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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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MEMORANDUM **Date: January 28, 2010**

SUBJECT: Metrafenone: Ecological Risk Assessment For Proposed Use on Grapes

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This memorandum transmits the Environmental Fate and Effects Division's (EFED) environmental risk assessment for the active ingredient, metrafenone, as a fungicide for uses on grapes to treat powdery mildew; it is used for preventive but not curative measures. The proposed end-use product Metrafenone 300 is a suspension concentrate containing 25.2% metrafenone. Ground (liquid) application is the proposed method of application. The maximum single application rate is 0.300 lbs a.i./A; at 6 applications per season, the seasonal maximum rate is 1.80 lbs a.i./A. The minimum application interval is 14 days.

While metrafenone is expected to be slightly mobile, its major routes of degradation are aqueous photolysis and aquatic metabolism, in both aerobic and anaerobic conditions. Soil metabolism is slow, with laboratory half-lives of 6 months to a year and accumulation over two years observed in field studies. In aquatic environments, the

measured photolysis half-life was 6.4 days and metabolism in both aerobic and anaerobic occurred with half-lives of one to four weeks. Exposure is expected to be dominated by runoff and spray drift. Metrafenone exposure may also result from off-site movement in runoff water and on metrafenone -bearing soil particulates to adjacent fields (soil erosion). The moderate K_{oc}'s (K_{oc} 1073 to 22517 L/kg-oc) of metrafenone suggest that leaching to ground water/recharge to surface water would not be a route of exposure. Long-range transport of metrafenone in the gas phase is not considered a significant route of exposure. While the high log K_{ow} (4.3 at 25^{o}C, pH 4) would suggest the potential for bioaccumulation, the lipid normalized BCF (between 140 and 530) indicates that metrafenone is not expected to accumulate in tissues of aquatic organisms.

The results of this screening-level assessment indicate a potential for direct adverse effects to non-target mammals (dose-based RQs 1.14-2.91) following chronic exposure; these RQs also exceed the federally listed species LOC. Due to the potential for direct adverse effects to mammals associated with the application of metrafenone on grapes, indirect effects may consequently affect other aquatic and terrestrial species. To reduce chronic risk to mammals, several components of the application protocol would have to change. For example, in order to have no chronic LOC exceedances (*i.e.*, all RQs < 1) for mammals, the minimum single application rate (0.2 lbs a.i./A) would have to be cut by 25% (*i.e.*, to 0.15 lbs a.i./A) yet considered the maximum instead, the application interval would have to nearly double (from 14 days up to 26 days), and the maximum allowed number of applications would have to be cut from 6 to 5. Alternatively, in order to have no chronic LOC exceedances for mammals, the minimum single application rate (0.2 lbs a.i./A) can still be applied yet considered the maximum instead, but the application interval would have to nearly double (from 14 days up to 26 days), and the maximum number of applications would have to be cut by 50% (*i.e.*, from 6 to 3). Furthermore, in order to have no chronic LOC exceedances in nearly all cases for mammals –the one exception being an exceedance for the 15g size glass consuming short grass where the calculated RQ is 1.02 – the minimum single application rate (0.2 lbs a.i./A) would be changed to the maximum single application rate and the application interval (14 days) could remain as currently prescribed by the label, but the maximum number of applications would have to be reduced from 6 to 2.

Data were either not submitted or were deemed invalid for freshwater invertebrates and marine/estuarine fish via chronic exposure. Without data, risk cannot be ruled out for these taxa (either non-listed or federally listed species).

Non-definitive endpoints – in this case, where total concentrations were estimated instead of the preferred dissolved concentrations in the majority of aquatic studies on metrafenone – suggest potential chronic risk to federally listed freshwater fish and acute risk to federally listed marine/estuarine invertebrates. More detailed risk conclusions are provided in the environmental risk assessment document.

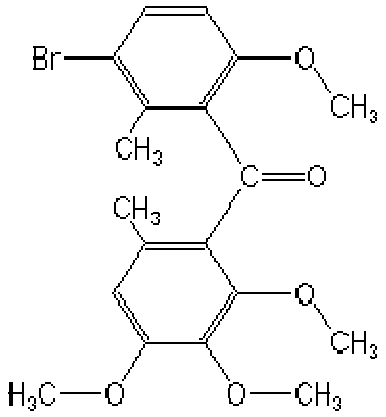
Data Needs

The ecotoxicity and environmental fate data needs are summarized in Tables 1 and 2 below. Further explanation and characterization of these data needs can be found in the executive summary of the ecological risk assessment.

Table 1. Ecological Toxicity Data Gaps	
Study	**Reason**
850.1075 Acute toxicity freshwater fish	Non-definitive endpoint w/effects observed
850.1400 Freshwater fish early-life stage (chronic)	Non-definitive endpoint w/effects observed
850.1300 Aquatic freshwater invertebrate life-cycle (chronic)	Invalid, CFR data gap
850.1025 Acute marine/estuarine invertebrate (oyster) Or 850.1035 Acute marine/estuarine invertebrate (mysid)	Non-definitive endpoint w/effects observed
OECD 218 Freshwater invertebrate whole sediment study (chronic)	Invalid, CFR data gap
850.4400 Aquatic vascular plant growth, Tier II	Non-definitive endpoint w/effects observed
850.5400 Aquatic non-vascular plant growth, Tier II	Non-definitive endpoint w/effects observed
850.2100 Avian acute oral toxicity test (passerine species)	Not submitted, CFR data gap
850.3020 Honeybee acute contact toxicity (on TEP)	Address formulation uncertainty
850.4100 Seedling Emergence, Tier II (on TEP)	Address formulation uncertainty
850.4150 Vegetative Vigor, Tier II (on TEP)	Address formulation uncertainty

Table 2. Environmental Fate Data Gaps
835.6100 Independent Laboratory Validations

NEW CHEMICAL REGISTRATION
(Section 3)
ECOLOGICAL RISK ASSESSMENT
Metrafenone: Fungicide
USEPA PC # 000325



Chemical Name(s): (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)methanone (CAS)
3'-bromo-2,3,4,6'-tetramethoxy-2',6-dimethylbenzophenone (IUPAC)

Chemical Abstracts Service (CAS) Number: 220899-03-6

Chemical Family: Benzophenone fungicide

Pesticidal Mode of Action: Inhibits growth of mycelium on the leaf surface, leaf penetration, formation of haustoria, and sporulation. Likely affects actin proteins which play a role in cell function and cell division.

Proposed End-use Product: Metrafenone 300 SC (EPA Reg.No.7969-xxx); Suspension Concentrate, 25.2% (2.5 lbs a.i./gallon)

Target Pest(s): Powdery mildew produced by *Uncinula necator*

Proposed Target Crop(s): Grapes

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I. Executive Summary

A. Nature of the Chemical Stressor

Metrafenone (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)methanone; CAS Reg. No. 220899-03-6) is a new pesticide active ingredient submitted for registration in the United States. Metrafenone is a benzophenone fungicide with resistance code U8 (unknown mode of action). What is known is that the chemical inhibits growth of mycelium on the leaf surface, leaf penetration, formation of haustoria, and sporulation of the target fungus, *Uncinula necator* (EPA Pesticide Fact Sheet 2006). Metrafenone likely affects actin proteins which play a role in cell function and cell division (Opalski *et al.* 2006). It has a local inhibition effect; in other words, it is not systemic and not a contact killer.

As a new pesticide active ingredient, the actual usage of metrafenone is not known. The end-use product Metrafenone 300 SC is proposed for use on grapes to treat powdery mildew; it is used for preventive but not curative measures. Ground (liquid) application is the proposed method of application. The maximum single application rate is 0.300 lbs a.i./A; at 6 applications per season, the seasonal maximum rate is 1.80 lbs a.i./A. The minimum application interval is 14 days.

B. Potential Risks to Non-target Organisms

The results of this screening-level assessment indicate a potential for direct adverse effects to non-target mammals (dose-based RQs 1.14-2.91) following chronic exposure; these RQs also exceed the federally listed species LOC. Due to the potential for direct adverse effects to mammals associated with the application of metrafenone on grapes, indirect effects may consequently affect other aquatic and terrestrial species. Data were either not submitted or were deemed invalid for freshwater invertebrates and marine/estuarine fish via chronic exposure. Without data, risk cannot be ruled out for these taxa (either non-listed or federally listed species). Non-definitive endpoints – in this case, where total concentrations were estimated instead of the preferred dissolved concentrations in the majority of aquatic studies on metrafenone – suggest potential chronic risk to federally listed freshwater fish and acute risk to federally listed marine/estuarine invertebrates. Comparison of the non-definitive endpoints with aquatic EECs indicates potential risk to the given federally listed taxa (see Section I.F below, Table 3) but not the non-listed taxa summarized in **Table 1** and **Table 2**.

Table 1. Summary of Environmental Risk Conclusions for Aquatic Animals and Plants from Metrafenone Use on Grapes at the Maximum Proposed Application Rate (0.3 lb a.i./A, Assuming 6 Applications/Year)*		
Taxonomic Group	**Assessment Endpoint**	**Summarized Risk Characterization and Important Uncertainties**
Freshwater Fish and Aquatic Phase Amphibians	Mortality	Acute risk is not expected (from technical grade active ingredient, metabolites, and EU/UK formulation).
	Reproduction, growth etc.	Chronic risk is not expected.
Freshwater Invertebrates	Mortality	Acute risk is not expected (from technical grade active ingredient, metabolites, and EU/UK formulation).
	Reproduction, growth etc.	No acceptable studies available. Chronic risk cannot be precluded.
Marine/ Estuarine Fish	Mortality	Acute risk is not expected.
	Reproduction, growth etc.	No studies submitted. Chronic risk cannot be precluded.
Marine/ Estuarine Invertebrates	Mortality	Acute risk is not expected.
	Reproduction, growth etc.	Chronic risk is not expected.
Aquatic Plants	Acute Risk	Risk to vascular species is not expected from technical grade active ingredient. In addition, risk to non-vascular species is not expected (from the technical grade active ingredient, metabolites, and EU/UK formulation).
* Consult 'Risk Description' section for further details. Also, risk in this table implies risk to technical grade active ingredient unless otherwise specified that metabolites and formulations were assessed as well.		

Table 2. Summary of Environmental Risk Conclusions for Terrestrial Animals and Plants from Metrafenone Use on Grapes at the Maximum Proposed Application Rate (0.3 lbs a.i./A, Assuming 6 Applications/Year)*		
Taxonomic Group	**Risk Endpoint**	**Summarized Risk Characterization and Important Uncertainties**
Birds, Reptiles and Terrestrial Phase Amphibians	Mortality	Acute risk is not expected.
	Reproduction, growth etc.	Chronic risk is not expected.
Mammals	Mortality	Acute risk is not expected.
	Reproduction, growth etc.	Chronic risk is expected.
Non-target Invertebrates	Acute Risk	Acute risk to honeybees is not expected. Acute risk to earthworms (from the technical grade active ingredient, a metabolite, and EU/UK formulation) is not expected.
Terrestrial Plants	Acute Risk	Risk to terrestrial plants is not expected (from EU/UK formulation).
* Consult 'Risk Description' section for further details. Also, risk in this table implies risk to technical grade active ingredient unless otherwise specified that metabolites and formulations were assessed as well.		

C. Environmental Fate Summary

While metrafenone is expected to be slightly mobile, its major routes of degradation are aqueous photolysis and aquatic metabolism, in both aerobic and anaerobic conditions.

Soil metabolism is slow, with laboratory half-lives of 6 months to a year and accumulation over two years observed in field studies. In aquatic environments, the measured photolysis half-life was 6.4 days and metabolism in both aerobic and anaerobic occurred with half-lives of one to four weeks.

Soil photolysis and anaerobic aquatic metabolism of metrafenone led to one major degradate, CL 377160 [Methanone, (3-bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)-], and anaerobic aquatic metabolism also led to six other major degradates, but all were either unidentified or only tentatively identified. In addition, there were many minor degradates, which were formed at low levels individually but reached substantial amounts as groups.

Tier II aquatic exposure modeling was conducted for parent metrafenone and metrafenone residues (metrafenone+ extractable residues) (Table 11). This modeling approach was used to address uncertainties associated with identification of metrafenone residues in soil and sediment. The highest aquatic EECs for metrafenone were 11.70 µg/L for the daily peak, 8.82 µg/L for the 21 day average, and 6.21 µg/L for the 60 day average. The highest aquatic EECs for total metrafenone residues were 20.22 µg/L for the daily peak, 17.67 µg/L for the 21 day average, and 16.98 µg/L for the 60 day average. No monitoring data are available to compare with model estimates. Bioaccumulation modeling was conducted because metrafenone has a log octanol:water coefficient > 4. Maximum residue concentrations in fish are expected to range from 17,636 to 19,586 µg/kg-ww.

D. Ecological Effects Summary

Aquatic Organisms

The greatest amount of uncertainty in the assessment stems from aquatic studies which were largely based on total concentrations (both dissolved and undissolved) of the test compound. In all cases (except for the chronic study on the saltwater mysid, but including aquatic plants), the risk quotient values were not calculated. However, given comparisons of the total concentrations for most available studies and soluble concentrations for the saltwater mysid (chronic) and sheepshead minnow (acute) to respective EEC values the implication is that acute and chronic risk (including sublethal effects) to aquatic organisms (including aquatic plants) is not expected as a result of metrafenone use on grapes. However, the taxa for which studies were either submitted and deemed unacceptable (chronic freshwater invertebrate studies) or not submitted at all (chronic marine/estuarine fish), the risk as a result of metrafenone use cannot be precluded.

Terrestrial Organisms

The acute oral avian studies indicated no effects; the acute dietary avian studies indicated a significant change in body weight that was not associated with a dose-response pattern. In the acute mouse study on the technical grade active ingredient, only one mortality was reported. Therefore, no implications with lethal or sublethal acute effects can be made

for either avian or mammalian taxa. Metrafenone is classified as 'practically non-toxic' to honeybees and non-lethal to earthworms up to the limit concentration. Therefore, the implication is that acute risk to terrestrial invertebrates is not expected as a result of metrafenone use on grapes. In addition, no terrestrial plant risks are expected as a result of use of the EU/UK formulation; however, no data on terrestrial plants are available on the U.S. formulation.

The avian chronic toxicity endpoint (NOAEC 848 mg a.i./kg diet) based on egg production and hatchability did not yield chronic LOC exceedances. The most sensitive chronic mammalian endpoint (NOAEL 35.9 mg/kg bw/day) based on decreased body weights and body weight gain in F_1 males as well as body weights in F_1 and F_2 females, however, exceeded the chronic LOC, which implies that sublethal chronic effects on mammals are expected under field conditions. Therefore, given the LOC exceedance chronic risk to mammals is expected as a result of metrafenone use on grapes.

E. Uncertainties and Data Gaps

1. Environmental Fate and Exposure

All but one of the environmental fate studies required to support the registration of metrafenone for use on grapes were submitted. Independent Laboratory Validations for the methods submitted for analysis of metrafenone in soil and water remain a data gap. These data gaps fall under the terrestrial field dissipation study guideline (835.6100). For a list of submitted environmental fate studies for metrafenone see **Appendix F**.

All of the environmental fate studies for the parent compound were determined to be scientifically valid and therefore results from all of the studies can be used to characterize the mobility and rates of transformation of metrafenone. However, many of the metabolism studies have major uncertainties in the identification and pattern of formation and decline of transformation products. In all of the aquatic metabolism studies, between 57% and 65% of the applied radioactivity remains unidentified with incomplete characterization, and in two aerobic soil metabolism studies, 15% and 44% of the applied radioactivity is unidentified. This includes at least four major degradates that individually reach levels of 11% to 35% of the applied. Other transformation products appear as groups of up to 15 components, in some cases characterized as each being <5% of the applied radioactivity, but in other cases, some individual components make up 9% to 10% of the applied. Even when individual components can all be classified as minor degradates, these groups represent such a large portion of the applied radioactivity overall that the possibility that they may have some impact as a group cannot be precluded despite their lower individual levels. This is especially true given that the degradation pathways suggest that groups of degradates may have a high degree of structural similarity and so may have similar fate and effects behavior. Without information to adequately characterize the degradates, it was necessary to assume that they are of equal toxicity to the parent in order to quantify risks for metrafenone extractable residues.

In addition to the studies submitted by the registrant, EFED was provided with a Draft Assessment Report (DAR) on Metrafenone written by the United Kingdom. That assessment reports that the long dissipation time observed in field studies triggered a requirement for longer term field accumulation studies. Two years into those five year studies, results show that metrafenone appears to be accumulating, and that metrafenone residues have been detected as deep as 20-30 cm below the surface. These studies would provide useful information regarding the environmental fate of metrafenone and would assist in reducing uncertainties.

2. Ecological Effects Data

The submitted ecotoxicity database is incomplete. The greatest amount of uncertainty in the assessment stems from aquatic studies which were largely based on total (both dissolved and undissolved) concentrations of test compound; that is, the concentrations were measured without centrifugation even though precipitate was observed or test was conducted at the solubility limit of the metrafenone technical grade active ingredient; the solubility limits of the metabolites are unknown. To reduce uncertainty the majority of the toxicity studies, which are considered supplemental, and cannot be used in a quantitative risk estimation would need to be redone; especially those where mortality or sublethal effects were observed. These aquatic studies are specified below. For a list of submitted ecological effects studies for metrafenone see **Appendix G**.

The following studies are considered data needs:

Aquatic studies

Given the freshwater fish studies on metrafenone technical grade active ingredient and assuming that the concentrations in the environment reach the solubility limit, the effect of the compound is likely to be low. However, according to model estimated EECs (which include metrafenone and metrafenone residue scenarios: 0.00153 - 0.02 mg/L), levels of metrafenone (TGAI, TEP)[1] at the solubility limit (0.2-0.5 mg/L at 12^{o}C) and metrafenone metabolites at the tested concentrations are not expected to occur in the environment given the proposed grape use. Therefore, acute risk to freshwater fish and aquatic-phase amphibians is not expected as a result of metrafenone use on grapes. In addition, although the EU/UK formulation – with which the submitted studies were conducted – closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on freshwater fish and aquatic-phase amphibians is not known.

To reduce uncertainty in characterizing risk, which is currently based on total concentrations, the freshwater fish studies on bluegill sunfish (*Lepomis macrochirus*) are requested to make same species comparisons for acute and chronic values. Bluegill was chosen over the rainbow trout due to greater sensitivity (on an acute basis) of the bluegill to the TGAI relative to the trout. Since the acute marine/estuarine fish study is acceptable and given the above two studies, the acute to chronic ratio can be utilized to estimate

[1] TGAI: technical grade active ingredient; TEP: typical end-use product (formulation)

marine/estuarine chronic endpoints, negating the need to fill this data gap. Special emphasis must be placed on centrifuging the test samples prior to analytical determination of the test compound.

- Acute: Freshwater fish toxicity (96-hour LC_{50} for *Lepomis macrochirus*) (850.1075; 72-1), TGAI
- Chronic: Fish early-life stage (freshwater: *Lepomis macrochirus*) (850.1400; 72-4), TGAI

Although acute freshwater invertebrate studies were based on total concentrations, no effects were observed with the TGAI, negating the need to request additional data. However, a chronic freshwater invertebrate data gap still exists since the two reviewed studies were deemed invalid.

- Chronic: Aquatic invertebrate life cycle (freshwater: *Daphnia magna*) (850.1300; 72-4), TGAI

Effects were observed in both acute marine/estuarine invertebrate studies with the TGAI on the eastern oyster (*Crassostrea viginica*) and saltwater mysid (*Americamysis bahia*) but the endpoints are based on total concentrations. Therefore, should concentrations in the environment reach the solubility limit, the acute risk to marine/estuarine invertebrates may be expected. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Given these studies and assuming that metrafenone concentrations in the environment are not likely to reach the solubility limit, the acute risk to marine/estuarine invertebrates is not expected. However, to reduce uncertainty in characterizing risk, either study is requested to be redone with special emphasis on centrifuging the test samples prior to analytical determination of the test compound.

