



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: *Fluopicolide*: Human Health Risk Assessment for Imported Grapes. PC Code: 027412, Petition No: 5E6903, DP Number: 315502.

Regulatory Action: Section 3 Action
Risk Assessment Type: Single Chemical/ No Aggregate

FROM: Nancy Dodd, Chemist
Registration Action Branch 3
Health Effects Division (7509P)

AND

Amelia Acierto, Chemist
Myron Ottley, Toxicologist
Registration Action Branch 3
Health Effects Division (7509P)

THROUGH: Mary Elissa Reaves, Ph.D., Toxicologist
Christine Olinger, Chemist
Risk Assessment Review Committee

AND

Paula Deschamp, Branch Chief
Registration Action Branch 3
Health Effects Division (7509P)

TO: Janet Whitehurst/Tony Kish, RM Team #22
Fungicide Branch
Registration Division (7505P)

Bayer CropScience AG has submitted a petition for tolerances for the fungicide fluopicolide in/on imported grapes and raisins. The Registration Division of the Office of Pesticide Programs (OPP) has requested that HED evaluate hazard and exposure data and conduct dietary exposure assessments to estimate the risk to human health that will result from importation of fluopicolide-treated grapes. Occupational, residential, and aggregate exposure assessments have not been conducted since HED has determined that they are not needed for fluopicolide on imported grapes.

Fluopicolide is a new fungicide with no established U.S. tolerances. This request for tolerances for imported grapes and raisins is being followed by petitions for domestic uses on grapes and other crops.

The residue chemistry and the toxicological databases support the establishment of tolerances for the fungicide fluopicolide of 2.0 ppm in/on *grape* and 6.0 ppm in/on *grape, raisin*. Provided the deficiencies regarding the Sections B and F and the method validation which are specified in Section 10.0 of this document are resolved, the tolerances can be granted; deficiencies regarding storage stability can be resolved after establishment of the tolerances.

One of the formulations used in the crop field trials, the 4.44% water-dispersible granule (WDG) formulation of fluopicolide, also contains fosetyl-aluminum. A tolerance with regional registration is established in 40 CFR §180.415(c) for residues of fosetyl-aluminum on grapes at 10 ppm. Based on a substantially lower use rate and comparable preharvest interval for imported grapes as compared to the registered fosetyl-aluminum use, residues of fosetyl-aluminum on imported grapes are not likely to exceed the established 10 ppm tolerance.

As part of every pesticide risk assessment, HED considers a large variety of consumer subgroups. These are broken into two main categories: 1) subgroups based on dietary consumption patterns, and 2) subgroups based on activity patterns in a residential setting. In the course of assessing the potential exposures resulting from the use of fluopicolide, HED considered to the extent possible whether or not there are population groups that may have unusually high exposure compared to the general population. HED did not identify any additional specialized subgroup that would not be included in the generic models and approaches utilized for this risk assessment.

A summary of the findings and an assessment of human risk resulting from fluopicolide on imported grapes are provided in this document. The residue chemistry assessment was provided by Amelia Acierto, the dietary exposure assessment and risk assessment by Nancy Dodd, and the hazard characterization by Myron Ottley of RAB3/HED.

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1.0 Executive Summary

Use Profile: Fluopicolide is a fungicide to be used on imported grapes. Three foliar applications are to be made to grapes in Europe at the maximum seasonal application rate of 0.36 lb ai/A. Minimum retreatment intervals of 10 days and a preharvest interval of 21 days are to be observed.

One of the formulations used in the crop field trials, the 4.44% water-dispersible granule (WDG) formulation of fluopicolide, also contains fosetyl-aluminum. A tolerance with regional registration is established in 40 CFR §180.415(c) for residues of fosetyl-aluminum on grapes at 10 ppm. Based on a substantially lower use rate and comparable preharvest interval for imported grapes as compared to the registered fosetyl-aluminum use, residues of fosetyl-aluminum on imported grapes are not likely to exceed the established 10 ppm tolerance.

Fluopicolide controls a wide range of *Oomycete* (Phycomycete) diseases including downy mildews (*Plasmopara*, *Pseudoperonospora*, *Peronospora*, *Bremia*), late blight (*Phytophthora*), and some *Pythium* species.

The mode of action of fluopicolide has not been determined; however, it is a mode of action unlike the known modes of action of other registered fungicides.

Fluopicolide is a mesosystemic fungicide; it translocates toward the stem tips via the xylem but it does not translocate toward the roots.

2,6-Dichlorobenzamide (BAM) is a metabolite and/or environmental degradate of both fluopicolide and dichlobenil. As determined by the HED Risk Assessment Review Committee (RARC1) on 12/21/06, BAM will not be included in the tolerance or risk assessment for fluopicolide on imported grapes because 1) as both a plant and rat metabolite of fluopicolide, it has been included in the toxicology studies and fluopicolide endpoint selections; and 2) residues of BAM in food resulting from fluopicolide on imported grapes are expected to be negligible since BAM is only 2.0% of the total radioactive residue in the fluopicolide grape metabolism study and is a maximum of only 0.047 ppm in the fluopicolide grape field trials. However, both parent fluopicolide and BAM will be included in risk assessments for future uses of fluopicolide on domestic crops since more exposure to BAM is expected with domestic uses.

Human Health Risk Assessment:

Toxicity/Hazard: An appropriate endpoint was identified for the chronic dietary exposure scenario based on a NOAEL in a developmental toxicity study in rabbits and uncertainty factors of 10x for extrapolation from animals to humans (interspecies variation) and 10x for potential variation in sensitivity among members of the human population (intraspecies variation). A LOAEL in that study was based on death, abortions/premature deliveries, decreased food consumption, and decreased body weight gain. No appropriate endpoint was identified for an acute dietary assessment. Incidental oral, dermal, and inhalation endpoints were selected but are not applicable to this risk assessment because residential and occupational exposures are not anticipated for an imported crop. Fluopicolide is not likely to be carcinogenic to humans.

Dietary Exposure (Food Only): A dietary exposure assessment was conducted using the Dietary Exposure Evaluation Model DEEM-FCID™, Version 2.03, which uses food consumption data from the U.S. Department of Agriculture's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. The dietary exposure assessment was conducted for residues of fluopicolide (parent only) in food (only). Since U.S. registration is not required for an imported crop and there are no existing U.S. registrations for fluopicolide, no fluopicolide residues are expected to occur in drinking water.

The chronic dietary (food only) exposure assessment for fluopicolide on imported grapes was a conservative assessment using the recommended tolerance levels and assuming that 100% of the crop was treated and 100% of the crop was imported. An adequate processing study was conducted on grapes indicating no concentration in grape juice but concentration in raisins. No default processing factors were used since an adequate processing study was available; tolerance levels of 2.0 ppm and 6.0 ppm were used for grapes and raisins, respectively. Since grapes are imported, no fluopicolide residues are expected to occur in rotational crops. Since no livestock feed items are associated with grapes, no fluopicolide residues are expected to occur in livestock commodities.

The chronic dietary (food only) exposure to fluopicolide is below HED's level of concern for the general U.S. population and all population subgroups. The chronic dietary exposure estimates are <1% cPAD for the general U.S. population and 3% cPAD for children 1-2 years old, the most highly exposed subgroup.

Residential Exposure: There are no U.S. registrations for fluopicolide; therefore, no residential exposure is expected.

Aggregate Risk: No aggregate exposure is expected to occur in the U.S. as a result of fluopicolide on imported grapes since exposure is expected to occur only from food.

Occupational Exposure/Risk: No occupational exposure to fluopicolide is expected to occur in the U.S. as a result of fluopicolide on imported grapes.

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 (as it relates to an imported crop), HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup’s food consumption. 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 proposed/registered food uses/tolerances 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. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure from traditional dietary patterns among specific subgroups.

Review of Human Research:

This risk assessment does not rely on any data from studies in which human subjects were intentionally exposed to a pesticide or other chemical.

Additional Data Needs:

Pending the resolution of Residue Chemistry Deficiencies #'s 1a and 1b (pertaining to directions for use), Deficiency # 2 (pertaining to the requirement for a proposed confirmatory method or an interference study), Deficiency #3 (pertaining to the need for the proposed enforcement method, Method 00782/M002, to undergo a successful petition method validation by ACB/BEAD), and Deficiency #6 (pertaining to a revised Section F), there are no Residue Chemistry data gaps that would preclude permanent tolerances for residues of fluopicolide as follows:

Grape.....	2.0 ppm
Grape, raisin.....	6.0 ppm

This decision is based on use of water dispersible granular (WDG) or emulsifiable concentrate (EC) formulations without adjuvants. To use other formulations (other than WDG and EC formulations) or spray adjuvants, additional residue data (or review of additional residue data) would be required as indicated in Deficiencies 1c and 1d.

Deficiency #4 (pertaining to storage stability data for fluopicolide in juice or must [i.e., the unfiltered liquid that results from pressing grapes] and raisins) and Deficiency #5 (pertaining to

length of storage information for the processed commodities) are confirmatory data requirements which must be resolved but can be resolved after establishment of the tolerances.

Residue Chemistry Deficiencies

860.1200 Directions for Use

- 1a. Residue data were submitted which reflected use of a 4.44% WDG formulation (WG71) and a 95 g/L suspo-emulsion formulation (SE10), which is similar to an emulsifiable concentrate (EC) formulation. The petitioner should submit representative labels or a revised Section B to indicate the types of formulations to be used on imported grapes.
- 1b. A Section B was submitted which provided some information regarding the proposed use pattern on imported grapes, including the maximum number of applications per season (3), the maximum seasonal application rate (0.36 lb ai/A), the minimum preharvest interval (PHI; 21 days), and retreatment intervals (10-14 days). The petitioner should submit a representative label or a revised Section B to more fully describe the use pattern(s) to be applied to grapes and raisins to be exported to the USA. The additional information to be provided to the Agency should include the maximum single application rate, application timing (as it relates to the plant growth stage), names and quantities of stickers, spreaders, and other adjuvants (if any) to be added to the spray solution, application tank-mix preparation, volume of spray mix per unit area (hectare or acre), and type of application equipment.
- 1c. No spray adjuvants were used in the crop field trials submitted to support this petition. If the petitioner intends to recommend use of spray adjuvants, residue data reflecting use of spray adjuvants should be submitted.
- 1d. The submitted residue data reflect use of WDG and EC types of formulations. If other types of formulations are to be used on grapes to be imported, additional residue data would be needed to reflect use of those other types of formulations.

860.1340 Residue Analytical Methods

2. The petitioner must propose confirmatory procedures for the proposed enforcement method, or submit an interference study for fluopicolide.
3. The proposed enforcement method, Method 00782/M002, must be validated as an adequate enforcement method by ACB/BEAD.

860.1380 Storage Stability

4. The petitioner must submit data demonstrating the stability of residues of fluopicolide in grape juice (or must) and raisins stored frozen for 29 months or the maximum storage interval for each of these commodities.

860.1520 Processed Food and Feed

5. The petitioner should submit the actual dates of collection, extraction, and analysis for each sample of grape juice (or must) and raisins from the processing studies to determine the storage interval required for the storage stability study.

860.1550 Proposed Tolerances

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

Toxicology Deficiencies

None.

2.0 Ingredient Profile

Fluopicolide (2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide; AE C638206; V10161) is a fungicide which belongs to the benzamide class and the pyridine class. Another fungicide in the benzamide class is zoxamide.

Fluopicolide controls a wide range of *Oomycete* (Phycomycete) diseases including downy mildews (*Plasmopara*, *Pseudoperonospora*, *Peronospora*, *Bremia*), late blight (*Phytophthora*), and some *Pythium* species.

The mode of action of fluopicolide has not been determined; however, it is a mode of action unlike the known modes of action of other registered fungicides.

Fluopicolide is a mesosystemic fungicide; it translocates toward the stem tips via the xylem but it does not translocate toward the roots.

2.1 Summary of Registered/Proposed Uses

Table 2.1. Summary of Directions for Use of Fluopicolide.						
Applic. Timing, Type, and Equip.	Formulation [EPA Reg. No.]	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [g ai/ha]	PHI (days)	Use Directions and Limitations
Grapes						
Foliar (application timing and equipment not specified)	Not specified	Not specified	3	0.36 [400]	21	A minimum retreatment interval of 10 days is specified.

2.2 Structure and Nomenclature

Table 2.2. Fluopicolide Nomenclature.	
Chemical structure	
Empirical Formula	C ₁₄ H ₈ Cl ₃ F ₃ N ₂ O
Common name	Fluopicolide
Company experimental name	AE C638206
IUPAC name	2,6-dichloro- <i>N</i> -[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide
CAS name	2,6-dichloro- <i>N</i> -[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide
CAS Registry Number	239110-15-7
End-use products (EPs)	<ol style="list-style-type: none"> 1. WG71 Formulation (4.44% AE C638206 + 66.7% fosetyl-aluminum) 2. SE10 (Suspo-Emulsion; similar to an emulsifiable concentrate; 95 g/L)
Chemical Class	Fungicide
Known Impurities of Concern	None

2.3 Physical and Chemical Properties

The physical/chemical properties of fluopicolide as they affect inhalation or dermal exposure are not relevant for an imported crop.

Table 2.3. Physicochemical Properties of Fluopicolide.		
Parameter	Value	Reference
Molecular Weight	383.59	
Melting point/range	149 °C	MRID 46474015
pH	6.5 at 22.0 °C	MRID 46474013
Density	1.65 g/cc	MRID 46474016
Water solubility (20 °C)	2.86 mg/L at pH 4 2.80 mg/L at pH 7 2.80 mg/L at pH 9	MRID 46474021
Solvent solubility (g/L at 20 °C)	n-Hexane: 0.20 Ethanol: 19.2 Toluene: 20.5 Ethyl acetate: 37.7 Acetone: 74.7 Dichloromethane: 126 Dimethyl sulfoxide: 183	MRID 46474022
Vapor pressure at 25 °C	8.03×10^{-7} Pa	MRID 46474023
Dissociation constant (pKa)	No evidence of ionization in the pH range of 1.9 to 9.8	MRID 46474017
Octanol/water partition coefficient Log(K _{ow})	Log P _{ow} = 3.26 at pH 7.8 and 22 ± 1 °C	MRID 46474018
	Log P _{ow} = 2.9 at pH 4.0, 7.3 and 9.1 and 40 °C	MRID 46474019
UV/visible absorption spectrum	Absorption maxima wavelengths (nm): In methanol: 203 and 271 In methanol/HCl: 202 and 270 In methanol/NaOH: 219 and 271	MRID 46474014

3.0 Hazard Characterization/Assessment

3.1 Hazard and Dose-Response Characterization

3.1.1 Database Summary

The toxicology database for fluopicolide (AC 638206) submitted by Bayer CropScience AG is complete and deemed adequate for hazard assessment and for FQPA evaluation.

An important fluopicolide metabolite, 2,6-Dichlorobenzamide (BAM) is considered in this risk assessment, and its Hazard characterization/Assessment appears in Appendix E.

3.1.1.1 Studies available and considered (animal, human, general literature)

Fluopicolide (AC638206)

Acute- oral, dermal, inhalation, eye irritation, skin irritation, dermal sensitization

Subchronic- oral 90-day rat, oral 90-day mouse (2 studies), oral 90-day dog

Chronic- oral rat (combined chronic/carcinogenicity) and oral dog

Reproductive/developmental- oral developmental rat and rabbit, rat reproduction/fertility

Other- acute and subchronic rat neurotoxicity, oral mouse carcinogenicity, mutagenicity studies (*in vitro and in vivo*), metabolism/pharmacokinetics studies and phenobarbital 28-day hepatotoxicity mouse studies (2 studies)

3.1.1.2 Mode of action, metabolism, toxicokinetic data

Fluopicolide is a fungicide that is effective in controlling plant disease caused by *Oomycetes*. The biological activity is mesosystemic in that it controls pathogens on contact through translocation toward the stem tips and not the roots. The exact mode of action of disease control has not been fully determined. The test substance is mostly used on grapes and raisins. For detailed metabolism and toxicokinetic data, please refer to Section 3.2.

3.1.1.3 Sufficiency of studies/data

The toxicity database is complete for fluopicolide (see Appendixes A-2 through A-4 for toxicity profile tables) and is adequate for risk assessment evaluations and determination of FQPA. All studies evaluated were deemed acceptable and met guideline criteria except for one reverse gene mutation study. This study was unacceptable because purity of the test material was not provided; however, there were enough adequate studies for gene mutation that this does not constitute a data gap.

3.1.2 Toxicological Effects

NOAEL and LOAEL: The no-observed-adverse-effect level (NOAEL) is the dose level in which no adverse effects were noted. The lowest-observed-adverse-effect level (LOAEL) is the dose level at which effects of toxicological significance are observed. These two parameters are adequately provided in the studies for fluopicolide.

Acute toxicity: Fluopicolide has moderate toxicity with no deaths noted in male or female rats at doses of > 2000 mg/kg when given orally, and > 4000 mg/kg dermally. Following inhalation exposure, an LC₅₀ of >1.789 to < 5.16 mg/L was calculated. Toxicity was observed primarily in the inhalation studies and included a decrease in body weight, decrease in mean body temperature and signs of irritation (piloerection, hunched posture, reddened nostrils). Moderate eye irritation occurred in the form of chemosis and corneal opacities, but all effects were gone by 72 hours. Slight dermal irritation occurred, but the test substance was not a skin sensitizer.

Subchronic toxicity: The most common effect observed in the 90 day studies was a decrease in body weight gain. Weight gain was markedly decreased in male and female rats in a subchronic study at doses that exceeded the limit dose (1668-1673 mg/kg/day), and male and female rats in a subchronic neurotoxicity study had reduced body weight gain at doses of 780.6 and 125.2 mg/kg/day, respectively. There was no effect on weight gain in dogs or mice in subchronic studies. Besides effects on body weight and body weight gain, no definitive cross-species target organ was identified in subchronic studies with fluopicolide. No organ lesions were found in dogs administered up to 1000 mg/kg/day for 90 days. Male rats had hypertrophy of the zona glomerulosa in the adrenal gland, trabecular hyperostosis of the bone joint, and decreased bone marrow cellularity after exposure to 1668 mg/kg/day for 90 days. Similar lesions in the adrenal gland and bone marrow were found in female rats administered 119 mg/kg/day for 90 days. In mice, females administered 965 mg/kg/day showed an increased incidence of hepatic oval cell proliferation.

Chronic toxicity: As in the subchronic studies, the main effect in the chronic studies was a decrease in body weight gain with no definitive cross-species target organ identified. Male dogs had reduced weight gain after exposure to 1000 mg/kg/day for one year; body weight of females was not affected. Mice had severely decreased body weight and body weight gain with administration of 551.0 and 772.3 mg/kg/day to males and females, respectively, for 18 months. Male and female rats had decreased weight gain after exposure to 109.4 and 142.2 mg/kg/day for 2 years, respectively. No organ lesions were found in dogs administered up to 1000 mg/kg/day for 52 weeks. Thyroid cystic follicular hyperplasia was seen in male rats after 109.4 mg/kg/day for two years. In mice, altered liver cell foci were seen in males and females given 551.0 or 772.3 mg/kg/day, respectively, for 18 months.

Carcinogenicity: No evidence for carcinogenicity was seen in rats administered fluopicolide in food for 24 months. Treatment of rats did not result in an increase in overall tumor incidence or an increase in the incidence of any specific type of tumor. In contrast, mice had an increased incidence of hepatocellular adenoma following administration of 3200 ppm in the diet for 18 months (551.0 and 772.3 mg/kg/day for males and females, respectively).

Developmental toxicity: In developmental toxicity studies, maternal toxicity was clearly evident only in rabbits as increased mortality, abortion, and decreased body weight gain at 60 mg/kg/day, the highest dose tested. Minimal maternal toxicity was observed in rats dosed with 700 mg/kg/day; slightly reduced body weight gain did not result in lower absolute body weight. At the same dose affecting the dam, 700 mg/kg in rats and 60 mg/kg in rabbits, fetal growth was affected in both species and observed as decreases in body weight and crown-rump length. Also, at 700 mg/kg, delays in fetal ossification and increased incidence of skeletal malformations were observed in rat fetuses, with neither of these effects seen in rabbit fetuses. No external or visceral abnormalities were observed in either species. In rats the adverse effect was judged to be greater in the fetus than in the dam, suggesting a greater susceptibility in the fetus compared to that of the dam.

Reproductive toxicity: Reproductive performance was not affected in a two-generation reproduction toxicity study in which fluopicolide was administered to male and female rats at nominal dietary concentrations of 0, 100, 500, or 2000 ppm (0, 7.4-8.8, 36.4-43.7, 144.6-179.9 mg/kg/day, respectively, for males and 0, 8.1-9.4, 41.0-46.9, 159.7-193.9 mg/kg/day, respectively, for females). Evidence of parental toxicity in the high-dose groups included decreased body weight gain in F₀ females and kidney toxicity in F₀ and F₁ males and females. Kidney lesions consisted of cortical tubular basophilia or dilation, medullary granular casts, cortical scarring, interstitial inflammation, and/or corticomedullary mineralization. Body weight of the high-dose F₁ and F₂ pups was significantly less than that of the controls beginning on lactation day 14. The high-dose pups had decreased weight gain throughout the 28-day lactation interval. Overall weight gain during lactation was decreased by 8-9% of the control level in the high-dose F₁ male and female pups and by 11-14% in the high-dose F₂ male and female pups. No other effects on offspring growth or survival were noted in either generation.

Neurotoxicity: No evidence of neurotoxicity was seen in acute or subchronic oral rat neurotoxicity studies with fluopicolide. A transient decrease in body temperature was the only finding in male and female rats given a single dose of 2000 mg/kg. Brain weight, brain morphometry, and neuropathology were not affected by treatment.

Dermal toxicity: Acute dermal toxicity studies showed that fluopicolide was only a slight dermal irritant (Tox. Category IV). A dermal subchronic toxicity study showed no systemic or local effects at the limit dose.

3.1.3 Dose-response

HED has selected the most sensitive and protective endpoints from the database to develop the risk assessment. Appropriate endpoints were chronic dietary exposure scenario, incidental oral short-term and intermediate-term, dermal all time periods, and inhalation all time periods. Further discussions in regards to the studies chosen for each endpoint are included in Section 3.5.

3.1.4 FQPA.

Data are adequate for evaluation of effects resulting from *in utero* and post-natal exposure. Acceptable developmental toxicity studies were conducted in rodents and non-rodents, and a reproductive toxicity study in rodents was available. Developmental toxicity was found in both rats and rabbits at doses equal to those resulting in maternal toxicity. In the rat developmental study, the developmental effects, developmental delays and skeletal defects, were judged to be qualitatively more severe than the minimal maternal toxicity (decreased body weight gain) observed. In the multigeneration study, neither quantitative nor qualitative susceptibility was observed. Although there was evidence of increased qualitative susceptibility in the rat developmental study, the concern is low and there are no residual uncertainties. The 10X FQPA Safety Factor is reduced to 1X. (See section 3.3.6.2 for details.)

3.2 Absorption, Distribution, Metabolism, Excretion (ADME)

Several studies were available on metabolism and disposition for fluopicolide in rats. The studies demonstrated rapid absorption, metabolism and excretion within 72 hours after oral dosing. The main metabolites were oxidative N-dealkylation cleavage products. The primary routes of excretion for the parent compound were fecal (68.8 - 72.4%) and urinary (18.8 - 21.4%) with metabolites identified in both urine and feces. Up to 49 metabolites were identified in the urine, while the main compound in the feces was identified as the parent. No gender-related variability was observed in any of the studies. Following administration of the fluopicolide, highest concentrations were found in the intestines and its contents. The next highest concentrations were found in the liver, kidneys and adrenals. However, based upon tissue burden, neither the parent compound nor its metabolites appear to undergo any significant tissue sequestration.

A metabolite of fluopicolide, 2,6-dichlorobenzamide or BAM, is formed following hydroxylation of the parent molecule and cleavage of the straight chain bridge to form the amide. Following oral administration of BAM itself, it is found in the kidney and liver, and most of the radioactivity (82%) is found in the urine, with 13% found in the feces. Existing data show that BAM produces toxicity at lower levels than fluopicolide.

BAM also is a plant and soil metabolite of other compounds such as dichlobenil, and concern has been expressed about the contribution of fluopicolide to total environmental levels of BAM. When fluopicolide is administered to the rat, only about 0.09% of the total administered radioactivity 8 hrs post dosing is identified as BAM, mitigating against this concern.

3.3 FQPA Considerations

3.3.1 Adequacy of the Toxicity Database

Data for fluopicolide are adequate for evaluation of FQPA.

3.3.2 Evidence of Neurotoxicity

Acute and subchronic neurotoxicity studies have been conducted with fluopicolide in rats. The only notable functional observational battery (FOB) finding was a lower body temperature in males and females six hours after a single oral dose of 2000 mg/kg/day. No clinical signs of toxicity or effects on motor activity were observed in either study. In acute inhalation lethality studies, there were no treatment-related effects on a battery of reflex measurements evaluated the day after exposure; however, mean body temperature was decreased after exposure.

Acute Neurotoxicity in rats- fluopicolide

In an acute neurotoxicity study (MRID 46474218; summarized in MRID 46474217), groups of fasted, 6- to 7-week old CD rats (10/sex) were given a single oral dose of AE C638206 (95.9% a.i., batch/lot #OP2050046) in 1% methylcellulose at doses of 0, 10, 100, or 2000 mg/kg bw and observed for 15 days. Doses were based on a range-finding study in which single doses of 50 mg/kg induced behavioral changes (MRID 46474219). Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed in 10 animals/sex/group pretreatment, on Day 1 (at six hours post-dosing, the time of peak effect), and on Days 8 and 15. 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, the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There was no effect of treatment on body weight, body weight gain, food consumption, food efficiency, brain weight, brain measurements (cerebral hemispheres), or incidence of gross or microscopic lesions. Lower body temperature in the high-dose males and females at the time of peak effect (6 hours post-dosing) on the day of treatment (Day 1) was the only treatment-related observation during the FOB. This sign was not observed on Days 8 or 15. A statistically significant decrease in forelimb grip strength in females in the 2000 mg/kg group on Day 8, reduced motor activity of males in the 2000 mg/kg treatment group on Day 1, and increased motor activity in females in the 2000 mg/kg group on Day 15 were considered incidental to treatment as these effects were not clearly dose-related and were not observed in the other sex.

The LOAEL for AE C638206 in male and female rats was 2000 mg/kg, based on the transient effect of lower body temperature. The NOAEL for male and female rats was 100 mg/kg.

