80-0		
CGA-205375 [CGA-211391] 1-[2-Chloro-4-(4-chlorophenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol alpha-[2-Chloro-4-(4-chlorophenoxy)phenyl]-1H-1,2,4-triazole-1-ethanol. CAS #: 117018-19-6		Mobility data available
CGA-142856 [1,2,4]Triazol-1-yl-acetic acid 1H-1,2,4-Triazole-1-acetic acid. CAS #: 28711-29-7		DW assessment completed in 2006
CGA-71019 1-H-(1,2,4)-Triazole 1H-1,2,4-Triazole 4H-[1,2,4]Triazole CAS #: 288-88-0		DW assessment completed in 2006

Name(s)	Structure	Known Chemical and Fate Parameters
CGA-107069 1-H-(1,2,4)-Triazole-1-methanol CAS #: 74205-82-6		
CGA-131013 2-Amino-3-[1,2,4]triazol-1-yl-propionic acid alpha-Amino-1H-1,2,4-triazole-1-propanoic acid CAS #: 86362-20-1		

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