- Acute: Marine/estuarine invertebrate either *Crassostrea virginica* (850.1025; 72-3) **or** *Americamysis bahia* (850.1035; 72-3), TGAI

A non-guideline chronic midge study (*Chironomus riparius*) with metrafenone technical (97.1%) was deemed invalid on the basis of low negative control emergence. Therefore, a chronic freshwater invertebrate whole sediment study is requested. A protocol should be submitted for approval prior to study initiation. The protocol should include spiked sediment. The whole sediment study is requested because metrafenone has a high $\log K_{ow}$ value (4.3 at 25^{o}C, pH 4) and a high aerobic soil half life (> 4 months), which indicates that the compound may partition to sediment and persist.

- *Chironomus dilutus* (freshwater) using TGAI. Consult EPA Test Method 100.5 Life-cycle Test for Measuring the Effects of Sediment Associated Contaminants on *Chironomus dilutus* (formerly *Chironomus tentans*) and OECD Guideline 218 Sediment-Water Chironomid Toxicity Test Using Spiked Sediment

Non-target aquatic plant studies were also based on total concentrations leading to uncertainty in exposure concentrations and thus endpoint values. Had valid endpoints (*i.e.*, those based on dissolved concentrations) been determined using these studies, they would likely be greater than the highest model predicted concentration in the environment, which implies that risk to vascular and non-vascular species is not expected as a result of metrafenone use on grapes. However, without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot accurately be determined. Therefore, vascular and non-vascular aquatic plant studies are requested with special emphasis placed on centrifuging the test samples prior to analytical determination of the test compound. In addition, cyanobacteria yielded no effects given total concentrations and does not need to be redone.

- Aquatic vascular plant growth (*Lemna* spp.), Tier II (850.4400), TGAI
- Aquatic non-vascular plant growth, Tier II (850.5400), TGAI

Terrestrial Studies

An avian acute oral toxicity test in passerine species is required. Passerines are the most common birds (in terms of numbers and number of species) in the United States. Many utilize agricultural fields, forests, residential areas and surrounding areas, and, therefore, have the potential to be exposed to pesticides used in agricultural, forest, and residential settings. It is likely that, for the requested use patterns, passerines are more likely to be exposed to metrafenone than upland game species and waterfowl. Passerines are smaller and have a higher energy requirement than larger-sized birds. As such, passerines may be more sensitive than other birds.

- Passerine bird toxicity study (EPA approved protocol is required prior to study initiation): Avian acute oral toxicity test (850.2100, 71-1), TGAI

Formulated Product Testing

The available formulation product studies were conducted using three different UK/EU formulated products (*i.e.*, BAS 560 00F, BAS 560 01F, and BAS 560 02F), one of which (BAS 560 00F) closely matches the U.S. formulation (*i.e.*, BAS 560 03F). Formulation studies in this assessment are based on BAS 560 00F because data on the U.S. formulation were not submitted. In order to eliminate uncertainty in effects characterization of formulated products used within the U.S. on given taxa, future registrant submitted studies should be based on the U.S. formulation. At this time the effect of the U.S. formulation on given taxa is not known, however, greater toxicity of the EU/UK formulation on the honeybee relative to the TGAI was observed. Similarly, data submitted for terrestrial plants is based on the EU/UK formulation. As a result, the following studies are requested:

- Honeybee acute contact toxicity (850.3020, 141-1), TEP
- Seedling emergence, Tier II (850.4225, 123-1), TEP
- Vegetative vigor, Tier II (850.4250, 123-1), TEP

F. Endangered Species Considerations

Table 3 summarizes the listed species at risk associated with either direct or indirect effects following application of metrafenone for the proposed uses.

Concerns For Federally Listed as Endangered and/or Threatened Species

Table 3. Listed Species Risks Associated With Direct or Indirect Effects from Metrafenone use on Grapes at the Maximum Proposed Application Rate (0.3 lbs a.i./A, Assuming 6 Applications/Year)

Listed Taxon	Direct Effects	Indirect Effects
Terrestrial and semi-aquatic plants - monocots	No	Yes from effects to mammals
Terrestrial and semi-aquatic plants – dicots	No	Yes from effects to mammals
Terrestrial invertebrates	No	Yes from effects to mammals
Birds	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Terrestrial-phase amphibians	No	Yes from effects to mammals
Reptiles	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Mammals	Yes for chronic[1]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic non-vascular plants	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic vascular plants	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Freshwater (FW) fish	Yes for chronic[2]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic-phase amphibians	Yes for chronic[3]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Freshwater (FW) invertebrates	Yes for chronic[4]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Marine/estuarine (M/E) fish	Yes for chronic[4]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Marine/estuarine (M/E) invertebrates (mollusk)	Yes for acute[5], No for chronic[6]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)

[1] The chronic LOC is exceeded on a dose basis for mammals in all size classes eating short grass, for the 15 and 35 gram size classes eating tall grass and broadleaf plants / small insects. The chronic LOC on a dietary basis is not exceeded for any of the food categories.
[2] The total concentration based endpoint (NOAEC: 0.118 mg total a.i./L) for the chronic freshwater fish (fathead minnow) study (MRID 47267449) with an effect on post-hatch survival is approximately 7x greater than the highest estimated EEC (0.016 mg/L), hence risk to federally listed freshwater fish cannot be precluded.
[3] Results from freshwater fish used as surrogate for assessing risk to aquatic-phase amphibians
[4] Studies not submitted or invalid for which risk cannot be precluded.
[5] Mollusk (Eastern oyster); [6] Saltwater mysid

II. Problem Formulation

The purpose of this problem formulation is to provide the foundation for the ecological risk assessment being conducted for the fungicide metrafenone. As such, it articulates the purpose and objectives of the risk assessment, evaluates the nature of the problem, and provides a plan for analyzing the data and characterizing the risk (EPA, 1998).

A. Nature of Regulatory Action

Metrafenone is a new pesticide active ingredient being proposed as a fungicide to control powdery mildew on grapes. As a new active ingredient submitted for registration, there are no previously prepared ecological risk assessments by the Agency for metrafenone uses. However, the European Commission has issued a Dossier for Metrafenone[2] (DAR 2005).

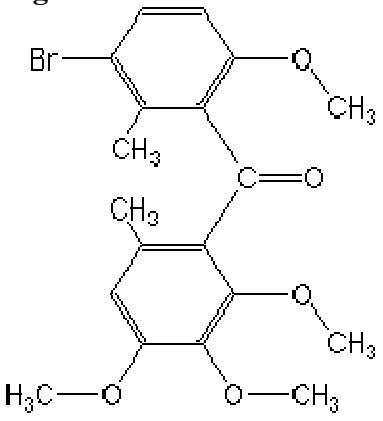
B. Stressor Source and Distribution

The stressor is the metrafenone and its unidentified, extractable degradation products when applied to grapes to control powdery mildew. Therefore, metrafenone could potentially be used anywhere in the United States where grapes are grown.

1. Nature of the Chemical Stressor

Figure 1 provides the chemical structure of metrafenone. **Table 4** identifies the physical and chemical properties of metrafenone from experimental data.

Figure 1. Chemical Structure of Metrafenone



[2] EC Directive 91/414.

Table 4. Environmental Fate Properties of Metrafenone			
PARAMETER	**VALUE(S) (units)**	**SOURCE**	**COMMENT**
Chemical Name	(3-Bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-methanone	–	–
Molecular Formula	$C_{19}H_{21}BrO_5$.		
Molecular Weight	409	–	–
Solubility (20 °C)	0.474 mg/L or ppm	MRID: 46415711	"Slightly soluble" according to FAO Classification
Vapor Pressure (20 °C)	1.15×10^{-6} mmHg	MRID: 46415713	–
Henry's Law constant	1.3×10^{-6} atm-m^3/mole		Estimated from vapor pressure and water solubility.
pKa (20 °C)	None	MRID: 46415714	–
Octanol-Water Partition Coefficient (Log K_{OW}, at 25 °C, pH 4)	4.3	MRID: 46415715	–
Hydrolysis Half-life (pH 5, 7, 9; (50 °C))	Stable	MRID: 47267422	Stable at all pHs.
Aqueous Photolysis Half-life (pH 5)	$t_{1/2}$ = 6.4 days	MRID: 47267423	Value corrected to represent natural sunlight at 40°N latitude; uncorrected lab half-life of 3.2 days (continuous irradiation; xenon lamp).
Soil Photolysis Half-life	$t_{1/2}$ = 31 days	MRID: 47267424	–
Aerobic Soil Metabolism Half-life	Silt loam: $t_{1/2}$ = 178-277 d Sandy loam: $t_{1/2}$ = 277, 365 d Clay loam: $t_{1/2}$ = 299, 330 d Loamy sand: $t_{1/2}$ = 182 d DT_{50} = 160 – 270 d	MRIDs: 47267425 47267426 47267427	8 systems, 4 conducted with foreign soils. In 2 studies metrafenone residues exceeded 50% at study termination (120 & 210 d)
Anaerobic Aquatic Metabolism Half-life	$t_{1/2}$ = 21.6 d, 18.0 d DT_{50} = 15 d, 3 d	MRIDs: 47267429 47267431	–
Aerobic Aquatic Metabolism Half-life	$t_{1/2}$ = 27.3 d, 24.1 d DT_{50} = 10 d, 11 d	MRID: 47267430	–
Organic Carbon Partition Coefficient (K_{OC})	1073, 2230, 2331, 22517, 2792 mL/g_{OC}	MRID: 47267420	"Slightly mobile" according to FAO Classification
Soil Partition Coefficient (K_d)	15.1, 36.5, 40.1, 42.0, 86.5 mL/g	MRID: 47267420	--
Terrestrial Field Dissipation	$t_{1/2}$ =144 d, 161 d, 210 d, 161 d	MRID 47267432	Field studies were conducted in WA, ON,CA, FL
Bioaccumulation in Fish	BCF: 140 to 530	MRID 47267450	Lipid Normalized BCF

a. Mode of Action (MoA) of Metrafenone

Metrafenone is a benzophenone fungicide. What is known is that the chemical inhibits growth of mycelium on the leaf surface, leaf penetration, formation of haustoria, and sporulation of the target fungus, *Uncinula necator* (EPA Pesticide Fact Sheet 2006). Metrafenone likely affects actin proteins which play a role in cell function and cell division (Opalski *et al.* 2006).

b. Reactions of Metrafenone in the Environment

Metrafenone is expected to be slightly mobile. Its major routes of degradation are aqueous photolysis and aquatic metabolism, in both aerobic and anaerobic conditions. Soil metabolism is slow, with laboratory half-lives of 6 months to a year and accumulation over two years observed in field studies. In aquatic environments, the measured photolysis half-life was 6.4 days and metabolism in both aerobic and anaerobic occurred with half-lives of one to four weeks.

Soil photolysis and anaerobic aquatic metabolism of metrafenone led to one major degradate, CL 377160, and anaerobic aquatic metabolism also led to six other major degradates, but all were either unidentified or only tentatively identified. In addition, there were many minor degradates, which were formed at low levels individually but reached substantial amounts as groups.

2. Overview of Pesticide Usage

As a new pesticide active ingredient, the actual usage of metrafenone is not known. The end-use product Metrafenone 300 SC is proposed for use on grapes.

Ground application is the proposed method of application. The label states, "do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark." The chemical is to be applied at most 6 times per season at a maximum application rate of 0.3 lbs a.i./A (**Table 5**). The label also states, "do not make more than 2 sequential applications of Metrafenone 300 SC fungicide before alternating to a labeled fungicide with a different mode of action."

Table 5. Metrafenone use and application information based on the proposed label for Metrafenone 300 SC

Use	Max. Single App. Rate (lbs a.i./A)	# of App. / Season	Seasonal Max. Rate (lbs a.i./A)	Minimum App. Interval (days)
Grapes	0.300	6	1.80	14 days

C. Receptors

1. Aquatic and Terrestrial Effects

The receptor is the biological entity that is exposed to the stressor (EPA, 1998). Based on the proposed uses for metrafenone, it is expected that the aquatic and terrestrial receptors will include freshwater fish and invertebrates, marine/estuarine fish and invertebrates,

aquatic plants, terrestrial plants, birds, mammals, and terrestrial invertebrates.

Consistent with the process described in the Overview Document (EPA, 2004), this risk assessment uses a surrogate species approach in its evaluation of metrafenone. Toxicological data generated from surrogate test species, which are intended to be representative of broad taxonomic groups, are used to extrapolate to potential effects on a variety of species (receptors) included under these taxonomic groupings.

Acute and chronic toxicity data from studies submitted by pesticide registrants are used to evaluate the potential direct effects of metrafenone to the aquatic and terrestrial receptors identified in this section. This includes toxicity data on the technical grade active ingredient, any major transformation products, and when available, formulated products (e.g. "Six-Pack" studies).

Table 6 provides a summary of the taxonomic groups and the surrogate species tested to help understand potential acute ecological effects of pesticides to these non-target taxonomic groups. In addition, the table provides a preliminary overview of the potential acute toxicity of metrafenone by providing the acute toxicity classifications.

Table 6. Test Species Evaluated for Assessing Potential Ecological Effects of Metrafenone and the Associated Acute Toxicity Classification

Taxonomic Group	Surrogate Species	Acute Toxicity Classification
Birds[1]	Mallard Duck (*Anas platyrhynchos*) Bobwhite Quail (*Colinus virginianus*) Passerine species	Practically nontoxic Practically nontoxic No available study
Mammals	Laboratory rat (Rattus norvegicus) Laboratory mouse	Practically non-toxic Practically non-toxic
Insects	Honey bee (*Apis mellifera L.*)	Practically non-toxic
Freshwater fish[2]	Bluegill sunfish (*Lepomis macrochirus*) Rainbow trout (*Oncorhynchus mykiss*)	Highly toxic Highly toxic
Freshwater invertebrates	Water flea (*Daphnia magna*)	At most, highly toxic
Marine/estuarine fish	Sheepshead minnow *(Cyprinodon variegatus)*	Highly toxic
Marine/estuarine invertebrates	Mysid shrimp *(Americamysis bahia)* Eastern oyster (*Crassostrea virginica)*	Highly toxic Highly toxic
Terrestrial plants[3]	Monocots – most sensitive species Dicots – most sensitive species	No Classification
Aquatic plants and algae	Duckweed (*Lemna gibba*) Cyanobacteria/blue-green algae (*Anabaena flos-aquae*) Marine diatom (*Skeletonema costatum*) Freshwater diatom (*Navicula pelliculosa*) Algae (*Pseudokirchneriella subcapitata;* previously known as *Selenastrum capricornutum)*	No classification

[1] Birds represent surrogates for terrestrial-phase amphibians and reptiles.

[2] Freshwater fish may be surrogates for aquatic-phase amphibians.

[3] Normally four species of two families of monocots, of which one is corn; six species of at least four dicot families, of which one is soybeans.

2. Ecosystems Potentially at Risk

The ecosystems at risk are often extensive in scope, and as a result it may not be possible to identify specific ecosystems during the development of a baseline risk assessment. However, in general terms, terrestrial ecosystems potentially at risk could include the treated field and areas immediately adjacent to the treated field that may receive drift or runoff. Areas adjacent to the treated field could include cultivated fields, fencerows and hedgerows, meadows, fallow fields or grasslands, woodlands, riparian habitats, and other uncultivated areas.

Aquatic ecosystems potentially at risk might include but are not necessarily limited to water bodies adjacent to, or down stream from, the treated field and might include impounded bodies such as ponds, lakes and reservoirs, or flowing waterways such as streams or rivers. For uses in coastal areas, aquatic habitat also includes marine ecosystems, including estuaries.

D. Assessment Endpoints

Assessment endpoints represent the actual environmental value that is to be protected, defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (EPA, 1998). Generally, the ecological entities may include the following: freshwater as well as marine/estuarine fish and invertebrates, aquatic and terrestrial plants, birds, reptiles, amphibians, mammals, and non-target insects. For metrafenone: chronic risk to mammals is expected. The attributes for each of these entities may include growth, reproduction, and survival.

E. Conceptual Model

A conceptual model provides a written description and visual representation of the predicted relationships between metrafenone residues, potential routes of exposure, and the predicted effects for the assessment endpoint. A conceptual model consists of two major components: risk hypothesis and a conceptual diagram (EPA, 1998).

1. Risk Hypothesis

For a pesticide to pose an ecological risk, it must reach ecological non-target organisms (receptors) at biologically significant concentrations. An exposure pathway is the means by which a pesticide moves in the environment from the application site to non-target organisms. The evaluation of the ecological exposure pathways in this assessment includes an examination of the source and potential transport pathways for metrafenone and the determination of exposure routes of non-target species.

Metrafenone, when used in accordance with the label, results in potential direct adverse effects upon the growth and reproduction of mammals. As a result, given the persistence of metrafenone, there is potential for indirect effects to terrestrial and/or aquatic

organisms.

2. Conceptual Diagram

The conceptual model diagram is a generic graphic depiction of the risk hypotheses identified in the previous section. It is assumed that metrafenone is capable of affecting exposed terrestrial and aquatic organisms if environmental concentrations are sufficiently elevated as a result of proposed label uses. Through a preliminary process of examining fate and effects data, the risk hypotheses and conceptual model have been refined to reflect possible exposure pathways and the organisms that are most relevant and applicable to this assessment (**Figure 2**). If exposed at sufficient levels, mortality may occur, as well as sublethal effects. Direct effects on a taxonomic group may result in indirect effects (i.e., loss of habitat, food resources) to other taxonomic groups. This assessment will examine the potential for these effects to occur within the surrogate taxa with the intent to extrapolate to actual effects within the environment.

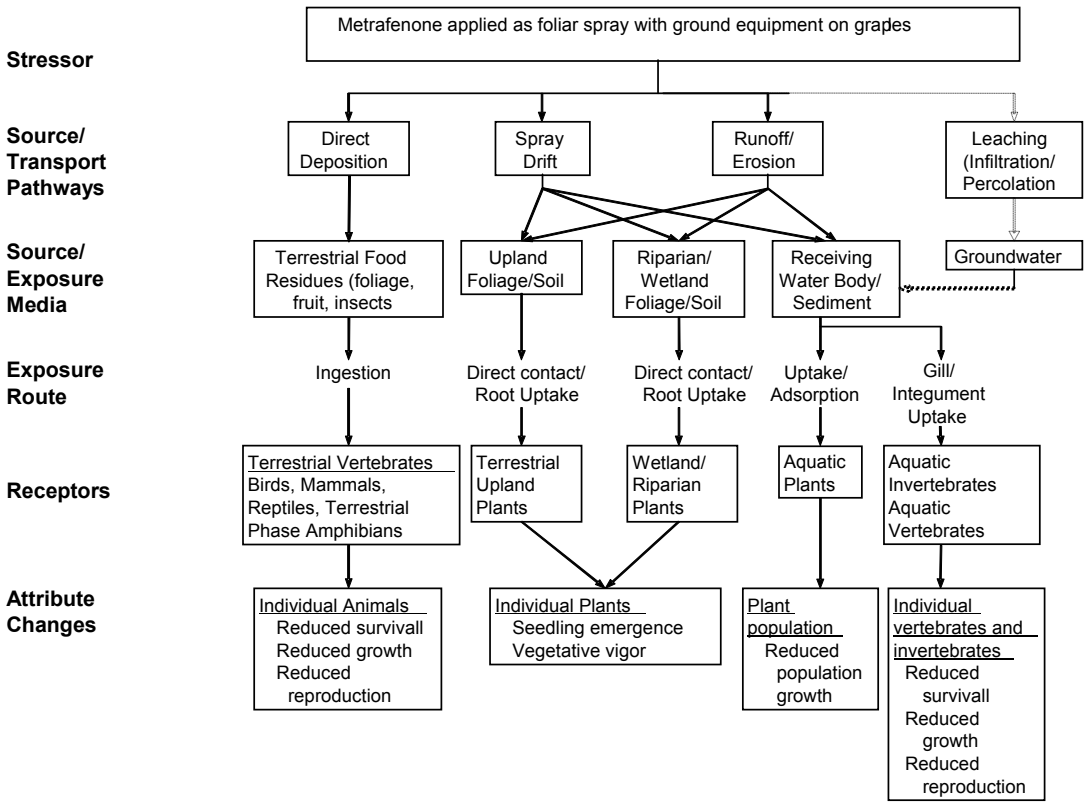
In order for a chemical to pose an ecological risk, it must reach ecological receptors in biologically significant concentrations. An exposure pathway is the means by which a contaminant moves in the environment from a source to an ecological receptor. For this pathway to be complete, it must have a source, a release mechanism, an environmental transport medium for metrafenone and/or its transformation products, a point of exposure for ecological receptors, and a feasible route of exposure. The assessment of these pathways thus includes an examination of the sources and potential migration pathways for constituents, and the determination of potential exposure routes.