This neurotoxicity study is classified as **Acceptable/Nonguideline**. Upon receipt of provided positive control neuropathology data are submitted by the conducting laboratory, this study can be upgraded to Acceptable/Guideline, satisfying the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

Subchronic Neurotoxicity in rats- fluopicolide

In a subchronic neurotoxicity study (MRID 46474221), Technical Grade AE C638206 (97.8% a.i., Batch # OP2050046) was administered to 10 CD rats/sex at dietary concentrations of 0, 200, 1400, or 10,000 ppm for 13 weeks. Time-weighted average doses were 0, 15.0, 106.6, or 780.6 mg/kg/day, respectively, for males and 0, 18.0, 125.2, or 865.8 mg/kg/day, respectively, for females. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals pre-test and at weeks 4, 8, and 13. At study termination, 6 animals/sex/group were euthanized and perfused in situ for neuropathological examination. Of the perfused animals, control and high-dose rats were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Positive control data for FOB and motor activity testing were submitted in MRID 46474222 and were summarized in MRID 46474220.

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity or gross lesions were observed in any group. FOB findings and motor activity were similar between the treated and control groups.

Mean body weight of the low-dose males and females was similar to the controls throughout the study. Mid- and high-dose males and females had slightly lower body weight than that of the control group beginning at week 1 but these data were not analyzed statistically. Overall body weight gain by the high-dose males and females and mid-dose females was 81%, 72%, and 87% (p # 0.05 or 0.01), respectively, of the respective control levels. The most pronounced effect on body weight gain in the high-dose groups was during weeks 0-1 when males and females gained 56% and 63%, respectively, of the control level. Weight gain by the mid-dose groups appeared to be consistently less than that of controls at each weekly interval. Food consumption by the high-dose males and females was slightly less than that of the controls for most weekly intervals of the study. Excessive food scatter was observed by the mid- and high-dose males and by all treated female groups. Overall food conversion efficiency by the high-dose males and females and mid-dose females was 87%, 79%, and 89%, respectively, of the respective control levels. The most pronounced effect on food efficiency in the high dose groups was during week 1 when males and females were 62% and 69%, respectively, of the control level.

Treatment-related lesions observed in the liver (hypertrophy) of males and females and the male kidney (hyaline droplets) were not considered adverse or relevant to humans.

Therefore, the systemic and neurotoxicity LOAEL for AE C638206 in male and female rats is 10,000 and 1400 ppm, respectively (780.6 and 125.2 mg/kg/day for males and females, respectively) based on decreased body weight gain, food consumption, and food efficiency. The NOAEL for males and females was 1400 and 200 ppm, respectively (106.6 and 18.0 mg/kg/day for males and females, respectively).

This neurotoxicity study is classified as **Acceptable/Nonguideline**. Upon receipt of provided positive control neuropathology data are submitted by the conducting laboratory, this study can be upgraded to Acceptable/Guideline, satisfying the guideline requirement for a subchronic neurotoxicity study in rats (870.6200; OECD 424).

3.3.3 Developmental Toxicity Studies

Developmental toxicity studies have been conducted with fluopicolide in the rat and rabbit. The high dose approached the limit dose for rats but was well below the limit dose for rabbits. Minimal maternal toxicity was evident in rats but was marked in rabbits. At the highest dose tested, pregnant rats had slightly reduced body weight gain that did not affect absolute body weight. In contrast, rabbits demonstrated increased mortality, abortion/premature delivery, decreased food consumption, and decreased body weight gain or weight loss at the highest dose. Developmental toxicity was observed at the highest dose tested in both species. In rats, maternal administration of 700 mg/kg, resulted in delayed fetal growth and skeletal malformations but no treatment-related structural external or visceral abnormalities. In fetal rabbits, maternal treatment with 60 mg/kg/day resulted in increased abortion/premature delivery and delayed fetal growth with no treatment-related structural external, visceral, or skeletal abnormalities.

Developmental Toxicity in rats- fluopicolide

In a developmental toxicity study (MRID 46474120), AE C638206 (97.6 and 97.8% a.i., lot/batch # PP/241024/2 & PP241067/1) was administered to 23 female Sprague-Dawley rats/dose by gavage at dose levels of 0, 5, 60, or 700 mg/kg bw/day from days 7 through 20 of gestation. On gestation day (GD) 21, dams were sacrificed and subjected to gross necropsy. Approximately one-half of the fetuses were fixed in alcohol, examined for external defects, checked for visceral anomalies, and then fixed and examined for skeleton and cartilage defects. The remaining one-half of the fetuses were examined for external defects and then examined for visceral abnormalities by Wilson's slicing technique. The total number of fetuses examined (number of litters) was 284(22), 291(21), 297(22), and 274(21) for the 0, 5, 60, and 700 mg/kg bw/day groups, respectively.

Treatment with 700 mg/kg bw/day was only minimally toxic to the pregnant dams. Mean absolute body weight values were statistically decreased ($p < 0.05$) at several time points as compared to controls, but were not biologically relevant at only 97-98% of control levels. No statistically significant differences were noted in body weight gain at any intervals. However, body weight gain over GD 7-21, both corrected and not corrected for the gravid uterine weight, was bordering on biological significance at 92% and 88%, respectively, of controls. No significant differences were noted in clinical signs and feed consumption, or during gross necropsy.

Therefore, the maternal toxicity LOAEL for AE C638206 in rats is 700 mg/kg bw/day based on marginally reduced body weight gain, and the maternal toxicity NOAEL is 60 mg/kg bw/day.

No adverse, treatment-related, statistically significant effects on pregnancy rates, number of corpora lutea, pre- or post implantation losses, resorptions/dam, fetuses/litter, or fetal sex ratio were observed in the treated groups compared with the controls. No dams had complete litter resorption. No treatment-related malformations or external or visceral variations were observed in any group.

Decreased fetal growth was noted in the high-dose group as evidenced by significant decreases in mean fetal weight (3.4 g vs. 3.7 g for controls), crown/rump length (34.8 mm vs. 36.2 mm for controls), mean placental weight (0.52 g vs. 0.57 for controls), and delays in ossification of sacral vertebra (arch/centra), sternebra, and 5th metacarpal or 5th metatarsal of the forepaw or hindpaw, respectively. The high-dose group also had slightly elevated litter incidences of skeletal defects of the thoracic vertebra (arch: aplasia, dysplasia, fused, fused with attached rib; 4 fetuses from 3/21 litters affected), thoracic vertebra (centra: aplasia, dysplasia, fragmented, fused, dislocated; 10 fetuses from 6/21 litters affected), and ribs (aplasia, dysplasia, shortened, fused, anlage of only 9; 6 fetuses from 3/21 litters affected) compared to the control incidence of 0/22 litters affected.

Therefore, the developmental toxicity LOAEL for AE C638206 in rats is 700 mg/kg bw/day based on delays in fetal growth (decreased fetal weight, crown/rump length, delays in ossification) and skeletal defects of the thoracic vertebra, and ribs and the developmental toxicity NOAEL is 60 mg/kg bw/day.

The developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

Developmental Toxicity in rabbits- fluopicolide

In a developmental toxicity study (MRID 46474122) AE C638206 [Fluopicolide; 97.8% a.i.; batch numbers PP/241024/2 and PP241067/1 (mixed sample)] was administered to 23 mated female Chbb:HM(SPF) Kleinrusse (Himalayan) rabbits/dose by gavage in 1% (w/v) methylcellulose in deionized water at dose levels of 0, 5, 20, or 60 mg/kg bw/day on gestation days (GDs) 6 through 28, inclusive. On GD 29, the surviving dams were sacrificed and necropsied. Gravid uterine weight, corpora lutea counts, and the numbers and positions of live and dead fetuses, early resorptions and late resorptions, empty implantation sites and “conceptuses” were recorded. Fetuses were weighed, measured crown-to-rump, subjected to external, visceral, and skeletal examinations, including cross-sectioning of the eyes, brain, heart, and kidneys. The number of fetuses (litters) examined in the control, low-, mid-, and high-dose groups was 157 (22), 132 (20), 147 (21), and 32 (5), respectively.

Treatment-related clinical signs included deaths of 3 high-dose animals (on GDs 24, 25, and 29) following hypoactivity, decreased defecation and/or decreased hay consumption over the preceding 1-5 days; one decedent also had a bristling haircoat and red discoloration of the urine on the day prior to death. Fifteen high-dose animals aborted or delivered prematurely (during GD 22-28); five of these also showed hypoactivity, decreased defecation, decreased hay consumption, abnormal (“pultaceous”) feces, and/or red discoloration of the urine. One surviving high-dose animal had increased salivation on GD 14. At the highest dose level, there were treatment-related decreases in body weight gain during GD 10-23 (approximately 54-70% of the control levels) and mean weight loss by this group during GD 23-29 (-41.4 g. vs. +123.6 g. for controls). Mean daily food consumption of the high dose-animals (in g/100 g bw) was decreased to 73-89% of controls during GD 8-23 (n.s) and to 46-57% of controls during GD 23-

29 ($p < 0.05$). Red discoloration of the urine was noted from two additional high-dose animals at necropsy for a total of five affected (3 in life and 2 post mortem); this finding is considered treatment-related and possibly adverse.

The maternal LOAEL for Fluopicolide in Himalayan rabbits is 60 mg/kg bw/day, based on death, abortions/premature deliveries, decreased food consumption, and decreased body weight gain. The maternal NOAEL is 20 mg/kg bw/day.

At the highest dose level, there were significant decreases in mean fetal crown-rump length (94% of controls; $p < 0.05$) and mean fetal weight (86%; $p < 0.05$). There were no treatment-related effects on live litter size, numbers of dead fetuses or resorptions, or postimplantation loss. Fetal sex ratio and placental weight were not affected by treatment. There were no treatment-related increases in the fetal or litter incidences of major or minor defects, variations, or retardations, and no evidence of altered ossification was seen.

The developmental LOAEL for Fluopicolide in Himalayan rabbits is 60 mg/kg bw/day, based on abortions, premature deliveries, and decreased fetal body weight and crown-rump length. The developmental NOAEL is 20 mg/kg bw/day.

This developmental toxicity study in the rabbit is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in the rabbit. Excessive maternal toxicity was seen at the highest dose level; however, the dose levels were appropriately spaced, and the small number of litters did not preclude the evaluation of the potential developmental toxicity of fluopicolide.

3.3.4 Reproductive Toxicity Study

Reproductive performance was not affected in a two-generation reproduction toxicity study in which fluopicolide was administered to male and female rats. The most common effect was a decrease in body weight gain in both the parental animals and offspring.

Reproductive Toxicity in rats- fluopicolide

In a two-generation reproduction study (MRID 46474124 and 46474125), AE C638206 (95.9% a.i., batch/lot # OP2050046) was administered to 28 F₀ generation and 24 F₁ generation male and female Crl:CD®(SD)IGS BR rats at concentrations of 0, 100, 500, or 2000 ppm. The dietary levels corresponded to doses of 0, 7.4, 36.4, and 144.6 mg/kg bw/day, respectively, for F₀ males; 0, 8.8, 43.7, and 179.9 mg/kg bw/day for F₁ males; 0, 8.1, 41.0, and 159.7 mg/kg bw/day for F₀ females; and 0, 9.4, 46.9, and 193.9 mg/kg bw/day for F₁ females. The pre-mating period was 10 weeks. The males received the treated or control diets continuously until sacrificed when almost all their litters were weaned, and the females received the diets during pre-mating, mating, gestation, and lactation until sacrifice after weaning their litters.

No treatment-related effects were observed on survival or clinical signs in any group of parental male or female rats in either generation. Absolute body weight and weight gain were

significantly decreased but were within 10% of that of controls in F₀ and F₁ males during pre mating/postmating periods and in female rats during pre mating except as noted below. High-dose F₀ females gained up to 14% (p<0.01) less weight than controls and high-dose F₁ males weighed 11% (p<0.01) less than controls on day 4 of pre mating because of the significantly decreased male pup weight at weaning. Food consumption was significantly decreased during a few weekly intervals in high-dose F₀ and F₁ males and females, but was within 10% of that of controls. Food efficiency was not significantly affected by treatment of male or female rats in either generation. No treatment-related effect was observed on body weight, weight gain, food consumption, or food efficiency in low- or mid-dose male or female rats of either generation.

In high-dose pregnant females, body weight was significantly decreased by 7% on GD 6 and 13 in the F₀ generation and by 10-11% throughout gestation in the F₁ generation compared with that of controls. Both generations gained 14-16% (p<0.01) less weight than controls during the first 13 days of gestation, but weight gain was similar to or greater than that of controls after GD 13. A 13% decrease in body weight gain in mid-dose F₀ females during GD 0-6 was not accompanied by a decrease in body weight. High-dose F₀ and F₁ lactating females had body weight up to 8% and 13% (p<0.01) less, respectively, than controls, but weight gain was not significantly affected. High-dose F₀ and F₁ females consumed up to 12% (p<0.01) less food than controls during the first 13 days of lactation.

Postmortem evaluation showed treatment-related and toxicologically significant effects only in the kidneys. High-dose F₀ and F₁ males had small, statistically significant increases in absolute and relative kidney weights and high-dose F₀ and F₁ females had significant increases in relative kidney weight. No treatment-related gross lesions were observed in male or female rats in either generation. Treatment-related and toxicologically significant histopathologic lesions were observed in the kidneys of high-dose F₀ and F₁ male and female rats. The incidences of cortical tubular basophilia, medullary granular casts, and cortical scarring were significantly increased in high-dose F₀ and F₁ males compared with the control incidences. The incidence of interstitial inflammation was significantly increased in high-dose F₀ males. The increased incidences of cortical tubular dilatation and cortical granular casts in high-dose F₁ males did not reach statistical significance but were considered treatment related. In high-dose F₀ and F₁ female rats, the incidences of cortical tubular basophilia and cortical tubular dilatation were significantly increased and the increased incidence of corticomedullary mineralization was not statistically significant but was considered treatment related.

The lowest-observed-adverse-effect level (LOAEL) for systemic toxicity of AE C638206 in rats is 2000 ppm (144.6-179.9 mg/kg bw/day in males and 159.7-193.3 mg/kg bw/day in females) based on decreases in weight gain in F₀ females and kidney toxicity in F₀ and F₁ males and females. The no-observed-adverse-effect level (NOAEL) is 500 ppm (36.4-43.7 mg/kg bw/day in males and 41.0-46.9 mg/kg bw/day in females).

Evaluation of reproductive parameters showed no treatment-related effects on estrous cycle periodicity or length, sperm measures (motility or sperm count), pre-coital interval, gestation length, or reproductive indices (mating, conception, fertility, and gestation) in either generation. The numbers of implantation sites and viable litters were similar in the treated and control

groups in both generations. No treatment-related gross or microscopic lesions were observed in reproductive organs.

The lowest-observed-adverse-effect level (LOAEL) for reproductive toxicity of AE C638206 in rats was not determined; therefore the no-observed-adverse-effect level (NOAEL) is >2000 ppm (>179.9 mg/kg bw/day in males and >193.3 mg/kg bw/day in females).

No treatment-related effects were observed on the behavior or other clinical signs of offspring of either generation. No treatment-related effects were observed on litter size, sex ratio, or any survival index (postimplantation survival, live birth, viability, and lactation indices) in F₁ or F₂ offspring. The day of attainment of sexual maturation and the body weight at attainment were not affected by treatment with the test material in male or female F₁ offspring. Body weight was significantly reduced by 7-13% in high-dose group F₁ and F₂ male and female pups 14, 21, and 28 days old. Weight gain over the 28-day postnatal period was significantly decreased by 8-9% in high-dose F₁ male and female pups and by 11-14% in high-dose F₂ male and female pups compared with that of controls due primarily to decreases in weight gain occurring after postnatal day 7. Statistically significant changes in organ weights in F₁ and F₂ weanlings (absolute and/or relative spleen, thymus and/or brain) were not accompanied by gross lesions in these organs and microscopic examinations were not conducted.

The lowest-observed-adverse-effect level (LOAEL) for offspring toxicity of AE C638206 in rats is 2000 ppm (144.6-179.9 mg/kg bw/day in males and 159.7-193.3 mg/kg bw/day in females) based on decreases in body weight and weight gain F₁ and F₂ male and female pups. The no-observed-adverse-effect level (NOAEL) is 500 ppm (36.4–43.7 mg/kg bw/day for males and 41.0-46.9 mg/kg bw/day in females).

Kidney toxicity was observed in the parental animals at the high-dose level; therefore, the animals in this study were adequately dosed to assess both reproductive and offspring toxicity.

This study is **Acceptable/Guideline** and it satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800); OECD 416 in rats.

3.3.5 Additional Information from Literature Sources

No additional information on the toxicity of fluopicolide was identified in the open literature.

3.3.6 Pre-and/or Postnatal Toxicity

It is concluded that there is concern for prenatal toxicity resulting from exposure to fluopicolide.

3.3.6.1 Determination of Susceptibility

There was no prenatal susceptibility in the developmental rabbit study with fluopicolide; developmental effects occurred only at doses that caused maternal toxicity. There was no

prenatal susceptibility in the rat multi-generation reproduction study either; adverse effects in the offspring occurred at doses that also caused maternal toxicity.

There was qualitative, but not quantitative susceptibility developmental rat study with fluopicolide. At a dose of 700 mg/kg/day, pregnant rats showed only minimally decreased body weight gain, while the fetuses showed reduced growth and skeletal defects.

3.3.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Postnatal Susceptibility

Since there was evidence of increased susceptibility of offspring following exposure to fluopicolide in rat developmental study, a Degree of Concern Analysis was performed to: 1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and 2) identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment for this chemical. If residual uncertainties are identified, an examination is made whether these residual uncertainties can be addressed by an FQPA safety factor and, if so, the size of the factor needed.

It is concluded that there is low concern for the qualitative susceptibility because: the offspring toxicity was well characterized and was accompanied by maternal toxicity; there was a clear NOAEL/LOAEL for offspring toxicity; and because the dose/endpoint selected for long-term risk assessments is considerably lower and would address the concerns for offspring toxicity seen in this study. Therefore, there are no residual uncertainties for pre- and/or postnatal toxicity.

3.3.7 Recommendation for a Developmental Neurotoxicity Study

The available data on the toxicity of fluopicolide do not support the recommendation for a developmental neurotoxicity study. Prenatal exposure resulting in delayed growth and skeletal effects did not result in central nervous system malformations. While offspring growth was affected at the same dose that also affected parental animals, no functional or behavioral changes were reported in adults or pre- or post-weaning pups (complete neurotoxicity evaluation not done). Clinical signs suggestive of neurotoxicity were not observed in any study at doses that caused systemic toxicity such as decreased body weight or histopathologic lesions. No gross or microscopic pathology was found in neurologic tissues from animals on acute and subchronic neurotoxicity studies or on general subchronic and chronic studies.

3.4 Safety Factor for Infants and Children

Based on the hazard and exposure data, the fluopicolide risk assessment team has recommended that the FQPA Safety Factor be reduced to 1X because there is a complete toxicity database for fluopicolide and exposure data are complete or are estimated based on data that reasonably account for potential exposures. There is no evidence of susceptibility following *in utero* and/or postnatal exposure in the rabbit developmental toxicity study or in the 2-generation rat reproduction study. There is low concern for qualitative susceptibility observed in the rat

developmental toxicity study because the offspring effects (reduced growth and skeletal defects) are well characterized and accompanied by maternal toxicity. There are no residual uncertainties concerning pre- and post-natal toxicity and no neurotoxicity concerns. The dietary food exposure assessment utilizes tolerance level residues and 100% CT. There is no potential for drinking water exposure. There is no potential for residential exposure. Based on these data and conclusions, the FQPA Safety Factor can be reduced to 1X.

3.5 Hazard Identification and Toxicity Endpoint Selection

3.5.1 Acute Reference Dose (aRfD) - Females age 13-49

Study Selected: None. An endpoint attributable to a single dose was not identified from the available data.

MRID No: None

Executive summary: None

Dose and Endpoint for Risk Assessment: None

Comments on Study/Endpoint/Uncertainty Factors: None

3.5.2 Acute Reference Dose (aRfD) - General Population

Study Selected: None. An endpoint attributable to a single dose was not identified from the available data.

MRID No: None

Executive summary: None

Dose and Endpoint for Risk Assessment: None

Comments on Study/Endpoint/Uncertainty Factors: None

3.5.3 Chronic Reference Dose (cRfD)

Study Selected: developmental toxicity -- rabbit OPPTS 3700b

MRID No: 46474122

Executive summary: See Section 3.3.3.

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 20 mg/kg/day, based on death, abortions/premature deliveries, decreased food consumption and decreased body weight at 60

mg/kg/day.

Comments on Study/Endpoint/Uncertainty Factors: A chronic study in rats (MRID 46474139) provided a NOAEL of 31.5/41.0 mg/kg/day (M/F) based on decreased weight gain in males and females and thyroid lesions in males at 109.4/142.2 mg/kg/day (M/F). The duration of dosing and the chronic endpoint are appropriate for this scenario, and represents the highest level at which toxicity is not seen, based on the available data. However, this rabbit developmental study provides valid endpoints which are more protective of populations than any other available study.

$$\text{Chronic RfD} = \frac{20.0 \text{ mg/kg/day}}{100} = 0.2 \text{ mg/kg/day}$$

3.5.4 Incidental Oral Exposure (Short- and Intermediate-Term)

Study Selected: developmental toxicity -- rabbit OPPTS 870.3700b

MRID No: 46474122

Executive summary: See Section 3.3.3.

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 20 mg/kg/day, based on death, abortions/premature deliveries, decreased food consumption and decreased body weight at 60 mg/kg/day.

Comments on Study/Endpoint/Uncertainty Factors: This rabbit developmental study provides endpoints appropriate of short and intermediate term exposure which are more protective of populations than any other available study.

3.5.5 Dermal Absorption

Two studies are available (MRID 46708638 and 46708637 summarized below) which show that 1) dermal absorption through rat skin (in vivo) is as high as 37%, and 2) that human skin (in vitro) is about eight times less permeable than rat skin.

In a dermal penetration study (MRID 46708638), [¹⁴C-Phenyl]-AE C638206 (Fluopicolide; 99.8% radiochemical purity; Batch No. SEL/1200) in a commercial concentrate (or aqueous dilution of a concentrate for the low dose) was applied to the skin of 5 male Sprague-Dawley rats/time point/dose. The dose (1.43 or 659 µg/cm² skin) was applied to 12 cm² skin and removed after 8 hours. The animals were sacrificed at 8, 24, 72, or 144 hours after application. Additionally, 2 male rats/time point/dose were treated similarly in a preliminary study and were sacrificed at 24, 72, or 144 hours, except only 1 rat was treated with the low dose in the 144 hour group.

Recovery of the applied dose was 91-109%. The distribution profile of radioactivity was qualitatively similar between the two dose groups. The majority of the administered dose (41-

69% of the low dose and 87-91% of the high dose) was recovered from the swabs used to remove the test compound from the skin after 8 hours of treatment. A total of 56-81% (low dose) or 92-95% (high dose) was considered not absorbed. After 144 hours, only 2-7% remained at the dose site and was considered available for absorption. Estimates of dermal absorption were based on the sum of urine + feces + cage wash + tissues + treated skin + stratum corneum. **Dermal absorption ranged from 3-8% (low dose) to 22-37% (high dose).** In the main studies, dermal absorption was greatest at 24 hours after application, but there was no clear evidence for increased dermal absorption with time at either dose. Although there was not a time-dependent increase in total dermal absorption at either dose, there was a time-dependent increase in absorption through the stratum corneum at the low dose (but not the high dose).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.7600; OECD none) for a dermal penetration study in rats.

In a non-guideline *in vitro* dermal penetration study (MRID 46708637), [¹⁴C-Phenyl]-AE C638206 (Fluopicolide; 99.8% radiochemical purity; Batch No. SEL/1200) was applied to excised human and rat skin in a suspension concentrate formulation (EXP 11120A) at 2 dose concentrations, 1.9 and 744 µg/cm² skin. Flow-through diffusion cells were prepared for each skin type at each dose level (n=7/group). Dermatomed membranes of approximately 300 µm thickness were tested for permeability prior to treatment. Receptor fluid samples were collected each hour after treatment for 24 hours. At 8 hours after test compound application, the skin was swabbed with a mild detergent solution. After 24 hours, the experiment was terminated, and the skin membranes were tape stripped. The initial 2 tape strips were assumed to represent the residual (non-absorbed) dose. Subsequent tape strips, the remaining skin, and the receptor fluid remaining in the cell and outlet tubing at the end of the experiment were also assayed. Radioactivity was determined by liquid scintillation counting. Results for 5-7 skin samples/species/dose were reported.

Total recovery was 92.3-96.5%. The total amounts of applied radioactivity absorbed within 24 hours at the high dose level were 0.022% in humans and 0.172% in rats, while at low dose levels the amounts absorbed were 1.454% in humans and 14.26% in rats. Therefore, **the amount of radioactive material absorbed was 7.8 times greater for rat skin than for human skin at the high dose level, and 9.8 times greater for rat skin than human skin at the low dose level.** These data indicate that dermal penetration studies in the rat will provide a very conservative estimate of dermal absorption in humans for risk assessment.

This study is **acceptable/non-guideline**.

3.5.6 Dermal Exposure (Short-, Intermediate- and Long-Term)

Study Selected: developmental toxicity -- rabbit OPPTS 870.3700b

MRID No: 46474122

Executive summary: See Section 3.3.3.

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 20 mg/kg/day, based on death, abortions/premature deliveries, decreased food consumption and decreased body weight at 60 mg/kg/day.

Comments on Study/Endpoint/Uncertainty Factors: This rabbit developmental study provides endpoints appropriate of short, intermediate, and long-term exposure. A rat dermal subchronic study (MRID 46708614) showed no local or systemic effects at dose levels up to 1000 mg/kg/day, indicating that the use of the rabbit developmental study endpoint(s) provides adequate protection to all populations.

3.5.7 Inhalation Exposure (Short-, Intermediate- and Long-Term)

Study Selected: developmental toxicity -- rabbit OPPTS 870.3700b

MRID No: 46474122

Executive summary: See Section 3.3.3.

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 20 mg/kg/day, based on death, abortions/premature deliveries, decreased food consumption and decreased body weight at 60 mg/kg/day.

Comments on Study/Endpoint/Uncertainty Factors: An inhalation study is not available for this risk assessment. The rabbit developmental study provides endpoints appropriate of short, intermediate, and long-term exposure and is considered appropriate for this risk assessment. 100% absorption rate is assumed.

3.5.8 Level of Concern for Margin of Exposure

There is no concern for margins of exposure because there is no occupational or residential exposure anticipated in this risk assessment. The following MOEs would apply to the non-dietary endpoints selected in this section (Section 3.5) but not used for this risk assessment.

Table 3.5.8 Summary of Levels of Concern for Risk Assessment.			
Route	Short-Term (1 - 30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	100	100	100
Inhalation	100	100	100
Incidental oral	100	100	100
Residential Exposure			
Dermal	100	100	100
Inhalation	100	100	100

3.5.9 Recommendation for Aggregate Exposure Risk Assessments

Not applicable.