The conceptual model for both potential aquatic and terrestrial risk is shown in **Figure 2**. Exposure routes shown in dashed lines are not quantitatively considered because the resulting exposures are expected to be very low when compared to the major routes of exposure.

Exposure is expected to be dominated by runoff and spray drift. Metrafenone exposure may also result from off-site movement in runoff water and on metrafenone -bearing soil particulates to adjacent fields (soil erosion). The moderate K_{oc}'s (K_{oc} 1073 to 22517 L/kg-oc) of metrafenone suggest that leaching to ground water/recharge to surface water would not be a route of exposure. Long-range transport of metrafenone in the gas phase is not considered a significant route of exposure.

This screening-level assessment for ground spray applications of metrafenone only considered dietary exposure. Other routes of exposure that were not considered in the assessment are incidental soil ingestion exposure, inhalation exposure, dermal exposure, and drinking water exposure. These routes are not represented in the diagram (**Figure 2**).

Figure 2. Conceptual Model Depicting Sources of Exposure from Metrafenone as well as Metrafenone Residues, Potential Receptors, and Adverse Effects from the Proposed Uses of Metrafenone on Grapes.



F. Analysis Plan

The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA, 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA, 2004).

1. Conclusions from Previous Risk Assessments

There are no previous ecological risk assessments because this is the first registration petition for metrafenone for use on grapes in the United States.

2. Preliminary Identification of Data Gaps

Review of the submitted studies indicated the following points:

Environmental Fate

All but one of the environmental fate studies required to support the registration of metrafenone for use on grapes were submitted. Independent Laboratory Validations for the methods submitted for analysis of metrafenone in soil and water remain a data gap. For a list of submitted environmental fate studies for metrafenone see **Appendix F**.

All of the environmental fate studies for the parent compound were determined to be scientifically valid and therefore results from all of the studies can be used to characterize the mobility and rates of transformation of metrafenone. However, many of the metabolism studies have major uncertainties in the identification and pattern of formation and decline of transformation products. In all of the aquatic metabolism studies, between 57% and 65% of the applied radioactivity remains unidentified with incomplete characterization, and in two aerobic soil metabolism studies, 15% and 44% of the applied radioactivity is unidentified. This includes at least four major degradates that individually reach levels of 11% to 35% of the applied. Other transformation products appear as groups of up to 15 components, in some cases characterized as each being <5% of the applied radioactivity, but in other cases, some individual components make up 9% to 10% of the applied. Even when individual components can all be classified as minor degradates, these groups represent such a large portion of the applied radioactivity overall that the possibility that they may have some impact as a group cannot be precluded despite their lower individual levels. This is especially true given that the degradation pathways suggest that groups of degradates may have a high degree of structural similarity and so may have similar fate and effects behavior. Without information to adequately characterize the degradates, it may be necessary to assume that they are of equal toxicity to the parents in order to quantify risks.

In addition to the studies submitted by the registrant, EFED was provided with a Draft Assessment Report (DAR) on Metrafenone written by the United Kingdom. That assessment reports that the long dissipation time observed in field studies triggered a requirement for longer term field accumulation studies. Two years into those five year studies, results show that metrafenone appears to be accumulating, and that metrafenone residues have been detected as deep as 20-30 cm below the surface. These studies would provide useful information regarding the environmental fate of metrafenone and would assist in reducing uncertainties.

Ecotoxicity

The submitted ecotoxicity database is incomplete. The greatest amount of uncertainty in the assessment stems from aquatic studies which were largely based on total (both dissolved and undissolved) concentrations of test compound; that is, the concentrations were measured without centrifugation even though precipitate was observed or test was conducted at the solubility limit of the metrafenone technical grade active ingredient; the solubility limits of the metabolites are unknown. To reduce uncertainty the majority of the toxicity studies, which are considered supplemental, and cannot be used in a quantitative risk estimation would need to be redone; especially those

where mortality or sublethal effects were observed. These aquatic studies are specified below. For a list of submitted ecological effects studies for metrafenone see **Appendix G**.

The following studies are considered data needs:

Aquatic studies

Given the freshwater fish studies on metrafenone technical grade active ingredient and assuming that the concentrations in the environment reach the solubility limit, the effect of the compound is likely to be low. However, according to model estimated EECs (which include metrafenone and metrafenone residue scenarios: 0.00153 - 0.02 mg/L), levels of metrafenone (TGAI, TEP)[3] at the solubility limit (0.2-0.5 mg/L at 12°C) and metrafenone metabolites at the tested concentrations are not expected to occur in the environment given the proposed grape use. Therefore, acute risk to freshwater fish and aquatic-phase amphibians is not expected as a result of metrafenone use on grapes. In addition, although the EU/UK formulation – with which the submitted studies were conducted – closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on freshwater fish and aquatic-phase amphibians is not known.

To reduce uncertainty in characterizing risk, which is currently based on total concentrations, the freshwater fish studies on bluegill sunfish (*Lepomis macrochirus*) are requested to make same species comparisons for acute and chronic values. Bluegill was chosen over the rainbow trout due to greater sensitivity (on an acute basis) of the bluegill to the TGAI relative to the trout. Since the acute marine/estuarine fish study is acceptable and given the above two studies, the acute to chronic ratio can be utilized to estimate marine/estuarine chronic endpoints, negating the need to fill this data gap. Special emphasis must be placed on centrifuging the test samples prior to analytical determination of the test compound.

- Acute: Freshwater fish toxicity (96-hour LC_{50} for *Lepomis macrochirus*) (850.1075; 72-1), TGAI
- Chronic: Fish early-life stage (freshwater: *Lepomis macrochirus*) (850.1400; 72-4), TGAI

Although acute freshwater invertebrate studies were based on total concentrations, no effects were observed with the TGAI, negating the need to request additional data. However, a chronic freshwater invertebrate data gap still exists since the two reviewed studies were deemed invalid.

- Chronic: Aquatic invertebrate life cycle (freshwater: *Daphnia magna*) (850.1300; 72-4), TGAI

[3] TGAI: technical grade active ingredient; TEP: typical end-use product (formulation)

Effects were observed in both acute marine/estuarine invertebrate studies with the TGAI on the eastern oyster (*Crassostrea viginica*) and saltwater mysid (*Americamysis bahia*) but the endpoints are based on total concentrations. Therefore, should concentrations in the environment reach the solubility limit, the acute risk to marine/estuarine invertebrates may be expected. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Given these studies and assuming that metrafenone concentrations in the environment are not likely to reach the solubility limit, the acute risk to marine/estuarine invertebrates is not expected. However, to reduce uncertainty in characterizing risk, either study is requested to be redone with special emphasis on centrifuging the test samples prior to analytical determination of the test compound.

- Acute: Marine/estuarine invertebrate either *Crassostrea virginica* (850.1025; 72-3) **or** *Americamysis bahia* (850.1035; 72-3), TGAI

A non-guideline chronic midge study (*Chironomus riparius*) with metrafenone technical (97.1%) was deemed invalid on the basis of low negative control emergence. Therefore, a chronic freshwater invertebrate whole sediment study is requested. A protocol should be submitted for approval prior to study initiation. The protocol should include spiked sediment. The whole sediment study is requested because metrafenone has a high $\log K_{ow}$ value (4.3 at 25^{o}C, pH 4) and a high aerobic soil half life (> 4 months), which indicates that the compound may partition to sediment and persist.

- *Chironomus dilutus* (freshwater) using TGAI. Consult EPA Test Method 100.5 Life-cycle Test for Measuring the Effects of Sediment Associated Contaminants on *Chironomus dilutus* (formerly *Chironomus tentans*) and OECD Guideline 218 Sediment-Water Chironomid Toxicity Test Using Spiked Sediment

Non-target aquatic plant studies were also based on total concentrations leading to uncertainty in exposure concentrations and thus endpoint values. Had valid endpoints (*i.e.*, those based on dissolved concentrations) been determined using these studies, they would likely be greater than the highest model predicted concentration in the environment, which implies that risk to vascular and non-vascular species is not expected as a result of metrafenone use on grapes. However, without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot accurately be determined. Therefore, vascular and non-vascular aquatic plant studies are requested with special emphasis placed on centrifuging the test samples prior to analytical determination of the test compound. In addition, cyanobacteria yielded no effects given total concentrations and does not need to be redone.

- Aquatic vascular plant growth (*Lemna* spp.), Tier II (850.4400), TGAI
- Aquatic non-vascular plant growth, Tier II (850.5400), TGAI

Terrestrial Studies

An avian acute oral toxicity test in passerine species is required. Passerines are the most common birds (in terms of numbers and number of species) in the United States. Many utilize agricultural fields, forests, residential areas and surrounding areas, and, therefore, have the potential to be exposed to pesticides used in agricultural, forest, and residential settings. It is likely that, for the requested use patterns, passerines are more likely to be exposed to metrafenone than upland game species and waterfowl. Passerines are smaller and have a higher energy requirement than larger-sized birds. As such, passerines may be more sensitive than other birds.

- Passerine bird toxicity study (EPA approved protocol is required prior to study initiation): Avian acute oral toxicity test (850.2100, 71-1), TGAI

Formulated Product Testing

The available formulation product studies were conducted using three different UK/EU formulated products (*i.e.*, BAS 560 00F, BAS 560 01F, and BAS 560 02F), one of which (BAS 560 00F) closely matches the U.S. formulation (*i.e.*, BAS 560 03F). Formulation studies in this assessment are based on BAS 560 00F because data on the U.S. formulation were not submitted. In order to eliminate uncertainty in effects characterization of formulated products used within the U.S. on given taxa, future registrant submitted studies should be based on the U.S. formulation. At this time the effect of the U.S. formulation on given taxa is not known, however, greater toxicity of the EU/UK formulation on the honeybee relative to the TGAI was observed. Similarly, data submitted for terrestrial plants is based on the EU/UK formulation. As a result, the following studies are requested:

- Honeybee acute contact toxicity (850.3020, 141-1), TEP
- Seedling emergence, Tier II (850.4225, 123-1), TEP
- Vegetative vigor, Tier II (850.4250, 123-1), TEP

3. Measures of Exposure and Effects

EFED uses a tiered system of pesticide exposure modeling to assess ecological risk following a registered application of that pesticide. This tiered system is designed to minimize the amount of analysis which is required to register any given chemical. Each of the tiers is designed to screen out pesticides by requiring higher, more complex levels of investigation only for those that have not passed the next lower tier. Each tier screens out a percentage of pesticides from having to undergo a more rigorous review prior to registration or re-evaluation.

a. Aquatic Exposure Models

Tier II PRZM and EXAMS[4] simulation models were used to estimate the exposure concentrations of metrafenone in surface water for the proposed use on grapes. The results are presented in **Appendix B.**

The data used as input parameters come solely from the environmental fate studies and proposed product label submitted by the petitioner to support in the United States the registration of metrafenone as a new pesticide-active ingredient for grapes.

KABAM ($\underline{K}_{OW}$ (based) $\underline{A}$quatic $\underline{B}$io$\underline{A}$ccumulation $\underline{M}$odel) v.1.0 is used to estimate potential bioaccumulation of hydrophobic organic pesticides such as metrafenone (log $K_{ow} > 4$) in freshwater aquatic food webs and subsequent risks to mammals and birds via consumption of contaminated aquatic prey. The bioaccumulation portion of KABAM is based upon work by Arnot and Gobas (2004) who parameterized a bioaccumulation model based on PCBs and some pesticides (e.g., lindane, DDT) in freshwater aquatic ecosystems. KABAM relies on a chemical's octanol-water partition coefficient (KOW) to estimate uptake and elimination constants through respiration and diet of organisms in different trophic levels. Pesticide tissue residues are calculated for different levels of an aquatic food web. The model then uses pesticide tissue concentrations in aquatic animals to estimate dose- and dietary-based exposures and associated risks to mammals and birds consuming aquatic organisms, using an approach that is similar to the T-REX model (USEPA 2008).

KABAM incorporates 7 trophic levels to describe bioaccumulation of a pesticide in a model aquatic food web: phytoplankton, zooplankton (e.g., *Daphnia* sp.), benthic invertebrates (e.g., *Chironomus* sp., crayfish), filter feeders (e.g., mussels, clams), small fish (e.g., young of the year), medium sized fish (e.g., adult bluegill), and larger upper-trophic level fish (e.g., largemouth bass).

Metrafenone concentrations in organisms of the aquatic trophic levels listed above were used to estimate acute and chronic exposures of piscivorous mammals and birds consuming fish. Applicable and available acute and chronic toxicity data on metrafenone (mammals and birds) were used to calculate risk quotients for estimated exposures due to bioaccumulation of metrafenone in an aquatic ecosystem.

b. Terrestrial Exposure Models

T-REX Model

The focus of terrestrial wildlife exposure estimates is for birds (also acting as surrogate for reptiles and terrestrial-phase amphibians) and mammals with an exposure route emphasis on uptake through the diet. The residues in or on potential dietary sources for mammals and birds (e.g., vegetation, insects, and seeds) were estimated using the Tier I

[4] PRZM 3.1.2.2 (5/16/05) and EXAMS 2.98.04(4/25/04) were used rather than GENEEC2 (4/25/04) in anticipation of toxicity concerns for aquatic organisms.

model T-REX (Version 1.4.1, 2008). In this Tier I assessment, it was assumed that organisms are exposed to one active ingredient in a given exposure scenario. In all screening-level assessments, the organisms are assumed to consume 100% of their diet as one food type and one food source. The T-REX output is presented in the Risk Characterization section of this document as well as an example in **Appendix C**.

The approach used to estimate exposure of terrestrial animals to metrafenone was based on potential foliar applications of metrafenone. Upper-bound exposure levels were calculated for spray applications of metrafenone using maximum proposed application rates for one application for the proposed uses. The exposure estimates are based on a database of pesticide residues on wildlife food sources associated with specified application rates (Kenaga, 1972; Fletcher et al., 1994). Essentially, for a single application, there is a linear relationship between the amount of pesticide applied and the amount of pesticide residue present on a given food item. Food item residue levels are then linearly adjusted based on application rate. The upper-bound estimates are used to estimate risks since these values represent the high-end exposure that may be encountered for terrestrial species that consume food items that have received label-specified pesticide application. Although these represent higher-end estimates, they do not represent the highest possible exposure estimates.

T-REX is a simulation model that, in addition to incorporating the relationship between application rate and food item residue concentrations, accounts for pesticide degradation in the estimation of terrestrial EECs. T-REX calculates pesticide residues on each type of food item on a daily interval for one year. A first-order decay function is used to calculate the residue concentration at each day based on the concentrations present from both initial and all subsequent applications. The decay rate is dependent on the foliar dissipation half-life. The food item concentration on any given day is the sum of all residues up to that day, taking into account the first-order degradation. The initial application occurs on day 0 (t=0) and the model runs for 365 days. Over the 365-day run, the highest residue concentration is the measure of exposure (EEC) used to calculate risk quotients (RQs).

The foliar dissipation half-life and residue decline studies can be important in estimating exposure because they essentially determine how long the pesticide remains in or on food items after application. In many cases, neither empirically determined foliar dissipation nor residue decline half-life (with a day 0 residue) values are available, in which case the default value of 35 days is used (Willis and McDowell, 1987). That was the case for this assessment.

TERRPLANT Model

The TerrPlant (Ver.1.2.2) model is used to predict EECs from terrestrial uses for terrestrial plants located in dry and semi-aquatic areas adjacent to the treated field or treated water body. TerrPlant assumes 100% efficiency in ground and aerial applications. A semi-aquatic area (wetland) is defined as a low-lying area of terrestrial habitat that is wet but may dry up at times throughout the year. TerrPlant incorporates two similar

conceptual models for depicting dry and semi-aquatic areas of terrestrial habitats. For both models, a non-target area is adjacent to the target area. Pesticide exposures to plants in the non-target area are estimated to receive runoff and spray drift from the target area.

For a dry area adjacent to the treatment area, runoff exposure is estimated as sheet runoff. Sheet runoff is the amount of pesticide in water that runs off of the soil surface of a target area of land which is equal in size to the non-target area (1:1 ratio of areas). In the sheet runoff scenario, the treated area generating runoff is assumed to drain into an area with equal size containing seedlings, resulting in 1, 2, or 5% of the application rate being deposited. For semi-aquatic areas, runoff exposure is estimated as channel runoff. Channel runoff is the amount of pesticide that runs off of a target area 10 times the size of the non-target area (10:1 ratio of areas). In the channel runoff scenario, a ten-to-one ratio of watershed area to receiving area results in 10, 20, or 50% of the application rate being deposited on soil with emerging or emerged seedlings. The magnitude of runoff is assumed to be dependent on the water solubility of the pesticide active ingredient. For pesticides with a solubility of <10, 10 to 100, or >100 ppm, runoff fractions of 0.01, 0.02 or 0.05 respectively are selected by the model user.

Exposures through runoff and spray drift are then compared to measures of survival and growth (e.g. effects to seedling emergence and vegetative vigor) to develop RQ values. The model compares the combined deposition estimates from runoff and spray drift to adverse effect levels measured in seedling emergence studies. In addition, RQs are derived for plants with consideration for spray drift exposures. For monocots and for dicots, TerrPlant compares estimated spray drift deposition, without a runoff exposure component, to the more sensitive measure of effect, either seedling emergence or vegetative vigor (USEPA 2005).

Table 7 summarizes the measures of ecological effects and exposure used to assess ecological risk following exposure to metrafenone with the proposed uses.