3.5.10 Classification of Carcinogenic Potential

In accordance with the EPA’s Final Guidelines for Carcinogen Risk Assessment (March, 2005), the HED Cancer Assessment Review Committee classified Fluopicolide as **“not likely to be carcinogenic to humans”** based on convincing evidence that a non-genotoxic, mitogenic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely at doses that do not cause perturbations of the liver. Quantification of carcinogenic potential is not required. The cRfD, which is based on the developmental rabbit study, is protective of both chronic and carcinogenic effects.

3.5.11 Summary of Toxicological Doses and Endpoints for Fluopicolide for Use in Human Risk Assessments

Table 3.5.11.1 Summary of Toxicological Doses and Endpoints for Fluopicolide for Use in Dietary and Non-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
Acute Dietary (All Populations)	None	None	None	An endpoint attributable to a single dose was not identified from the available data.
Chronic Dietary (All Populations)	Maternal NOAEL=20 mg/kg/day	UF _A =10x UF _H =10x FQPA SF = 1X	Chronic RfD = 0.2 mg/kg/day cPAD = 0.2 mg/kg/day	Developmental Toxicity Study in Rabbits LOAEL = 60 mg/kg/day based death, abortions/ premature deliveries, decreased food consumption, decreased body weight gain.
Incidental Oral Intermediate-Term (1 - 6 months)	maternal NOAEL = 20 mg/kg/day	UF _A =10x UF _H =10x FQPA SF = 1X	MOE = 100 (occupational) MOE = 100 (residential)	Developmental Toxicity Study in Rabbits LOAEL = 60 mg/kg/day based death, abortions/ premature deliveries, decreased food consumption, decreased body weight gain.
Dermal Short-Intermediate- and Long-Term (1-30 days and 1-6 months)	maternal NOAEL = 20 mg/kg/day	UF _A =10x UF _H =10x FQPA SF = 1X	MOE = 100 (occupational) MOE = 100 (residential)	Developmental Toxicity Study in Rabbits LOAEL = 60 mg/kg/day based death, abortions/ premature deliveries, decreased food consumption, decreased body weight gain.
Inhalation Short-Intermediate- and Long-term (1-30 days and 1-6 months)	maternal NOAEL = 20 mg/kg/day	UF _A =10x UF _H =10x FQPA SF = 1X	MOE = 100 (occupational) MOE = 100 (residential)	Developmental Toxicity Study in Rabbits LOAEL = 60 mg/kg/day based death, abortions/ premature deliveries, decreased food consumption, decreased body weight gain.
Cancer (oral, dermal, inhalation)	Classification: “Not Likely to be Carcinogenic to Humans” .			

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. 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_L = use of a LOAEL to extrapolate a NOAEL. UF_S = use of a short-term study for long-term risk assessment. UF_{DB} = to account for the absence of key

data (i.e., lack of a critical study). FQPA SF = FQPA Safety Factor. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

3.6 Endocrine disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate. Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

4.0 Public Health and Pesticide Epidemiology Data.

4.1 Incident Reports

No information is available since fluopicolide is not registered for use in the U.S.

4.2 National Health and Nutritional Examination Survey (NHANES)

No information is available since fluopicolide is a new pesticide.

4.3 Agricultural Health Study (AHS)

No information is available since fluopicolide is a new pesticide.

4.4 Other Pesticide Epidemiology Published Literature

No information is available since fluopicolide is a new pesticide.

5.0 Dietary Exposure/Risk Characterization

5.1 Pesticide Metabolism and Environmental Degradation

5.1.1 Metabolism in Primary Crops

The qualitative nature of the residue of fluopicolide in grapes is adequately understood. As determined by the HED Risk Assessment Review Committee (RARC1) on 12/21/06, the residue of concern for the tolerance and the risk assessment for fluopicolide on imported grapes (only) is fluopicolide (parent). 2,6-Dichlorobenzamide (BAM) is a metabolite and/or environmental degradate of both fluopicolide and dichlobenil. As determined by the RARC1, BAM will not be included in the tolerance or risk assessment for fluopicolide on imported grapes because 1) as both a plant and rat metabolite, it has been included in the toxicology studies and fluopicolide endpoint selections; and 2) residues of BAM in food resulting from fluopicolide on imported grapes are expected to be negligible since BAM is only 2.0% of the total radioactive residue in the fluopicolide grape metabolism study and is a maximum of only 0.047 ppm in the fluopicolide grape field trials. However, both parent fluopicolide and BAM will be included in risk assessments for future uses of fluopicolide on domestic crops since more exposure to BAM is expected with domestic uses.

Metabolism studies were conducted on greenhouse-grown grapes using suspension concentrate formulations of [2,6-¹⁴C-pyridinyl]fluopicolide and [U-¹⁴C-phenyl]fluopicolide. The formulations were applied to grape vines as three sequential foliar applications at nominal rates of 0.149, 0.104, and 0.104 lb ai/A or 1.49, 1.04, and 1.04 lb ai/A. The total application rates were 0.357 lb ai/A (1x the proposed maximum seasonal rate) and 3.56 lb ai/A (10x the proposed maximum seasonal rate). In mature samples (21-day PHI) at the 1X rate, total radioactive residues (TRR) were 11.754-24.397 ppm in/on forage and 1.012-1.344 ppm in/on fruit. TRR in samples treated at the higher rate were approximately 10x greater than in samples treated at the lower rate.

The majority of radioactivity was found to be on the surface of fruit and foliage samples. Surface washes with acetonitrile (ACN) released ~50-75% TRR from mature foliage samples and ~46-79% TRR from mature fruit samples. Fluopicolide was the primary residue identified in the fruit, accounting for 87-91% TRR (0.91-1.15 ppm) in/on fruit at the 1x treatment rate. Three minor metabolites were identified in fruit at 1X: 2,6-dichlorobenzamide (BAM; AE C653711) at 2.0% TRR (0.026 ppm); 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA; AE C657188) at 2.3% TRR (0.024 ppm), and 2,6-dichloro-*N*-[(3-chloro-5-trifluoromethylpyridin-2-yl)methyl]-3-hydroxybenzamide (AE C643890) at 0.2% TRR (0.002 ppm). Based on these grape metabolism studies, fluopicolide appears to be metabolized slowly in grapes to BAM and PCA, via cleavage of the amide bond, and to AE C643890 by hydroxylation of the phenyl ring in the parent compound.

5.1.2 Metabolism in Rotational Crops

No data pertaining to rotational crops are required for an imported crop (HED SOP 98.6, *Data Requirements for Import Tolerances*, Table 3, 12/3/98).

5.1.3 Metabolism in Livestock

Since no livestock feedstuffs are associated with grapes (Table 1 Feedstuffs, October 2006), no livestock metabolism data are required.

5.1.4 Analytical Methodology

The petitioner has proposed an LC/MS/MS method, Method 00782/M002, for the enforcement of tolerances in grapes and raisins. This method separately determines residues of fluopicolide, BAM, PCA, and AE 1344122 (PIX, a rotational crop metabolite; 3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid). The validated limit of quantitation (LOQ) is 0.01 ppm for each analyte in each commodity. Confirmatory procedures for the method, or an interference study, are needed. Method 00782/M002 has been forwarded to the Analytical Chemistry Branch (ACB/BEAD) for petition method validation. Acceptable data collection methods, Methods 00782 and 00782/M001 (earlier versions of the proposed enforcement method), were used in the storage stability, field trial, and processing studies.

Since there are no livestock feedstuffs associated with grapes, no livestock enforcement methods are required to support use on grapes.

5.1.5 Environmental Degradation

Because the proposed use of fluopicolide is on an imported crop, the environmental degradation of fluopicolide is not relevant to this risk assessment. Since U.S. registration is not required for an imported crop and there are no existing U.S. registrations for fluopicolide, no fluopicolide residues are expected to occur in drinking water.

5.1.6 Comparative Metabolic Profile

In the rat, fluopicolide was readily absorbed and rapidly excreted. The major metabolites identified appeared to be oxidative N-dealkylation cleavage products, including BAM at 0.09% of the total administered dose in rats. The radioactivity concentrations in any given tissue consistently represented considerably less than 1% of the administered dose within 24 hours of administration.

The metabolic profiles of fluopicolide in ruminants and poultry are not relevant to this risk assessment since no livestock feedstuffs are associated with grapes.

In grapes, fluopicolide (parent) was the major residue identified in the fruit, accounting for ~87-91% TRR at the 1X treatment rate. Three minor metabolites were identified in the fruit at 1X:

2,6-dichlorobenzamide (BAM) at 2.0% TRR; 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA) at 2.3% TRR, and 2,6-dichloro-*N*-[(3-chloro-5-trifluoromethylpyridin-2-yl)methyl]-3-hydroxybenzamide (AE C643890) at 0.2% TRR. Fluopicolide appeared to be metabolized slowly in grapes to BAM and PCA, via cleavage of the amide bond, and to AE C643890 via hydroxylation of the phenyl ring in the parent compound.

The metabolic profile of fluopicolide in rotational crops is not relevant to this risk assessment since grapes are an imported crop.

The degradation of fluopicolide in the environment is not relevant to an imported crop. No residues are expected to occur in drinking water.

5.1.7 Toxicity Profile of Major Metabolites and Degradates

The major residue from use of fluopicolide on grapes is parent. Three minor metabolites have been identified: 2,6-dichlorobenzamide (BAM; AE C653711), 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA; AE C657188), and 2,6-dichloro-*N*-[(3-chloro-5-trifluoromethylpyridin-2-yl)methyl]-3-hydroxybenzamide (AE C643890).

BAM is a metabolite and/or environmental degradate of both fluopicolide and dichlobenil. The toxicity of BAM has been recently reviewed (Appendix E, R. Mitkus, 7/27/06). The acute and chronic studies were sufficient to evaluate human hazard potential. BAM demonstrated moderate acute toxicity (Category III) via the oral route of exposure. The small cPAD (0.0045 mg/kg/day) is based on decreased body weight and decreased body weight gain in the chronic oral toxicity study (dog). There was no evidence that BAM was mutagenic or clastogenic in either *in vitro* or *in vivo* assays. A statistically marginal increase ($p < 0.049$) in the incidence of adenomas was observed in female high-dose rats. In the absence of carcinogenicity study data for a second species, HED considers the carcinogenic potential of BAM to be similar to that of a parent compound, dichlobenil (possible human carcinogen); an RfD approach is appropriate for quantification of human cancer risk. BAM is considered to be neurotoxic. No evidence of endocrine modulation was observed in any study with BAM. An FQPA SF of 10X for database uncertainty is applied to all exposure scenarios, in most cases for absence of key data.

As determined by the HED Risk Assessment Review Committee (RARC1) on 12/21/06, BAM will not be included in the tolerance or risk assessment for fluopicolide on imported grapes because 1) as both a plant and rat metabolite of fluopicolide, it has been included in the toxicology studies and fluopicolide endpoint selections; and 2) residues of BAM in food resulting from fluopicolide on imported grapes are expected to be negligible since BAM is only 2.0% of the total radioactive residue in the fluopicolide grape metabolism study and is a maximum of only 0.047 ppm in the fluopicolide grape field trials. However, both parent fluopicolide and BAM will be included in risk assessments for future uses of fluopicolide on domestic crops since more exposure to BAM is expected to occur with domestic uses.

Based on its structure, PCA is not expected to be more toxic than parent.

5.1.8 Pesticide Metabolites and Degradates of Concern

Table 5.1.8 Summary of Fluopicolide Residues to be included in the Risk Assessment and Tolerance Expression for Imported Grapes			
Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Imported Grapes	Primary Crop	fluopicolide <i>per se</i> ¹	fluopicolide <i>per se</i>
	Rotational Crop	Not applicable for an import ²	Not applicable for an import ²
Livestock	Ruminant	Not applicable since no livestock feedstuffs are associated with grapes. ^{2, 3}	Not applicable since no livestock feedstuffs are associated with grapes. ^{2, 3}
	Poultry		
Drinking Water		Not applicable for an import	Not applicable

¹ The risk assessment for domestic crops will include both fluopicolide (parent) and 2,6-dichlorobenzamide (BAM).

² HED SOP 98.6, *Data Requirements for Import Tolerances*, Table 3, 12/3/98.

³ OPPTS 860.1000, Table 1 Feedstuffs (October 2006)

5.1.9 Drinking Water Residue Profile

Since U.S. registration is not required for an imported crop and there are no existing U.S. registrations for fluopicolide, no residues from fluopicolide are expected to occur in drinking water.

5.1.10 Food Residue Profile

Reference:

PP#5E6903. *Petition for Tolerances on Imported Grapes and Raisins. Summary of Analytical Chemistry and Residue Data. DP Number 321209, Amelia Acierto, 1/23/07.*

In a grape metabolism study, the majority of radioactivity was found to be on the surface of fruit and foliage samples; surface washes with ACN released ~97-99% of the total radioactive residues (TRR) from foliage samples collected immediately after application, ~73-93% TRR from foliage samples collected 26-28 days after application, ~50-75% TRR from mature foliage samples, and ~46-79% TRR from mature fruit samples.

In the crop field trials, detectable residues were found in grapes. The majority of the residue was fluopicolide (parent). Residues are not expected to exceed 2.0 ppm in grapes and 6.0 ppm in raisins based on field trials and processing studies summarized below.

Adequate field trial data for grapes are available. A total of 34 grape field trials were conducted in Europe during 2000, 2001, and 2002, with 14 trials conducted in northern Europe (6 trials in Germany and 8 trials in northern France) and 20 trials conducted in southern Europe (8 trials in southern France, 3 trials in Italy, 5 trials in Spain, and 4 trials in Greece). Maximum residues of fluopicolide in/on grapes treated at total rates of 0.34-0.37 lb ai/A and harvested at the proposed

21-day PHI were 1.2 ppm; maximum residues of BAM and PCA were 0.047 ppm and 0.06 ppm, respectively, in/on the same samples.

The submitted grape processing data are adequate, pending submission of supporting storage stability data and information pertaining to individual sample storage intervals. The data indicate that fluopicolide residues do not concentrate in must. Must, which is the unfiltered liquid that results from pressing grapes, is considered to be a surrogate for juice. Fluopicolide residues do concentrate in raisins, with average processing factors of 3.4x for fluopicolide, 4x for BAM, and 4x for PCA.

No rotational crop data are required for an imported crop (HED SOP 98.6, *Data Requirements for Import tolerances*, Table 3, 12/3/98).

No residues are expected to occur in livestock commodities since no livestock feedstuffs are associated with grapes (Table 1 Feedstuffs, October 2006).

5.1.11 International Residue Limits

No Codex, Canadian, or Mexican maximum residue limits (MRLs) or tolerances have been established for fluopicolide.

5.2 Dietary Exposure and Risk

References:

Fluopicolide Chronic Dietary Exposure and Risk Assessment for the Section 3 Registration Action on Imported Grapes, DP Number 334710, N. Dodd, 1/31/07.

Dietary exposure assessments were conducted using the Dietary Exposure Evaluation Model DEEM-FCID™, Version 2.03, which uses food consumption data from the U.S. Department of Agriculture's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. Since U.S. registration is not required for an imported crop and there are no existing U.S. registrations for fluopicolide, no fluopicolide residues are expected to occur in drinking water.

5.2.1 Acute Dietary (Food Only) Exposure/Risk

An appropriate endpoint for fluopicolide was not identified from the available data.

5.2.2 Chronic Dietary (Food Only) Exposure/Risk

The chronic dietary (food only) exposure assessment for fluopicolide on imported grapes was a conservative assessment using the recommended tolerance levels and assuming that 100% of the crop was treated and 100% of the crop was imported. The recommended tolerance levels of 2.0 ppm and 6.0 ppm were used for grapes and raisins, respectively. An adequate processing study was conducted on grapes indicating no concentration in grape juice but concentration in raisins. Since residues of fluopicolide do not concentrate in juice, 2.0 ppm was also used for grape juice

and wine. No default processing factors were used since an adequate processing study was available. No residues are expected to occur in rotational crops since the grapes are imported. Since no livestock feed items are associated with grapes, no residues are expected to occur in livestock commodities.

The results of the chronic dietary exposure analysis for fluopicolide on imported grapes are reported in Table 5.2.2 below. The chronic dietary (food only) exposure to fluopicolide is below HED’s level of concern for the general U.S. population and all population subgroups. The chronic dietary exposure estimates are <1% cPAD for the general U.S. population and 3% cPAD for children 1-2 years old, the most highly exposed subgroup.

Table 5.2.2. Result of Chronic Dietary (Food Only) Exposure and Risk Estimates for Fluopicolide.			
Population Subgroup	PAD, mg/kg/day	DEEM-FCID	
		Exposure, mg/kg/day	% PAD
Chronic Dietary Estimates			
U.S. Population	0.20	0.001129	<1
All infants (< 1 yr)		0.001742	<1
Children 1-2 yrs		0.006272	3
Children 3-5 yrs		0.003827	2
Children 6-12 yrs		0.001456	<1
Youth 13-19 yrs		0.000513	<1
Adults 20-49 yrs		0.000697	<1
Adults 50+ yrs		0.000828	<1
Females 13-49 yrs		0.000748	<1
Cancer Dietary Estimate			
U.S. Population	Classification: “Not likely to be Carcinogenic to Humans”		

5.2.3 Cancer Dietary Risk

Fluopicolide is not likely to be carcinogenic to humans; therefore, a cancer risk assessment was not conducted.

5.3 Anticipated Residue and Percent Crop Treated (%CT) Information

Anticipated residues/percent crop treated (% CT) data were not needed to refine the risk assessment so they were not used.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

There are no U.S. registrations for fluopicolide; therefore, no residential exposure is expected.

7.0 Aggregate Risk Assessments and Risk Characterization

Since exposure is expected to occur only from food, no aggregate exposure is expected to occur in the U.S. as a result of fluopicolide on imported grapes.

8.0 Cumulative Risk Characterization/Assessment

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to fluopicolide and any other substances and fluopicolide does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that fluopicolide has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at <http://www.epa.gov/pesticides/cumulative/>.

9.0 Occupational Exposure/Risk Pathway

No occupational exposure to fluopicolide is expected to occur in the U.S. as a result of fluopicolide on imported grapes.

10.0 Data Needs and Label Requirements

10.1 Toxicology

None

10.2 Residue Chemistry

Pending the resolution of Residue Chemistry Deficiencies #'s 1a and 1b (pertaining to directions for use), Deficiency # 2 (pertaining to the requirement for a proposed confirmatory method or an interference study), Deficiency #3 (pertaining to the need for the proposed enforcement method, Method 00782/M002, to undergo a successful petition method validation by ACB/BEAD), and Deficiency #6 (pertaining to a revised Section F), there are no Residue Chemistry data gaps that would preclude permanent tolerances for residues of fluopicolide as follows:

Grape.....	2.0 ppm
Grape, raisin.....	6.0 ppm

This decision is based on use of water dispersible granular (WDG) or emulsifiable concentrate (EC) formulations without adjuvants. To use other formulations (other than WDG and EC formulations) or spray adjuvants, additional residue data (or review of additional residue data) would be required as indicated in Deficiencies 1c and 1d.

Deficiency #4 (pertaining to storage stability data for fluopicolide in juice or must and raisins) and Deficiency #5 (pertaining to length of storage information for the processed commodities) are confirmatory data requirements which must be resolved but can be resolved after establishment of the tolerances.

Residue Chemistry Deficiencies

860.1200 Directions for Use

- 1a. Residue data were submitted which reflected use of a 4.44% WDG formulation (WG71) and a 95 g/L suspo-emulsion formulation (SE10), which is similar to an emulsifiable concentrate (EC) formulation. The petitioner should submit representative labels or a revised Section B to indicate the types of formulations to be used on imported grapes.
- 1b. A Section B was submitted which provided some information regarding the proposed use pattern on imported grapes, including the maximum number of applications per season (3), the maximum seasonal application rate (0.36 lb ai/A), the minimum preharvest interval (PHI; 21 days), and retreatment intervals (10-14 days). The petitioner should submit a representative label or a revised Section B to more fully describe the use pattern(s) to be applied to grapes and raisins to be exported to the USA. The additional information to be provided to the Agency should include the maximum single application rate, application timing (as it relates to the plant growth stage), names and quantities of stickers, spreaders, and other adjuvants (if any) to be added to the spray solution, application tank-mix preparation, volume of spray mix per unit area (hectare or acre), and type of application equipment.
- 1c. No spray adjuvants were used in the crop field trials submitted to support this petition. If the petitioner intends to recommend use of spray adjuvants, residue data reflecting use of spray adjuvants should be submitted.
- 1d. The submitted residue data reflect use of WDG and EC types of formulations. If other types of formulations are to be used on grapes to be imported, additional residue data would be needed to reflect use of those other types of formulations.

860.1340 Residue Analytical Methods

2. The petitioner must propose confirmatory procedures for the proposed enforcement method, or submit an interference study for fluopicolide.
3. The proposed enforcement method, Method 00782/M002, must be validated as an adequate enforcement method by ACB/BEAD.

860.1380 Storage Stability

4. The petitioner must submit data demonstrating the stability of residues of fluopicolide in grape juice (or must) and raisins stored frozen for 29 months or the maximum storage interval for each of these commodities.

860.1520 Processed Food and Feed

5. The petitioner should submit the actual dates of collection, extraction, and analysis for each sample of grape juice (or must) and raisins from the processing studies to determine the storage interval required for the storage stability study.

860.1550 Proposed Tolerances

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

10.3 Occupational and Residential Exposure

None.

References:

PP#5E6903. Petition for Tolerances on Imported Grapes and Raisins. Summary of Analytical Chemistry and Residue Data. DP Number 321209, Amelia Acierto, 1/23/07.

Fluopicolide Chronic Dietary Exposure and Risk Assessment for the Section 3 Registration Action on Imported Grapes, DP Number 334710, N. Dodd, 1/31/07.

Appendix A: Toxicology Assessment

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for food use for fluopicolide are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Test	Technical	
	Required	Satisfied
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	yes	yes
870.3150 Oral Subchronic (nonrodent)	yes	yes
870.3200 21-Day Dermal	no	-
870.3250 90-Day Dermal	no	-
870.3465 90-Day Inhalation	no	-
870.3700a Developmental Toxicity (rodent)	yes	yes
870.3700b Developmental Toxicity (nonrodent)	yes	yes
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (rodent)	yes	yes ¹
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes ¹
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5375 Mutagenicity—Structural Chromosomal Aberrations ...	yes	yes
870.5395 Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a Acute Delayed Neurotox. (hen)	no	-
870.6100b 90-Day Neurotoxicity (hen)	no	-
870.6200a Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b 90-Day Neuro. Screening Battery (rat)	yes	yes
870.6300 Develop. Neuro	no	-
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	no	-
Special Studies for Ocular Effects		
Acute Oral (rat)	no	-
Subchronic Oral (rat)	no	-
Six-month Oral (dog)	no	-

¹ Endpoint is met with combined chronic toxicity/carcinogenicity study in rats.

A.2 Toxicity Profiles

Table A.2.1 Acute Toxicity Profile - Test Substance				
Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral [rat]	46709903 46708601 46709803	LD ₅₀ ≥ 2000 mg/kg (m/f) LD ₅₀ ≥ 2000 mg/kg (f) LD ₅₀ ≥ 2000 mg/kg (m/f)	III III III
870.1100	Acute oral [rat] - studies with metabolites 1) 2,6-dichlorobenzamide 2) 1,3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid 3) 3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid	46708602 46708603 46708604	LD ₅₀ ≥ 2000 mg/kg (m) and LD ₅₀ ≥ 300 mg/kg (f) LD ₅₀ ≥ 2000 mg/kg (m/f) LD ₅₀ ≥ 2000 mg/kg (f)	II III III
870.1200	Acute dermal [rat]	46709904 46708605 46709804	LD ₅₀ ≥ 4000 mg/kg LD ₅₀ ≥ 5000 mg/kg LD ₅₀ ≥ 4000 mg/kg	III IV III
870.1300	Acute inhalation [rat]	46709905 46708606 46709805	LC ₅₀ ≥ 1.789 mg/L LC ₅₀ ≥ 5.16 mg/L LC ₅₀ ≥ 3.195 mg/L	III IV IV
870.2400	Acute eye irritation [rabbit]	46709906 46709806	chemosis/corneal opacity in both studies	III III
870.2500	Acute dermal irritation [rabbit]	46709907 46709807	slight (PDII = 0.08) slight (PDII = 0.25)	IV IV
870.2600	Skin sensitization [guinea pig]	46709908 46708608 46709808	Negative (Buehler method) Negative (Magnusson-Kligman Design method) Negative (modified Buehler method)	non-sensitizer non-sensitizer non-sensitizer

Table A.2.2 Subchronic, Chronic and Other Toxicity Profile			
Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100	90-Day oral toxicity (rat)	46474112 (2000) Acceptable/guideline M: 0, 7.4, 109, 1668 mg/kg/d F: 0, 8.4, 119, 1673 mg/kg/day	NOAEL = 109 mg/kg/day for males; 8.4 mg/kg/day for females LOAEL = 1668 mg/kg/day for males and 119 mg/kg/day for females based on hypertrophy of the zona glomerulosa in the adrenal gland (M/F), decreased cellularity of the bone marrow (M/F), and trabecular hyperostosis of the bone joint (M)
870.3100	90-Day oral toxicity (mouse)	46474114 (2000) Acceptable/guideline M/F: 0, 5.5, 53, 545 or 1092 mg/kg/day	NOAEL = >1092 mg/kg/day (M/F) LOAEL = not identified
870.3100	90-Day oral toxicity (mouse)	46474116 (2001) Acceptable/guideline M: 0, 10.4, 37.8, 161, 770 mg/kg/d F: 0, 12.6, 52.8, 207, 965 mg/kg/day	NOAEL = >770 mg/kg/day for males; 207 mg/kg/day for females LOAEL = not identified for males; 965 mg/kg/day for females based on increased incidence of liver oval cell proliferation
870.3150	90-Day oral toxicity (dog)	46474118 (2000) Acceptable/guideline M&F: 0, 5, 70, 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (M/F) LOAEL = not identified (M/F)
870.3200	21/28-Day dermal toxicity (species)	46708614 (2003) Acceptable Guideline 0, 100, 250, 500, 1000 mg/kg/day	NOAEL = 1000 mg/kg/day LOAEL > 1000 mg/kg/day No local or systemic toxicity observed
870.3700a	Prenatal developmental in (rat)	46474120 (2001) Acceptable/guideline F: 0, 5, 60, 700 mg/kg/day (GD 7-20)	Maternal NOAEL = 60 mg/kg/day LOAEL = 700 mg/kg/day based on decreased body weight gain Developmental NOAEL = 60 mg/kg/day LOAEL = 700 mg/kg/day based on delayed fetal growth and skeletal malformations
870.3700b	Prenatal developmental in (rabbit)	46474122 (2001) Acceptable/guideline F: 0, 5, 20, 60 mg/kg/day (GD 6-28)	Maternal NOAEL = 20 mg/kg/day LOAEL = 60 mg/kg/day based on death, abortion/premature delivery, decreased food consumption and weight gain Developmental NOAEL = 20 mg/kg/day LOAEL = 60 mg/kg/day based on abortion/premature delivery, decreased fetal body weight and crown-rump length

Table A.2.2 Subchronic, Chronic and Other Toxicity Profile			
Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3800	Reproduction and fertility effects (rat)	46474124 (2003) 46474125 (additional data, 2004) 46474126 (range-finding, 2002) Acceptable/guideline M: 0, 7.4, 36.4, 144.6 mg/kg/d F: 0, 8.1, 41.0, 159.7 mg/kg/day	Parental/Systemic NOAEL = 36.4/41.0 mg/kg/day (M/F) LOAEL = 144.6/159.7 mg/kg/day (M/F) based on kidney toxicity in males and females and decreased weight gain in females. Reproductive NOAEL = >144.6/159.7 mg/kg/day (M/F) LOAEL = not identified. Offspring NOAEL = 36.4/41.0 mg/kg/day (M/F) LOAEL = 144.6/159.7 mg/kg/day (M/F) based on decreased body weight and weight gain.
870.4100b	Chronic toxicity (dog)	44674128 (2002) Acceptable/guideline M&F: 0, 70, 300, 1000 mg/kg/day	NOAEL = 300 mg/kg/day (M); >1000 mg/kg/day (F) LOAEL = 1000 mg/kg/day based on decreased body weight gain (M); not identified (F)
870.4200b	Carcinogenicity (mouse)	46474130 (2003) Acceptable/guideline M: 0, 7.9, 64.5, 551.0 mg/kg/d F: 0, 11.5, 91.9, 772.3 mg/kg/day	NOAEL = 64.5/91.9 mg/kg/day (M/F) LOAEL = 551.0/772.3 mg/kg/day (M/F) based on decreased body weight and weight gain and liver lesions. no evidence of carcinogenicity
870.4300	Chronic/ Carcinogenicity (rat)	46474139 (2003) Acceptable/guideline M: 0, 2.1, 8.4, 31.5, 109.4 mg/kg/day F: 0, 2.8, 10.8, 41.0, 142.2 mg/kg/day	NOAEL = 31.5/41.0 mg/kg/day (M/F) LOAEL = 109.4/142.2 mg/kg/day based on decreased body weight gain (M/F) and thyroid toxicity (M). no evidence of carcinogenicity

Table A.2.2 Subchronic, Chronic and Other Toxicity Profile			
Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5100	Gene Mutation (<i>Salmonella typhimurium</i>)	<p>46474146 (2001) Unacceptable/guideline 1.6- 5000 µg/plate</p> <p>46474202 (2001) Acceptable/guideline 1.6- 5000 µg/plate</p> <p>46474148 (2001) Acceptable/guideline 1.6- 5000 µg/plate</p> <p>46474144 (2001) Acceptable/guideline 1.6- 5000 µg/plate</p> <p>46474142 (2004) Acceptable/guideline AE638206 (batch mixture of PP/241067/1 and PP/241024) 5 - 5000 µg/plate</p>	<p>negative (non-mutagenic) Upgradable if purity for test material is given.</p> <p>negative (non-mutagenic)</p> <p>negative (non-mutagenic)</p> <p>negative (non-mutagenic)</p> <p>positive (mutagenic)</p>
870.5300	Gene mutation (Chinese hamster lung cells)	<p>46474204 (2000) Acceptable/guideline AE638206 (batch mixture of PP/241067/1 and PP/241024) 1.2- 3820 µg/mL</p>	negative (non-mutagenic)
870.5375	Cytogenetics	<p>46474208 (2001) Acceptable/guideline 1.22 to 625 µg/mL</p> <p>46474206 (2004) Acceptable/guideline AE638206 (batch mixture of PP/241067/1 and PP/241024) 3.2 to 100 µg/mL</p>	<p>negative for chromosome aberrations</p> <p>positive for aberrations without S9 activation</p>

Table A.2.2 Subchronic, Chronic and Other Toxicity Profile			
Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5395	Micronucleus (mouse)	46474214 (2003) Acceptable/guideline 150, 300 or 600 mg/kg/day 46474210 (2005) Acceptable/guideline AE638206 (batch mixture of PP/241067/1 and PP/241024) 200, 600 or 2000 mg/kg/day	negative at doses up to 600 mg/kg negative at doses up to 2000 mg/kg

Table A.2.2 Subchronic, Chronic and Other Toxicity Profile			
Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.5550	Unscheduled DNA Synthesis (rat hepatocytes)	42169839 (1989) Acceptable/guideline	negative at concentration up to 300 µg/mL in cultured rat hepatocytes
(no OPPTS no./ FIFRA test guideline 84-2)	Other Genotoxicity Unscheduled DNA synthesis (rat hepatocytes)	46474216 (2000) Acceptable/guideline AE638206 (batch mixture of PP/241067/1 and PP/241024) 600 or 2000 mg/kg	negative at concentrations up to 2000 mg/kg in hepatocytes from treated rats
870.6200a	Acute neurotoxicity screening battery (rat)	46474218 (2002) 46474219 (range-finding, 2002) Acceptable/guideline M/F: 0, 10, 100, 2000 mg/kg	NOAEL = 100 mg/kg (M/F) LOAEL = 2000 mg/kg (M/F) based on transiently lowered body temperature.
870.6200b	Subchronic neurotoxicity screening battery	46474221 (2002) 46474222 (positive control, 2002) Acceptable/guideline M: 0, 15.0, 106.6, 780.6 mg/kg/day F: 0, 18.0, 125.2, 865.8 mg/kg/day	NOAEL = 106.6/18.0 mg/kg/day (M/F) LOAEL = 780.6/125.2 mg/kg/day based on decreased body weight gain, food consumption, and food efficiency.
870.6300	Developmental neurotoxicity	None	
870.7485	Metabolism and pharmacokinetics (rat)	46474242 (2004)-main studies 46474241 (2001) 46474244 (2003) 46474226 (2003) 46474239 (2003)	rapid absorption, metabolism and excretion; main metabolites were oxidative N-dealkylation cleavage products. Primary route of excretion is fecal and urinary with little accumulation in the tissues.
870.7600	Dermal penetration (rat)	46708638 (2003) Acceptable Guideline 1.43, 659 ug/cm ² skin	In vivo study Dermal Penetration rate: 37%
870.7600	Dermal penetration (comparative)	46708637 (2003) Acceptable Non-guideline 1.9, 744 ug/cm ² skin	In vitro study Rat skin dermal penetration rate is 7.8 times greater than human skin.

A.3 Executive Summaries

A.3.1 Subchronic Toxicity

870.3100 90-Day Oral Toxicity – Rat

In a 90-day oral toxicity study (MRID 46474112) Fluopicolide (Lot # AE C638206 00 1C99 0005; 97.2% a.i.) was administered to groups of 10 male and 10 female Sprague Dawley rats in a diet containing 0, 100, 1400 or 20,000 ppm (equivalent to 0, 7.4, 109 or 1668 mg/kg/day for males, and 8.4, 119 or 1673 mg/kg/day for females) for 13 weeks. Ten additional rats/sex from the control and high dose group were maintained on control diet for a further four weeks to determine the reversibility of any effects seen.

Two nontreatment-related mortalities were noted in the high dose group. Body weight gain over the course of the 20,000 ppm treatment was reduced by 41% in males and 29% in females, while the corresponding mean food consumption was reduced by 22% and 19% ($p < 0.01$). Body weight gain was dramatically affected the first week of the study as evidenced by essentially no weight gain at the highest dose as compared to controls that gained an average of 58 g for males and 39 g for females. Reduced food consumption was also most dramatic during this week at about 50% for both sexes. Water consumption was 43% higher for females relative to the controls ($p < 0.01$) during this same time frame and was somewhat higher for the remainder of the study. An increase in urinary volume and a slight decrease in specific gravity was observed in females only which corresponds to the increased water intake. No toxicologically relevant hematological or clinical chemistry findings were noted. Microscopic examination showed a minimal to slight hypertrophy of the zona glomerulosa in the adrenal of 17/20 of the rats at the highest dose level compared to one of each sex in the controls, and minimal changes were seen in 3/10 females at the 1400 ppm level. Minimum to slight trabecular hyperostosis of the bone joint was observed in 7/10 males and all females at the 20,000 ppm level compared to 0/10 males and 3/10 females in the control group. Decreased cellularity of the bone marrow was observed for 7/10 males and 9/10 females at 20,000 ppm, and in 8/10 females at 1400 ppm compared to 0/10 males and 1/10 females in the control group. No treatment-related effects were observed at the 100 ppm dose level.

Following the four week off-dose period there was a complete or partial recovery of all treatment-related effects.

The LOAEL is 20,000 ppm in the diet (1668 mg/kg/day) for males based on hypertrophy of the zona glomerulosa in the adrenal, trabecular hyperostosis of the bone joint, and decreased cellularity of the bone marrow. The LOAEL for females is 1400 ppm in the diet (119 mg/kg/day) based on hypertrophy of the zona glomerulosa in the adrenal and decreased cellularity of the bone marrow. The NOAEL is 1400 ppm (109 mg/kg/day) for males and 100 ppm (7.9 mg/kg/day) for females.

This 90-day oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

870.3100 90-Day Oral Toxicity – Mouse

In a 90-day oral toxicity study (MRID 46474114), Fluopicolide (>96.9% a.i., batch/lot #AE C638206 00 1C99 0005) was administered to 10 Crl: CD-1 (ICR) BR mice/sex/dose in diet at concentrations of 0, 32, 320, 3200, or 6400 ppm (equivalent to 0, 5.5, 53, 545, or 1092 mg/kg bw/day).

There were no compound related effects on mortality, clinical signs of toxicity or measured hematological parameters. The overall body weight gain was reduced by 22%-32% in females at 3200 ppm and 20% in males at 6400 ppm. At 3200 ppm, there were statistically significant increases in alanine aminotransferase activity in males and females (79%-98% and 116%-147%, respectively), and in aspartate aminotransferase (39%-69%) activity in males. A statistically significant increase was also noted at 6400 ppm in alkaline phosphatase activity (106%) in males.

At 3200 ppm, both absolute and relative liver weights increased (33%-60% and 36%-78%, respectively) in both sexes. The microscopic examination revealed hepatocellular hypertrophy in all males of the top two dose groups, all females in the 6400 ppm dose group, and 9/10 females in the 3200 ppm dose group. These findings were accompanied by the presence of slight focal hepatocytic necrosis in 2/10 females at 3200 ppm and 3/10 males and 3/10 females at 6400 ppm. Minimal to slight centrilobular hepatocytic hypertrophy in the liver was also seen in 9/10 males and minimal hypertrophy in 1/10 females at 320 ppm. **A LOAEL was not identified in this study. The NOAEL is the highest dose tested, 6400 ppm (1092 mg/kg/day).**

This 90-day oral toxicity study in the mouse is **Acceptable/Guideline**, and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice.

In a 90-day oral toxicity study (MRID 46474116, summarized in MRID 46474115), AE C638206 (Fluopicolide, 95.9% a.i., Batch # OP2050046) was administered to 10 C57BL/6JICO mice/sex/dose in the diet at concentrations of 0, 50, 200, 800, or 3200 ppm (approximately 10.4, 37.8, 161, or 770 mg/kg/day for males and 12.6, 52.8, 207, or 965 mg/kg/day for females, respectively). Doses were selected based on previous results from a 90-day mouse dietary study with AE C638206 using Crl:CD1 (1 CR) Br mice (MRIDs 46474114 and 46474113).

There were nine deaths that appeared unrelated to treatment (no dose-response relationship). There were no adverse effects on clinical signs or neurological parameters noted for the surviving animals. Although body weight of males and females in the 3200 ppm group was lower by 7-10% early in the study, final mean body weights were comparable with the controls (both 97% of controls). The overall weight gain was slightly reduced in males in the 800 and 3200 ppm groups and in females in the 3200 ppm group (86-93% of control gain). There were some clinical chemistry variations such as slight decreases in the concentration of albumin and total cholesterol in animals treated with 800 ppm of AE C638206 and slightly increased alkaline phosphatase enzyme activity in males in the 3200 ppm group.

There was a slight dose-related increase in absolute (110 - 125% of control) and relative (114 - 130% of control) liver weight in animals treated with 800 ppm of AE C638206. These weight changes were associated with a diffused centrilobular hepatocellular liver hypertrophy. Microscopic examination revealed this lesion in 4/8 and 8/8 surviving male mice (control: 0/8) and in 8/9 and 10/10 surviving female mice (control: 0/8) at 800 and 3200 ppm of AE C638206, respectively. In addition, there was a dose-related increase in liver oval cell proliferation in females: 2/9, 2/9, 3/10, 4/9, and 8/10 in the control through the high dose groups, respectively. The toxicological significance of dark coloration of the liver in 4/8 males and 9/10 females treated with 3200 ppm was not determined.

Under the conditions of this study, the LOAEL for AE C638206 in male mice is not established; the LOAEL for female is 3200 ppm based on liver oval cell proliferation. The NOAEL for AE C638206 in male mice is 3200 ppm and for female mice is 800 ppm.

This 90-day oral toxicity study in the mouse is **Acceptable/Guideline**, and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408).

870.3150 90-Day Oral Toxicity – Dog

In a subchronic oral toxicity study (MRID 46474118) AE C638206 (97.7%, Lot Nos. PP/241024/2 and PP/241067/1) was administered to 4 beagle dogs/sex/dose by oral gavage at concentrations of 0, 5, 70 and 1000 mg/kg/day for 13 weeks.

There were no significant compound-related effects on mortality, clinical signs, food consumption, food efficiency, body weight/body weight gain, ophthalmoscopic examinations, urinalysis, hematology, clinical chemistry, organ weight, gross pathology or histopathology. Increased liver weight in males and females receiving 1000 mg/kg/day AE C 638206 was considered an exposure-related effect, however, there were no correlative changes in clinical pathology or histopathology. A marginal effect on body weight gain was also seen in males and females dosed with 70 and 1000 mg/kg/day.

A LOAEL was not determined for AE C638206 in male and female dogs in this study. The NOAEL was 1000 mg/kg/day, the highest dose administered.

This 90-day oral toxicity study in the dog is **Acceptable/Guideline** and satisfies the requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in a non-rodent.

870.3200 21/28-Day Dermal Toxicity – Rat

In a 28-day dermal toxicity study (MRID 46708614) AE C638206 (fluopicolide; 97.7% a.i., Batch # 2050190/PP241024/2) was applied to the shaved skin of 10 Wistar rats/sex/dose at dose levels of 0, 100, 250, 500, or 1000 mg/kg/day (limit dose), 6 hours/day for 5days/week during a 28-day period.

No compound-related effects were observed in mortality, clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic exams, hematology, clinical chemistry, absolute or relative organ weights, or gross or microscopic pathology in either sex.

The LOAEL was not observed. The NOAEL for local and systemic toxicity is 1000 mg/kg/day.

This study is classified as **acceptable guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

870.3465 90-Day Inhalation – Rat

No studies available.

A.3.2 Prenatal Developmental Toxicity

870.3700a Prenatal Developmental Toxicity Study – Rat

In a developmental toxicity study (MRID 46474120), AE C638206 (97.6 and 97.8% a.i., lot/batch # PP/241024/2 & PP241067/1) was administered to 23 female Sprague-Dawley rats/dose by gavage at dose levels of 0, 5, 60, or 700 mg/kg bw/day from days 7 through 20 of gestation. On gestation day (GD) 21, dams were sacrificed and subjected to gross necropsy. Approximately one-half of the fetuses were fixed in alcohol, examined for external defects, checked for visceral anomalies, and then fixed and examined for skeleton and cartilage defects. The remaining one-half of the fetuses were examined for external defects and then examined for visceral abnormalities by Wilson's slicing technique. The total number of fetuses examined (number of litters) was 284(22), 291(21), 297(22), and 274(21) for the 0, 5, 60, and 700 mg/kg bw/day groups, respectively.

Treatment with 700 mg/kg bw/day was only minimally toxic to the pregnant dams. Mean absolute body weight values were statistically decreased ($p < 0.05$) at several time points as compared to controls, but were not biologically relevant at only 97-98% of control levels. No statistically significant differences were noted in body weight gain at any intervals. However, body weight gain over GD 7-21, both corrected and not corrected for the gravid uterine weight, was bordering on biological significance at 92% and 88%, respectively, of controls. No significant differences were noted in clinical signs and feed consumption, or during gross necropsy.

Therefore, the maternal toxicity LOAEL for AE C638206 in rats is 700 mg/kg bw/day based on slightly reduced body weight gain, and the maternal toxicity NOAEL is 60 mg/kg bw/day.

No adverse, treatment-related, statistically significant effects on pregnancy rates, number of corpora lutea, pre- or post implantation losses, resorptions/dam, fetuses/litter, or fetal sex ratio were observed in the treated groups compared with the controls. No dams had complete litter

resorption. No treatment-related malformations or external or visceral variations were observed in any group.

Decreased fetal growth was noted in the high-dose group as evidenced by significant decreases in mean fetal weight (3.4 g vs. 3.7 g for controls), crown/rump length (34.8 mm vs. 36.2 mm for controls), mean placental weight (0.52 g vs. 0.57 for controls), and delays in ossification of sacral vertebra (arch/centra), sternebra, and 5th metacarpal or 5th metatarsal of the forepaw or hindpaw, respectively. The high-dose group also had slightly elevated litter incidences of skeletal defects of the thoracic vertebra (arch: aplasia, dysplasia, fused, fused with attached rib; 4 fetuses from 3/21 litters affected), thoracic vertebra (centra: aplasia, dysplasia, fragmented, fused, dislocated; 10 fetuses from 6/21 litters affected), and ribs (aplasia, dysplasia, shortened, fused, anlage of only 9; 6 fetuses from 3/21 litters affected) compared to the control incidence of 0/22 litters affected.

Therefore, the developmental toxicity LOAEL for AE C638206 in rats is 700 mg/kg bw/day based on delays in fetal growth (decreased fetal weight, crown/rump length, delays in ossification) and skeletal defects of the thoracic vertebra, and ribs and the developmental toxicity NOAEL is 60 mg/kg bw/day.

The developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

870.3700b Prenatal Developmental Toxicity Study – Rabbit

In a developmental toxicity study (MRID 46474122) AE C638206 [Fluopicolide; 97.8% a.i.; batch numbers PP/241024/2 and PP241067/1 (mixed sample)] was administered to 23 mated female Chbb:HM(SPF) Kleinrusse (Himalayan) rabbits/dose by gavage in 1% (w/v) methylcellulose in deionized water at dose levels of 0, 5, 20, or 60 mg/kg bw/day on gestation days (GDs) 6 through 28, inclusive. On GD 29, the surviving dams were sacrificed and necropsied. Gravid uterine weight, corpora lutea counts, and the numbers and positions of live and dead fetuses, early resorptions and late resorptions, empty implantation sites and “conceptuses” were recorded. Fetuses were weighed, measured crown-to-rump, subjected to external, visceral, and skeletal examinations, including cross-sectioning of the eyes, brain, heart, and kidneys. The number of fetuses (litters) examined in the control, low-, mid-, and high-dose groups was 157 (22), 132 (20), 147 (21), and 32 (5), respectively.

Treatment-related clinical signs included deaths of 3 high-dose animals (on GDs 24, 25, and 29) following hypoactivity, decreased defecation and/or decreased hay consumption over the preceding 1-5 days; one decedent also had a bristling haircoat and red discoloration of the urine on the day prior to death. Fifteen high-dose animals aborted or delivered prematurely (during GD 22-28); five of these also showed hypoactivity, decreased defecation, decreased hay consumption, abnormal (“pultaceous”) feces, and/or red discoloration of the urine. One surviving high-dose animal had increased salivation on GD 14. At the highest dose level, there were treatment-related decreases in body weight gain during GD 10-23 (approximately 54-70%

of the control levels) and mean weight loss by this group during GD 23-29 (-41.4 g. vs. +123.6 g. for controls). Mean daily food consumption of the high dose-animals (in g/100 g bw) was decreased to 73-89% of controls during GD 8-23 (n.s) and to 46-57% of controls during GD 23-29 (p<0.05). Red discoloration of the urine was noted from two additional high-dose animals at necropsy for a total of five affected (3 in life and 2 post mortem); this finding is considered treatment-related and possibly adverse.

The maternal LOAEL for Fluopicolide in Himalayan rabbits is 60 mg/kg bw/day, based on death, abortions/premature deliveries, decreased food consumption, and decreased body weight gain. The maternal NOAEL is 20 mg/kg bw/day.

At the highest dose level, there were significant decreases in mean fetal crown-rump length (94% of controls; p<0.05) and mean fetal weight (86%; p<0.05). There were no treatment-related effects on live litter size, numbers of dead fetuses or resorptions, or postimplantation loss. Fetal sex ratio and placental weight were not affected by treatment. There were no treatment-related increases in the fetal or litter incidences of major or minor defects, variations, or retardations, and no evidence of altered ossification was seen.

The developmental LOAEL for Fluopicolide in Himalayan rabbits is 60 mg/kg bw/day, based on abortions, premature deliveries, and decreased fetal body weight and crown-rump length. The developmental NOAEL is 20 mg/kg bw/day.

This developmental toxicity study in the rabbit is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in the rabbit. Excessive maternal toxicity was seen at the highest dose level; however, the dose levels were appropriately spaced, and the small number of litters did not preclude the evaluation of the potential developmental toxicity of fluopicolide.

A.3.3 Reproductive Toxicity

870.3800 Reproduction and Fertility Effects – Rat

In a two-generation reproduction study (MRID 46474124 and 46474125), AE C638206 (95.9% a.i., batch/lot # OP2050046) was administered to 28 F₀ generation and 24 F₁ generation male and female Crl:CD®(SD)IGS BR rats at concentrations of 0, 100, 500, or 2000 ppm. The dietary levels corresponded to doses of 0, 7.4, 36.4, and 144.6 mg/kg bw/day, respectively, for F₀ males; 0, 8.8, 43.7, and 179.9 mg/kg bw/day for F₁ males; 0, 8.1, 41.0, and 159.7 mg/kg bw/day for F₀ females; and 0, 9.4, 46.9, and 193.9 mg/kg bw/day for F₁ females. The pre-mating period was 10 weeks. The males received the treated or control diets continuously until sacrificed when almost all their litters were weaned, and the females received the diets during pre-mating, mating, gestation, and lactation until sacrifice after weaning their litters.

No treatment-related effects were observed on survival or clinical signs in any group of parental male or female rats in either generation. Absolute body weight and weight gain were significantly decreased but were within 10% of that of controls in F₀ and F₁ males during

prematuring/postmaturing periods and in female rats during prematuring except as noted below. High-dose F₀ females gained up to 14% (p<0.01) less weight than controls and high-dose F₁ males weighed 11% (p<0.01) less than controls on day 4 of prematuring because of the significantly decreased male pup weight at weaning. Food consumption was significantly decreased during a few weekly intervals in high-dose F₀ and F₁ males and females, but was within 10% of that of controls. Food efficiency was not significantly affected by treatment of male or female rats in either generation. No treatment-related effect was observed on body weight, weight gain, food consumption, or food efficiency in low- or mid-dose male or female rats of either generation.

In high-dose pregnant females, body weight was significantly decreased by 7% on GD 6 and 13 in the F₀ generation and by 10-11% throughout gestation in the F₁ generation compared with that of controls. Both generations gained 14-16% (p<0.01) less weight than controls during the first 13 days of gestation, but weight gain was similar to or greater than that of controls after GD 13. A 13% decrease in body weight gain in mid-dose F₀ females during GD 0-6 was not accompanied by a decrease in body weight. High-dose F₀ and F₁ lactating females had body weight up to 8% and 13% (p<0.01) less, respectively, than controls, but weight gain was not significantly affected. High-dose F₀ and F₁ females consumed up to 12% (p<0.01) less food than controls during the first 13 days of lactation.

Postmortem evaluation showed treatment-related and toxicologically significant effects only in the kidneys. High-dose F₀ and F₁ males had small, statistically significant increases in absolute and relative kidney weights and high-dose F₀ and F₁ females had significant increases in relative kidney weight. No treatment-related gross lesions were observed in male or female rats in either generation. Treatment-related and toxicologically significant histopathologic lesions were observed in the kidneys of high-dose F₀ and F₁ male and female rats. The incidences of cortical tubular basophilia, medullary granular casts, and cortical scarring were significantly increased in high-dose F₀ and F₁ males compared with the control incidences. The incidence of interstitial inflammation was significantly increased in high-dose F₀ males. The increased incidences of cortical tubular dilatation and cortical granular casts in high-dose F₁ males did not reach statistical significance but were considered treatment related. In high-dose F₀ and F₁ female rats, the incidences of cortical tubular basophilia and cortical tubular dilatation were significantly increased and the increased incidence of corticomedullary mineralization was not statistically significant but was considered treatment related.

The lowest-observed-adverse-effect level (LOAEL) for systemic toxicity of AE C638206 in rats is 2000 ppm (144.6-179.9 mg/kg bw/day in males and 159.7-193.3 mg/kg bw/day in females) based on decreases in weight gain in F₀ females and kidney toxicity in F₀ and F₁ males and females. The no-observed-adverse-effect level (NOAEL) is 500 ppm (36.4-43.7 mg/kg bw/day in males and 41.0-46.9 mg/kg bw/day in females).

Evaluation of reproductive parameters showed no treatment-related effects on estrous cycle periodicity or length, sperm measures (motility or sperm count), precoital interval, gestation length, or reproductive indices (mating, conception, fertility, and gestation) in either generation. The numbers of implantation sites and viable litters were similar in the treated and control

groups in both generations. No treatment-related gross or microscopic lesions were observed in reproductive organs.

The lowest-observed-adverse-effect level (LOAEL) for reproductive toxicity of AE C638206 in rats was not determined; therefore the no-observed-adverse-effect level (NOAEL) is >2000 ppm (>179.9 mg/kg bw/day in males and >193.3 mg/kg bw/day in females).

No treatment-related effects were observed on the behavior or other clinical signs of offspring of either generation. No treatment-related effects were observed on litter size, sex ratio, or any survival index (postimplantation survival, live birth, viability, and lactation indices) in F₁ or F₂ offspring. The day of attainment of sexual maturation and the body weight at attainment were not affected by treatment with the test material in male or female F₁ offspring. Body weight was significantly reduced by 7-13% in high-dose group F₁ and F₂ male and female pups 14, 21, and 28 days old. Weight gain over the 28-day postnatal period was significantly decreased by 8-9% in high-dose F₁ male and female pups and by 11-14% in high-dose F₂ male and female pups compared with that of controls due primarily to decreases in weight gain occurring after postnatal day 7. Statistically significant changes in organ weights in F₁ and F₂ weanlings (absolute and/or relative spleen, thymus and/or brain) were not accompanied by gross lesions in these organs and microscopic examinations were not conducted.