Table 7. Measures of Ecological Effects and Exposure for Metrafenone

Assessment Endpoint		Surrogate Species and Measures of Ecological Effect[1,2]	Measures of Exposure
Birds[3]	Survival	Bobwhite Quail acute oral LD_{50}, subacute dietary LC_{50} Mallard Duck acute oral LD_{50}, subacute dietary LC_{50}	Upper bound residues on food items (foliar)
	Reproduction and growth	Bobwhite Quail reproduction NOAEC/LOAEC	
Mammals	Survival	Laboratory rat acute oral LD_{50} Laboratory mouse acute oral LD_{50}	
	Reproduction and growth	Laboratory rat reproduction NOAEL/LOAEL	

Table 7. Measures of Ecological Effects and Exposure for Metrafenone			
Assessment Endpoint		**Surrogate Species and Measures of Ecological Effect[1,2]**	**Measures of Exposure**
Freshwater fish[4]	Survival	Rainbow trout 96-hr LC_{50} Bluegill sunfish 96-hr LC_{50}	Peak EEC[5]
	Reproduction and growth	Fathead minnow NOAEC/LOAEC	60-day average EEC[5]
Freshwater invertebrates	Survival	Water flea 48-hr EC_{50}	Peak EEC[5]
	Reproduction and growth	No acceptable study available	21-day average EEC[5]
Marine/estuarine fish	Survival	Sheepshead minnow 96-hr LC_{50}	Peak EEC[5]
	Reproduction and growth	No study available	60-day average EEC[5]
Marine/estuarine invertebrates	Survival	Eastern oyster 96-hr EC_{50} Saltwater mysid 96-hr LC_{50}	Peak EEC[5]
	Reproduction and growth	Saltwater mysid NOAEC/LOAEC	21-day average EEC[5]
Terrestrial plants[6]	Survival and growth	Monocot Seedling emergence EC_{25}, NOAEC or EC_{05} Monocot Vegetative Vigor EC_{25}, NOAEC or EC_{05} Dicot Seedling emergence EC_{25}, NOAEC or EC_{05} Dicot Vegetative Vigor EC_{25}, NOAEC or EC_{05}	Estimates of runoff and spray drift to non-target areas
Insects	Survival	Honey bee acute contact 48-hr LD_{50}	Maximum application rate
Aquatic plants and algae	Survival and growth	Duckweed 7-day EC_{50}, NOAEC Cyanobacteria 96-hr EC_{50}, NOAEC Marine diatom 96-hr EC_{50}, NOAEC Freshwater diatom 96-hr EC_{50}, NOAEC Green algae 96-hr EC_{50}, NOAEC Algae 72-hr EC_{50}, NOAEC Green algae 72-hr EC_{50}, NOAEC	Peak EEC[5]

[1] LD_{50} = Lethal dose to 50% of the test population; NOAEC = No observed adverse effect concentration; LOAEC = Lowest observed adverse effect concentration; LC_{50} = Lethal concentration to 50% of the test population; EC_{50}/EC_{25} = Effect concentration to 50%/25% of the test population.

[2] If species listed in this table represent most commonly encountered species from registrant-submitted studies, risk assessment guidance indicates most sensitive species tested within taxonomic group are to be used for baseline risk assessments.

[3] Birds represent surrogates for amphibians (terrestrial phase) and reptiles.

[4] Freshwater fish may be surrogates for amphibians (aquatic phase).

[5] One in 10-year return frequency.

[6] Four species of two families of monocots - one is corn, six species of at least four dicot families, of which one is soybeans.

III. Analysis

A. Use Characterization

The proposed end-use product is "Metrafenone 300 SC" (EPA Reg. No. 7969-XXX), a suspension concentrate containing 25.2% (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)methanone (metrafenone) at a concentration of 2.5 lbs metrafenone per gallon. The product is claimed to provide "optimum disease control when applied in a regularly scheduled protective fungicide program and when used in a spray program that rotates fungicides with different modes of action." The product is intended to control powdery mildew of grapes.

Ground application is the proposed method of application. The label states, "do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark." The chemical is to be applied at most 6 times per season at a maximum application rate of 0.3 lbs a.i./A (Table 5). The label also states, "do not make more than 2 sequential applications of Metrafenone 300 SC fungicide before alternating to a labeled fungicide with a different mode of action."

B. Exposure Characterization

1. Environmental Fate and Transport

Summary

Metrafenone is a new chemical of the benzophenone class that is expected to be slightly mobile. Its major routes of degradation are aqueous photolysis and aquatic metabolism, in both aerobic and anaerobic conditions. Soil metabolism is slow, with laboratory half-lives of 6 months to a year and accumulation over two years observed in field studies. In aquatic environments, the measured photolysis half-life was 6.4 days and metabolism in both aerobic and anaerobic occurred with half-lives of one to four weeks.

Soil photolysis and anaerobic aquatic metabolism of metrafenone led to one major degradate, CL 377160 [Methanone, (3-bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)-]., and anaerobic aquatic metabolism also led to six other major degradates, but all were either unidentified or only tentatively identified. In addition, there were many minor degradates, which were formed at low levels individually but reached substantial amounts as groups.

Tier II aquatic exposure modeling was conducted for parent metrafenone and metrafenone residues (metrafenone+ extractable residues) (Table 11). This modeling approach was used to address uncertainties associated with identification of metrafenone residues in soil and sediment. The highest aquatic EECs for metrafenone were 11.70 µg/L for the daily peak, 8.82 µg/L for the 21 day average, and 6.21 µg/L for the 60 day average. The highest aquatic EECs for metrafenone were 20.22 µg/L for the daily peak, 17.67 µg/L for the 21 day average, and 16.98 µg/L for the 60 day average.

No monitoring data are available to compare with model estimates. Bioaccumulation modeling was conducted because metrafenone has a log octanol:water coefficient > 4. Maximum residue concentrations in fish are expected to range from 17,636 to 19,586 µg/kg-ww.

Persistence

Metrafenone biodegrades slowly in terrestrial environments. In laboratory studies in a variety of soils, aerobic soil metabolism linear half-lives ranged from 178 to 365 days. In four of the eight soils tested, data collection extended long enough to determine an empirical DT_{50}, of 124 to 188 days in two silt loam soils and 272 to 362 days in a clay loam and sandy loam soil. In these two soils, metabolism appeared to slow after 272 days. No data are available for anaerobic soil metabolism rates. Photolysis on soil was observed in laboratory studies to occur with a linear half-life of 31 days.

In aquatic environments, metrafenone biodegrades in weeks to months in both aerobic and anaerobic conditions (linear half-lives of 24-27 days and 18-22 days, respectively). Considering abiotic degradation, metrafenone is photolyzed in water (half-life of 6.4 days), but is stable to hydrolysis at pH values from 4 to 9.

Degradation Products

The only positively identified major degradate of metrafenone was CL 377160 [Methanone, (3-bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)-]. CL 377160 was formed through soil photolysis at up to 18.9% AR and through anaerobic aquatic metabolism at up to 10.8% AR in the total system. It was also formed as a minor degradate through aquatic photolysis and aerobic aquatic metabolism. A compilation of identified degradation products are shown in **Appendix A**.

In one anaerobic aquatic metabolism study, another six degradates were formed at >9%, but these were either unidentified or only tentatively identified. These included TN (tentatively identified as a lactone compound; maximum 3.3%, 26.9% and 28.0% in the water layer, sediment and total system, respectively), MB (tentatively identified as a hydroxylation product of TN; maximum 1.1%, 11.6% and 11.7% in the water layer, sediment and total system, respectively), and four unidentified degradates formed in the total system at 9.3% to 13% AR.

Carbon dioxide was also a major degradate, formed through aquatic photolysis at up to 25% AR and through all other measured processes as a minor degradate.

Most processes also resulted in formation of a substantial amount of minor degradates, some individually measured and identified, but most not. Although all of these degradates were present at less than 10%, and some at very low levels, they still may be of exposure concern because of the large overall amounts. In some cases minor degradates, mostly unidentified, make up more than 50% of the applied radioactivity in a single sampling event. In addition, based on the structure of the parent compound and

the proposed degradation pathways, many of the minor degradates may have a high degree of structural similarity to each other and/or the parent and so may have cumulative effects.

Mobility

Batch equilibrium data on metrafenone suggest that the compound will sorb to organic surfaces and would be considered "slightly mobile" according to the FAO classification scheme (organic carbon partition coefficients range from 1,073 to 2,792 ml/g-oc). Also according to FAO classification, metrafenone's solubility in water of 0.447 mg/L is considered "slightly soluble." It showed low volatility from soil and water with <0.5% volatilization in any laboratory study, consistent with the vapor pressure of 1.15×10^{-6} mmHg. Therefore, dissipation in the environment is expected to occur predominantly via runoff of suspended soil and sediments to which metrafenone is adsorbed. Based on terrestrial field dissipation studies in the UK , metrafenone was detected at a soil depth of 20-30 cm after 2 years.

In aquatic systems, partitioning of metrafenone to sediment occurred rapidly. In one anaerobic system, half of the applied metrafenone was detected in sediment on the first day, and in three other systems (one anaerobic, two aerobic), at least 50% of the parent compound had partitioned to sediment within three to seven days. Including degradates, partitioning was rapid in anaerobic systems but slower in aerobic systems. In two anaerobic aquatic metabolism studies, more than half of the applied radioactivity (AR) was found in sediment within one week and radioactivity in the water layer was down to <5% AR in two to six weeks. However, in aerobic aquatic metabolism studies in two systems, from 8% to 28% AR remained in the water layer at study termination (100 days).

Field Dissipation Studies

Field dissipation studies were conducted on bare plots in California (CA), Washington (WA), Ontario (ON), and Florida (FL). The first-order dissipation half-life for metrafenone in surface soils (0-7.5 cm) was 144 days (DT 50 14-30 days; DT 90 272-360 days) in CA, 161.2 days (DT 50 181-269 days; DT 90 540-900 days) in WA, 210 days (DT 50 59-272 days; DT 90 598-710 days) in ON, and 161 days (DT 50 14-30 days; DT 90 360-451 days) in FL. No degradation products were detected above the limit of quantification (LOQ) in the field dissipation studies.

The reference degradation product in the field study was CL377160. Degradation was not a clear route of metrafenone dissipation in field studies because of the lack of detectable degradation products in soil. Leaching is possible route of dissipation. There were sporadic detections of metrafenone with soil depth in the Florida study with maximum detection depth of 15-30 cm, Ontario study with maximum detection depth of 60-75 cm, Washington study with a maximum depth depth of 7.5-15 cm, and California study with

a maximum detection depth of 15-30 cm. The field studies were not designed to assess runoff and volatilization of metrafenone residues.

Bioaccumulation in Fish

Bluegill sunfish exposed to metrafenone, at 5 and 50 µg/L, accumulated metrafenone residues during a 28 day accumulation period. After the accumulation phase, the fish were transferred to metrafenone-free water for a 14 day depuration phase. For the bromophenyl labeled metrafenone, lipid normalized bioconcentration factors (BCFs) ranged 140 to 460 in whole fish. For trimethoxybenzene labeled metrafenone, lipid normalized bioconcentration factors (BCFs) ranged 490 to 530 in whole fish. The lipid normalized BCF range indicates that metrafenone is not expected to accumulate in tissues of aquatic organisms. Major bioaccumulated residues (>10% of TRR) were identified as [(3-Bromo-6-methoxy-2-methylphenyl)(4-hydroxy-2,3-dimethoxy-6-ethylphenyl)methanone (CL 434223), CL1500699 (3- bromo-6-methoxy-2-methylphenyl)[4-(beta-D-gluco pyran uronosyloxy)-2,3-dimethoxy-6-methyphenyl]-methanone), and CL377160. These degradation products were detected in whole fish, viscera, fillet, and in the exposure water. The bioaccumulation rate at 90% steady-state of total radioactive residue and metrafenone ranged from 1.3 to 1.8 days and 1.8 to 2.1 days, respectively. The 95% depuration rate of total radioactive residue and metrafenone ranged from 1.8 to 2.3 days and 1.8 to 2.1 days, respectively.

2. Measures of Aquatic Exposure

a. Aquatic Exposure Modeling

The estimated environmental concentrations (EECs) reported in the assessment were calculated using the Tier II model for surface water (PRZM/EXAMS). Sample inputs and outputs of the model are presented in **Appendix B.**

PRZM (v3.12.2, May 2005) and EXAMS (v2.98.4.6, April 2005) are screening simulation models coupled with the input shell pe5.pl (Aug 2007) to generate daily exposures and 1-in-10 year EECs of metrafenone and total metrafenone residues that may occur in surface water bodies adjacent to application sites receiving metrafenone residues through runoff and spray drift for specific scenarios. PRZM simulates pesticide application, movement and transformation on an agricultural field and the resultant pesticide loadings to a receiving water body via runoff, erosion and spray drift. EXAMS simulates the fate of the pesticide and resulting concentrations in the water body. The standard scenario used for ecological pesticide assessments assumes application to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2-meters deep (20,000 m^3 volume) with no outlet. PRZM/EXAMS was used to estimate screening-level exposure of aquatic organisms to metrafenone residues. The measure of exposure for aquatic species is the 1-in-10 year return peak or rolling mean concentration. The 1-in-10 year peak is used for estimating acute exposures of direct effects to aquatic organisms as well as indirect effects. The 1-in-10-year 60-day mean concentration is used for assessing chronic exposure. The 1-in-10 year 21-day mean concentration is used for assessing

chronic exposures to aquatic invertebrates.

Input Parameters
The appropriate input parameters were selected from the physical/chemical properties and environmental fate data submitted by the petitioner to support registration of metrafenone. Input parameters were selected in accordance with US EPA-OPP EFED water model parameter selection guidelines, Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version 2.1, November 10, 2009. Expanded information about the models, selection of input parameters and scenarios can be obtained from http://www.epa.gov /oppefed1/models/water/index.htm.

The use input parameters were selected from label for the end-use product "Metrafenone 300 SC." The physical-chemical properties and environmental fate input parameters were obtained from studies submitted and reviewed by the Agency.

Total Residues Modeling Input Parameters
Calculation of total residue half-lives for metrafenone are based on the concentration (expressed as percent of applied radioactivity) of extractable residues (**Table 8**). Extractable residues include metrafenone, CL 377160, tentatively identified compounds, and unidentified compounds (**Appendix A**).

Table 8. Total Extractable Metrafenone Residue Half-lives from Laboratory Degradation Studies

Study Type	MRID[1]	Half-life (days)	Environmental Matrix	Extractable Residues
Aqueous Photolysis	-23	27		Total Mass Balance - VOC – CO_2
Soil Photolysis	-24	47.8		Acetonitrile, acetonitrile:0.2NHCl, 0.5 N NaOH
Aerobic Soil	-27	169.1	sandy loam soil	Acetonitrile, acetonitrile:acetone, acetonitrile:water
Aerobic Soil	-26	210.0	clay loam soil	Acetone, methanol:water
Aerobic Soil	-25	277.2	silty loam soil	Acetone, methanol :water, acetonitrile:0.5NHCl
Aerobic Soil	-27	277.3	clay loam soil	Acetonitrile, acetonitrile:acetone, acetonitrile:water
Aerobic Soil	-27	301.4	silt loam soil	Acetonitrile, acetonitrile:acetone, acetonitrile:water
Aerobic Soil	-26	315.1	sandy loam soil	Acetone, methanol:water
Aerobic Soil	-27	330.1	silt loam soil	Acetonitrile, acetonitrile:acetone, acetonitrile:water
Aerobic Soil	-26	364.8	loamy sand soil	Acetone, methanol:water
Anaerobic Aquatic	-29	182.0	DI water- silty clay loam	Water + Acetone, methanol acetic acid, acetonitrile, 0.5N NaOH:acetonitrile, ammonium hydroxide:acetonitrile
Anaerobic Aquatic	-31	577.6	pond-sandy loam	Water + acetone:acetonitrile, acetonitrile:formic acid, acetonitrile;water formic acid, acetonitrile:water:triethylamine, acetonitrile:0.05 M sodium phosphate dibasic, 0.5N ammonium hydroxide
Aerobic Aquatic	-30	91.2	river-loam	Water + acetone, methanol:acetic acid, methylene chloride, acetonitrile:0.5N NaOH
Aerobic Aquatic	-30	123.8	pond-sand	Total Mass Balance - VOC – CO_2

1- Prefix MRID number is 472674-

Surface water
Scenarios used to run PRZM and EXAMS (Tier II) for the proposed use are shown in

Table 9. Input parameters for modeling in Tier II are shown in **Table 10**.

Table 9. Scenarios used to estimate Metrafenone concentrations in surface water.			
Agricultural Commodity	**Crop Scenario**	**Met File**	**Scenario Characterization**
Grapes	NY CA	W14860.dvf W93193.dvf	Standard Scenarios for assessing pesticides use on grapes

Table 10. PRZM/EXAMS Input Parameters for Metrafenone and Total Extractable Metrafenone Residues

PARAMETER	Metrafenone	Total Extractable Metrafenone Residues	COMMENT	SOURCE
Application Rate per Event *Lb a.i./A (kg a.i./ha)*	0.3 lb a.i./A	0.3 lb a.i./A		Metrafenone 300 SC Label
No. of Applications (Interval)	6 (14 day)	6 (14 day)		Metrafenone 300 SC Label
CAM (Chemical App. Method)	2	2	Broadcast spray	Metrafenone 300 SC Label
Depth of Incorporation	0	0	Default	
Spray Drift Fraction / Application Efficiency	0.01 / 0.99	0.01 / 0.99	Assume ground spray.	EFED Input Guidance
Aerobic Soil Metabolism $t_{1/½}$	303 days	313 days	Upper 90th percentile of the mean half-life (n=8)	MRID 47267425 MRID 47267426 MRID 47267427
Aerobic Aquatic Degradation $t_{1/½}$	28.2 days	158 days	Upper 90th percentile of the mean half-life (n=2)	MRID 47267430
Anaerobic Aquatic Degradation $t_{1/½}$	25.3 days	989 days	Upper 90th percentile of the mean half-life (n=2)	MRID 47267429 MRID 47267431
Aqueous Photolysis $t_{1/½}$	6.4 days	27.0 days	Corrected for dark	MRID 47267423
Hydrolysis $t_{1/½}$	0 days	0 days	Stable	MRID 47267422
Soil Partition Coefficient (K_{oc})	2188 mL/g_{oc}	2188 mL/g_{oc}	Average Koc	MRID 47267420
Molecular Weight	409 g/mole	409 g/mole		MRID 47267423
Water Solubility @ 25°C	0.474 mg/L	0.474 mg/L		MRID 47267423
Vapor Pressure	1.15 x 10^{-6} mmHg	1.15 x 10^{-6} mmHg		EPA Fact Sheet (9/06)

Tier II- PRZM/EXAMS

Tier II estimated environmental concentrations for metrafenone and total metrafenone residues are shown in **Table 11**.

Table 11. Estimated Exposure Concentrations for Metrafenone and Total Metrafenone Residues from Surface Water. Concentrations are in µg/L (ppb)

Agricultural Commodity/Scenario	1-in-10 yr Peak	1-in-10 yr 21-Day Average	1-in-10 yr 60-Day Average
Metrafenone			
NY Grapes	11.70	8.82	6.21
CA Grapes	1.53	1.10	0.75
Total Metrafenone Residues*			
NY Grapes	20.22	17.67	16.98
CA Grapes	2.25	1.89	1.58
*Parent metrafenone + identified and unidentified extractable residues			

b. Aquatic Exposure Monitoring and Field Data

Monitoring data for metrafenone in surface water and ground water are not available in the United States or Europe.

c. Aquatic Bioaccumulation Assessment

Available data on the octanol-water partition coefficient (K_{ow}) for metrafenone indicates that this pesticide may accumulate in aquatic food webs. Because the Log K_{ow} is > 4.0, KABAM v.1.0 was used to estimate concentrations of metrafenone in tissues of aquatic organisms resulting from bioaccumulation. Input parameters are provided in **Table 12** and estimated concentrations of metrafenone in fish are provided in **Table 13**. Sample KABAM output is provided in **Appendix D**.

Table 12. Input Parameters and Chemical Characteristics of Metrafenone Used in KABAM		
Characteristic	**Value**	**Comments**
Log K_{OW}	4.3	
K_{OW}	19953	
K_{OC} (L/kg OC)	2188	Input value used in PRZM/EXAMS to derive EECs.
Time to steady state (T_S; days)	8	This value is calculated automatically from the Log K_{OW} value entered above.
Pore water EEC (µg/L)	16.8	Value generated by PRZM/EXAMS benthic file for New York Grapes scenario for **metrafenone total residue and high runoff scenario**. PRZM/EXAMS EEC represents the freely dissolved concentration of the pesticide in the pore water of the sediment. The appropriate averaging period of the EEC is dependent on the specific pesticide being modeled and is based on the time it takes for the chemical to reach steady state. 21-day average concentration (EEC) was used as averaging period closest to the time to steady state calculated above.
Water Column EEC (µg/L)	17.7	Value generated by PRZM/EXAMS water column file for New York Grapes scenario for **metrafenone total residue and high runoff scenario**. PRZM/EXAMS EEC represents the freely dissolved concentration of the pesticide in the water column. 21-day average concentration (EEC) was used as averaging period closest to the time to steady state calculated above (as discussed above for pore water).

Table 13. Estimated Concentrations of Metrafenone in Fish (based on metrafenone total residue scenario)				
Ecosystem Component	**Total concentration (µg/kg-ww)**	**Lipid normalized concentration (µg/kg-lipid)**	**Contribution due to diet (µg/kg-ww)**	**Contribution due to respiration (µg/kg-ww)**
Small Fish	17,636	440909	862.94	16,773.41
Medium Fish	18,305	457626	1,651.07	16,653.98
Large Fish	19,586	489661	3,063.04	16,523.40

3. Measures of Terrestrial Exposure

a. Terrestrial Exposure Modeling

T-REX

Exposure of free-ranging terrestrial animals is a function of the timing and extent of pesticide application with respect to the location and behavior of those species. OPP's terrestrial exposure model generates exposure estimates assuming that the animal is present on the use site at the time that pesticide levels are highest. The upper-bound pesticide residue concentration on food items is calculated from both initial applications and any additional applications, taking into account pesticide degradation between applications. Although this approach is conservative, it is reasonable, particularly when considering acute risks. For acute risks, the assumption is that the duration of exposure is a single day and, again, occurs when residue levels are highest. In evaluating chronic risks, longer-term exposure estimates are also based on the assumption that the animal is present on the use site when residue levels are highest and furthermore that it repeatedly forages on the use site.

The current screening-level approach does not directly relate timing of exposure to critical or sensitive population, community, or ecosystem processes. Given that the application timing and location is crop-dependent, it is difficult to address the temporal and spatial co-occurrence of metrafenone use and sensitive ecological processes. However, pesticides are frequently used from spring through fall; crop cultivation frequently starts in the spring, hence uses of metrafenone are likely to occur in spring and perhaps summer depending on the region. Spring and early summer are typically seasons of active migrating, feeding, and reproduction for many wildlife species. The increased energy demands associated with these activities (as opposed to hibernation, for example) can increase the potential for exposure to pesticide-contaminated food items since agricultural areas can represent a concentrated source of relatively easily obtained, high-energy food items. In this assessment, the spatial extent of exposure for terrestrial animal species is limited to the use area only and the area immediately surrounding the use area.

Currently, the Agency does not require toxicity studies on reptiles and amphibians in support of pesticide registrations. To accommodate this data gap, birds are used as surrogates for terrestrial-phase amphibians and reptiles. It is assumed that, given the usually lower metabolic demands of reptiles and amphibians compared to birds, exposure to birds would be greater due to higher relative food consumption. While this assumption is likely true, there are no supported relationships regarding the relative toxicity of a compound to birds and herpetofauna. The lack of toxicity data on reptiles and amphibians represents a source of uncertainty in this assessment.

Tables 14 and **15** list selected predicted EECs for birds, reptiles, terrestrial amphibians, and mammals obtained from T-REX simulations for the proposed use of metrafenone at the maximum label rates.

Table 14. Terrestrial Food-Item Residue Estimates for Birds with Metrafenone Proposed Use on Grapes at 0.3 lbs a.i./A (6 apps./year; 14 day app. interval) with a Foliar Dissipation Half-life default value of 35 Days.

Crop	Food Item	Maximum Dose-Based EECs (mg/kg)[1]	Maximum Dose-Based EECs (mg/kg)[2]	Maximum Dose-Based EECs (mg/kg)[3]	Dietary-Based EECs (ppm)[4]
Grapes	Short grass	274.49	156.52	70.08	241.01
	Tall grass	125.81	71.74	32.12	110.46
	Broadleaf plants/ small insects	154.40	88.04	39.42	135.57
	Fruits, pods, seeds, lg. insects	17.16	9.78	4.38	15.06

[1] Based on 20 gram birds (acute)
[2] Based on 100 gram birds (acute)
[3] Based on 1000 gram birds (acute)
[4] Dietary-based EECs apply to both acute and chronic exposure

Table 15. Terrestrial Food-Item Residue Estimates for Mammals with Metrafenone Proposed Use on Grapes at 0.3 lbs a.i./A (6 apps./year; 14 day app. interval) with a Foliar Dissipation Half-life default value of 35 Days.

Size Class (grams)	Adjusted LD_{50}[1]	EECs				
		Short Grass	Tall Grass	Broadleaf Plants/ Small Insects	Fruits/Pods/ Seeds/ Large Insects	Granivore
		Dose-Based	Dose-Based	Dose-Based	Dose-Based	Dose-Based
15	2225.56	229.78	105.32	129.25	14.36	3.19
35	1800.71	158.81	72.79	89.33	9.93	2.21
1000	778.86	36.82	16.88	20.71	2.30	0.51
Dietary-Based EECs[2]		241.01	110.46	135.57	15.06	Not applicable

[1] Herbivores/ insectivores; Granivores
[2] Dietary-based EECs apply to both acute and chronic exposure

TERRPLANT

Effects on non-target terrestrial plants are most likely to occur as a result of spray drift and/or runoff from ground applications. These are important factors in characterizing the risk of metrafenone to non-target plants, which is assumed to reach off-site soil. The TerrPlant (Ver.1.2.2) model predicts EECs for terrestrial plants located in dry and semi-aquatic areas adjacent to the treated field. The EECs are based on the application rate and solubility of the pesticide in water and drift characteristics. The amount of metrafenone that runs off is a proportion of the application rate and is assumed to be 1%, based on metrafenone's solubility of <10 ppm (i.e. 0.474 mg/L) in water. Drift from ground applications are assumed to be 1% the application rate. An incorporation depth was not referenced in the label setting the default value to 1 inch for ground applications. For a standard scenario on an agricultural field, the runoff scenario for terrestrial plants inhabiting dry areas adjacent to a field is characterized as "sheet runoff" (one treated acre to an adjacent acre; a 1:1 ratio) and inhabiting semi-aquatic or wetland areas adjacent to a field is characterized as "channelized runoff" (10 treated acre to an adjacent low-lying acre; a 10:1 ratio). The TerrPlant model EECs are presented in **Table 16**.

Table 16. Estimated Environmental Concentrations of Metrafenone for Terrestrial Plants from Grape Use				
Application Method	**Application Rate (lbs a.i./A)**	**Total Loading to Dry areas (lb/A) [1]**	**Total Loading to Semi-Aquatic Areas (lb/A)[2]**	**Drift (lb/A)[3]**
Ground	0.3*	0.006	0.033	0.003
Ground	1.8**	0.036	0.198	0.018

[1] EEC = Sheet Runoff + Drift (1% for ground)
[2] EEC = Channelized Runoff + Drift = 1% for ground
[3] EEC for ground (appl rate x 1% drift)
* Maximum single application rate
**Maximum seasonal application rate

C. Ecological Effects Characterization

1. Aquatic Effects Characterization

a. Aquatic Animals
(1) Acute Effects
Freshwater Fish and Aquatic-Phase Amphibians -Technical
The freshwater fish studies on rainbow trout (*Oncorhynchus mykiss*, MRID 47267443) and bluegill sunfish (*Lepomis macrochirus*, MRID 47267442) are classified as supplemental for several reasons. The water samples were not centrifuged prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown. This is especially a problem as some of the testing concentrations were conducted above the water solubility limit of the technical grade active ingredient (0.2-0.5 mg a.i./L at 12^{o}C). Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Freshwater Fish and Aquatic-Phase Amphibians – Metabolites / Degradates
Similar to freshwater fish studies on the technical, the freshwater fish studies on degradates of metrafenone on rainbow trout (*O. mykiss* MRID 47267444, 47267445) are also classified supplemental. The water solubility limit was not given, and there was no report of whether the test solutions were centrifuged prior to analytical determination of the test concentrations to ensure that the measured concentrations represent bioavailable material. For example, in the latter study (MRID 47267445), undissolved test substance (likely the result of exceeding the limit of water solubility of the test material) was noted at the bottom of the test vessel in all treatment vessels throughout the definitive test. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Freshwater Fish and Aquatic-Phase Amphibians -Formulations
Similar to the freshwater fish technical grade and metabolite/degradate studies, the freshwater fish study on rainbow trout (*O. mykiss* MRID 47267605) is also classified as

supplemental. There was no report of whether the test solutions were centrifuged prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown; neither is the water solubility for this formulation active ingredient. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Table 17. Freshwater Fish Acute Toxicity Data

Common Name	%AI	Study parameters	LC_{50}/NOAEC/LOAEC	MRID	Classification/ Category
Technical AC 375839 (a.k.a. BAS 560F)					
Rainbow trout *Oncorhynchus mykiss*	97.1	96 hour flow-through study 2 reps / 10 fish per rep. Mean-measured: <0.000498 (negative, solvent), 0.065, 0.13, 0.25, 0.43, and 0.82 mg total a.i./L	96-hr LC_{50} > 0.82 mg total a.i./L NOAEC: 0.25 mg total a.i./L Endpoint(s) affected: mortality, sublethal effects. *Mortality* Cumulative mortality after 96 hours was 0% among all fish in the negative and solvent control groups, and in the treatment groups exposed to 0.13 and 0.25 mg a.i./L of AC 375839. Cumulative mortality was 5% among fish exposed to 0.065, 0.43 and 0.82 mg a.i./L of AC 375839 after 96 hours. *Sublethal effects* No sublethal effects were observed among fish in the negative or solvent controls, 0.065, 0.13, 0.25 or 0.43 mg a.i./L treatment groups exposed to AC 375839 after 96 hours. Within the 0.82 mg a.i./L treatment group, 16% of fish still living were lethargic and 5% exhibited dark discoloration after 96 hours.	47267443	Supplemental (not to be used in risk estimation)/ At most, Highly toxic[1]

Table 17. Freshwater Fish Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LC_{50}/NOAEC/LOAEC**	**MRID**	**Classification/ Category**
Bluegill sunfish *Lepomis macrochirus*	97.1	96 hour flow-through study 2 reps / 10 fish per rep. Mean-measured: <0.000498 (negative, solvent), 0.066, 0.14, 0.25, 0.45, and 0.87 mg total a.i./L	96-hr LC_{50} > 0.87 mg total a.i./L NOAEC: 0.45 mg total a.i./L Endpoint(s) affected: mortality, sublethal effects. *Mortality* Cumulative mortality after 96 hours was 0% among all fish in the negative and solvent control groups and all treatment groups except the 0.87 mg a.i./L treatment group in which 15% of fish died. *Sublethal effects* No sublethal effects were observed among fish in the negative or solvent controls, 0.066, 0.14, 0.25 or 0.45 mg a.i./L treatment groups exposed to AC 375839 after 96 hours. Within the 0.87 mg a.i./L treatment group, 18% of fish still living were lethargic and 12% were lying on the bottom of the test chamber with little motion other than gill movement after 96 hours.	47267442	Supplemental (not to be used in risk estimation)/ At most, Highly toxic[1]
Metabolites/ Degradates					
Rainbow trout *Oncorhynchus mykiss*	99.5	Reg. No. 4074484 (Metabolite of BAS 560 F) 96 hour static study; limit test 2 reps (neg. control), 3 reps (treatment) / 10 fish per rep. Mean-measured: <1 (negative) and 99 mg total a.i./L	96-hr LC_{50} > 99 mg total a.i./L NOAEC ≥ 99 mg total a.i./L Endpoint(s) affected: none.	47267444	Supplemental (not to be used in risk estimation)/ At most, Slightly toxic[1]

Table 17. Freshwater Fish Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LC_{50}/NOAEC/LOAEC**	**MRID**	**Classification/ Category**
Rainbow trout *Oncorhynchus mykiss*	98.2	Reg. No. 4084564 (Metabolite of BAS 560F) 96 hour static study 1 rep / 10 fish per rep. Mean-measured: <1 (negative), 4.4, 9.2, 20.3, 35.2 and 58.4 mg total a.i./L	96-hr LC_{50} 15.8 (9.2-35.2) mg total a.i./L NOAEC: 9.2 mg total a.i./L Endpoint(s) affected: mortality, sublethal effects *Mortality* Cumulative mortality at 96 hours was 80% in the 20.3 mg total a.i./L concentration and 100% in the two highest concentrations. *Sublethal effects* Tottering and distended abdomen were observed after 4 hours in the group exposed to 58.4 mg total a.i./L; also observed among those in the group exposed to 20.3 mg total a.i./L between 24 and 72 hours. Apathy and distended abdomen were observed after 24 hours among those in the 35.2 mg total a.i./L treatment group and after 96 hours among those in the group exposed to 20.3 mg total a.i./L.	47267445	Supplemental (not to be used in risk estimation)/ Slightly toxic[1]
Formulations					
Rainbow trout *Oncorhynchus mykiss*	24.7% a.i.; 294 g BAS 560 00F/L	BAS 560 00F (SF 10358, RLF 12359) 96 hour static study 2 reps. / 10 fish per rep. Mean-measured: <4.05 (neg. control), 12, 20, 34, 56, and 94 mg form/L [<1 (neg. control), 3.0, 4.9, 8.3, 13.7, and 23.3 mg total a.i./L]	96-hr LC_{50} >94 mg form/L [>23.3 mg total a.i./L] NOAEC ≥ 94 mg form/L [23.3 mg total a.i./L] Endpoint(s) affected: none.	47267605	Supplemental (not to be used in risk estimation)/ At most, Slightly toxic[1]
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic					

Freshwater Invertebrates -Technical
The freshwater invertebrate study using the technical grade active ingredient on water flea (*Daphnia magna*, MRID 47267437) is classified as supplemental. The water samples were not centrifuged prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown. This is especially a problem as some of the testing concentrations were conducted at or above the water solubility limit of the technical grade active ingredient (0.2-0.5 mg a.i./L at 12^{o}C). Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Freshwater Invertebrates –Metabolites / Degradates
Similar to freshwater invertebrate study on the technical, the freshwater invertebrate studies on degradates of metrafenone on the water flea (*D. magna* MRID 47267438, 47267439) is also classified supplemental. The water solubility limit was not given, and there was no report of whether the test solutions were centrifuged prior to analytical determination of the test concentrations to ensure that the measured concentrations represent bioavailable material. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Freshwater Invertebrates –Formulations
Similar to the freshwater invertebrate technical grade and metabolite/degradate studies, the freshwater invertebrate study on water flea (*D. magna* MRID 47267604) is also classified as supplemental. There was no report of whether the test solutions were centrifuged prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown; neither is the water solubility for this formulation active ingredient. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Table 18. Freshwater Invertebrate Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**EC_{50}/NOAEC/LOAEC**	**MRID**	**Classification / Category**
Technical AC 375839 (a.k.a. BAS 560F)					
Water flea *Daphnia magna*	97.1	48 hour static study 2 reps.; 10 inverts. per rep Mean-measured: <0.000498 (negative, solvent), 0.059, 0.12, 0.22, 0.45, and 0.92 mg total a.i./L	48-hr EC_{50} > 0.92 mg total a.i./L Slope: N/A NOAEC ≥ 0.92 mg total a.i./L Endpoint(s) affected: none.	47267437	Supplemental (not to be used in risk estimation)/ At most, Highly toxic[1]
Metabolites/ Degradates					
Water flea *Daphnia magna*	99.5	CL 375816 48 hour static study 4 reps.; 5 inverts per rep. Mean-measured: <1 (negative), 12.9, 25.8, 51.4, and 102.5 mg total a.i./L	48-hr EC_{50} > 102.5 mg total a.i./L Slope: N/A NOAEC ≥ 102.5 mg total a.i./L Endpoint(s) affected: none.	47267438	Supplemental (not to be used in risk estimation)/ Practically non-toxic[1]
Water flea *Daphnia magna*	98.2	CL 4084564 48 hour static study 4 reps.; 5 inverts per rep. Mean-measured: <1 (negative), 6.4, 12.5, 23.2, 49.6, 66.4 mg a.i./L	48-hr EC_{50} = 50.9 (42.1-63.5) mg total a.i./L Slope: 4.59 (2.52-6.66) NOAEC = 23.2 mg total a.i./L Endpoint(s) affected: immobility. *Immobility* Cumulative immobility was 0% among all daphnids exposed to 0 (negative control), 6.4 and 12.5 mg total a.i./L of CL 4084564 (Metabolite of BAS 560 F) after 48 hours. Cumulative immobility was 5% among animals exposed to 23.2 mg total a.i./L, 55% among daphnids exposed to 49.6 mg total a.i./L and 65% among animals exposed to 66.4 mg total a.i./L of CL 4084564 (Metabolite of BAS 560 F) after 48 hours.	47267439	Supplemental (not to be used in risk estimation)/ Slightly toxic[1]

Table 18. Freshwater Invertebrate Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**EC_{50}/NOAEC/LOAEC**	**MRID**	**Classification / Category**
Formulations					
Water flea *Daphnia magna*	24.7% a.i.; 294 g BAS 560 00 F/L	BAS 560 00 F (SF 10358, RLF 12359) 48 hour static study 2 reps.; 10 inverts per rep. Mean-measured: <0.810 (neg. control), 1.7, 3.1, 6.1, 12.2, 24.3, and 47.1 mg form/L [<0.20 (neg. control), 0.42, 0.76, 1.5, 3.0, 6.0, and 11.6 mg total a.i./L]	48-hr EC_{50}: 17.1 (12.8-24.3) mg form/L [4.2 (3.2-6.0) mg total a.i./L] NOAEC: 6.1 mg form/L [1.5 mg total a.i./L] Endpoint(s) affected: mortality and sublethal effects (immobility) *Mortality* Cumulative mortality after 48 hours was 0% among animals exposed to mean-measured concentrations of 0 (negative control), 1.7, 3.1, 6.1 and 12.2 mg form/L. Cumulative mortality was 65% among animals exposed to 24.3 mg form/L and 90% among animals exposed to 47.1 mg form/L after 48 hours. *Immobility* Cumulative immobility aft 48 hours was 0% at mean-measured concentrations of 1.7 and 47.1 mg form/L; 5% at neg. control, 3.1, and 6.1 mg form/L; 15% at 12.2 mg form/L; and, 25% at 24.3 mg form/L.	47267604	Supplemental (not to be used in risk estimation) / Moderately toxic[1]
[1]Based on EC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic					

Marine/Estuarine Fish

Unlike the majority of the aquatic studies, which were supplemental on account of not having centrifuged samples which clearly exceeded the water solubility limit, the marine/estuarine fish study on the sheepshead minnow (*Cyprinodon variegatus*, MRID 47267446) is acceptable on account of having centrifuged the two highest concentrations, both of which were above the solubility limit of 0.3 mg a.i./L (in saltwater). The results reveal no effects on mortality or sublethal effects.