The lowest-observed-adverse-effect level (LOAEL) for offspring toxicity of AE C638206 in rats is 2000 ppm (144.6-179.9 mg/kg bw/day in males and 159.7-193.3 mg/kg bw/day in females) based on decreases in body weight and weight gain F₁ and F₂ male and female pups. The no-observed-adverse-effect level (NOAEL) is 500 ppm (36.4–43.7 mg/kg bw/day for males and 41.0-46.9 mg/kg bw/day in females).

Kidney toxicity was observed in the parental animals at the high-dose level; therefore, the animals in this study were adequately dosed to assess both reproductive and offspring toxicity.

This study is **Acceptable/Guideline** and it satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800); OECD 416 in rats.

A.3.4 Chronic Toxicity

870.4100a (870.4300) Chronic Toxicity – Rat

A combined chronic toxicity/carcinogenicity study in rats is included in section A.3.5 below.

870.4100b Chronic Toxicity – Dog

In a chronic toxicity study (MRID 46474128) AE C638206 (95.9%, Lot No. OP2050046) was administered to 5 beagle dogs/sex/dose by oral gavage at concentrations of 0, 70, 300 and 1000 mg/kg/day for 52 weeks.

There were no significant compound-related effects based on mortality, clinical signs, food consumption, ophthalmoscopic examinations, urinalysis, hematology, clinical chemistry, organ weight, gross pathology or histopathology. Body weight gain was inhibited in males treated with 1000 mg fluopicolide/kg/day. Although increased cholesterol concentrations at the end of the study were statistically significant and slightly above the historical range in females treated with 1000 mg/kg/day, there were no correlative changes indicating an exposure-related effect on lipid metabolism in these animals. One female in the 300 mg/kg/day dosage group died during the study, however, the death was not conclusively associated with exposure to fluopicolide.

The LOAEL for AE C638206 in male dogs was 1000 mg/kg/day based on decreased body weight gain. The NOAELs were 300 and 1000 mg/kg/day for males and females, respectively.

This chronic study in the dog is **Acceptable/Guideline** and satisfies the requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in a non-rodent.

A.3.5 Carcinogenicity

870.4200a Carcinogenicity Study – rat

In a combined chronic toxicity/carcinogenicity study (MRID 46474139), AE C638206 (Fluopicolide, 95.9%, a.i.; Batch No. OP2050046) was administered to 60 Crl:CD (SD) IGS BR rats/sex/dose in the diet at concentrations of 0 (controls), 50, 200, 750 or 2500 ppm (equivalent to 0, 2.1, 8.4, 31.5 or 109.4 mg/kg bw/day in males and 0, 2.8, 10.8, 41.0 or 142.2 mg/kg bw/day in females) for up to 104 weeks. An additional 20 animals/sex/dose were administered the same concentration and sacrificed after 52 weeks of treatment for a interim sacrifice. A third set of 10 animals/sex/dose were fed the treated diet at the same concentrations for 52 weeks followed by 13 weeks of being fed basal diet prior to sacrifice in a recovery study. A report, MRID 46474138, which consisted of a summary of the study profile was provided as an additional source of information.

Statistical analysis showed no increased incidence of mortality in any of the treated groups compared to controls. The only clinical signs observed were in the females rats and consisted of yellow perigenital staining, brown staining of the pinna and brown staining of the dorsum. Statistical significance was not evaluated for these clinical signs. Yellow perigenital staining and the brown staining of the pinna was observed primarily in the 2500 ppm females in the main study with these signs beginning around week 13 and increasing to weeks 47-53 when they were observed in 21-31% of the females at 2500 ppm. Both effects then started to diminish and were seen in few animals (<5%) by the end of the second year. Similar results were observed in the 52 week study. Brown staining of the dorsum was observed but was not seen in a concentration-related increase, affecting controls as well as treated females. These clinical signs appear to be of low toxicological significance due to a lack of corresponding urinalysis, clinical chemistry or histopathology effects identified, and they were transient with most effects minimizing after the first year. While palpable masses were observed and monitored, there was not a treatment-related increase in the incidence of these masses.

There was no statistically significant difference in body weight in any of the treated groups. A statistically significant ($p < 0.05$ or $p < 0.01$) decrease in mean body weight gain was observed in weeks 0-1 in both studies at the highest dose in males (33%) and females (28%), compared to controls. In the main study, a statistically significant ($p < 0.01$) decrease was also seen in the females at 200 (20%) and 750(32%) ppm groups. The only significant decrease in body weight gain in weeks 1-2 of the main study was in males at 2500 ppm and females at 50 and 2500 ppm, and these gains were decreased 11% in the males and 15 and 42% in the females, respectively, compared to controls. After this time, body weight and body weight gain remained lower than controls (n.s.) with the overall body weight gain of the 2500 ppm group being 11% and 17% less than controls in the males and females, respectively. In the animals dosed for 52 weeks, the similar effect of decreased body weight gain in the highest dosed males and females was observed with statistical significance in the first 2 weeks. Both male and female rats had comparable body weight gain by the end of the recovery period. A corresponding decrease in food consumption and food efficiency was observed in the highest dosed group of males and females during the first two weeks of treatment; however, statistical analysis was not included in the report.

Statistical differences in hematology and clinical chemistry were not toxicologically significant. Those observed were minor, sporadic and did not have a clear treatment-related association.

Statistically significant increases ($p < 0.01$ or 0.05) in relative (to body weight) and absolute kidney (122- 137%), thyroid (154-163%) and liver (122-134%) weights were observed in the males at 2500 ppm in the main study. These same increases were observed in the males at 2500 ppm in the 52 week study, except for absolute kidney weight. Females at 2500 ppm in the 52 week study had statistically significant increases in relative, but not absolute, liver and kidney weights; this was associated with a significant decrease in terminal body weight.

Males had a statistically significant increase in the incidence of and severity of non-neoplastic microscopic lesions in the thyroid, kidney and liver in the main study. A corresponding increase ($p < 0.05$) in the incidence of enlarged kidneys and thyroids were present in the males at 2500 ppm compared to controls on gross observation. On histopathological examination, an increased incidence of thyroid cystic follicular hyperplasia was present in the males and observed in 0/60, 1/37, 0/37, 4/35 and 7/60 ($p < 0.05$) in the controls, 50, 200, 750 or 2500 ppm males. Lesions in the kidney were those associated with the alpha -2u-globulin accumulation normally present in male aged rats and are not considered adverse or applicable to human risk assessment. The liver effect, centrilobular hepatocyte hypertrophy, observed both at 52 and 104 weeks was considered to be an adaptive change due to treatment. During the recovery period, all lesions present were reversed except for a slight increase in the severity of the renal cortical tubular basophilia in the males. Females had no statistically significant differences in lesions in any of the dose groups in either the toxicity or the main study.

The lowest-observed-adverse effect level (LOAEL) for AE C638206 in rats is 2500 ppm (109.4 and 142.2 mg/kg/day for males and females, respectively) based on decreases in body weight gain (M and F) and an increase in thyroid organ weight with corresponding

increases in the incidence of thyroid lesions (M only). The no-observed-adverse effect level (NOAEL) for AE C638206 is 750 ppm (31.5 and 41.0 in males and females, respectively).

At the doses tested, there was not a treatment related increase in tumor incidence of any type in animals dosed with up to 2500 ppm AE C638206 for up to 104 weeks. Dosing was considered adequate based on the decreased body weight gain in the male and female rats at 2500 ppm, and the non-neoplastic lesions observed at 2500 ppm in males. While effects were minimal in the female, a reproductive study, MRID 46474124 (main study) and 46474125 (supplemental study histopathological evaluation of liver and kidneys) indicated kidney toxicity (microscopic lesions) in male and female rats in both parental generations and decreased body weight gain in the F₀ females treated with AC638206 for 16 weeks at 2000 ppm indicating adequate dosing in this study at 2500 ppm.

This chronic/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a chronic/carcinogenicity study [(OPPTS 870.4300); OECD 453] in rats.

870.4200b Carcinogenicity (feeding) – Mouse

In a carcinogenicity study (MRID 46474130) AE C638206 (Fluopicolide) (95.9% a.i., batch #OP2050046) was administered to 50 C57BL/6 mice/sex/dose in the diet at dietary levels of 0, 50, 400, or 3200 ppm (equivalent to 0, 7.9, 64.5, 551.0 mg/kg bw/day for males, and 0, 11.5, 91.9, and 772.3 mg/kg bw/day for females) for 18 months. Satellite groups of 10 C57BL/6 mice/sex/dose were similarly treated for 12 months. Historical control incidences of hepatocellular lesions were provided (MRID 46474135).

The incidence of mortality and clinical signs was similar in treated and control groups. Body weights and body weight gains of only the 3200 ppm animals were significantly decreased throughout the study. After 78 weeks, the body weight of 3200 ppm males was 20% lower and of females was 16% lower than of the control group, and overall body weight gains were 45% lower for males and 35% lower for females. Food consumption was decreased in the 3200 ppm satellite and main group animals up to 18% throughout the study. The overall (week 1-78) food efficiency was decreased 40% for males and 30% for females at 3200 ppm. Hematology evaluations were not conducted, and there were no treatment-related changes in serum enzyme activities. After 52 weeks, absolute and relative liver weights were significantly increased in 400 ppm males (15-30%), and in 3200 ppm males and females (35-99%). After 78 weeks, liver weights were increased in both sexes at 400 ppm (15-33%) and 3200 (46-81%). At both 400 and 3200 ppm, the liver weight increases were correlated with a significant increase in the incidence of hepatocyte hypertrophy after 52 and 78 weeks, in males and females. The hypertrophy is likely a non-specific adaptive response to a xenobiotic and is not considered adverse. The 3200 ppm animals had statistically significant increases in the incidence of enlarged liver and altered liver cell foci (most common type was acidophilic) after 78 weeks, and a non-significant increase after 52 weeks (#2/10 for each lesion).

The LOAEL for AE C638206 in mice is 3200 ppm for both sexes (551.0 mg/kg/day for males, 772.3 mg/kg/day for females), based on severely decreased body weights and body

weight gains and liver lesions in both sexes. The NOAEL is 400 ppm in both sexes (64.5 mg/kg/day for males, 91.9 mg/kg/day for females).

At the doses tested, there was a treatment related increase in the incidence of hepatocellular adenoma when compared to controls. The 3200 ppm animals had statistically significant increases in hepatocellular adenoma in both sexes after 78 weeks, and a small increase after 52 weeks. The adenoma incidence after 78 weeks at 0, 50, 400 and 3200 ppm was 5/50, 0/50, 5/50, and 11/50, respectively, for males, and 1/50, 2/50, 0/50, and 16/50, respectively, for females. After 52 weeks, hepatocellular adenoma was found in 3/10 high-dose females but no males. The adenomas were correlated with an increased incidence of liver masses and nodules at necropsy. Dosing was considered adequate based on decreased body weight gains, decreased food efficiency, and liver lesions seen in both sexes at the high dose.

This carcinogenicity study is **Acceptable/Guideline** and satisfies guideline requirements for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice.

A.3.6 Mutagenicity

Gene Mutation

Guideline 84-2, Reverse gene mutation MRID 46474146 Unacceptable/guideline	dose range: 1.6 to 5000 µg/plate No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 µg/plate. Therefore, AEC638206 00 IC99 0005 is considered nonmutagenic in the conventional battery of bacterial strains. This study was considered unacceptable/guideline because purity information for the test material was not provided.
Guideline 84-2, Reverse gene mutation MRID 46474202 acceptable/guideline	dose range: 1.6 to 5000 µg/plate No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 µg/plate. Therefore, AEC638206 00 IC99 0001 is considered nonmutagenic in the conventional battery of bacterial strains.
Guideline 84-2, Reverse gene mutation MRID 46474148 acceptable/guideline	dose range: 1.6 to 5000 µg/plate No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 µg/plate. Therefore, AEC638206 00 IB99 0002 is considered nonmutagenic in the conventional battery of bacterial strains.
Guideline 84-2, Reverse gene mutation MRID 46474144 acceptable/guideline	dose range: 1.6 to 5000 µg/plate No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 µg/plate. Therefore, AEC638206 Technical is considered nonmutagenic in the conventional battery of bacterial strains.
Guideline 84-2, Reverse gene mutation MRID 46474142 acceptable/guideline	dose range: 5 to 5000 µg/plate AE C638206 (fluopicolide) is considered mutagenic at precipitating concentrations in the conventional battery of bacterial strains.

Cytogenetics

Guideline 84-2, <i>in vitro</i> mammalian cells in culture/gene mutation assay in Chinese hamster lung cells MRID 46474204 Acceptable/guideline	1.2 to 3820 µg/mL, 0.4 to 120 µg/mL and 0.313 to 60 µg/mL with and without activation No concentration in any of the three experiments was a biologically relevant, reproducible increase in mutant colonies found at concentrations up to the highest subcytotoxic levels. Both positive controls showed marked increases.
Guideline 84-2, <i>in vivo</i> mammalian chromosome aberrations MRID 46474208 Acceptable/guideline	1.25 to 625 µg/mL without activation and 4.88 to 625 µg/mL with activation In the presence of ≥50% reduction in the MI, no statistically significant increases in the structural or numerical (polyploidy) aberrant metaphases were found at any test concentration in either trial, compared to marked (p≤0.001) increases in both positive controls.
Guideline 84-2, <i>in vitro</i> mammalian chromosome aberrations MRID 46474206 Acceptable/guideline	3.2 to 100 µg/mL with and without activation; 0.1 to 6.3 µg/mL without activation This batch mixture of AE C638206 is considered a clastogen in the <i>in vitro</i> Chinese hamster lung (V79) cell system in the absence of S9 activation.
Guideline 84-2, <i>in vivo</i> mammalian cytogenetics MRID 46474214 Acceptable/guideline	IP injection of 150, 300 or 600 mg/kg/day No dose up to the HDT was a significantly increased number of mPCEs recorded, in the presence of a statistically increased ratio of PCEs to NCEs (evidence of interference with erythropoiesis), either when compared to vehicle controls, or to the laboratory's 8-year historical control data base. The positive control registered a marked increase in mPCEs, in the absence of any alteration of erythropoietic effects.
Guideline 84-2, <i>in vivo</i> mammalian cytogenetics MRID 46474210 Acceptable/guideline	Two oral doses of 200, 600, or 2000 mg/kg/day, 24 hours apart No adverse clinical signs were observed during the main study. The ratio of polychromatic to normochromatic erythrocytes was unaffected by treatment. Additionally, at no dose level up to the limit dose, (2000 mg/kg/day), were increased numbers of mPCEs induced by the test article, compared with the marked increases observed in CPA-treated cells.

Other Genotoxicity

Guideline (no # given), Unscheduled DNA synthesis in hepatocytes MRID 46474216 acceptable/guideline	600 or 2000 mg/kg There was no evidence (or a dose-related positive response) that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts) was induced at either timed sacrifice in rats exposed to the test material up to the limit dose (2000 mg/kg).
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A.3.7 Neurotoxicity

870.6100 Delayed Neurotoxicity Study – Hen

Not required for this chemical.

870.6200 Acute Neurotoxicity Screening Battery

In an acute neurotoxicity study (MRID 46474218; summarized in MRID 46474217), groups of fasted, 6- to 7-week old CD rats (10/sex) were given a single oral dose of AE C638206 (95.9% a.i., batch/lot #OP2050046) in 1% methylcellulose at doses of 0, 10, 100, or 2000 mg/kg bw and observed for 15 days. Doses were based on a range-finding study in which single doses of 50 mg/kg induced behavioral changes (MRID 46474219). Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed in 10 animals/sex/group pretreatment, on Day 1 (at six hours post-dosing, the time of peak effect), and on Days 8 and 15. 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, the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There was no effect of treatment on body weight, body weight gain, food consumption, food efficiency, brain weight, brain measurements (cerebral hemispheres), or incidence of gross or microscopic lesions. Lower body temperature in the high-dose males and females at the time of peak effect (6 hours post-dosing) on the day of treatment (Day 1) was the only treatment-related observation during the FOB. This sign was not observed on Days 8 or 15. A statistically significant decrease in forelimb grip strength in females in the 2000 mg/kg group on Day 8, reduced motor activity of males in the 2000 mg/kg treatment group on Day 1, and increased motor activity in females in the 2000 mg/kg group on Day 15 were considered incidental to treatment as these effects were not clearly dose-related and were not observed in the other sex.

The LOAEL for AE C638206 in male and female rats was 2000 mg/kg, based on the transient effect of lower body temperature. The NOAEL for male and female rats was 100 mg/kg.

This neurotoxicity study is classified as **Acceptable/Guideline**, and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424) provided positive control neuropathology data are submitted by the conducting laboratory.

870.6200 Subchronic Neurotoxicity Screening Battery

In a subchronic neurotoxicity study (MRID 46474221), Technical Grade AE C638206 (97.8% a.i., Batch # OP2050046) was administered to 10 CD rats/sex at dietary concentrations of 0, 200, 1400, or 10,000 ppm for 13 weeks. Time-weighted average doses were 0, 15.0, 106.6, or 780.6 mg/kg/day, respectively, for males and 0, 18.0, 125.2, or 865.8 mg/kg/day, respectively, for females. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals pre-test and at weeks 4, 8, and 13. At study termination, 6 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, control and high-dose rats were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Positive control

data for FOB and motor activity testing were submitted in MRID 46474222 and were summarized in MRID 46474220.

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity or gross lesions were observed in any group. FOB findings and motor activity were similar between the treated and control groups.

Mean body weight of the low-dose males and females was similar to the controls throughout the study. Mid- and high-dose males and females had slightly lower body weight than that of the control group beginning at week 1 but these data were not analyzed statistically. Overall body weight gain by the high-dose males and females and mid-dose females was 81%, 72%, and 87% ($p \# 0.05$ or 0.01), respectively, of the respective control levels. The most pronounced effect on body weight gain in the high-dose groups was during weeks 0-1 when males and females gained 56% and 63%, respectively, of the control level. Weight gain by the mid-dose groups appeared to be consistently less than that of controls at each weekly interval. Food consumption by the high-dose males and females was slightly less than that of the controls for most weekly intervals of the study. Excessive food scatter was observed by the mid- and high-dose males and by all treated female groups. Overall food conversion efficiency by the high-dose males and females and mid-dose females was 87%, 79%, and 89%, respectively, of the respective control levels. The most pronounced effect on food efficiency in the high dose groups was during week 1 when males and females were 62% and 69%, respectively, of the control level.

Treatment-related lesions observed in the liver (hypertrophy) of males and females and the male kidney (hyaline droplets) were not considered adverse or relevant to humans.

Therefore, the systemic and neurotoxicity LOAEL for AE C638206 in male and female rats is 10,000 and 1400 ppm, respectively (780.6 and 125.2 mg/kg/day for males and females, respectively) based on decreased body weight gain, food consumption, and food efficiency. The NOAEL for males and females was 1400 and 200 ppm, respectively (106.6 and 18.0 mg/kg/day for males and females, respectively).

The study is classified as **Acceptable/Guideline** and does satisfy the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b) provided positive control neuropathology data are submitted by the conducting laboratory.

870.6300 Developmental Neurotoxicity Study

Not required for this chemical.

A.3.8 Metabolism

870.7485 Metabolism - Rat

Five studies (MRIDs 46474226, 46474239, 46474241, 46474242, and 46474244) were conducted to examine the metabolism and disposition of AE C638206 (fluopicolide) in male and

female Sprague-Dawley CD rats following single doses of [¹⁴C-2,6-pyridyl]-AE C638206 at 10 mg/kg bw or [¹⁴C-2,6-pyridyl]-AE C538206 and [¹⁴C-U-phenyl]-AE C638206 at 10 and 100 mg/kg bw. Rats were subjected to the dosing regimens above using [¹⁴C-2,6-pyridyl]-AE C538206 (lot nos. 903AE-3 and GAR 2034-4; >99% and 97% radiochemical purity) or [¹⁴C-U-phenyl]-AE C538206 (lot no. CFQ 12747; 99.1% radiochemical purity) and nonlabeled test article (batch no. R001737, 99.3% chemical purity). Excretion, tissue distribution, pharmacokinetics (blood/plasma), and metabolite profiles were determined. In MRID 46474242, metabolite profiles were assessed in urine and fecal extracts of male and female rats given a single oral 10 mg/kg bw dose of [¹⁴C-2,6-pyridyl]-AE C638206 and sacrificed at 48 hours post-dosing.

There were no biologically significant treatment-related effects noted during the course of the study. Overall recovery of administered radioactivity was an acceptable 93.9-103.6%. The data supported the contention that AE C538206 is readily absorbed and rapidly excreted within 72 hours following a single oral dose of 10 mg/kg. Fecal elimination accounted for 68.8-72.4% of the administered radioactivity whereas urinary excretion accounted for only 18.8-21.4% of the administered radioactivity. In the bile excretion study (MRID 46474244), 51.7% of the administered radioactive dose in both sexes was excreted by the cannulated bile duct indicating a significant portion of the radioactivity recovered in feces is derived from hepatic metabolism of AE C638206. Time-course blood/plasma and tissue studies revealed rapid absorption and distribution of administered radioactivity to all organs and tissues followed by moderately rapid excretion with reduction to background levels in most tissues and organs within 72 hours. Tissue concentrations peaked at 6-7 hours post-dosing and about 96% of the peak tissue concentrations were dissipated between 6-7 hours and 168 hours post-dose. Absorption and excretory patterns did not exhibit gender-related variability, but blood/plasma kinetic studies (MRID 46474226) suggest near-saturation of absorption at the high dose (100 mg/kg bw). Based upon tissue burden data, neither AE C638206 nor its metabolites appear to undergo any significant tissue sequestration. With the exception of transiently higher levels in the liver, kidneys, and intestines during the elimination phase, radioactivity concentrations in any given tissue consistently represented considerably less than 1% of the administered dose within 24 hours of administration of AE C638206.

Both urinary and fecal metabolites were quantified by HPLC and most were identified using HPLC or HPLC/MS in conjunction with known standards. The major metabolites identified appeared to be oxidative N-dealkylation cleavage products. Extraction efficiency appeared to be excellent and most components in both of the matrices examined (urine and feces) were adequately quantified and characterized. The available data, based upon studies using [¹⁴C-2,6-pyridyl]-AE C638206, affirmed the metabolism pathway (Appendix, Figure 1) proposed by the investigators.

These metabolism studies (MRID 46474226, 46474239, 46474241, 46474242, and 46474244) are, collectively, **Acceptable/Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)]. The studies were properly designed, conducted and reported.

870.7600 Dermal Absorption – Rat

In a dermal penetration study (MRID 46708638), [¹⁴C-Phenyl]-AE C638206 (Fluopicolide; 99.8% radiochemical purity; Batch No. SEL/1200) in a commercial concentrate (or aqueous dilution of a concentrate for the low dose) was applied to the skin of 5 male Sprague-Dawley rats/time point/dose. The dose (1.43 or 659 µg/cm² skin) was applied to 12 cm² skin and removed after 8 hours. The animals were sacrificed at 8, 24, 72, or 144 hours after application. Additionally, 2 male rats/time point/dose were treated similarly in a preliminary study and were sacrificed at 24, 72, or 144 hours, except only 1 rat was treated with the low dose in the 144 hour group.

Recovery of the applied dose was 91-109%. The distribution profile of radioactivity was qualitatively similar between the two dose groups. The majority of the administered dose (41-69% of the low dose and 87-91% of the high dose) was recovered from the swabs used to remove the test compound from the skin after 8 hours of treatment. A total of 56-81% (low dose) or 92-95% (high dose) was considered not absorbed. After 144 hours, only 2-7% remained at the dose site and was considered available for absorption. Estimates of dermal absorption were based on the sum of urine + feces + cage wash + tissues + treated skin + stratum corneum. **Dermal absorption ranged from 3-8% (low dose) to 22-37% (high dose).** In the main studies, dermal absorption was greatest at 24 hours after application, but there was no clear evidence for increased dermal absorption with time at either dose. Although there was not a time-dependent increase in total dermal absorption at either dose, there was a time-dependent increase in absorption through the stratum corneum at the low dose (but not the high dose).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.7600; OECD none) for a dermal penetration study in rats.

In a non-guideline *in vitro* dermal penetration study (MRID 46708637), [¹⁴C-Phenyl]-AE C638206 (Fluopicolide; 99.8% radiochemical purity; Batch No. SEL/1200) was applied to excised human and rat skin in a suspension concentrate formulation (EXP 11120A) at 2 dose concentrations, 1.9 and 744 µg/cm² skin. Flow-through diffusion cells were prepared for each skin type at each dose level (n=7/group). Dermatomed membranes of approximately 300 µm thickness were tested for permeability prior to treatment. Receptor fluid samples were collected each hour after treatment for 24 hours. At 8 hours after test compound application, the skin was swabbed with a mild detergent solution. After 24 hours, the experiment was terminated, and the skin membranes were tape stripped. The initial 2 tape strips were assumed to represent the residual (non-absorbed) dose. Subsequent tape strips, the remaining skin, and the receptor fluid remaining in the cell and outlet tubing at the end of the experiment were also assayed. Radioactivity was determined by liquid scintillation counting. Results for 5-7 skin samples/species/dose were reported.

Total recovery was 92.3-96.5%. The total amounts of applied radioactivity absorbed within 24 hours at the high dose level were 0.022% in humans and 0.172% in rats, while at low dose levels the amounts absorbed were 1.454% in humans and 14.26% in rats. Therefore, **the amount of radioactive material absorbed was 7.8 times greater for rat skin than for human skin at the**

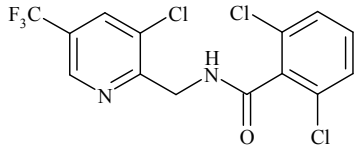
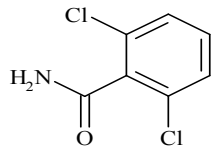
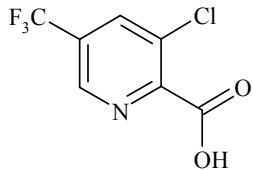
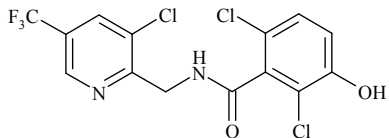
high dose level, and 9.8 times greater for rat skin than human skin at the low dose level. These data indicate that dermal penetration studies in the rat will provide a very conservative estimate of dermal absorption in humans for risk assessment.

This study is **acceptable/non-guideline**.

A.3.9 Special/Other Studies

None.

Appendix B: Metabolism Assessment

Table B. Tabular Summary of Fluopicolide and its Metabolites and Degradates in Plants				
Chemical Name (other names in parenthesis)	Matrix	Percent TRR (PPM) ¹		Structure
		Matrices - Major Residue, >10%TRR	Matrices - Minor Residue, <10%TRR	
Parent fluopicolide 2,6-dichloro- <i>N</i> -[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]-benzamide (AE C638206)	Grapes (fruit)	91.2 % (1.152 ppm) (phenyl label) 87.4% (0.910 ppm) (pyridinyl label)		
Metabolite 1: 2,6-dichlorobenzamide (BAM) (AE C653711)	Grapes (fruit)		2.0% (0.026 ppm) (phenyl label only)	
Metabolite 2: 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA) (AE C657188)	Grapes (fruit)		2.3% (0.024 ppm) (pyridinyl label only)	
Metabolite 3: 2,6-dichloro- <i>N</i> -[(3-chloro-5-trifluoromethylpyridin-2-yl)methyl]-3-hydroxybenzamide (AE C643890)	Grapes (fruit)		0.2% (0.002 ppm) (phenyl label only)	
Grapes: MRID 46474025; three foliar applications for a total seasonal rate of 0.357 lb ai/A (1x); 21-day PHI				

Appendix C: Tolerance Assessment Summary and Table

HED has determined that the terminal residue of concern in grape for the tolerance expression is fluopicolide *per se* [2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide]. The tolerance expression (fluopicolide) proposed in the petition is appropriate.

No Codex, Canadian, or Mexican maximum residue limits (MRLs) or tolerances have been established for fluopicolide.

Adequate field trial data for grapes are available. The available field trial data will support a tolerance for fluopicolide in/on *grape* of 2.0 ppm.

Adequate processing data for raisins are available pending submission of the requested storage stability data/information. The available data indicate that residues of fluopicolide are not likely to concentrate in juice but do concentrate in raisins. The processing data indicate that a tolerance of 6.0 ppm for *grape, raisin* is appropriate.

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

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments; <i>Correct Commodity Definition</i>
Grape (imported)	2	2.0	<i>Grape</i>
Grape, raisin (imported)	6	6.0	<i>Grape, raisin</i>

Appendix D: Review of Human Research

None.

Appendix E: Sections 3.0, 4.0, 8.0, and 10.0 for BAM (from R. Mitkus, RAB1, updated 7/27/06)

3.0 Hazard Characterization/Assessment

3.1 Hazard and Dose-Response Characterization

3.1.1 Studies Considered in the Toxicity and Dose-Response Evaluation

Dichlobenil

Data from the following studies were used to evaluate the hazard potential of dichlobenil:

- **Acute (for olfactory toxicity):** one intraperitoneal (single dose) toxicity study (mouse, rat) and one subcutaneous (single dose, neonates)/intraperitoneal (single dose, adults) toxicity study (mouse)
- **Subchronic:** Two oral (hamster, rat), one dermal (rabbit), one dermal (mouse), and two inhalation (rat) toxicity studies
- **Chronic:** Two oral toxicity (dog), two oral carcinogenicity (hamster), and one combined oral toxicity/carcinogenicity (rat) studies
- **Reproduction/developmental:** Two developmental (rat, rabbit) and one two-generation reproduction (rat) studies
- **Other:** Nine genotoxicity screens (*in vivo/in vitro*) and five metabolism/toxicokinetics studies (rat)

BAM

Data from the following studies were used to evaluate the hazard potential of dichlobenil:

- **Acute (for olfactory toxicity):** one intraperitoneal (single dose) olfactory toxicity study (mouse)
- **Subchronic:** One oral (rat) toxicity study
- **Chronic:** One oral toxicity (dog) study and one combined oral toxicity/carcinogenicity (rat) study
- **Reproduction/developmental:** One developmental (rabbit) toxicity study and one three-generation reproduction (rat) study
- **Other:** Three genotoxicity screens (*in vivo/in vitro*)

3.1.2 Sufficiency of studies/data

Dichlobenil

The acute and chronic studies were sufficient to determine whether human hazard could exist within the context of dose, duration, timing, and route of exposure. Data quality is acceptable. There was no evidence that dichlobenil was either

mutagenic or clastogenic in either *in vitro* or *in vivo* assays. Dichlobenil was determined to be non-mutagenic in bacteria and mouse lymphoma cells, negative in an *in vivo* chromosomal aberration assay, and did not cause unscheduled DNA synthesis (repair of DNA damage) in or transformation of mammalian cells *in vitro*. The HED Cancer Peer Review Committee (1995) classified dichlobenil as a “Group C, possible human carcinogen.” An RfD approach was recommended for the quantification of human cancer risk.

Based on olfactory toxicity observed following dermal (Deamer et al. 1994), inhalation (Guideline study), and intraperitoneal (i.p.) (Brandt et al. 1990; Eriksson and Brittebo 1995) exposures of adult animals and subcutaneous exposure (s.c.) of neonatal mice (Eriksson and Brittebo 1995), **the Agency requires a comparative study of olfactory toxicity by the oral route in neonates and adults. The registrant is encouraged to consult with the Agency to discuss the protocol.**

BAM

The acute and chronic studies were sufficient to evaluate human hazard potential, and data quality is acceptable. There was no evidence that BAM was either mutagenic or clastogenic in either *in vitro* or *in vivo* assays. In addition, BAM did not cause unscheduled DNA synthesis (repair of DNA damage) in mammalian cells *in vitro*. The carcinogenic potential of BAM was evaluated in the rat. An increased incidence of hepatocellular adenomas was observed at the high dose in females only in the study. However, the statistical significance of the effect was marginal ($P=0.049$), dosing was considered adequate in the study, and the tumors were non-cancerous. In the absence of data for a second species, HED considers the carcinogenic potential of BAM to be similar to that of the parent compound, dichlobenil (possible human carcinogen).

BAM-mediated olfactory toxicity was observed in one study (i.p.) collected from the open literature (Brittebo et al. 1991). However, olfactory epithelial necrosis was observed in adult animals at a dose that was eight times higher than that which caused the same effect using dichlobenil. In the absence of studies via other routes of exposure, potential BAM-mediated olfactory toxicity *in offspring* is considered by HED to be similar to that of dichlobenil.

3.1.3 Herbicidal Mode of Action

Dichlobenil is a systemic herbicide that inhibits cellulose biosynthesis in plants, thereby leading to alteration of cell wall structure and function (Sabba et al. 1999). Dichlobenil is primarily converted to 2,6-dichlorobenzamide (BAM) in the soil by way of microbial degradation and is then taken up by the roots of exposed plants (Verloop 1972).

3.1.4 Mammalian Toxicology

3.1.4.1 Dichlobenil

Dichlobenil technical demonstrated moderate acute toxicity (Category II or III) via the oral, dermal, and inhalation routes. It is neither a dermal irritant (Category IV), eye irritant (Category IV), nor a dermal sensitizer (Table 3.1a).

A summary of the subchronic and chronic toxicity and genotoxicity databases for dichlobenil is found in Table 3.1b. In the subchronic and chronic oral toxicity studies in hamsters, rats, and dogs, liver toxicity was the adverse effect most often observed at the LOAEL. For example, in a 90-day oral toxicity study in rats, inflammation and necrosis were observed in the liver of males, and increased liver weight and liver histopathology (swelling and vacuolation of hepatocytes) were observed in females. In a 90-day oral toxicity study in hamsters, increased liver weight, enlarged liver (with rough surface), and swollen hepatocytes were observed in females. In addition, decreased weight of the prostate and mineralization of the prostate were reported in males. Increased liver weights and hepatic enzymes, as well as liver histopathology, were observed at lower doses in both chronic dog toxicity studies, as well as in the combined chronic toxicity/carcinogenicity study in the rat.

In addition to the liver, the nose is considered a target organ for dichlobenil. Olfactory toxicity was observed in short-term dermal and inhalation toxicity studies in mice and rats. **Because olfactory toxicity was not assayed following acute or subchronic oral exposure to dichlobenil, the absence of this information is considered a data gap.** Olfactory toxicity was not observed in the chronic oral (capsule) toxicity study in the dog.

In a high-dose carcinogenicity study in the hamster, decreased body weight gain was observed in males and females, while liver histopathology (finely vacuolated hepatocytes, hepatitis, and brown pigment in the hepatocytes) was observed in males. According to the Health Effects Division 2nd Carcinogenicity Peer Review (1995), there was a treatment-related increase in liver adenomas and combined adenomas/carcinomas in males only at the highest dose tested, when compared to controls. However, dosing was considered excessive at this dose in both sexes, based on decreased body weight gains and severe hepatotoxicity.

In a second carcinogenicity study performed in hamsters at lower doses, reduced secretion of the prostate and seminal vesicles was observed at the LOAEL in males, whereas generalized systemic toxicity was observed in females, as evidenced by decreased body weight gain, peritonitis, and hyperplasia of the adrenal cortex, small intestine, and bone marrow (sternum). According to the Health Effects Division 2nd Carcinogenicity Peer Review (1995), there was no treatment-related increase in the incidence of any tumor type in this study. Dosing was considered adequate, based on decreased body weight gains and hyperplasia in various tissues in both sexes.

In a combined chronic toxicity/carcinogenicity study in the rat, adverse effects on clinical chemistry, gross pathology, and histopathology confirmed dichlobenil hepatotoxicity. Nephrosis

(kidney damage) was also observed in males, followed by parathyroid hyperplasia, which was considered a compensatory mechanism to maintain normal blood calcium levels. According to the Health Effects Division 2nd Carcinogenicity Peer Review (1995), a treatment-related increase in the incidence of hepatocellular adenomas and combined adenomas/carcinomas was observed in females only at the highest dose tested. Dosing was considered adequate in females, but excessive in males, at this dose. Based on the weight of the evidence, the HED 2nd Carcinogenicity Peer Review (1995) classified dichlobenil as a Group C, possible human carcinogen, and recommended that an RfD approach should be used for quantification of human cancer risk.

Olfactory toxicity was observed following dermal and inhalation exposures in three toxicity studies that were either published in the open literature (one dermal) or submitted to the Agency (two inhalation). In each study, degeneration of the olfactory epithelium was observed. Since the olfactory epithelium is composed of olfactory sensory neurons, damage to the olfactory epithelium is considered a neurotoxic effect that is confined to this organ only.

HED concluded that there was no evidence of increased susceptibility to offspring following pre-natal exposure to rats or rabbits in developmental toxicity studies. Evidence of increased pre-/post-natal susceptibility was observed in the two-generation reproduction study in rats. However, the degree of concern for this susceptibility is low, since the NOAEL from this study is six times lower than the dose (LOAEL) at which adverse effects (decreased pup body weight) were observed, and is therefore protective.

Delayed maturity of the uterus was observed in all high-dose females tested in the chronic oral (capsule) toxicity study in the dog. A marked decrease in mean uterine weight at the high dose confirmed this finding. Ovarian weights were also decreased in high-dose females, but no alterations were observed microscopically. These results are suggestive of modulation of the female endocrine system in this study; however, the dose utilized in this risk assessment for the chronic RfD is almost forty times lower than that at which the effects were observed and is considered protective of any potential endocrine modulation.

Table 3.1a. Acute toxicity profile for dichlobenil technical^a			
Guideline No./Study Type	MRID No.	Results	Toxicity Category
870.1100/Acute oral toxicity (rat)	44866902	>2000 mg/kg	III
870.1200/Acute dermal toxicity (rabbit)	43250401	<2000 mg/kg	II
870.1300/Acute inhalation toxicity (rat)	40425401	>0.250 mg/L	II
870.2400/Primary eye irritation (rabbit)	40425403	Not an eye irritant	IV
870.2500/Primary dermal irritation (rabbit)	40425402	Not a dermal irritant	IV
870.2600/Dermal sensitization (guinea pig)	40548501	Not a skin sensitizer	---

^a Based on Memo, Dupuy JL, D257750, 11/12/1999

Table 3.1b. SUBCHRONIC, CHRONIC, AND GENO- TOXICITY PROFILE FOR DICHLOBENIL TECHNICAL		
Guideline No./ Study Type	MRID No. (Year)/Doses/ Classification	Results
870.3100 90-day oral (hamster; dietary)	40600701 (1987) 0, 41, 209, 1289, or 7500/4648 ppm (adjusted due to evaporation; equivalent to 0, 3, 16, 79, or 395/263 mg/kg bw/day) Acceptable/Non-guideline	NOAEL = 3 (M) and 16 (F) mg/kg/day LOAEL = 16 mg/kg/day (M) based on decreased weight of the prostate and mineralization of the prostate; and 79 mg/kg/day (F) based on increased liver weight, enlarged liver (with rough surface), and swollen hepatocytes
870.3100 90-day oral (rat; dietary)	00107106 (1961) 0, 100, 1000, 3000, or 10000 ppm (equivalent to 0, 4.5, 45, 135, or 453 mg/kg/day; adjusted for purity) Acceptable/Non-guideline	NOAEL = 4.5 (M) and 45 (F) mg/kg/day LOAEL = 45 mg/kg/day (M) based on hepatocytic inflammation and necrosis; and 135 mg/kg/day (F) based on increased liver weight and liver histopathology (swelling and vacuolation of hepatocytes)
870.3200 21-day dermal (rabbits)	43879301 (1995) 0, 100, 300, or 1000 mg/kg/day Acceptable/Non-guideline	NOAEL = 1000 mg/kg/day (HDT) LOAEL was not observed.

Non-guideline (literature) 5-day dermal (mouse)	Deamer et al. (1994); no MRID 0, 10, 25, 50, 100, 150, or 200 mg/kg/day Acceptable/Non-guideline	NOAEL = 25 mg/kg/day LOAEL = 50 mg/kg/day based on olfactory epithelial damage following single (1-day) and repeated (5-day) dosing
870.3465 90-day inhalation (rat)	46398701 (2002), 46653001 (2002) 0, 2.3, 5.1, or 12 mg/m ³ (equivalent to 0, 0.03, 0.07, or 0.17 mg/kg/day, respectively ¹) for 28 days (6 hrs/day; 5 days/week) Unacceptable/Guideline due to inadequate dosing for 28 days	NOAEL (28 days) = 12 mg/m ³ (HDT) LOAEL (28 days) was not observed.
870.3465 90-day inhalation (rat)	46653001 (2002) 0, 21, 77, or 200 mg/m ³ (equivalent to 0, 0.3, 1.1, or 2.9 mg/kg/day, respectively ²) for 7 days (range finding; 6 hrs/day) Acceptable/Non-guideline	NOAEL (7 days, range finding) was not observed. LOAEL (7 days, range finding) = 21 mg/m ³ (0.3 mg/kg/day) based on an increased incidence of nasal degeneration
870.4100 Chronic toxicity oral (dog; dietary)	00067649 (1969) 0, 20, 50, or 350 ppm (equal to 0, 0.5, 1.25, or 8.75 mg/kg/day) Acceptable/Non-guideline	NOAEL = 1.25 mg/kg/day LOAEL = 8.75 mg/kg/day based on increased liver weight (M&F), serum alkaline phosphatase (M&F), and serum alanine aminotransferase (F); liver histopathology [leukocytic infiltration around the central veins (M&F) and necrosis (M)]; and an increase in the number of erythrocytes in the urine (F)
870.4100 Chronic toxicity oral (dog; capsule)	43969701 (1995) 0, 1, 6, or 36 mg/kg/day Acceptable/Guideline	NOAEL = 1 mg/kg/day LOAEL = 6 mg/kg/day based on increased liver weights and increased serum cholesterol, triglycerides, phospholipids, and alkaline phosphatase (M&F) and increased serum γ -GT and periportal hypertrophy of hepatocytes (M)
870.4200 Carcinogenicity oral (hamster;	41988301 (1991), 42015101 (1991), 42563601 (1992)	NOAEL = 1.69 (M) and 9.20 (F) mg/kg/day LOAEL = 9.39 mg/kg/day (M) based on reduced secretion of the prostate and seminal vesicles; and 48.85 mg/kg/day (F)

¹ Calculated as follows: [(Concentration in mg/m³) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where MW=172.

² Calculated as follows: [(Concentration in mg/m³) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where MW=172.

<p>dietary)</p>	<p>0, 5, 26, 132, or 675 ppm [equal to 0/0, 0.34/0.35, 1.69/1.78, 9.39/9.20, or 45.64/48.85 mg/kg/day (M/F)]</p> <p>Acceptable/Non-guideline</p>	<p>based decreased body weight gain, peritonitis, and hyperplasia of the adrenal cortex, small intestine, and bone marrow (sternum)</p> <p>No evidence of carcinogenicity.</p>
<p>870.4200 Carcinogenicity oral (hamster; dietary)</p>	<p>42221201 (1992), 42563601 (1992)</p> <p>0, 675, 1500, or 3375 ppm [equal to 0/0, 51/55, 117/121, 277/277 mg/kg/day (M/F)]</p> <p>Acceptable/Non-guideline</p>	<p>NOAEL was not observed. LOAEL = 51/55 mg/kg/day (M/F) based on decreased body weight gain (M&F), and liver histopathology (finely vacuolated hepatocytes, hepatitis, and brown pigment in the hepatocytes) (M)</p> <p>Statistically significant increase in hepatocellular adenomas and combined adenomas/carcinomas at 277 mg/kg/day (M); dosing considered excessive in M&F</p>
<p>870.4300 Combined Chronic Toxicity/ Carcinogenicity oral (rat; dietary)</p>	<p>00147438 (1983), 40401101 (1987), 40823801 (1988)</p> <p>0, 50, 400, or 3200 ppm (equal to 0, 2.3, 18.9, or 173.1 mg/kg/day)</p> <p>Acceptable/Non-guideline</p>	<p>NOAEL = 2.3 mg/kg/day LOAEL = 18.9 mg/kg/day based on changes in clinical chemistry (increased blood urea nitrogen, cholesterol), gross pathology (enlarged liver, enlarged kidney), and histopathology (nephrosis, parathyroid hyperplasia) in males; and increased liver weight, enlarged liver, and cytologic alterations (polyploidy with hepatocytic swelling) in the liver in females.</p> <p>Statistically significant increase in hepatocellular adenomas and combined adenomas/carcinomas in at 173.1 mg/kg/day (F); dosing considered adequate in females</p> <p>Statistically significant increasing trend for hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas at 173.1 mg/kg/day (M); dosing considered excessive in males</p>
<p>870.3700 Developmental toxicity oral (rat; gavage)</p>	<p>00147437 (1984)</p> <p>0, 20, 60, or 180 mg/kg/day</p> <p>Acceptable/Non-guideline</p>	<p>Maternal NOAEL = 20 mg/kg/day Maternal LOAEL = 60 mg/kg/day based on decreased body weight gain, food consumption, and food efficiency during dosing</p> <p>Developmental NOAEL = 180 mg/kg/day Developmental LOAEL was not identified.</p>
<p>870.3700 Developmental toxicity oral (rabbit; gavage)</p>	<p>41257302 (1989)</p> <p>0, 15, 45, or 135 mg/kg/day</p>	<p>Maternal NOAEL = 45 mg/kg/day Maternal LOAEL = 135 mg/kg/day based on decreased body weight gain and food consumption during dosing</p> <p>Developmental NOAEL = 45 mg/kg/day Developmental LOAEL = 135 mg/kg/day, based on increased number of total resorptions/dam and post-implantation loss; and increased incidences of fetal external (cleft palate, adactly, bilateral open eye), visceral (abnormal cystic gallbladder, distended ureter with bilateral severe hydronephrosis), and skeletal (malformed and malpositioned right scapula, right radius absent with malpositioned ulna and</p>

		humerus, fused cervical vertebral arches, asymmetrically ossified and fused cervical vertebra centra, abnormally shaped cranium with enlarged and misshapen fontanelle, enlarged fontanelle, misshapen frontals, skull and frontals foreshortened and nasal malpositioned, and major fusion of sternbrae) anomalies
	Acceptable/Non-guideline	
870.3800 2-generation reproduction oral (rat; dietary)	41257303 (1989), 42239101 (1992) 0, 60, 350, or 2000 ppm (equivalent to 0, 3, 17.5, or 100 mg/kg/day)	Parental NOAEL = 17.5 mg/kg/day Parental LOAEL = 100 mg/kg/day based on decreased body weight gains during premating (M&F) and gestation (F) in both generations and decreased food consumption during premating in both generations (M&F) Reproductive NOAEL = 17.5 mg/kg/day Reproductive LOAEL = 100 mg/kg/day based on decreased number of implantations/dam in F ₁ (unreported for P) Offspring NOAEL = 3 mg/kg/day Offspring LOAEL = 17.5 mg/kg/day based on decreased body weight during weaning in both generations
	Acceptable/Non-guideline	
Non-guideline (literature) Fetal and neonatal mouse olfactory study (single injections; s.c. in neonates, i.p. in adults)	Eriksson and Brittebo (1995); no MRID 0, 12, or 25 mg/kg/day (s.c. in neonates, i.p. in adults)	Parental NOAEL not observed. Parental LOAEL (i.p.) = 12 mg/kg/day based on many vacuolated, degenerated, or necrotic Bowman's glands and no periodic acid-Schiff (PAS) staining of contents of Bowman's glands Offspring NOAEL not observed. Offspring LOAEL (PND 8; s.c.) = 12 mg/kg/day based on no periodic acid-Schiff (PAS) staining of contents of Bowman's glands (measure of mucus production)
	Acceptable/Non-guideline	
Non-guideline (literature) Adult mouse olfactory study (single injection; i.p.)	Brandt et al. (1990); no MRID 0, 6, 12, 25, or 50 mg/kg/day (i.p.)	NOAEL (i.p.) = 6 mg/kg/day LOAEL (i.p.) = 12 mg/kg/day based on necrosis in Bowman's glands and olfactory epithelium
	Acceptable/Non-guideline	
870.5100 <u>In vitro</u> bacterial reverse mutation (Ames test)	00153579 (1984) At concentrations up to 5000 µg/plate (-/+ activation) in <u>S. typhimurium</u>	Negative with or without activation.
	Acceptable/Guideline	
870.5100 <u>In vitro</u> bacterial reverse mutation (Ames test)	00153586 (1981) At concentrations up to 5000 µg/plate (-/+ activation) in <u>E. coli</u> and <u>S. typhimurium</u> ; and 5000 µg/disk (- activation) in <u>B. subtilis</u>	Negative with or without activation.

	Acceptable/Guideline	
870.5300 <u>In vitro</u> mouse lymphoma (L5178Y) mutation	00153576 (1984) At concentrations up to 280 µg/ml (- activation) and 50 µg/ml (+ activation) Acceptable/Guideline	Negative with or without activation.
870.5395 <u>In vivo</u> mouse erythrocyte micronucleus assay	00153578 (1983) 0, 300, 600, or 1200 mg/kg Unacceptable/Guideline due to insufficient sampling times	Negative.
870.5375 <u>In vitro</u> chromosomal aberrations (human lymphocytes)	00153577 (1984) At concentrations up to 1 µg/ml (-/+ activation) (limit of solubility) Acceptable/Guideline	Negative with or without activation.
870.5375 <u>In vitro</u> chromosomal aberrations (CHO cells)	43191501 (1990) At concentrations up to 100 µg/ml (-/+ activation) Acceptable/Guideline	Negative with or without activation.
870.5550 <u>In vitro</u> Unscheduled DNA synthesis (human HeLa epithelioid cells)	00153580 (1984) At concentrations up to 102.4 µg/ml (-/+ activation) Acceptable/Guideline	Negative with or without activation.
<u>In vitro</u> transformation assay (BALB/3T3 cells)	00153581 (1984) At concentrations up to 7500 µg/ml (+ activation) Acceptable/Guideline	Negative.
<u>In vitro</u> recombination assay	00153586 (1981) At concentrations up to 5000 µg/disk (- activation) in <u>B. subtilis</u> Acceptable/Non-guideline	Negative.

870.7485 Metabolism and toxicokinetics (rats)	41227401-04 (1989), 41299401 (1987) 2.5 mg/kg (single dose; oral); 5 mg/kg (single dose; oral, i.v.); 3.75, 30, or 240 mg/kg (single and repeated dose; oral) Acceptable/Guideline	After 7 days of i.v. exposure, 65-71% and 25-31% of the dose was excreted in the urine and feces, resp., in M&F; similar results were obtained for the oral route of exposure (therefore efficient GI absorption). 79% and 20% oral dose was measured in bile and urine, resp., 24 hrs. after exposure; 20-30% oral dose eliminated in feces via bile (supports enterohepatic recirculation). 95% of urinary metabolites were excreted 24 hrs. after dosing. Recovery (96%) 7 days after a single i.v. dose indicates low residence time in tissues; slightly longer residence time following oral exposure is suggested by 84-86% recovery 7 days after a single oral dose. Tissue levels peaked 1-3 hrs. after oral dosing. Dichlobenil is metabolized via hydroxylation at the 3 or 4 position of the phenyl group followed by sulfation or glucuronidation; or via displacement of a Cl atom followed by glutathione conjugation. Glutathione conjugation appears to be saturable during repeated dosing.
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3.1.4.2 2,6-Dichlorobenzamide (BAM)

The dichlobenil soil metabolite, 2,6-dichlorobenzonitrile (BAM) demonstrated moderate acute toxicity (Category III) via the oral route of exposure (Table 3.1c). Because the subchronic and chronic toxicity of BAM is less than or equal to that of dichlobenil (parent compound), the acute toxicity of BAM via the inhalation and dermal routes is expected to be less than or equal to that of dichlobenil.

A summary of the subchronic and chronic toxicity and genotoxicity databases for BAM is found in Table 3.1d. In the subchronic and chronic oral toxicity studies in the rat and dog, respectively, body weight change was the toxicological effect most often observed at the LOAEL. In the combined chronic toxicity/carcinogenicity study in the rat, toxicologically significant changes in body weight were also observed at the LOAEL, as were eosinophilic foci in the liver. In general, adverse liver effects were observed in the submitted studies at doses of BAM that were higher than those of dichlobenil (parent).

There was no evidence of carcinogenicity in the combined chronic toxicity/carcinogenicity study of BAM in the rat. An increased incidence of hepatocellular adenomas was observed at the high dose in females only in the study. However, the statistical significance of the effect was marginal (P=0.049), dosing was considered adequate in the study, and the tumors were non-cancerous. In addition, there was no evidence that BAM was either mutagenic or clastogenic. However, in the absence of a second species, HED considers the carcinogenic potential of BAM to be similar to that of dichlobenil (possible human carcinogen) and that it is appropriate to use an RfD approach for quantification of human cancer risk.

BAM-mediated olfactory toxicity was observed in one study collected from the open literature (Brittebo et al. 1991). Although olfactory epithelial necrosis was observed after i.p. administration of BAM, the dose was eight times higher than that which caused the same effect using dichlobenil. In the absence of studies via other routes of exposure, BAM-mediated olfactory toxicity is considered by HED to be less than that of dichlobenil (parent).

HED concluded that there was no evidence of increased susceptibility to offspring following pre-natal exposure to rabbits in a developmental toxicity study and pre-/post-natal exposure in the two-generation reproduction study in the rat.