Table 19. Marine/ Estuarine Fish Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LC_{50}/NOAEC/LOAEC**	**MRID**	**Classification/ Category**
Technical AC 375839 (a.k.a. BAS 560F)					
Sheepshead Minnow *Cyprinodon variegatus*	94.2	96 hour flow-through study 2 reps / 10 fish per rep. Mean-measured: <0.04 (negative, solvent), 0.072, 0.13, 0.24, 0.32 (0.13 mg a.i./L based on centrifuged samples) and 0.65 (0.35 mg a.i./L based on centrifuged samples) mg a.i./L	96-hr LC_{50} > 0.35 mg dissolved a.i./L NOAEC ≥ 0.35 mg dissolved a.i./L Endpoint(s) affected: none.	47267446	Acceptable/ At most, Highly toxic[1]
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic					

Marine/Estuarine Invertebrates – Technical

The marine/estuarine invertebrate study using the technical grade active ingredient on eastern oyster (*Crassostrea virginica*, MRID 47267440) is classified as supplemental. Given that centrifugation was not performed on all concentration test levels, it is possible that the endpoint retrieved from this study will provide an underestimation of risk. For example, we know that after centrifugation of the highest test level the mean-measured concentration decreased by 42% relative to the pre-centrifugation mean-measured concentration. The reduction in soluble substance is likely due to the concentration being above the limit of solubility (*i.e.*, 0.3 mg a.i./L). Nevertheless, it is unknown from the study results whether a similar reduction will occur at lower concentrations, including one more above the limit of solubility and the three levels remaining below the limit of solubility. Similarly, the saltwater mysid study (*Americamysis bahia*, MRID 47267441) is classified supplemental on account of no centrifugation of water samples at any test concentration level prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown. Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Table 20. Marine/ Estuarine Invertebrate Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**EC_{50}/LC_{50}/NOAEC/LOAEC**	**MRID**	**Classification/ Category**
Technical AC 375839 (a.k.a. BAS 560F)					
Eastern oyster *Crassostrea virginica*	94.2	96 hour flow-through study 20 bivalves per level Time-weighted average: <0.04 (negative, solvent), 0.0522, 0.104, 0.203, 0.287, and 0.573 mg total a.i./L (highest concentration centrifuged yielded 0.33 mg dissolved a.i./L)	96-hr EC_{50}: 0.22 (0.20-0.25) mg total a.i./L[2] NOAEC: 0.0522 mg total a.i./L Endpoint(s) affected: shell deposition. *Shell deposition* Relative to the negative control, the mean percent reduction in shell growth starting with the negative solvent is as follows: 17.4, 13.9, 26.3, 44.3, 84.2, and 100%, respectively. No significant differences (p= 0.05) were detected between the two controls.	47267440	Supplemental (not to be used in risk estimation)/ Highly toxic[1]
Saltwater mysid *Americamysis bahia*	94.2	96 hour flow-through study 2 reps./ 10 mysids per rep. Time-weighted average: <0.04 (negative, solvent), 0.0749, 0.129, 0.240, 0.416, and 0.663 mg total a.i./L	96-hr LC_{50}: 0.487 (0.428-0.575) mg total a.i./L[2] NOAEC: 0.0749 mg total a.i./L Endpoint(s) affected: mortality, sublethal effects. *Mortality* At 96 hours, cumulative mortality at the TWA concentrations 0.129 and 0.240 mg total a.i./L was 5%, at 0.416 mg total a.i./L was 15%, and at 0.663 mg total a.i./L was 95%. *Sublethal effects* No sub-lethal effects were observed in the controls or TWA 0.0749-0.240 mg total ai/L treatment levels. At test termination 7 out of the surviving 17 mysids in the 0.416 mg total ai/L level and the single surviving mysid at the 0.663 mg total ai/L level were observed swimming erratically.	47267441	Supplemental (not to be used in risk estimation)/ Highly toxic[1]

[1]Based on EC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic

[2] Range is 95% confidence interval for endpoint

(2) Chronic Effects

Freshwater Fish – Technical
The freshwater fish study on fathead minnow (*Pimephales promelas*, MRID 47267449) is classified as supplemental. The solubility limit of the test compound is not reported; it is possible given previous reports that the limit was approximately 0.2-0.5 mg a.i./L at 12 ^{o}C, which implies that the two highest test concentrations (0.421 and 0.839 mg total a.i./L) potentially exceeded the solubility limit and yet centrifugation (or filtration) was not mentioned as part of the protocol prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown. Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Hatching occurred at all levels on Day 4, and hatching success averaged 88-94% for all levels, with no statistically significant differences observed. Post-hatch survival (28-days post-hatch) was statistically-reduced compared to the negative control at the 227, 421, and 839 µg total a.i./L levels ($p \leq 0.05$). Post-hatch survival averaged 96% at the negative control through 118 µg total a.i./L levels, and 87, 86, and 11% at the 227, 421, and 839 µg total ai/L levels, respectively. No clinical signs of toxicity were observed during the study in any treatment group. Fish length was significantly reduced relative to the average negative control length at levels above 421 µg total ai/L and wet and dry weight were significantly lower than the negative control weights at the 839 µg total ai/L level; however, significant impact on survival occurred at these levels.

Table 21. Freshwater Fish Chronic Toxicity Data

Common Name	%AI	Study parameters	NOAEC/LOAEC	MRID	Classification /Category
Technical AC 375839 (a.k.a. BAS 560F)					
Fathead minnow *Pimephales promelas*	97.1	32-day flow-through test 80 embryos per level, split into 20 embryos per cup, 1 cup per aquarium, 4 rep. aquaria per treatment Time-weighted average: <0.498 (negative, solvent), 57, 118, 227, 421, and 839 µg total a.i./L	NOAEC: 0.118 mg total a.i./L LOAEC: 0.227 mg total a.i./L Most sensitive endpoint: post-hatch survival Endpoint(s) affected: post-hatch survival and growth (total length, wet and dry weight)	47267449	Supplemental (not to be used in risk estimation)

Freshwater Invertebrates – Technical

A chronic freshwater invertebrate study (MRID 47267447) with metrafenone technical (97.1%) was deemed invalid due to instability of the chemical under test conditions. A non-guideline chronic midge study (*Chironomus riparius*, MRID 47267501) with metrafenone technical (97.1%) is also invalid on the basis of low negative control emergence.

Marine/Estuarine Fish

No chronic marine/estuarine fish studies were submitted for review.

Marine/Estuarine Invertebrates- Technical
The marine/estuarine invertebrate study on saltwater mysid (*Americamysis bahia*, MRID 47267448) is classified as acceptable. Although the solubility limit of the test compound is not reported; it is possible given previous reports that the limit was approximately 0.2-0.5 mg a.i./L at 12 ^{o}C. The mean-measured test concentrations of the total metrafenone present do not exceed the suggested solubility limit. In addition, the highest nominal concentration (0.05 mg a.i./L) is significantly below solubility (*i.e.*, ≥ 4 - 10 times); in this particular case, not centrifuging is considered to not affect acceptability of the study. Finally, given the dilution system design, where the stock solution was a solvent stock solution suggests that solubility of the compound is not an issue in this particular case.

The day of first brood release was not assessed. The number of offspring per surviving female was statistically-reduced compared to the negative control at the 45 µg ai/L level (1.4 versus 5.8 offspring/female). Similarly, the number of offspring per female per reproductive day was statistically-reduced compared to the negative control at the 45 µg ai/L (0.10 versus 0.44 offspring/female/day). Although not statistically-compared, the percentage of females producing young averaged ≥85% at the control through 22 µg ai/L treatment levels, but only 25% at the 45 µg ai/L level. The NOAEC for reproduction was reported to be 22 µg ai/L.

Table 22. Marine/ Estuarine Invertebrate Chronic Toxicity Data					
Common Name	**%AI**	**Study parameters**	**NOAEC/LOAEC**	**MRID**	**Classification /Category**
Technical AC 375839 (a.k.a. BAS 560F)					
Saltwater mysid *Americamysis bahia*	94.2	28-day flow-through test Before pairing: 60 mysids per level After pairing: 20 mysids per level Time-weighted average: <LOD (negative, solvent), 2.8, 6.2, 12, 22, and 44 µg total a.i./L	NOAEC: **0.022 mg a.i./L**[1] LOAEC: 0.044 mg a.i./L Most sensitive endpoint: reproduction (number of offspring/female/repro. day) Endpoint(s) affected: reproduction (number of offspring/female/repro. day)	47267448	Acceptable
[1] **Bold** value is the value that will be used to calculate risk quotients					

b. Aquatic Plants

Vascular Aquatic Plants

The vascular aquatic plant study (MRID 47267511) on duckweed (*Lemna gibba*) is classified as supplemental for several reasons, which are considered major guideline deviations. The test was conducted for 7 days instead of the guideline prescribed 14 days. More importantly, the dissolved or soluble concentrations (*i.e.*, post-centrifugation) of test material were not determined. As a result of the latter, the stability of exposure to the dissolved form throughout the test is unknown. This is especially a problem as some of the testing concentrations were conducted above the solubility limit of the technical grade active ingredient (0.457 mg/L in water; 0.3 mg/L in 20X AAP media both at pH9). Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Non-vascular Aquatic Plants - Technical
The non-vascular aquatic plant studies on cyanobacteria (*Anabaena flos-aquae*; MRID 47267512), marine diatom (*Skeletonema costatum*, MRID 47267513), freshwater diatom (*Navicula pelliculosa*, MRID 47267514), and green algae (*Pseudokirchneriella subcapitata*, MRID 47267515) are classified as supplemental for several reasons. The water samples were not centrifuged prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown. This is especially a problem as some of the testing concentrations were conducted above the solubility limit of the technical grade active ingredient (0.457 mg/L in freshwater; 0.3 mg/L in saltwater). Therefore, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Non-vascular Aquatic Plants – Metabolites / Degradates
Similar to the non-vascular aquatic plant technical grade studies, the non-vascular aquatic plant studies on the degradates are classified as supplemental for several important reasons. For example, the algae study (*Pseudokirchneriella subcapitata* MRID 47267516) did not report daily observations of test solution appearance, metabolite water solubility, and method of filtration of test solution, which leads to uncertainty in the measured concentrations as well as the relationship of mean-measured and nominal concentrations relative to the metabolite water solubility value. For this reason too, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description. In addition, the other algae study (*Pseudokirchneriella subcapitata* MRID 47267517) did not centrifuge the water samples prior to analytical determination. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown; neither is the metabolite water solubility. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Non-vascular Aquatic Plants - Formulations
Similar to the non-vascular aquatic technical grade and metabolite/degradate studies, the non-vascular aquatic plant study on the green algae (*Pseudokirchneriella subcapitata*) is also classified as supplemental. Although centrifugation was conducted on the water samples in this particular case the protocol states that it was used to remove algal cells, which suggests that the centrifugation method may not have removed the undissolved material. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Stability of exposure to the dissolved form throughout the test is unknown; neither is the water solubility for this formulation active ingredient. Again, the concentrations are reported as mg 'total' a.i./L and are not useful for RQ calculations, but can contribute to risk description.

Table 23. Aquatic Plant Toxicity Data					
Species	**%A.I.**	**Study Parameters**	**EC_{50}/NOAEC**	**MRID No.**	**Study Classification**
Technical AC 375839 (a.k.a. BAS 560F)					
Vascular Aquatic Plants					
Duckweed *Lemna gibba*	94.2	Tier II study 7 day static renewal study 3 reps. / 4 plants per rep. Mean measured: <0.04 (negative, solvent), 0.057, 0.10, 0.21, 0.41, and 0.76 mg a.i./L	Biomass (dry weight) EC_{50} > 0.76 mg total a.i./L NOAEC: 0.21 mg total a.i./L Frond density EC_{50} > 0.76 mg total a.i./L NOAEC: 0.41 mg total a.i./L Growth rate EC_{50} > 0.76 mg total a.i./L NOAEC: 0.41 mg total a.i./L Most sensitive endpoint: biomass based on NOAEC Endpoint(s) affected: biomass, frond density, growth rate	47267511	Supplemental (do not use in risk estimation)
Non-Vascular Aquatic Plants					
Cyanobacteria (blue-green algae) *Anabaena flos-aquae*	94.2	Tier II study 96 hour static study 3 reps. Initial measured: <0.04 (negative, solvent), 0.0883, 0.139, 0.217, 0.580, and 0.862 mg a.i./L	Biomass (area under growth curve), cell density, growth rate EC_{50} > 0.862 mg total a.i./L NOAEC ≥ 0.862 mg total a.i./L Endpoint(s) affected: none.	47267512	Supplemental (do not use in risk estimation)

Table 23. Aquatic Plant Toxicity Data					
Species	**%A.I.**	**Study Parameters**	**EC_{50}/NOAEC**	**MRID No.**	**Study Classification**
Marine Diatom *Skeletonema costatum*	94.2	Tier II study 96 hour static study 3 reps. Initial measured: <0.04 (negative, solvent), 0.0509, 0.109, 0.214, 0.272, and 0.680 mg a.i./L	Biomass (area under growth curve) EC_{50}: 0.57 (0.38-0.85) mg total a.i./L NOAEC: 0.0509 mg total a.i./L Cell density EC_{50} > 0.680 mg total a.i./L NOAEC: 0.272 mg total a.i./L Growth rate EC_{50} > 0.680 mg total a.i./L NOAEC: 0.272 mg total a.i./L Most sensitive endpoint: biomass Endpoint(s) affected: biomass, cell density, growth rate	47267513	Supplemental (do not use in risk estimation)
Freshwater Diatom *Navicula pelliculosa*	94.2	Tier II study 96 hour static study 4 reps. Initial measured: <0.04 (negative, solvent), 0.0761, 0.154, 0.276, 0.432, and 0.914 mg a.i./L	Biomass (area under growth curve) EC_{50} > 0.914 mg total a.i./L NOAEC: 0.432 mg total a.i./L Cell density EC_{50} > 0.914 mg total a.i./L NOAEC: ≥0.914 mg total a.i./L Growth rate EC_{50} > 0.914 mg total a.i./L NOAEC: ≥0.914 mg total a.i./L Endpoint(s) affected: biomass based on NOAEC	47267514	Supplemental (do not use in risk estimation)

Table 23. Aquatic Plant Toxicity Data					
Species	**%A.I.**	**Study Parameters**	**EC_{50}/NOAEC**	**MRID No.**	**Study Classification**
Green Algae *Pseudokirchneriella subcapitata*	97.1	Tier I study 72 hour static study 6 reps. (negative, solvent controls); 3 reps. (treatments) Mean-measured: <0.498 (negative, solvent), 60, 123, 232, 472, and 870 µg a.i./L	Biomass (area under growth curve) EC_{50}: 0.71 (0.65-0.77) mg total a.i./L NOAEC: 0.23 mg total a.i./L Cell density EC_{50}: 0.74 (0.67-0.82) mg total a.i./L NOAEC: 0.23 mg total a.i./L Growth rate EC_{50} > 0.87 mg total a.i./L NOAEC: 0.23 mg total a.i./L Most sensitive endpoint: biomass Endpoint(s) affected: biomass, cell density, growth rate	47267515	Supplemental (do not use in risk estimation)
Metabolites/ Degradates					
Non-Vascular Aquatic Plants					
Algae *Pseudokirchneriella subcapitata*	99.5	CL 375816 (Metabolite of BAS 560 F) Tier I study 72 hour static study 5 reps. (negative control); 3 reps. (treatments) Mean-measured: ND (negative), 6.50, 12.66, 25.80, 51.45, and 101.92 mg a.i./L	Biomass (area under growth curve), Chlorophyll-a, and Growth rate EC_{50} > 101.9 mg total a.i./L NOAEC ≥ 101.9 mg total a.i./L Endpoint(s) affected: none.	47267516	Supplemental (do not use in risk estimation)

Table 23. Aquatic Plant Toxicity Data					
Species	**%A.I.**	**Study Parameters**	**EC_{50}/NOAEC**	**MRID No.**	**Study Classification**
Algae *Pseudokirchneriella subcapitata*	98.2	CL 4084564 (Metabolite of BAS 560 F) Tier I study 72 hour static study 5 reps. (negative control); 3 reps. (treatments) Mean-measured: ND (negative), 3.21, 6.28, 9.9, 18.57, 38.78, and 58.03 mg a.i./L	Chlorophyll-a EC_{50}: 24 (21-27) mg total a.i./L NOAEC: 9.9 mg total a.i./L Biomass (area under growth curve) EC_{50}: 26 (22-30) mg total a.i./L NOAEC: 9.9 mg total a.i./L Growth rate EC_{50}: 44 (40-47) mg total a.i./L NOAEC: 9.9 mg total a.i./L Most sensitive endpoint: chlorophyll-a Endpoint(s) affected: chlorophyll-a, biomass, and growth rate	47267517	Supplemental (do not use in risk estimation)
Formulations					
Non-Vascular Aquatic Plants					
Green Algae *Pseudokirchneriella subcapitata*	24.7	BAS 560 00F (SF 10358, RLF 12359) Tier I study 72 hour static study 6 reps. (negative control); 3 reps. (treatments) Initial mean-measured: <0.05 (negative), 0.188, 0.371, 0.716, 1.383, 2.717, and 5.681 mg a.i./L	Cell density EC_{50}: 0.66 (0.47-0.91) mg total a.i./L NOAEC: 0.188 mg total a.i./L Biomass (area under growth curve) EC_{50}: 0.73 (0.53-0.99) mg total a.i./L NOAEC: 0.188 mg total a.i./L Growth rate EC_{50}: 5.2 (4.4-6.2) mg total a.i./L NOAEC: 0.188 mg total a.i./L Most sensitive endpoint: cell density Endpoint(s) affected: cell density, biomass, growth rate	47267607	Supplemental (do not use in risk estimation)

2. Terrestrial Effects Characterization

a. Terrestrial Animals

(1) Acute Effects

Birds – Technical

The acute avian oral studies (MRID 47267502, 47267503) on 23-week old Northern bobwhite quail (*Colinus virginianus*) and 20-week old mallard duck (*Anas platyrhynchos*), respectively, assessed over 14 days are classified as acceptable. AC 375839 Technical was administered to the birds via gelatin capsules at nominal levels of 0 (vehicle control), 400, 600, 900, 1350, and 2025 mg ai/kg bw (limit dose). The 14-day acute oral LD_{50} was >2025 mg ai/kg bw (>limit dose). The 14-day NOAEL was 2025 mg ai/kg bw, as there were no mortalities, clinical signs of toxicity, or treatment-related effects on body weight or food consumption during the 14-day study. In addition, no toxicological effects were observed at necropsy. AC 375839 Technical (metrafenone) would be classified as practically non-toxic to young adult Northern bobwhite quail (*C. virginianus*) as well as to young adult mallard duck (*A. platyrhynchos*) in accordance with the classification system of the U.S. EPA.

The acute avian dietary studies (MRID 47267504, 47267505) on 11-day old Northern bobwhite quail (*C. virginianus*) and 9-day old mallard duck (*A. platyrhynchos*), respectively, assessed over 8 days are classified as acceptable. AC 375839 Technical was administered to the birds in the diet at nominal concentrations of 0 (negative control), 100, 270, 729, 1968, and 5314 mg ai/kg diet (adjusted for purity). Mean-measured concentrations were <3.6 (<LOD, control), 98, 262, 809, 2130, and 6070 mg ai/kg diet, respectively. The 8-day acute dietary LC_{50} was >6070 mg ai/kg diet. Given there was no dose-response, the NOAEC was the highest concentration tested, 6070 mg a.i./kg diet. There were no treatment-related mortalities, clinical signs of toxicity, or effects on food consumption. Some gross pathological changes were observed. AC 375839 Technical (metrafenone) would be classified as practically non-toxic to juvenile bobwhite quail (*C. virginianus*) as well as to juvenile mallard duck (*A. platyrhynchos*) on an acute dietary basis, in accordance with the classification system of the U.S. EPA.