BAM is considered neurotoxic. Toxicity to the olfactory sensory neurons was observed following single i.p. exposures of mice to BAM (Brittebo et al. 1991). In the 90-day oral toxicity study in rats, reduced muscle tone was observed; however, the toxicological significance of this finding is unclear, given the lack of reproducibility of the effect in other studies, the age of the study itself (1967), and the fact that the purity of the compound was not reported in the study. Lethargy and ataxia were observed in mice tested orally in a dose-range finding study for the *in vivo* erythrocyte micronucleus assay. The effects were resolved after 24 hours. In addition, in a two-week dietary study recently submitted and not fully reviewed by the Agency (MRID 46892401), clinical signs of toxicity (slightly decreased muscle tone, slight loss of pinnae reflexes) were observed in mice at 2500 ppm (approximately equal to 375 mg/kg/day).

Therefore, HED is requesting Guideline acute and subchronic neurotoxicity screening batteries (OPPTS 870.6200) with BAM.

Table 3.1c. Acute toxicity profile for soil metabolite 2,6-dichlorobenzamide (BAM)^a			
Guideline No./Study Type	MRID No.	Results	Toxicity Category
870.1100/Acute oral toxicity (mouse)	42940201	1538/1144 mg/kg (M/F)	III

^a According to Reregistration Eligibility Decision (1998)

Table 3.1d. SUBCHRONIC, CHRONIC, AND GENO- TOXICITY PROFILE FOR METABOLITE, 2,6-DICHLOROBENZAMIDE (BAM)		
Guideline No./ Study Type	MRID No. (Year)/Doses/ Classification	Results
870.3100 90-day oral (rat; dietary)	00067654 (1967) 0, 50, 180, 600, or 2300 ppm (equal to 0, 4, 14, 49, or 172 mg/kg/day) Acceptable/Non-guideline	NOAEL = 14 mg/kg/day LOAEL = 49 mg/kg/day based on decreased body weight gain (M) and reduced skeletal muscle tone (day 4 only in males; days 91 and 92 only in females)
870.3150 90-day oral (dog; dietary)	00067655 (1967) 0, 100, 300, or 2000 ppm (equal to 0, 7.5, 22.5, or 150 mg/kg/day) Unacceptable/Guideline due to parasitic infections	NOAEL = 22.5 mg/kg/day LOAEL = 150 mg/kg/day based on clinical signs (thin appearance, dull coat, hair loss) and increased liver weight and serum alkaline phosphatase concentrations (F) and clinical signs (thin appearance, dull coat, hair loss) (M)

	(ascariasis) in most animals	
870.4700 Chronic toxicity oral (dogs; dietary)	42940203 (1971) 0, 60, 100, 180, or 500 ppm (equal to 0, 1.5, 2.5, 4.5, or 12.5 mg/kg/day) Acceptable/Non-guideline	NOAEL = 4.5 mg/kg/day LOAEL = 12.5 mg/kg/day based on decreased body weight and body weight gain
870.4300 Combined Chronic Toxicity/ Carcinogenicity oral (rat; dietary)	42940202 (1971), 44043601 (1996), 44052901 (1996) 0, 60, 100, 180, or 500 ppm [equal to 0, 2.2/2.8, 3.6/4.7, 6.5/8.5, or 19/25 mg/kg/day (M/F)] Acceptable/Non-guideline	NOAEL = 6.5 (M) and 4.7 mg/kg/day (F) LOAEL = 19 mg/kg/day (M) and 8.5 mg/kg/day (F) based on decreased body weight and body weight gain (≥ week 26) and an increased incidence of hepatocellular alteration (eosinophilic foci) Borderline statistically significant (P=0.049) increased incidence of hepatocellular adenomas at 25 mg/kg/day (F only); dosing considered adequate (M&F)
870.3700 Developmental toxicity oral (rabbit; gavage)	43003601 (1986), 43265201 (1994) 0, 10, 30, or 90 mg/kg/day Acceptable/Guideline	Maternal NOAEL= 30 mg/kg/day Maternal LOAEL= 90 mg/kg/day based on increased incidences of clinical signs (late abortion, thin appearance) and decreased (severe) body weight gain and food consumption during dosing Developmental NOAEL = 30 mg/kg/day Developmental LOAEL = 90 mg/kg/day based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies
870.3800 3-generation reproduction oral (rat; dietary)	42940204 (1971) 0, 60, 100, or 180 ppm (equivalent 0, 4.5, 7.5, or 13.5 mg/kg/day) Acceptable/Non-guideline	Parental NOAEL = 13.5 mg/kg/day Parental LOAEL was not observed. Reproductive NOAEL = 13.5 mg/kg/day Reproductive LOAEL was not observed. Offspring NOAEL = 13.5 mg/kg/day Offspring LOAEL was not observed.
Non-guideline (literature) Adult mouse olfactory study (single injection; i.p.)	Brittebo et al. (1991); no MRID 0, 25, 50, or 100 mg/kg/day (i.p.) Acceptable/Non-guideline	NOAEL (i.p.) not observed. LOAEL (i.p.) = 25 mg/kg/day based on decreased periodic acid-Schiff (PAS) staining of contents of Bowman's glands (measure of mucus production) Necrosis of Bowman's glands and olfactory epithelium observed at 100 mg/kg/day only
870.5100 <u>In vitro</u> bacterial reverse mutation	43003603 (1992) At concentrations up to 5000 µg/plate (-/+ activation) in <u>S.</u>	Negative with or without activation.

(Ames test)	<u>typhimurium</u> Acceptable/Guideline	
870.5395 <u>In vivo</u> mouse erythrocyte micronucleus assay	43003602 (1993), 43747101 (1995) 0, 250 mg/kg Acceptable/Guideline	Negative. Lethargy observed in dose-range finding (pilot) study at 100 mg/kg/day (=LOAEL; NOAEL not observed)
870.5550 <u>In vitro</u> Unscheduled DNA synthesis (rat hepatocytes)	43003604 (1993) At concentrations up to 1000 µg/ml Acceptable/Guideline	Negative.

3.2 Absorption, Distribution, Metabolism, Excretion (ADME)

The metabolism of [phenyl- $U^{14}C$]dichlobenil was studied in male and female Sprague-Dawley rats. Following oral administration of dichlobenil at 2.5 or 5 mg/kg, approximately 65-75% of the dose was eliminated in the urine and 20-30% in the feces (via biliary excretion) after seven days. There were no apparent differences between the sexes. Results from oral, intravenous, and biliary excretion studies indicated that the compound is readily absorbed from the gastrointestinal tract and eliminated in the bile. However, as a result of enterohepatic recirculation, the compound is reabsorbed and then eliminated primarily in the urine. Based on elimination patterns, it was concluded that animals dosed at 3.75, 30, or 240 mg/kg exhibited reduced absorption at the high dose (i.e., saturation). Specifically, the relative rate of urinary excretion varied inversely with dose level, and increased amounts of unchanged parent compound were excreted in the feces at high doses. No apparent sex-related differences were seen. Similar elimination patterns were noted following the administration of [phenyl- $U^{14}C$]dichlobenil on days 1 and 11 and of unlabeled compound on days 2-10, in an 11-day daily dosing experiment. No major differences in metabolic patterns between sexes were observed.

Dichlobenil is metabolized via hydroxylation at the 3 or 4 position of the phenyl group followed by sulfation or glucuronidation; or via displacement of a chlorine atom followed by glutathione conjugation. Glutathione conjugation appears to be saturable during repeated dosing. **The soil metabolite 2,6-dichlorobenzamide (BAM) was not identified as a metabolite in the rat.**

Radioactive residue levels were assayed in the liver, kidney, whole blood, and plasma. High residues were detected in the liver. In a time-course study of residue levels in various tissues from rats receiving 2.5 mg/kg, the highest levels were found in the liver, kidney, and some samples of kidney fat. The highest radioactive levels were found during 1-3 hours post dosing. Thereafter, residue levels decreased. Recovery (96%) seven days after a single i.v. dose indicates low residence time in tissues; slightly longer residence time following oral exposure is

suggested by 84-86% recovery 7 days after a single oral dose. No major sex-related differences were noted in tissue distribution.

No metabolism or absorption studies are available for dichlobenil via the dermal or inhalation routes. **The Agency is currently reviewing metabolism studies for BAM.**

3.3 FQPA Considerations

3.3.1 Adequacy of the Toxicity Data Base

The toxicology database used to assess pre- and/or post-natal exposure to dichlobenil is adequate. However, the toxicology database used to assess pre- and/or post-natal exposure to the soil metabolite, BAM, is incomplete. Data from a 2-week oral toxicity study has been requested to evaluate potential neurotoxic effects of BAM in mice. The following acceptable studies are available:

Dichlobenil

- One developmental toxicity study in rats
- One developmental toxicity study in rabbits
- One two-generation reproduction study in rats

2,6-Dichlorobenzamide (BAM)

- One developmental toxicity study in rabbits
- One three-generation reproduction study in rats

3.3.2 Evidence of Neurotoxicity (Dichlobenil and BAM)

Olfactory toxicity was observed following dermal, inhalation, and intraperitoneal (i.p.) exposures to dichlobenil in five toxicity studies that were either published in the open literature (one dermal, two i.p. studies) or submitted to the Agency (two inhalation studies). In each study, degeneration of the olfactory epithelium was observed. Since the olfactory epithelium is composed of olfactory sensory neurons, damage to the olfactory epithelium is considered a neurotoxic effect.

BAM is considered neurotoxic. Toxicity to the olfactory sensory neurons was observed following single i.p. exposures of mice to BAM (Brittebo et al. 1991). In the 90-day oral toxicity study in rats, reduced muscle tone was observed; however, the toxicological significance of this finding is unclear, given the lack of reproducibility of the effect in other studies, the age of the study itself (1967), and the fact that the purity of the compound was not reported in the study. Lethargy and ataxia were observed in mice tested orally in a dose-range finding study for the *in vivo* erythrocyte micronucleus assay. The effects were resolved after 24 hours. In addition, in a two-week dietary study recently submitted and not fully reviewed by the Agency (MRID 46892401), clinical signs of toxicity (slightly decreased muscle tone, slight loss of pinnae

reflexes) were observed in mice at 2500 ppm (approximately equal to 375 mg/kg/day). **Therefore, HED is requesting Guideline acute and subchronic neurotoxicity screening batteries (OPPTS 870.6200) with BAM.**

3.3.3.1 Developmental Toxicity Studies (Dichlobenil)

Rat

In an Acceptable/Non-guideline, prenatal developmental toxicity study (MRID 00147437), dichlobenil technical (purity not reported; batch# FUN82B07A/FUX003000) was administered by gavage in 1% gum tragacanth to 25 pregnant Wistar Cpb:WU rats/sex/dose from gestation day (GD) 6-15 inclusive at daily dose levels of 0, 20, 60, or 180 mg/kg/day. No treatment-related mortality or clinical signs of toxicity were observed in pregnant does in the study.

A 29% (P<0.01) and 36% (P<0.01) decrease in mean body weight gain was observed during the dosing period in does treated at 60 and 180 mg/kg/day, resp. This effect was accompanied by a 15% (P<0.01) and 21% (P<0.01) decrease in food consumption at 60 and 180 mg/kg/day, respectively, as well as a respective 19% (P<0.05) and 24% (P<0.01) decrease in food efficiency. During the post-dosing period (GD 16-21), body weight gain, food consumption, and food efficiency values in mid- and high-dose animals rebounded to levels that were similar to or greater than those of controls. The maternal LOAEL is 60 mg/kg bw/day, based on decreased body weight gain, food consumption, and food efficiency during dosing. The maternal NOAEL is 20 mg/kg bw/day.

No treatment-related changes were observed in cesarean section parameters (death, altered growth) for either embryos or fetuses. It is unclear from the study report whether statistical analysis was performed for litter incidences of external, skeletal, or visceral observations. With regard to external anomalies, a shallow dose-dependent increase in the incidence of small subcutaneous hemorrhage or petechia was observed (0/23, 1/22, 2/22, and 3/21 litters at 0, 20, 60, and 180 mg/kg/day); however, the increase in the number of fetuses affected (0-3) was only slightly increased across dose and not statistically significant. Historical control incidences for this specific observation were not reported. However, the percentage of litters affected at all doses was within the historical control range (0-20%) of litter incidences of externally visible alterations.

An increased litter (13.6-14.3%) and fetal (3.1-3.5%) incidence of unilateral supernumerary rib (14th) was observed at \geq 60 mg/kg/day. The fetal incidences were not statistically significantly different from concurrent controls. The litter incidence (19%) of bilateral supernumerary rib (14th) was also increased at 180 mg/kg/day, as was the fetal incidence (4.7%; P<0.05). However, both the litter and fetal incidences of supernumerary (14th) rib were below the historical control incidences (32.26% and 10.86%, resp.). An increase (P<0.01) in the “degree” of absence of ossification of the sternbrae (expressed as transformed ossification values per litter) was also observed at 180 mg/kg/day (7.03 vs. 0.59 in controls). The increase was outside the historical control range (0-0.31). However, given that the concurrent control value (0.59)

was above the upper limit of the historical control range and dose-response was lacking in the effect, the calculated value is not considered toxicologically significant.

With respect to visceral anomalies, a very slight increase in the malformations, unilateral microphthalmia and intestinal alteration of the situs viscerum (combined with focal fibrosis of the peritoneum and mesentery) was observed at the high dose in one animal in 1/21 litters only. The malformation, soft consistency of the lens/unilateral folded retina, was observed in a different animal in 1/21 litters only. The incidence of each effect was above that of the historical controls (1/2691 fetuses; 1/401 litters); however, fetal incidences were not statistically significant and a dose-response was lacking at the doses tested. The developmental LOAEL was not identified. The developmental NOAEL is 180 mg/kg bw/day.

Rabbit

In an Acceptable/Non-guideline, prenatal developmental toxicity study (MRID 41257302), dichlobenil technical (98.5% a.i.; lot#s 3 and 6; batch# FUX010000) was administered by gavage in 1% gum tragacanth to 18 pregnant New Zealand white rabbits/sex/dose from gestation day (GD) 7-19 inclusive at daily dose levels of 0, 15, 45, or 135 mg/kg/day. No treatment-related mortality, clinical signs of toxicity (including abortions), or gross pathology were observed in the study.

A decrease (129%; $P < 0.05$) in body weight gain was observed during the dosing period only (GD 7-19) in does treated at 135 mg/kg/day. This effect was accompanied by a 30% ($P < 0.01$) decrease in food consumption at the high dose during the dosing period only. Body weight gain and food consumption in high-dose animals rebounded during the post-dosing period (GD 19-29). The maternal LOAEL is 135 mg/kg bw/day, based on decreased body weight gain and food consumption during dosing. The maternal NOAEL is 45 mg/kg bw/day.

Increases in total resorptions/dam (1.3) and post-implantation loss (17.9%) were observed at 135 mg/kg/day. Although the effects were not dose-dependent, the incidences were outside the historical control range and considered treatment-related. Although generally occurring at very low incidences (1-3 fetuses), several external, visceral, and skeletal defects anomalies were reported at 135 mg/kg/day. These effects were not observed in either concurrent or historical controls or were observed at incidences outside historical control ranges and were therefore considered toxicologically significant. External anomalies included bilateral open eye (3/115 fetuses; 3/14 litters), cleft palate, and adactyly. High-dose visceral anomalies included abnormal cystic gallbladder and distended ureter with bilateral severe hydronephrosis. Skeletal defects at 135 mg/kg/day were composed of malformed and malpositioned right scapula, right radius absent with malpositioned ulna and humerus, fused cervical vertebral arches, asymmetrically ossified and fused cervical vertebra centra, abnormally shaped cranium with enlarged and misshapen fontanelle, enlarged fontanelle (19/115 fetuses; 13/14 litters), misshapen frontals (2/115 fetuses; 2/14 litters), skull and frontals foreshortened and nasal malpositioned, and major fusion of sternbrae (3/115 fetuses; 3/14 litters).

The developmental LOAEL is 135 mg/kg bw/day, based on increased number of total

resorptions/dam and post-implantation loss; and increased incidences of external (cleft palate, adactyly, bilateral open eye), visceral (abnormal cystic gallbladder, distended ureter with bilateral severe hydronephrosis), and skeletal (malformed and malpositioned right scapula, right radius absent with malpositioned ulna and humerus, fused cervical vertebral arches, asymmetrically ossified and fused cervical vertebra centra, abnormally shaped cranium with enlarged and misshapen fontanelle, enlarged fontanelle, misshapen frontals, skull and frontals foreshortened and nasal malpositioned, and major fusion of sternbrae) anomalies. The developmental NOAEL is 45 mg/kg bw/day.

3.3.3.2 Reproductive Toxicity Study (Dichlobenil)

In an Acceptable/Non-guideline, two-generation reproduction study (MRIDs 41257303 and 42239101), dichlobenil technical (99.4% a.i.; lot#s 4 and 5; batch# F6N87EO8A/FUX011000) was administered in the diet to CrI:CD(SD)BR rats (30/sex/dose in the P generation; 25/sex/dose in the F₁ generation) at daily dose levels of 0, 60, 350, or 2000 ppm (equivalent to 0, 3, 17.5, or 100 mg/kg/day) for 2 consecutive generations. One (of 30) high-dose P males was found dead during week 14 of the study. The cause of death was liver necrosis and hemorrhage (MRID 42239101). One (of 25) F₁ high-dose males was sacrificed *in extremis* during week 12 of the study due after an accidental injury to the snout. Both deaths were considered incidental to treatment. There were no treatment-related clinical signs of toxicity in the study.

During the 10-week pre-mating period for P animals, mean cumulative body weight gains were decreased by 25-26% (P<0.05) at 2000 ppm in both males and females. Similarly, mean overall body weight gains for F₁ males were decreased by 25% during the 10-week pre-mating period and by 18% for F₁ females at 2000 ppm. During gestation, mean body weight gains were decreased by 13-14% in both P and F₁ females at 2000 ppm. Decrements in body weight gains were not observed in adult females of either generation during lactation. Mean food consumption at 2000 ppm was decreased by 11-33% (P<0.001) in P males and by 17-28% (P<0.001) in P females during pre-mating. In F₁ males, mean food consumption during pre-mating was decreased by 16-21% (P<0.001) and by 16-20% (P<0.001) in F₁ females at 2000 ppm. Mean food consumption for P and F₁ females during gestation and lactation was not reported.

No treatment-related effects were observed on fertility index, fecundity index, gestation index, or mean gestation length in either P or F₁ females. The mean number of implantations/dam was unreported for P females and decreased at 2000 ppm in F₁ females (12.3 vs. 14.6 in controls). The parental systemic LOAEL is 2000 ppm (100 mg/kg bw/day), based on decreased body weight gains during pre-mating (males and females) and gestation (females) in both generations, decreased food consumption during pre-mating in both generations (males and females), and decreased number of implantations/dam in F₁ females. The parental systemic NOAEL is 350 ppm (17.5 mg/kg bw/day). The reproductive LOAEL is 2000 ppm (100 mg/kg bw/day), based on decreased number of implantations/dam in F₁ females. The reproductive NOAEL is 350 ppm (17.5 mg/kg bw/day).

No treatment-related effects were observed on live birth index, viability index, lactation index, or sex ratio in either F₁ or F₂ offspring. In addition, the mean number of pups born live/litter was

unaffected in F₁ offspring but decreased (P<0.001) at 2000 ppm in F₂ offspring (11.0 vs. 13.7 in controls). This was likely due to the decreased number of implantations/F₁ dam at 2000 ppm. At ≥ 350 ppm, the mean pup body weight of F₁ offspring was decreased by 16-23% (P<0.05) from postnatal day (PND) 4 (pre-cull)-21. The effect was dose-dependent. In F₂ offspring, mean pup body weight was also dose-dependently decreased by 19-22% (P<0.05) from PND 14-21. An increased incidence of pelvic cavitation of the kidney at 2000 ppm (3% vs. 0%) was observed during necropsy in weanling F₂ offspring; however, the incidence was similar to the sporadic incidence observed in P and F₁ animals and was not considered toxicologically significant. The offspring LOAEL is 350 ppm (17.5 mg/kg bw/day), based on decreased body weight during weaning in both generations. The offspring NOAEL is 60 ppm (3 mg/kg bw/day).

3.3.4.1 Developmental Toxicity Study (BAM)

Rabbit

In an Acceptable/Non-guideline, prenatal developmental toxicity study (MRID 43003601), 2,6-dichlorobenzamide (99.4% a.i.; batch# FUX001000) was administered by gavage in 1% gum tragacanth to 16 pregnant New Zealand white rabbits/sex/dose from gestation day (GD) 7-19 inclusive at daily dose levels of 0, 10, 30, or 90 mg/kg/day. Five (of 16) females treated at 90 mg/kg/day were sacrificed *in extremis*. Three of the 5 sacrificed high-dose dams had late abortions (GD 19, 21, and 22, resp.). The incidence of abortion followed by sacrifice at 0, 10, and 30 mg/kg/day was 1/16, 1/16, and 0/16, resp. The other 2/5 high-dose animals were sacrificed moribund. Moribund condition followed by sacrifice was observed in 1/16, 0/16, and 2/16 animals at 0, 10, and 30 mg/kg/day, resp. The incidence of thin appearance was increased at 90 mg/kg/day (10/16), whereas the incidences at 0, 10, and 30 mg/kg/day were 1/16, 0/16, and 2/16, resp.

A decrease (129%) in mean body weight gain, relative to controls, was observed during the dosing period (GD 7-19) in does treated at 90 mg/kg/day. Similarly, food consumption at 90 mg/kg/day was decreased during the dosing period by 49%, relative to controls. Body weight gain and food consumption in high-dose animals rebounded above control levels during the post-dosing period (GD 20-28). No treatment-related gross pathology was observed. The maternal LOAEL is 90 mg/kg bw/day, based on increased incidences of clinical signs (late abortion, thin appearance) and decreased (severe) body weight gain and food consumption during dosing. The maternal NOAEL is 30 mg/kg bw/day.

No treatment-related effects were observed on several developmental endpoints, including the number of resorptions, post-implantation loss, litter size, and sex ratio. Mean fetal body weight at 90 mg/kg bw/day (33.9 g) was decreased by 6%, relative to controls; however, the change was not statistically significant and the mean value fell within the historical control range (27.7 g-39.4 g). No treatment-related changes were observed on the incidences of external defects.

An increase in the incidence of bipartite interparietal bone was observed at 90 mg/kg bw/day. Litter incidences were 1/14, 1/15, 2/14, and 3/11 litters at 0, 10, 30, and 90 mg/kg/day. Bipartite interparietal bone is considered a malformation in rats (Solecki et al. 2001). Historical control

data were not provided for the litter incidences of bipartite interparietal bone, and fetal incidences only were reported for the current study. However, the fetal incidences were not statistically significant different from concurrent controls, and the concurrent control fetal incidence (0.8%) exceeded that of the historical controls (0.3%). An increase in the incidence of postcaval lung lobe agenesis was also observed at 90 mg/kg bw/day. Litter incidences were 0/14, 0/15, 1/14, and 3/11 at 0, 10, 30, and 90 mg/kg/day. The fetal incidence of postcaval lung lobe agenesis at 90 mg/kg bw/day (3.2%) exceeded that of the historical controls (1.2%), whereas that at 30 mg/kg bw/day (0.9%) did not. The developmental LOAEL is 90 mg/kg bw/day, based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies. The developmental NOAEL is 30 mg/kg bw/day.

3.3.4.2 Reproductive Toxicity Study (BAM)

In an Acceptable/Non-guideline, 3-generation reproduction study (MRID 42940204), 2,6-dichlorobenzamide (99.5% a.i.; batch # 195) was administered in the diet to Long-Evans rats (10 males/dose and 20 females/dose in the P, F₁, and F₂ generations) at daily dose levels of 0, 60, 100, or 180 ppm (equivalent to 0, 4.5, 7.5, or 13.5 mg/kg/day) for 3 consecutive generations. Two litters were produced in each generation. The number of pups per litter was counted on postnatal days (PNDs) 1, 5, and 21 (also weighed). Litters were culled to ten animals each on PND 5. P, F₁, and F₂ generation parental animals were weighed and examined for gross pathology on the day of termination (unspecified). Organ weights were measured, and histopathology was performed on select F_{3b} weanlings only.

No treatment-related mortality was observed in the P, F_{1b}, or F_{2b} parental generations. A 6% (P<0.05) decrease in mean parental terminal body weight was observed at 180 ppm in the F_{2b} generation; however, the decrease was not biologically significant and not observed in P or F_{1b} parental animals. No treatment-related gross pathology was observed in P, F_{1b}, or F_{2b} parental animals. Fertility and gestation indices were similar across treatment groups in P, F₁, and F₂ generation dams. The parental systemic LOAEL was not observed. The parental systemic NOAEL is 180 ppm (13.5 mg/kg bw/day). The reproductive LOAEL was not observed. The reproductive NOAEL is 180 ppm (13.5 mg/kg bw/day).

No treatment-related differences were observed in the mean number of pups per litter on PNDs 1, 5 (pre-cull), or 21 in the F₁, F₂ or F₃ generations. Viability and lactation indices were similar across the treatment groups in F₁, F₂, and F₃ generation pups. Mean survival from birth to PND 5 (viability index) was slightly reduced (86.2%, P<0.01; vs. 95.7% in controls) at 180 ppm in the F_{3b} generation; however this decrease was not observed in any other generation. In addition, mean survival from birth to weaning was similar across dose for each of the three generations of offspring. Hyperexcitability was observed in pups (number not reported) from four litters in the F_{1b} generation only.

Mean weanling weights (calculated as entire litter weight divided by number of pups per litter) at 180 ppm were decreased by 15% (P<0.05), 12% (P<0.05), and 14% (P<0.01) in the F_{1b}, F_{3a}, and F_{3b} generations, respectively (Table 1); however, the decreases were not dose-dependent, not

observed in the F₂ generation, and not calculated from individual pup body weights. No treatment-related differences were observed in mean absolute brain weights or mean brain-to-body weight ratios in F_{3b} generation weanling rats of either sex. A 12% increase in mean absolute kidney weights and mean kidney-to-body weight ratios (P<0.01) was observed in F_{3b} generation female weanlings at 180 ppm. A 10% increase in mean absolute liver weights and mean liver-to-body weight ratios (P<0.05) in males at 180 ppm and at ≥100 ppm in females was observed in this same generation. Changes in F_{3b} generation weanling mean organ weights were not considered toxicologically significant, however, due to a lack of histopathological correlates in the liver and kidney. The offspring LOAEL was not observed. The offspring NOAEL is 180 ppm (13.5 mg/kg bw/day).

3.3.5 Additional Information from Literature Sources

Dichlobenil and its soil metabolite, BAM, have both been shown to be olfactory toxicants in adult animals following intraperitoneal (i.p.) exposure (Brandt et al. 1990; Brittebo et al. 1991; Eriksson and Brittebo 1995). Dichlobenil alone also causes olfactory toxicity in neonatal animals following subcutaneous (s.c.) exposure (Eriksson and Brittebo 1995). Olfactory toxicity has also been demonstrated after dermal exposure of dichlobenil (Deamer et al. 1994) to adult animals.

Experimental evidence suggests that dichlobenil and BAM are activated to the ultimate olfactory toxicant (currently unknown) by cytochrome P (CYP) 450 enzymes located in Bowman's glands of the nasal mucosa (Brandt et al. 1990; Brittebo 1997). It is suggested that damage to the olfactory epithelium is a secondary effect of damage to the Bowman's glands (primary lesion), which are located in the lamina propria of the olfactory mucosa (Brandt et al. 1990; Brittebo et al. 1991). Since CYP 450s are abundant in both the olfactory epithelium and Bowman's glands, both areas may be sites of bioactivation of dichlobenil and BAM (Harkema et al. 2006). Further, it has been hypothesized that damage to the olfactory epithelium, as well as the liver, may be mediated by hydroxylated metabolites of dichlobenil or BAM, the metabolites of which have been shown *in vitro* to be uncouplers of oxidative phosphorylation in rat liver mitochondria (Brittebo et al. 1991).

3.3.6 Pre-and/or Postnatal Toxicity (Dichlobenil and BAM)

3.3.6.1 Determination of Susceptibility

Dichlobenil

There was no evidence of increased prenatal susceptibility in the developmental toxicity studies in rats or rabbits. In the rat developmental toxicity study, no developmental effects were observed at the highest dose tested; maternal toxicity was observed at the mid dose. In the rabbit developmental toxicity study, an increase in total resorptions/dam, post-implantation loss, as well as external, visceral, and skeletal anomalies were observed at the high dose. However, maternal toxicity (decreased body

weight gain and food consumption) was also observed at the high dose.

Evidence of increased pre-/post-natal susceptibility was observed in the two-generation reproduction study in rats. In this study, toxicologically significant decreases in body weight gain (pre-mating and gestation) and food consumption (pre-mating) were observed at the high dose in both parental and F₁ generation adults. However, decreased body weight was observed during weaning in both F₁ (16-23%) and F₂ (19-22%) generation pups at the next lower (mid) dose.

Overall, HED concluded that there is evidence of increased susceptibility to offspring following pre-/post-natal exposure to rats in the two-generation reproduction study in rats.

BAM

There was no evidence of increased prenatal susceptibility in the developmental toxicity study in the rabbit or in the three-generation reproduction study in the rat. In the rabbit developmental toxicity study, an increase in the incidences of late abortion, as well as visceral and skeletal anomalies were observed at the high dose. However, severe maternal toxicity [decreased body weight gain (>15%) and food consumption (49%) and late abortion] was observed at the same dose. In the rat three-generation reproduction study, no adverse effects were observed up to the highest dose tested in either generation.

3.3.6.2 Degree of Concern Analysis

Dichlobenil

HED considers the concern for increased susceptibility to offspring following pre-/post-natal exposure to dichlobenil to be low. The Agency proposes to regulate potential incidental oral exposure by using the NOAEL from the two-generation reproduction study in rats. HED is confident in this NOAEL, which is six times lower than the dose (LOAEL) at which decreased pup body weight was observed in the two-generation reproduction study in rats.

BAM

Since there is no evidence of increased pre-/post-natal susceptibility in the developmental toxicity study in the rabbit or in the three-generation reproduction study in the rat, there is no concern.

3.3.7 Recommendation for a Developmental Neurotoxicity Study (Dichlobenil and BAM)

Abnormal offspring behavior, CNS malformations or neuropathology in offspring, effects on offspring brain weights, and effects on offspring sexual maturation were not observed in any study for dichlobenil. However, neuropathology (olfactory sensory neuron toxicity) was observed in both adult and neonatal animals following i.p. and s.c.

exposure, respectively, to dichlobenil (Eriksson and Brittebo 1995). Toxicity to olfactory sensory neurons was also observed in adult animals following a single i.p. dose of BAM (Brittebo et al. 1991).

Clinical signs of neurotoxicity were observed in three studies for BAM. In the subchronic oral toxicity study in the rat, decreased skeletal muscle tone was observed with increasing severity at the mid-high and high doses. However, the toxicological significance of this finding is unclear, given the lack of reproducibility of the effect in other studies, the age of the study itself (1967), and the fact that the purity of the compound was not reported in the study.

Lethargy and ataxia were observed after a single dose in mice in an oral dose-range finding study for the *in vivo* erythrocyte micronucleus assay for BAM. The effects were transient (resolved within 24 hours). In addition, in a two-week dietary study recently submitted and not fully reviewed by the Agency (MRID 46892401), clinical signs of toxicity (slightly decreased muscle tone, slight loss of pinnae reflexes) were observed in mice at 2500 ppm (approximately equal to 375 mg/kg/day). Until acute and subchronic neurotoxicity batteries are received and evaluated by the Agency, their absence is considered a data gap. **A developmental neurotoxicity study is pending, based on receipt and review of the requested acute and subchronic neurotoxicity screening batteries.**

3.4 FQPA Safety Factor (SF) for Infants and Children (Dichlobenil and BAM)

Dichlobenil

Data from the published literature (Eriksson and Brittebo 1995) indicate that olfactory toxicity following s.c. (neonates) or i.p. (adults) exposure is similar in both subpopulations of mice. However, a comparative assessment of the tissue dose of dichlobenil following s.c. or i.p. administration cannot be made based on the existing data. Therefore, the risk assessment team recommends that the 10X FQPA SF, in the form of a UF_{DB} be retained until the potential of dichlobenil to induce olfactory toxicity in neonates relative to adults via the oral route is assessed.

BAM

The risk assessment team recommends that the 10X FQPA SF, in the form of two UF_{DB} s, be retained for most exposure scenarios. The first UF_{DB} of 3X corresponds to the lack of data assessing the potential of dichlobenil (parent) to induce olfactory toxicity in neonates relative to adults via the oral route is assessed. HED believes that a value of 3X (rather than 10X) for the first UF_{DB} is warranted based on the observation that approximately 3-fold higher doses of BAM are needed to induce levels of olfactory toxicity that are similar to those caused by dichlobenil, following a single i.p. injection of either compound to adult mice (Brandt et al. 1990; Brittebo et al. 1991). The second UF_{DB} of 3X corresponds to the incompleteness of the database with regard to the systemic neurotoxic potential of BAM.

The risk assessment team also recommends that the FQPA SF be retained, but reduced to 3X for the acute dietary exposure scenario (general population) only, since a LOAEL based on clinical signs of neurotoxicity was utilized to extrapolate a NOAEL for this scenario. HED believes this reduction is warranted based on the decrease in severity of effects that was observed with decreasing dose in the pilot study for the *in vivo* mouse micronucleus assay.

3.5 Hazard Identification and Toxicity Endpoint Selection

Dichlobenil

A summary of the toxicological endpoints and doses chosen for the relevant exposure scenarios for human risk assessment is found in Table 3.2.

Table 3.2. Summary of Toxicological Doses and Endpoints for Dichlobenil for Use in Dietary and Non-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
Acute Dietary (General population, including infants and children)	N/A	N/A	N/A	An endpoint of concern (effect) attributable to a single dose was not identified in the database. Quantification of acute risk to general population, including infants and children, is not required.
Acute Dietary (Females 13-49 years of age)	NOAEL = 45 mg/kg/day	UF _A = 10X UF _H = 10X FQPA SF ⁴ = 10X (includes UF _{DB} = 10X)	aRfD = 0.45 mg/kg/day aPAD = 0.045 mg/kg/day	Developmental toxicity (rabbit) Offspring LOAEL = 135 mg/kg/day based on increased incidences of total resorptions/dam, post-implantation loss, and fetal external, visceral, and skeletal anomalies (see tox table)
Chronic Dietary (All populations)	NOAEL = 1 mg/kg/day	UF _A = 10X UF _H = 10X FQPA SF ⁴ = 10X (includes UF _{DB} = 10X)	cRfD = 0.01 mg/kg/day cPAD = 0.001 mg/kg/day	Chronic toxicity (dog) LOAEL = 6 mg/kg/day based on increased liver weights and increased serum cholesterol, triglycerides, phospholipids, and alkaline phosphatase (M&F) and increased serum γ -GT and periportal hypertrophy of hepatocytes (M); <i>olfactory toxicity was assayed and not observed in this study</i>
Incidental Oral Short- and Intermediate-Term	NOAEL = 3 mg/kg/day	UF _A = 10X UF _H = 10X FQPA SF ⁴ = 10X	Residential LOC for MOE = 1000	2-generation reproduction (rat) Offspring LOAEL = 17.5 mg/kg/day based on decreased

(1-30 days and 1-6 months)		(includes UF _{DB} = 10X)		body weight during weaning in both generations
Dermal Short-, Intermediate-, and Long-Term (1-30 days, 1-6 months, and > 6 months)	NOAEL = 25 mg/kg/day	UF _A = 10X UF _H = 10X FQPA SF ⁴ = 10X (includes UF _{DB} = 10X)	Residential LOC for MOE = 1000 Occupational LOC for MOE = 100	5-day dermal (mouse; literature study ¹) LOAEL = 50 mg/kg/day based on olfactory epithelial damage
Inhalation Short-, Intermediate-, and Long-Term (1-30 days, 1-6 months, and >6 months)	NOAEL = 0.17 mg/kg/day ²	UF _A = 10X UF _H = 10X FQPA SF ⁴ = 10X (includes UF _{DB} = 10X)	Residential LOC for MOE = 1000 Occupational LOC for MOE = 100	7-day inhalation (rat; dose-range finding) LOAEL = 0.30 mg/kg/day ³ based on nasal degeneration
Cancer	Classification: Group C, possible human carcinogen; RfD approach should be used for quantification of human risk (2 nd Carcinogenicity Peer Review, 1995)			

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), FQPA SF = FQPA Safety Factor, UF_{DB} = to account for the absence of key data, 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, N/A = Not Applicable

¹ [Deamer NJ, O'Callaghan JP, Genter MB](#). (1994). Olfactory toxicity resulting from dermal application of 2,6-dichlorobenzonitrile (dichlobenil) in the C57Bl mouse. *Neurotoxicology* 15(2):287-93

² Calculated as follows: [(NOAEL) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where NOAEL= 12 mg/m³ from 28-day inhalation toxicity study (rat) and MW=172

³ Calculated as follows: [(LOAEL) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where LOAEL= 21 mg/m³ from 7-day inhalation range finding study (rat) and MW=172

⁴ The 10X FQPA SF has been retained in the form of a UF_{DB} for the lack of olfactory toxicity data in neonates and/or adults following oral exposure to dichlobenil

3.5.1 aRfD - Females age 13-49

The aRfD for females 13-49 years of age was established based on the NOAEL (100 mg/kg/day) from the developmental toxicity study in rabbits. The LOAEL of 135 mg/kg/day is based on increased incidences of total resorptions/dam, post-implantation loss, and fetal external, visceral, and skeletal anomalies. This study and endpoint are the most appropriate for the population of concern, namely, women of childbearing age. The FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in adults and neonates following oral exposure to dichlobenil.

3.5.2 aRfD - General Population

An acute dietary endpoint for all populations, including infants and children, was not established since an endpoint of concern attributable to a single dose was not identified in the database.

3.5.3 cRfD

The cRfD was established based on the NOAEL (1 mg/kg/day) from the chronic toxicity study in the dog. The LOAEL of 6 mg/kg/day is based on increased liver weights and increased serum cholesterol, triglyceride, phospholipid, and alkaline phosphatase levels in males and females and increased serum γ -glutamyl transferase levels and periportal hypertrophy of hepatocytes in males. The NOAEL of 1 mg/kg is the lowest in the database. In addition, the study duration is appropriate for the duration of exposure. Although olfactory toxicity was assayed and not observed in adult dogs in this study, the 10X FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in neonates following oral exposure to dichlobenil.

3.5.4 Incidental Oral Exposure (Short- and Intermediate-Term)

The effects of concern that are relevant to the selection of the short- and intermediate-term incidental oral doses are decreased body weight observed during weaning in both generations at 17.5 mg/kg/day in the two-generation reproduction study in rats. The study length is appropriate for the durations of exposure, namely, 1-30 days (short-term) and 1-6 months (intermediate-term); and the NOAEL of 3 mg/kg/day is protective of the population of concern, namely, infants and children. However, the 10X FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in neonates following oral exposure to dichlobenil.

3.5.5 Dermal Absorption

No dermal absorption study is available in the database. Since a route-specific toxicity study (5-day dermal in mouse) is being used for dermal risk assessment, estimation of dermal absorption is not necessary.

3.5.6 Dermal Exposure (Short-, Intermediate-, and Long-Term)

The effects of concern that are relevant to the selection of the short-, intermediate-, and long-term dermal doses are olfactory epithelial damage observed in a published 5-day dermal toxicity study in rats (Deamer et al. 1994). The route of exposure of this study is ideal for these dermal exposure scenarios. However, the 10X FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in neonates following exposure to dichlobenil.

3.5.7 Inhalation Exposure (Short-, Intermediate-, and Long-Term)

The 7-day dose-range finding inhalation toxicity study in the rat was chosen for the short-, intermediate-, and long-term inhalation exposure scenarios. The effect of concern that is relevant to the selection of short-, intermediate-, and long-term inhalation endpoints is nasal degeneration observed at 0.3 mg/kg/day (NOAEL = 0.17 mg/kg/day) in this study. Although the route of exposure of this study is ideal for these exposure

scenarios, the 10X FQPA SF has been retained in the form of a UF_{DB} to account for the lack of olfactory toxicity data in neonates following exposure to dichlobenil.

BAM

A summary of the toxicological endpoints and doses chosen for the relevant exposure scenarios for human risk assessment is found in Table 3.3.

Table 3.3. Summary of Toxicological Doses and Endpoints for 2,6-Dichlorobenzamide (BAM) for Use in Dietary and Non-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
Acute Dietary (General population, including infants and children)	LOAEL = 100 mg/kg/day	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{4,5} = 10X (includes $UF_L = 3X$ and $UF_{DB} = 3X$)	aRfD = 1 mg/kg/day aPAD = 0.1 mg/kg/day	Dose-range finding assay for <i>in vivo</i> mouse erythrocyte micronucleus assay LOAEL = 100 mg/kg/day based on lethargy after a single oral dose
Acute Dietary (Females 13-49 years of age)	NOAEL = 30 mg/kg/day	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{5,6} = 10X (includes $UF_{DB} = 10X$)	aRfD = 0.30 mg/kg/day aPAD = 0.03 mg/kg/day	Developmental toxicity (rabbit) Offspring LOAEL = 90 mg/kg/day based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies
Chronic Dietary (All populations)	NOAEL = 4.5 mg/kg/day	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{5,6} = 10X (includes $UF_{DB} = 10X$)	cRfD = 0.045 mg/kg/day cPAD = 0.0045 mg/kg/day	Chronic toxicity (dog) LOAEL = 12.5 mg/kg/day based on decreased body weight and body weight gain
Incidental Oral Short- and Intermediate-Term (1-30 days and 1-6 months)	NOAEL = 14 mg/kg/day	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{5,6} = 10X (includes $UF_{DB} = 10X$)	Residential and Occupational LOC for MOE = 1000	90-day oral (rat) LOAEL = 49 mg/kg/day based on decreased body weight gain (M) and reduced skeletal muscle tone (day 4 only in males; days 91 and 92 only in females)
Dermal Short-, Intermediate-, and Long-Term (1-30 days, 1-6 months, and >6 months) ⁷	NOAEL = 25 mg/kg/day	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{5,6} = 10X (includes $UF_{DB} = 10X$)	Residential and Occupational LOC for MOE = 1000	5-day dermal using dichlobenil (mouse; literature study ¹) LOAEL = 50 mg/kg/day based on olfactory epithelial damage
Inhalation Short-, Intermediate-, and Long-Term (1-30	NOAEL = 0.17 mg/kg/day ²	$UF_A = 10X$ $UF_H = 10X$ FQPA SF ^{5,6} = 10X (includes $UF_{DB} =$	Residential and Occupational LOC for MOE = 1000	7-day inhalation using dichlobenil (rat; dose-range finding) LOAEL = 0.30 mg/kg/day ³ based on nasal

days, 1-6 months, and >6 months) ⁷		10X)		degeneration
Cancer	Classification: Formally unclassified; however, NOAEL in carcinogenicity study similar to that for parent (dichlobenil); therefore “Group C, possible human carcinogen” (former system); RfD approach appropriate for quantification of human risk			

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), FQPA SF = FQPA Safety Factor, UF_L = use of a LOAEL to extrapolate a NOAEL, UF_{DB} = to account for the absence of key data, 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, N/A = Not Applicable

¹ [Deamer NJ, O'Callaghan JP, Genter MB.](#) (1994). Olfactory toxicity resulting from dermal application of 2,6-dichlorobenzonitrile (dichlobenil) in the C57Bl mouse. *Neurotoxicology* 15(2):287-93

² Calculated as follows: [(NOAEL) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where NOAEL= 12 mg/m³ from 28-day inhalation toxicity study (rat) and MW=172

³ Calculated as follows: [(LOAEL) x (m³ / 1000 L) x (24,550 / MW) x (0.100 mg/kg/day / ppm for a young rat)], where LOAEL= 21 mg/m³ from 7-day inhalation range finding study (rat) and MW=172

⁴ The FQPA SF has been retained in the form of a UF_L, because a LOAEL was used to extrapolate a NOAEL

⁵ The FQPA SF has been retained in the form of a UF_{DB} for the lack of acute and subchronic olfactory toxicity data in neonates following oral exposure to dichlobenil

⁶ The FQPA SF has been retained in the form of a UF_{DB} for the lack of neurotoxicity data

⁷ In the absence of route-specific data, endpoints for all dermal and inhalation exposure scenarios were identical to those for dichlobenil (parent), since olfactory toxicity has been observed following i.p. administration of BAM in mice [[Brittebo EB, Eriksson C, Feil V, Bakke J, Brandt I.](#) (1991). Toxicity of 2,6-dichlorothiobenzamide (chlorthiamid) and 2,6-dichlorobenzamide in the olfactory nasal mucosa of mice. *Fundam Appl Toxicol* 17(1):92-102].

3.5.8 aRfD - Females age 13-49

The aRfD for females 13-49 years of age was established based on the LOAEL from the developmental toxicity study in rabbits. The LOAEL of 90 mg/kg/day is based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies. This study and endpoint are the most appropriate for the population of concern, namely, women of childbearing age. The 10X FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in adults and neonates following oral exposure to dichlobenil or BAM, as well as for the lack of systemic neurotoxicity data for BAM. It is noted that approximately 3-fold higher doses of BAM are needed to induce levels of olfactory toxicity that are similar to those caused by dichlobenil, following a single i.p. injection of either compound to adult mice (Brandt et al. 1990; Brittebo et al. 1991).

3.5.9 aRfD - General Population

The aRfD for the general population, including infants and children, was established based on the LOAEL from the dose-range finding assay for the *in vivo* mouse erythrocyte micronucleus assay. The LOAEL of 100 mg/kg/day is based on lethargy following a single oral dose of BAM. A NOAEL was not identified in this study. Since a LOAEL was used to estimate a point of departure for this exposure scenario, the FQPA SF has been retained in the form of a 3X UF_L.

The 10X FQPA SF is also composed of 3X UF_{DB} to account for the lack of olfactory toxicity data in adults and neonates following oral exposure to BAM or dichlobenil.

3.5.10 cRfD

The cRfD was established based on the NOAEL (4.5 mg/kg/day) from the chronic toxicity study in the dog. The LOAEL of 12.5 mg/kg/day is based on decreased body weight and body weight gain. The NOAEL of 4.5 mg/kg is the lowest in the BAM database. In addition, the study duration is appropriate for the duration of exposure. The FQPA SF has been retained in the form of a 10X UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in neonates following oral exposure to BAM or dichlobenil, as well as the lack of systemic neurotoxicity data for BAM.

3.5.11 Incidental Oral Exposure (Short- and Intermediate-Term)

The effects of concern that are relevant to the selection of the short- and intermediate-term incidental oral doses are decreased body weight gain observed in males and reduced skeletal muscle tone (observed on day 4 only in males and on days 91 and 92 only in females) at 49 mg/kg/day in the 90-day oral toxicity study in rats. The study length and effects are ideal for the durations of exposure, namely, 1-30 days (short-term) and 1-6 months (intermediate-term). The NOAEL of 14 mg/kg/day is protective of the population of concern, namely, infants and children. The 10X FQPA SF has been retained in the form of a UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in adults and neonates following oral exposure to dichlobenil or BAM, as well as for the lack of systemic neurotoxicity data for BAM.

3.5.12 Dermal Absorption

No dermal absorption study is available in the database. Since a route-specific toxicity study (5-day dermal in mouse using dichlobenil) is being used for dermal risk assessment, calculation of dermal absorption is not necessary.

3.5.13 Dermal Exposure (Short-, Intermediate-, and Long-Term)

The effects of concern that are relevant to the selection of the short-, intermediate-, and long-term dermal doses are olfactory epithelial damage observed in a published 5-day dermal toxicity study in rats (Deamer et al. 1994) using dichlobenil. The route of exposure of this study is ideal for these dermal exposure scenarios. However, the FQPA SF has been retained in the form of a 10X UF_{DB} for this exposure scenario to account for the lack of olfactory toxicity data in neonates following oral exposure to dichlobenil or BAM, as well as for the lack of systemic neurotoxicity data for BAM. Based on the results from two published literature studies of BAM via the intraperitoneal route, BAM is expected to cause olfactory toxicity at doses 3X higher than dichlobenil.

3.5.14 Inhalation Exposure (Short-, Intermediate-, and Long-Term)

The 7-day dose-range finding inhalation toxicity study using dichlobenil in the rat was chosen for the short-, intermediate-, and long-term inhalation exposure scenarios. The effect of concern that is relevant to the selection of short-, intermediate, and long-term inhalation endpoints is nasal degeneration observed at 0.3 mg/kg/day (NOAEL = 0.17 mg/kg/day) in this study. Although the route of exposure of this study is ideal for these exposure scenarios, the FQPA SF has been retained in the form of a 10X UF_{DB} to account for the lack of olfactory toxicity data in neonates following oral exposure to dichlobenil or BAM, as well as for the lack of systemic neurotoxicity data for BAM. Based on the results from two published literature studies of BAM via the intraperitoneal route, BAM is expected to cause olfactory toxicity at doses 3X higher than dichlobenil.

3.5.15 Level of Concern for Margin of Exposure (Dichlobenil and BAM)

The target MOEs for occupational and residential exposure risk assessments are as follows:

Route	Duration		
	Short-Term (1-30 days)	Intermediate-Term (1-6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	100/1000 ^a	100/1000	100/1000
Inhalation	100/1000	100/1000	100/1000
Residential (Non-Dietary) Exposure			
Oral	1000/1000	1000/1000	1000/1000
Dermal	1000/1000	1000/1000	1000/1000
Inhalation	1000/1000	1000/1000	1000/1000

^a MOEs expressed for dichlobenil/BAM

3.5.16 Recommendation for Aggregate Exposure Risk Assessments

Estimation of aggregate risk is currently not required, because a common toxicological effect across oral, dermal, and inhalation exposure scenarios was not observed for either dichlobenil or BAM.

3.5.17 Classification of Carcinogenic Potential

Dichlobenil was determined to be non-mutagenic in bacteria and mammalian cells, as well as non-clastogenic in several mammalian assays (*in vitro* and *in vivo*). The carcinogenic potential of dichlobenil was evaluated for the second time by the Health Effects Division Carcinogenicity Peer Review Committee (CPRC) in 1995. In a high-

dose carcinogenicity study in the hamster, a treatment-related increase in liver adenomas and combined adenomas/carcinomas was observed in males only at the highest dose tested. However, dosing was considered excessive at this dose in both sexes, based on decreased body weight gains and severe hepatotoxicity. In a second carcinogenicity study performed in hamsters at lower doses, no treatment-related increases in the incidence of any tumor type were observed. Dosing was considered adequate, based on decreased body weight gains and hyperplasia in various tissues in both sexes. In a combined chronic toxicity/carcinogenicity study in the rat, a treatment-related increase in the incidence of hepatocellular adenomas and combined adenomas/carcinomas was observed in females only at the highest dose tested. Dosing was considered adequate in females, but excessive in males, at this dose. Based on these data, the CPRC classified dichlobenil as a “Group C, possible human carcinogen” (former system), and an RfD approach to quantification of cancer risk was recommended.

BAM was determined to be non-mutagenic in bacteria and non-clastogenic in an *in vivo* mouse erythrocyte micronucleus assay. Like dichlobenil, BAM also did not induce unscheduled DNA synthesis in mammalian cells. **The Agency is currently reviewing additional genotoxicity studies for BAM.** There was no evidence of carcinogenicity in the combined chronic toxicity/carcinogenicity study of BAM in the rat. An increased incidence of hepatocellular adenomas was observed at the high dose in females only in the study. However, the statistical significance of the effect was marginal ($P=0.049$), dosing was considered adequate in the study, and the tumors were non-cancerous. However, since the carcinogenic potential of BAM was evaluated in only one species, HED considers the carcinogenic potential of BAM to be similar to that of dichlobenil (possible human carcinogen). Therefore, an RfD approach to quantification of cancer risk is also warranted for BAM.

3.6 Endocrine disruption

EPA is required under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate.” Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC’s recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP). When additional appropriate screening and/or testing protocols being considered under the Agency’s EDSP have been developed, dichlobenil or its soil

metabolite, BAM, may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption. It is noted that in the chronic dog toxicity study with dichlobenil, delayed maturity of the uterus was observed in all high-dose females. A marked decrease in mean uterine weight at the high dose confirmed this finding. Ovarian weights were also decreased in high-dose females, but no alterations were observed microscopically. These results are suggestive of modulation of the female endocrine system; however, the dose at which these effects were observed was forty times higher than that utilized for risk assessment, i.e., the NOAEL. The NOAEL utilized for risk assessment is therefore considered protective of any potential endocrine modulation by dichlobenil. In addition, no other reproductive parameters were affected in this or any other study in the database. No evidence of endocrine modulation was observed in any study with BAM.

4.0 Public Health and Pesticide Epidemiology Data

This is the function of the ORE assessor. It would be interesting to know if adverse nasal effects have been reported in human Ag workers exposed to dichlobenil.

8.0 Cumulative Risk Characterization/Assessment

Dichlobenil produces a toxic metabolite (BAM) that is also produced by chlorthiamid (2,6-dichlorothiobenzamide), and this should be considered. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at <http://www.epa.gov/pesticides/cumulative/>.

10.0 Data Needs and Label Requirements

10.1 Toxicology

- Guideline acute neurotoxicity screening battery (OPPTS 870.6200) with 2,6-dichlorobenzamide (BAM)
- Guideline subchronic neurotoxicity screening battery (OPPTS 870.6200) with 2,6-dichlorobenzamide (BAM)
- A comparative study of olfactory toxicity by the oral route in neonates and adults. The registrant is encouraged to consult with the Agency to discuss the protocol.
- A DNT is reserved pending receipt and review of the acute and subchronic neurotoxicity screening batteries