Table 24. Avian Acute Toxicity Data

Common Name	%AI	Study parameters	LD_{50}/LC_{50} NOAEL/ LOAEL	MRID	Classification /Category
Technical AC 375839 (a.k.a. BAS 560F)					
Northern Bobwhite Quail *Colinus virginianus*	95.86	Acute oral study 5 birds/sex/dose level 14 day observation period Nominal: 0 (vehicle), 400, 600, 900, 1350, and 2025 mg a.i./kg bw	LD_{50} > 2025 mg a.i./kg bw NOAEL: 2025 mg/kg bw Probit slope: N/A Endpoint(s) affected: none.	47267502	Acceptable/ Practically non-toxic[1]

Table 24. Avian Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LD_{50}/LC_{50} NOAEL/ LOAEL**	**MRID**	**Classification /Category**
Mallard Duck *Anas platyrhynchos*	95.86	Acute oral study 5 birds/sex/dose level 14 day observation period Nominal: 0 (vehicle), 400, 600, 900, 1350, and 2025 mg a.i./kg bw	LD_{50} >2025 mg a.i/kg bw NOAEL: 2025 mg a.i./kg bw Probit slope: N/A Endpoint(s) affected: none.	47267503	Acceptable/ Practically non-toxic[1]
Northern Bobwhite Quail *Colinus virginianus*	95.86	Acute dietary study 12 birds per rep.(neg. control & treatment); 3 reps. (neg. control), 1 rep. per treatment 5 days on treatment, 3 additional days observation Mean-measured: <3.6 (neg. control), 98, 262, 809, 2130, and 6070 mg a.i./kg diet	LC_{50} >6070 mg a.i./kg diet NOAEC: 6070 mg a.i./kg diet LOAEC >6070 mg a.i./kg diet Issues[3]: body weight change (significant reduction in body weight gain (39%, relative to Control 1) at the 262 mg ai/kg diet level). However, no concentration-response was observed so it may not be biologically significant.	47267504	Acceptable/ Practically non-toxic[2]
Mallard Duck *Anas platyrhynchos*	95.86	Acute dietary study 12 birds per rep.(neg. control & treatment); 3 reps. (neg. control), 1 rep. per treatment 5 days on treatment, 3 additional days observation Mean-measured: <3.6 (neg. control), 98, 262, 809, 2130, and 6070 mg a.i./kg diet	LC_{50} > 6070 mg a.i./kg diet NOAEC: 6070 mg a.i./kg diet LOAEC >6070 mg a.i./kg diet Issues[3]: body weight change (significant reduction in body weight gain (19%, relative to controls) at the 262 mg ai/kg diet level). However, no concentration-response was observed so it may not be biologically significant.	47267505	Acceptable/ Practically non-toxic[2]

[1] Based on LD_{50} (mg/kg) <10 very highly toxic; 10-50 highly toxic; 51-500 moderately toxic; 501-2000 slightly toxic; >2000 practically nontoxic

[2] Based on LC_{50} (mg/kg) <50 very highly toxic; 50-500 highly toxic; 501-1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically nontoxic

[3] The Northern bobwhite and mallard duck acute dietary studies were conducted at the same time in the same laboratory using the same feed. Therefore, the observance of body weight change at the same concentration level (262 mg a.i./kg diet) in both studies calls into question the validity of the effect as a result of the chemical versus some other factor.

Mammals - Technical

In an acute oral toxicity study (MRID 47267522), three per sex, fasted, young adult

C57BL mice [(age: 8- 13 weeks old, wt. males 23.0-25.7g, females 17.0-18.7g)] were given a single oral dose of the test material (BAS 560 F) prepared in 0.5% CMC solution in doubly distilled water in a sequential manner at a dose level of 2000 mg/kg bw by gavage and observed for 14 days. The three females were dosed in step 1, and the three males in step 2. The oral LD_{50} is > 2000 mg/kg in mice which classifies the product (metrafenone technical) in EPA Toxicity Category III for oral toxicity.

One male animal died on Day 4. This animal showed poor general health, dyspnea, tremor, piloerection and sunken flanks. The dead mouse showed discoloration of the lungs, and ulcer/erosion in stomach. All other male and female animals survived showed no obnormalities, and gained body weight during the first week post-exposure. No gross abnormalities were noted for any of the surviving animals when necropsied at the termination.

Mammals – Formulation
In an acute oral toxicity study (MRID 47267609), 5/sex of Sprague-Dawley derived (Crl:CD(SD)BR) albino rats (age: 8 weeks; weight: 206-229 g males and 165-184 g females) were given a single oral dose of AC 375839 300 g/L SC (RLF12359) (Lot No. R2066-048; 294 g/L and 25.21% AC 375839; density 1.19 g/mL; pH 6.6; viscous beige liquid) as received at a dose of 5000 mg/kg bw administered by oral gavage. The amount of test solution to be administered was calculated for each animal. Animals were observed for clinical signs of toxicity and mortality several times on the day of dosing and daily for 14 days. Individual body weights were recorded prior to dosing (day 0) and on days 7 and 14. A gross necropsy examination was performed on all animals at scheduled euthanasia. All animals survived and gained weight. No clinical signs of toxicity were observed. No gross pathological findings were observed at necropsy. The oral LD_{50} is > 5000 mg/kg in rats which classifies the product (metrafenone formulation) in EPA Toxicity Category IV for oral toxicity.

Table 25. Mammalian Acute Toxicity Data

Common Name	%AI	Study parameters	LD_{50} /NOAEL	MRID	Classification /Category
Technical AC 375839 (a.k.a. BAS 560F)					
Mouse	94.2	Acute oral study 2000 mg technical/kg bw (limit test) administered by gavage 3/sex/dose level 14-day observation period	Acute oral LD_{50} >2000 mg technical/kg bw (F, M, both)[2] NOAEL: No NOAEL LOAEL: No LOAEL	47267522	Acceptable/ Practically non-toxic[1]

Table 25. Mammalian Acute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LD_{50} /NOAEL**	**MRID**	**Classification /Category**
Formulation					
Laboratory albino rat	25.21%; AC 375839 300 g/ L SC (RLF 12359)	Acute oral study 5000 mg form/kg bw (limit test) administered by gavage 5/sex/dose level 14-day observation period	Acute oral LD_{50} >5000 mg form/kg bw (F, M, both) [> 1,260.5 mg a.i./kg bw] [3] NOAEL: No NOAEL LOAEL: No LOAEL	47267609	Acceptable[4]/ Practically non-toxic[1]

[1] Based on LD_{50} (mg/kg) <10 very highly toxic; 10-50 highly toxic; 51-500 moderately toxic; 501-2000 slightly toxic; >2000 practically nontoxic
[2] One male animal died on Day 4. This animal showed poor general health, dyspnea, tremor, piloerection and sunken flanks. The dead mouse showed discoloration of the lungs, and ulcer/erosion in stomach.
[3] All animals survived and gained weight. No clinical signs of toxicity were observed. No gross pathological findings were observed at necropsy.
[4] The study satisfies the OECD Guideline 401, which is no longer considered an acceptable protocol. The preferred protocol is OECD 425: Acute Oral Toxicity-Up-and-Down Procedure.

Terrestrial Invertebrates – Technical

The terrestrial invertebrate study on honey bees (*Apis mellifera*, MRID 47267508) classified as acceptable. The contact test had 4% mortality in the negative and solvent controls and 0% mortality in the 100 µg a.i./bee level by 48 hours; there were no sub-lethal effects in the contact test. The oral test also had 4% mortality in the negative control, 2% mortality in the solvent control, and 2% mortality in the 114 µg a.i./bee level at 24 and 48 hours; sub-lethal effects were 0% in the negative control, 2% in the solvent control, and 6% in the 114 µg a.i./bee level only at 4 hours.

In a 14 day acute limit toxicity study, earthworms (*Eisenia fetida*, MRID 47267518) were exposed to AC 375839 at a nominal concentration of 1000 mg a.i./kg dry weight of artificial soil. No concurrent reference chemical test was conducted in this study. The report indicated that the experiment was carried out in accordance with OECD 207. However, only one concentration was tested which did not fulfill the requirement of the guideline. No earthworm mortality was observed in the water control or test substance treatment. There was one mortality in the acetone control group. The 14 day LC_{50} was > 1000 mg a.i./kg dry soil, the concentration tested. No significant difference in earthworm burrowing time (*i.e.,* time needed for 10 earthworms to burrow into the soil after placement on soil surface) was observed. The average weight loss between Day 0 and Day 14 was 20.6, 3.5 and 39.7 mg in the test groups of water control, acetone control, and test substance treatment, respectively. There was a significant difference in weight change between acetone control and test substance treatment. However, there was no significant difference between the water control and the test substance. Therefore, the sub-lethal effect of AC 375839 at a concentration of 1000 mg a.i./kg dry wt soil on

weight loss is uncertain. The end-point toxicity concentration of AC 375839 can not be determined from this study, however, it is considered to be non-lethal to earthworms up to a concentration of 1000 mg a.i./kg dry soil. No other observable compound related toxicity effect was reported.

Terrestrial Invertebrates – Metabolites

In a 14 day acute toxicity study, earthworms (*E. fetida*, MRID 47267519) were exposed to CL 377160 at nominal concentrations of 0, 198, 296, 444, 667, and 1000 mg CL 377160/kg dry weight of artificial soil. No concurrent reference chemical test was conducted in this study, however, the facility conducts annual test with 2-chloroacetamide in a concentration range of 0 – 30 mg/kg dw of soil. The experiment was carried out in accordance with OECD 207. There were no observable compound related toxicity effects. No sub-lethal toxicity, specifically body weight loss, was observed. Calculations using the mean body weight for each treatment against the mean initial body weight (440 mg each) showed that there were body weight gains of 3.3%, 5.3%, 7.7%, 10.5%, 4.6%, and 2.7% for the treatments of 0, 198, 296, 444, 667, 1000 mg/kg dry soil weight. No other toxicity effect was reported. In addition, no earthworm mortality was observed in the water control or in test substance treatments. The 14 day LC_{50} was > 1000 mg/kg dw of soil. The 14 day NOEC, based on mortality and body weight, was 1000 mg/kg dw of soil, the highest concentration tested. The CL 377160 is considered to be non-toxic to earthworms up to a concentration of 1000 mg/kg dw of soil based on this study. However, a freeze storage stability study submitted by the applicant (PMRA # 1620213) showed that CL 377160 rapidly bound and degraded in soils even at – 5 ºC. Therefore, the LC_{50} obtained from this study is uncertain and the actual LC_{50} could be lower.

Terrestrial Invertebrates – Formulations

The terrestrial invertebrate study on honey bees (*A. mellifera*, MRID 47267606) classified as supplemental. The study uses a formulation to test toxicity. For honeybee acute contact toxicity studies, a TGAI test compound is required. Relative to a similar honeybee acute contact toxicity test (MRID 47267508), which shows no mortality at the treatment level (100 µg a.i./bee), this formulation appears to be more toxic at a lower nominal concentration (100 µg product./bee ≈ 24 µg a.i./bee) having 24% mortality. This information suggests that there is something in the formulation that is more toxic than the active ingredient acting alone. Finally, the concentrations of test substance in the dosing solutions were not determined. Therefore, the actual dose levels used are unknown.

The contact test had 2% mortality in the negative and solvent controls and 24% mortality in the 100 µg a.i./bee level by 48 hours; sub-lethal effects were 0% in the negative and solvent controls, and 10% in the 100 µg a.i./bee level only at 4 hours but not thereafter. The oral test also had 2% mortality in the negative control, 0% mortality in the solvent control, and 4% mortality in the 113.4 µg a.i./bee level by 48 hours; sub-lethal effects were 0% in the negative control, 2% in the solvent control, and 0% in the 113.4 µg a.i./bee level.

In a 14 day acute limit toxicity study, earthworms (*E. fetida*, MRID 47267608) were exposed to AC 375839 300 g/L SC RLF12359 (SF10358) at 1000 mg formulation/kg dry weight of artificial soil. No concurrent reference chemical test was conducted in this study. The report indicated that the experiment was carried out in accordance with OECD 207. However, only one concentration was tested which did not fulfill the requirement of the guideline. No earthworm mortality was observed in the water control or in test substance treatment. The 14 day LC_{50} was > 1000 mg formulation/kg dry soil. No significant difference in earthworm burrowing time (*i.e.,* time needed for 10 earthworms to burrow into the soil after placement on soil surface) was observed at day 7. The average weight loss between Day 0 and Day 14 was 20.6 and 87.9 mg in the test groups of water control and test substance treatment, respectively. The difference is statistically significant. The 14 day NOEC, based on body weight loss, was < 1000 mg/kg dry soil, the concentration tested. The end-point toxicity concentration of the test substance can not be determined from this study; however, it is considered to be non-lethal to earthworms up to a concentration of 1000 mg formulation/kg dry soil. No other observable compound related toxicity effect was reported.

Table 26. Terrestrial Invertebrate Acute/Subacute Toxicity Data

Common Name	%AI	Study parameters	LD_{50} /NOAEL	MRID	Classification /Category
Technical AC 375839 (a.k.a. BAS 560F)					
Honey bees *Apis Mellifera*	95.86	48 hour acute contact and oral toxicity tests 5 reps. / 10 bees per rep. Contact: 0 (negative, solvent), 100 µg a.i./bee; Oral: 0 (negative, solvent), 114 µg a.i./bee	48 hour <u>contact</u> LD_{50} > 100 µg ai/bee NOAEC: 100 µg a.i./bee LOAEC > 100 µg a.i./bee 48 hour <u>oral</u> LC_{50} > 114 µg ai/bee NOAEC: 114 µg a.i./bee LOAEC > 114 µg a.i./bee	47267508	Acceptable/ Practically non-toxic[1]
Earthworm *Eisenia fetida*	95.86	14 day acute limit toxicity 4 reps (treatment); 1 rep (control) / 10 earthworms per rep. Nominal: 1,000 mg a.i./kg dry soil	14 day LC_{50} >1,000 mg a.i./kg dry soil NOAEC < 1,000 mg a.i./kg dry soil LOAEC = 1,000 mg a.i./kg dry soil Endpoint(s) affected: possibly weight loss	47267518	Supplemental/ Non-GLN[2]

Table 26. Terrestrial Invertebrate Acute/Subacute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LD_{50} /NOAEL**	**MRID**	**Classification /Category**
Metabolites					
Earthworm *Eisenia fetida*	97	CL 377160 (hydrolytic metabolite of metrafenone) 14 day acute toxicity 4 reps (treatment); 1 rep (control) / 10 earthworms per rep. Nominal: 198, 296, 444, 667, and 1000 mg CL 377160/kg dry soil	14-day LC_{50} >1,000 mg CL377160/kg dry soil NOAEC: 1,000 mg CL 377160/kg dry soil LOAEC: 1,000 mg CL 377160/kg dry soil Endpoint(s) affected: none.	47267519	Supplemental/ Non-GLN[2]
Formulations					
Honey bees *Apis mellifera*	24.4% a.i.; 288 g/L	AC 375839 in a 300 g/L SC (SF10358/ RLF12359) 48 hour acute contact and oral toxicity tests 5 reps. / 10 bees per rep. Contact: 0 (negative, solvent), 100 µg test material[3]/bee; Oral: 0 (negative, solvent), 113.4 µg test material/bee	48 hour <u>contact</u> LD_{50} > 100 µg form/bee [>24.4 µg a.i./bee] NOAEC < 100 µg form/bee [<24.4 µg a.i./bee] <u>LOAEC: 100 µg form/bee [24.4 µg a.i./bee]</u> 48 hour <u>oral</u> LC_{50} > 113.4 µg form/bee [>27.7 µg a.i./bee] NOAEC: 113.4 µg form/bee [27.7 µg a.i./bee] LOAEC > 113.4 µg form/bee [> 27.7 µg a.i./bee]	47267606	Supplemental/ Practically non-toxic[1]

Table 26. Terrestrial Invertebrate Acute/Subacute Toxicity Data					
Common Name	**%AI**	**Study parameters**	**LD_{50} /NOAEL**	**MRID**	**Classification /Category**
Earthworm *Eisenia fetida*	288 g a.i./L	AC 375839 300 g/L SC RLF12359 (SF10358) 14 day acute limit toxicity 4 reps (treatment); 1 rep (control) / 10 earthworms per rep. Nominal: 1,000 mg form/kg dry soil	14 day LC_{50} >1,000 mg form[4]/kg dry soil NOAEC < 1,000 mg form/kg dry soil LOAEC < 1,000 mg form/kg dry soil Endpoint(s) affected: body weight (*i.e.*, weight loss)	47267608	Supplemental/ Non-GLN[2]

[1] Based on acute contact LD_{50} (µg a.i./bee) <2 highly toxic; 2-10.99 moderately toxic; ≥11 practically non-toxic
[2] Deemed acceptable by PMRA
[3] 'Test material' is assumed to mean 'formulation'
[4] Not enough information provided in the study to determine active ingredient content

(2) Chronic Effects

Birds - Technical

The one-generation reproductive toxicity study (MRID 47267506) using 20 pairs per level of 12.5-month old Northern bobwhite quail (*Colinus virginianus*) over 22 weeks. BAS 560F was administered to the birds in the diet at nominal concentrations of 0 (control), 185, 500, or 1350 mg ai/kg diet. Mean-measured concentrations were <3.75 (<LOD, control), 181, 486, and 1320 mg ai/kg diet, respectively. No treatment-related effects were observed on any reproductive parameters at any concentration level. There was, however, a significant reduction (p=0.02, 43%) in adult male body weight gain at the 486 mg ai/kg diet level. While this response was not dose-dependent, similar effects (non-dose-dependent) on bird body weights were observed in the acute avian dietary studies with this test material. The lack of dose response in this case indicates that both the NOAEC and LOAEC will be ≥1320 mg a.i./kg diet.

The one-generation reproductive toxicity study (MRID 47267507) used 16 pairs per level of *ca.* 5-month old mallard duck (*Anas platyrhynchos*) over 22 weeks. BAS 560 F was administered to the birds in the diet at nominal concentrations of 0 (control), 450, 900, or 1350 mg ai/kg diet. Mean-measured concentrations were <18.6 (<LOQ, control), 438, 848, and 1316 mg ai/kg diet, respectively. No treatment-related effects were observed on any adult parameter at any treatment level, or on any offspring parameter at the 438 and 848 mg ai/kg diet levels. At the 1316 mg ai/kg diet level, a statistically-significant reduction in the number of eggs laid per hen per week was observed compared to the control (3.3 versus 4.5 eggs/hen/week). Hatchability was also reduced at the 1316 mg

ai/kg level, where the percentage of chicks "dead-in-shell" of fertile eggs increased from 15.7% for the control level to 36.8% for the 1316 mg ai/kg diet level. As a direct result, the percentage of hatched chicks of fertile eggs was also statistically-different from the control (57.0 versus 77.0%). The study was deemed supplemental on account of several guideline deviations including lack of reporting for pre-test mortality, the initial age of the birds was below (*ca.* 5 months) recommended age (at least 7 months), and cage size was significantly smaller than recommended (OPPTS recommends at least 10,000 cm^2 per bird; instead, the floor space was only 4225 cm^2 per bird).

Table 27. Avian Chronic Toxicity Data

Common Name	%AI	Study Parameters	NOAEC/LOAEC	MRID	Classification
Technical AC 375839 (a.k.a. BAS 560F)					
Northern Bobwhite Quail *Colinus virginianus*	95.86	1-generation reproduction study Pre-laying exposure 10 weeks; egg laying exposure 12 weeks 2 birds per pen (1 ♂: 1♀); 20 pens per neg. control and treatment Mean measured: <3.75 (neg. control), 181, 486, and 1320 mg a.i./kg diet	NOAEC ≥ 1320 mg a.i./kg diet LOAEC ≥ 1320 mg a.i./kg diet Endpoint(s) affected: none.	47267506	Acceptable
Mallard Duck *Anas platyrhynchos*	99.4	1-generation reproduction study Pre-laying exposure 10 weeks; egg laying exposure 12 weeks 2 birds per pen (1 ♂: 1♀); 20 pens per neg. control and treatment Mean measured: <18.6 (neg. control), 438, 848, and 1316 mg a.i./kg diet	NOAEC: = **848 mg a.i./kg diet**[1] LOAEC: 1316 mg a.i./kg diet Endpoint(s) affected: egg production (eggs laid per ♀ per wk) and hatchability (% dead-in-shell of fertile eggs)	47267507	Supplemental

[1] **Bold** value is the value that will be used to calculate risk quotients

Mammals - Technical

In a rat 2-generation reproduction study (MRIDs 46415729, 46415728) and given the parental animals, no treatment-related effects were observed on mortality, clinical signs of toxicity, or macroscopic examinations. The LOAEL for parental toxicity is 1000 ppm (equivalent to 72.8/84.8 mg/kg bw/day [M/F]), based on decreased body weights and body weight gains in the F_1 males. The NOAEL is 500 ppm (equivalent to 35.9/42.9 mg/kg bw/day [M/F]). In the offspring, no effects of treatment were observed on clinical signs of toxicity, litter parameters, sexual maturation, anogenital distance, hematology, or macroscopic or microscopic pathology. The LOAEL for offspring toxicity is 10,000 ppm (equivalent to 759/864 mg/kg bw/day [M/F]), based on decreased body weights in the F_1 and F_2 pups. The NOAEL is 1000 ppm (equivalent to 72.8/84.8 mg/kg bw/day [M/F]).

No effects of treatment were observed on estrous cycle number or length, sperm parameters, primordial follicle count, or reproductive performance. Thus, the LOAEL for reproductive performance was not observed. The NOAEL for reproductive performance is 10,000 ppm (equivalent to 759/864 mg/kg bw/day [M/F]).

In a rat developmental toxicity study (MRID 46415726), no treatment-related effects were observed on mortalities, clinical signs, body weights, body weight gains, food consumption, hematology, liver weights, liver histology, or gross pathology relative to maternal toxicity. Therefore, the maternal LOAEL was not observed. The maternal NOAEL is 1000 mg/kg/day (limit dose). Regarding developmental toxicity: there were no treatment-related effects on the numbers of litters, fetuses (live or dead), resorptions (early, late, or complete litter) or on sex ratio or post-implantation loss, on fetal body weights or on skeletal ossification, indicating no effect on fetal growth or development; there were no treatment-related external, visceral, or skeletal variations on development or other malformations. Thus, the developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

In a rabbit developmental toxicity study (MRID 46415727), no treatment-related effect was observed on mortalities, hematology, or gross pathology relative to maternal toxicity. Therefore, the maternal LOAEL is 350 mg/kg/day, based on decreased body weight gains and food consumption; increased liver weights; and increased incidences and/or severity of periportal hepatocellular hypertrophy and diffuse hepatocellular vacuolation. The maternal NOAEL is 50 mg/kg/day. Regarding developmental toxicity: there were no treatment-related effects on the numbers of litters, fetuses (live or dead), resorptions (early, late, or complete litter) or on sex ratio or post-implantation loss, on fetal body weights or on skeletal ossification, indicating no effect on fetal growth or development; there were no treatment-related external, visceral, or skeletal variations on development or other malformations. Thus, the developmental LOAEL was not observed. The developmental NOAEL is 700 mg/kg/day.

Table 28. Mammalian Chronic Toxicity Data					
Common Name	**%AI**	**Study Parameters**	**NOAEC/ LOAEC**	**MRID**	**Classification/ Category**
Technical AC 375839 (a.k.a. BAS 560F)					
Rat	95.86	2-generation reproduction study 30 CD Sprague-Dawley rats/sex/group/generation, **by feeding (diet)**. 3 treatment groups; 1 untreated diet control group Nominal: 0, **500**[1], 1000, 10000 ppm M: 0, 35.9, 72.8, 759 mg BAS 560 F/kg/day F: 0, 42.9, 84.8, 864 mg BAS 560 F/kg/day	Parental systemic NOAEL (M/F): **35.9**/42.9 **mg/kg bw/day**[1] LOAEL (M/F): 72.8/84.8 mg/kg bw/day, based on decreased body wts and body wt gain F_1 males as well as body wts in F_1 and F_2 females Offspring/Develop-mental Toxicity NOAEL (M/F): 72.8/84.8 mg/kg bw/day LOAEL (M/F): 759/864 mg/kg bw/day, based on decreased body wts in F_1 and F_2 pups Reproductive Toxicity NOAEL (M/F): 759/864 mg/kg bw/day LOAEL (M/F): not attained	46415729 46415728	Acceptable / Guideline
Rat	95.86	Developmental toxicity study 25 rats per dose, **by gavage**. 3 treatment groups; 1 untreated control group 0, 50, 500, 1000 mg BAS 560 F/kg/day	*Maternal* NOAEL: 1000 mg/kg/day (HDT; limit dose) LOAEL: not attained *Developmental* NOAEL: 1000 mg/kg/day (HDT; limit dose) LOAEL: not attained	46415726	Acceptable/ Guideline

Table 28. Mammalian Chronic Toxicity Data					
Common Name	**%AI**	**Study Parameters**	**NOAEC/ LOAEC**	**MRID**	**Classification/ Category**
Rabbit	95.86	Developmental toxicity study 25 rabbits per group, **by gavage**. 3 treatment groups; 1 untreated control group 0, 50, 350, 700 mg BAS 560 F/kg/day	*Maternal* NOAEL: 50 mg/kg/day LOAEL: 350 mg/kg/day, based on decreased body wt gains and food consumption; increased liver wts; increased incidences and/or severity of periportal hepatocellular hypertrophy and diffuse hepatocellular vacuolation *Developmental* NOAEL: 700 mg/kg/day (HDT) LOAEL: not attained	46415727	Acceptable/ Guideline

[1] **Bold** value is the value that will be used to calculate risk quotients

b. Terrestrial Plants

The two tier I terrestrial plant studies, seedling emergence (MRID 47267509) and vegetative vigor (MRID 47267510), are both supplemental and are based on the UK/EU formulation (BAS 560 00F, 42.8% purity). The lower application rates in the studies (0.091 and 0.288 lbs a.i./A for the seedling emergence study; and, 0.091 and 0.285 lbs a.i./A for all but soybean [0.099 and 0.283 lbs a.i./A] for the vegetative vigor study) relative to the label application rate (0.3 lbs a.i./A) leads to uncertainty in the risk characterization especially as effects were noted, but the NOAEC and EC_{05} are undefined. The latter two endpoints are used for endangered species risk calculations, which cannot be done in this case. Tier II tests are requested to define the latter endpoints, to have a study available that is based on the U.S. formulation, and, subsequently, reduce uncertainty in risk characterization.

All species were not significantly affected by the two treatments in the seedling emergence study. The most sensitive monocot and dicot species could not be determined. The NOAEC for all species (monocot and dicot[5]) was 0.288 lbs ai/A. The EC_{05}, EC_{25} >0.288 lbs a.i./A. For the highest treatment level tested, the following effects were noted -- the % inhibition relative to control that is greater than 5% was observed in the following plants for the given endpoint: emergence (oat, tomato); survival (oat, onion, tomato), dry weight (onion, ryegrass), and height (cucumber, onion, soybean, tomato).

All species were not significantly affected by the two treatments in the vegetative vigor study. The most sensitive monocot and dicot species could not be determined. The NOAEC for all monocot species was 0.285 lbs ai/A. The EC_{05}, EC_{25} >0.285 lbs a.i./A. The NOAEC for all dicot species was the same as that for the monocots except for the soybean[6] which was 0.283 lbs a.i./A (with the EC_{05} and EC_{25} > 0.283 lbs a.i./A). For the highest treatment level tested, the following effects were noted -- the % inhibition relative to control that is greater than 5% was observed in the following plants for the given endpoint: dry weight (ryegrass) and height (none).

[5] Monocots include corn, *Zea mays*; oat, *Avena sativa*; onion, *Allium cepa*; and ryegrass, *Lolium Perenne*). Dicots include cucumber, *Cucumis sativa*; lettuce, *Lactuca sativa*; oilseed rape, *Brassica napus*; soybean, *Glycine max*; sugarbeet, *Beta vulgaris*; and tomato, *Lycopersicon esculentum*.

[6] Soybean was treated with the test substance on a different day than the other species, and the concentration of metrafenone in the test substance was analyzed on the same day, thus soybean had a different measured concentration than the other test species.

IV. Risk Characterization

A. Risk Estimation –Integration of Exposure and Effects Data

A quantitative estimation of risk integrates EECs and toxicity estimates and evaluates the likelihood of adverse ecological effects to non-target species. In a deterministic approach, an exposure estimate is divided by a single point estimate of toxicity to calculate a risk quotient (RQ). The RQ is then compared to Agency Levels of Concern (LOCs, **Appendix H**), which serve as criteria for categorizing potential risk to non-target organisms and the need to consider regulatory action.

1. Risk to Aquatic Animals and Plants

The greatest amount of uncertainty in the assessment stems from aquatic studies which were largely based on total concentrations (both dissolved and undissolved) of the test compound. In all cases (except for the chronic study on the saltwater mysid, but including aquatic plants), the risk quotient values were not calculated.

a. Aquatic Animals

1. *Risk following acute exposure*

Freshwater Fish and Aquatic-Phase Amphibians

The acute aquatic risk quotients (RQs) for freshwater fish and aquatic-phase amphibians were not calculated. Test compound in solution was not centrifuged and measured (post-centrifugation) in any of the acute freshwater fish studies (on the technical, metabolites, and formulations), even though higher concentrations tested exceeded or likely exceeded the solubility limit of the test compound. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Uncertainty in the level of dissolved test compound in the solution for all studies renders calculated endpoints suitable for qualitative use only.

Freshwater Invertebrates

The acute aquatic risk quotients (RQs) for freshwater invertebrates were not calculated. Test compound in solution was not centrifuged and measured (post-centrifugation) in any of the acute freshwater invertebrate studies (on the technical, metabolites, and formulations), even though higher concentrations tested exceeded or likely exceeded the solubility limit of the test compound. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Uncertainty in the level of

dissolved test compound in the solution for all studies renders calculated endpoints suitable for qualitative use only.

Marine/Estuarine Fish

The study on the technical active ingredient (MRID 47267446) yielded an LC_{50} greater than the highest concentration tested because there were no mortalities and no sublethal effects; therefore, RQs are not reported.

Marine/Estuarine Invertebrates

The acute aquatic risk quotients (RQs) for marine/estuarine invertebrates were not calculated. Test compound in solution was not consistently centrifuged and measured (post-centrifugation) in any of the acute marine/estuarine invertebrate studies (on the technical), even though higher concentrations tested exceeded or likely exceeded the solubility limit of the test compound. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Uncertainty in the level of dissolved test compound in the solution for all studies renders calculated endpoints suitable for qualitative use only.

2. *Risk following chronic exposure*

Freshwater Fish and Aquatic-Phase Amphibians

The chronic aquatic risk quotients (RQs) for freshwater fish and aquatic-phase amphibians were not calculated. Test compound in solution was not centrifuged and measured (post-centrifugation) in the chronic freshwater fish study (on the technical), even though higher concentrations tested exceeded or likely exceeded the solubility limit of the test compound. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Uncertainty in the level of dissolved test compound in the solution for this study renders calculated endpoints suitable for qualitative use only.

Freshwater Invertebrates

No acceptable guideline and non-guideline chronic studies on freshwater invertebrates are available. Therefore, a quantitative estimation of risk cannot be conducted. Chronic risk to freshwater invertebrates cannot be precluded.

Marine/Estuarine Fish

No chronic studies on marine/estuarine fish are available. Therefore, a quantitative estimation of risk cannot be conducted. Chronic risk to marine/estuarine fish cannot be precluded.

Marine/Estuarine Invertebrates

The chronic aquatic LOC was not exceeded for the proposed use of metrafenone for the technical tested. **Table 29** summarizes the RQ values and scenarios used to compare to chronic aquatic LOCs for marine invertebrates.

Table 29. Metrafenone: Chronic Risks to Marine/Estuarine Invertebrates (Application Rate 0.3 lbs a.i./A, 6 Applications/Year)

Species	Toxicity Endpoint (µg/L)	Scenario	21-Day EEC (µg/L)	Chronic Risk Quotient[1]	Levels of Concern Exceeded[2]
Saltwater mysid *Americamysis bahia*	NOAEC = 22 µg a.i./L Technical	Grapes (Metrafenone)			
		NY Grapes	8.82	0.40	No
		CA Grapes	1.10	0.05	No
Saltwater mysid *Americamysis bahia*	NOAEC = 22 µg a.i./L Technical	Grapes (Total Metrafenone Residues)			
		NY Grapes	17.67	0.80	No
		CA Grapes	1.89	0.09	No

[1] Chronic Risk Quotients are calculated using the following formula: EEC/NOAEC
[2] Chronic LOC for marine/estuarine invertebrates = 1

b. Aquatic Plants

The aquatic plant risk quotients (RQs) were not calculated. Test compound in solution was not centrifuged and measured (post-centrifugation) in these studies (on the technical, metabolites, and formulations), even though higher concentrations tested exceeded or likely exceeded the solubility limit of the test compound. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. Uncertainty in the level of dissolved test compound in the solution for these studies renders calculated endpoints suitable for qualitative use only.

2. Risk to Terrestrial Animals and Plants

a. Terrestrial Animals

To assess risks of metrafenone to non-target birds and mammals, EECs and acute and chronic RQs for residues on various forage categories (short grass, tall grass, broadleaf plants/small insects, fruits/pods/large insects, and seeds) were obtained from the Tier 1 model, T-REX v. 1.4.1 for foliar spray applications to the proposed crops. The model assumes initial concentrations on plant surfaces based on Kenaga predicted maximum residues as modified by Fletcher *et al.* (1994), and assumes first-order dissipation. In this case, six applications at 0.3 lbs a.i./A were used.

For birds, acute RQs are derived using dose-based and dietary-based acute toxicity values. For mammals, acute RQs are derived using a dose-based acute toxicity value, and chronic RQs are derived using a dose-based chronic toxicity value (the test material was administered by gavage) and a dietary-based chronic toxicity value using the standard FDA laboratory rat conversion value provided in the T-REX model. Dietary-based RQs are calculated using EECs expressed in terms of residue concentration for the various forage categories and toxicity values (LC_{50} or NOAEC) expressed in units of dietary concentration. Dose-based RQs are calculated using a body weight-adjusted LD_{50} and consumption-weighted equivalent dose sorted by food source and body size. For both birds and mammals, three weight categories (or sizes) are considered.

1. *Risk following acute exposure*

Birds

The acute oral and dietary endpoints are both greater than the highest concentrations tested (>2025 mg a.i./kg bw and >6070 mg a.i./kg diet, respectively). There were no mortalities or treatment related clinical signs of toxicity in the acute oral studies; one death in each dietary study was observed but was not considered treatment related. Therefore, RQ values are not reported. Further discussion will be provided in the risk description.

Potential risk to piscivorous birds

The potential risk to piscivorous birds considers exposure via consumption of fish contaminated with metrafenone total residues. However, the acute oral and dietary endpoints are both greater than the highest concentrations tested (>2025 mg a.i./kg bw and >6070 mg a.i./kg diet, respectively). There were no mortalities or treatment related clinical signs of toxicity in the acute oral studies; one death in each dietary study was observed but was not considered treatment related. Therefore, RQ values are not reported. Further discussion will be provided in the risk description.

Mammals

The acute endpoints for mammals are both greater than the highest concentrations tested (LD_{50}: >2000 mg technical/kg bw, mouse; >5000 mg form/kg bw, rat). There were no mortalities or treatment related clinical signs of toxicity in the acute oral rat study; one death was observed in the acute oral mouse study. Therefore, RQ values are not reported. Further discussion will be provided in the risk description.

Potential risk to piscivorous mammals

The potential risk to piscivorous mammals considers exposure via consumption of fish contaminated with metrafenone total residues. However, the mammalian acute endpoints

are both greater than the highest concentrations tested (LD_{50}: >2000 mg technical/kg bw, mouse; >5000 mg form/kg bw, rat). A male mouse died in the acute oral mouse study (MRID 47267522) on the technical; there were no mortalities in the acute oral rat study (MRID 47267609) on the formulation. Therefore, RQ values are not reported. Further discussion will be provided in the risk description.

Terrestrial invertebrates

Metrafenone is classified as 'practically non-toxic' to honey bees on an acute contact basis, based on available data for the TGAI and formulation (24.4% a.i.). Given a non-guideline acute earthworm study using the technical active ingredient, metrafenone is considered to be non-lethal to earthworms up to a concentration of 1000 mg a.i./kg dry soil. Similarly, given a non-guideline acute earthworm study using a metrafenone metabolite, the metabolite too is considered to be non-lethal to earthworms up to a concentration of 1000 mg/kg dry weight of soil. In addition, given a non-guideline acute earthworm study using a formulation, the formulation is also considered to be non-lethal to earthworms up to a concentration of 1000 mg formulation/kg dry soil. Additional discussion is provided in the risk description section.

2. *Risk following chronic exposure*

Birds

Utilizing the chronic endpoint (848 mg a.i./kg diet) from a 1-generation reproduction study (MRID 47267507) conducted with mallard duck and the T-REX model v.1.4.1, the chronic avian dietary-based RQs do not exceeded the chronic LOC for birds for any food category.

Table 30. Upper Bound Kenaga, Chronic Avian Dietary Based Risk Quotients
Grapes: 0.3 lbs a.i./A; 6 Applications/season

NOAEC (ppm)	EECs and RQs[1,2,3]							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
181	241.01	0.28	110.46	0.13	135.57	0.16	15.06	0.02

[1] Risk Quotients are calculated using the following formula: EEC / NOAEC
[2] Chronic risk LOC = 1
[3] Based on avian chronic NOAEC = 848 mg a.i./kg diet

Potential risk to piscivorous birds

The potential risk to piscivorous birds considers exposure via consumption of fish contaminated with metrafenone total residues. None of the RQs exceed the chronic LOC for birds. The following table provides estimated RQs from KABAM using the maximum application rate and the scenario yielding the highest aquatic EECs (New York Grapes scenario; metrafenone total residue and high runoff scenario).

Table 31. Chronic RQ values for Birds Consuming Fish Contaminated by Metrafenone (based on KABAM)[1]

Wildlife Species	Dose Based	Dietary Based[2,3]
sandpipers	N/A	0.016
cranes	N/A	0.016
rails	N/A	0.018
herons	N/A	0.019
small osprey	N/A	0.022
white pelican	N/A	0.023

N/A = Not applicable
[1] NY Grapes scenario (at 0.3 lbs a.i./A with 6 applications/year)
[2] Based on avian chronic NOAEC = 848 mg a.i./kg diet
[3] LOC for chronic risk = 1.0

Mammals

The chronic LOC is exceeded on a dose basis for mammals in all size classes eating short grass, for the 15 and 35 gram size classes eating tall grass and broadleaf plants / small insects. The chronic LOC on a dietary basis is not exceeded for any of the food categories. Risks to mammals following chronic exposure will be further discussed in the risk description section.

Table 32. Upper Bound Kenaga, Chronic Mammalian Dietary Based Risk Quotients Grapes: 0.3 lbs a.i./A; 6 Applications/season

NOAEC (ppm)	EECs and RQs[1,2,3]							
	Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects	
	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
500	241.01	0.48	110.46	0.22	135.57	0.27	15.06	0.03

[1] Risk Quotients are calculated using the following formula: EEC / NOAEC
[2] Chronic risk LOC = 1
[3] Based on mammalian chronic dietary NOAEL: 500 mg/kg diet

Table 33. Upper Bound Kenaga, Chronic Mammalian Dose-Based Risk Quotients											
Size Class (grams)	Adjusted NOAEL	EECs and RQs[1,2,3]									
		Short Grass		Tall Grass		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Granivore	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
15	78.90	229.78	**2.91**[4]	105.32	**1.33**	129.25	**1.64**	14.36	0.18	3.19	0.04
35	63.84	158.81	**2.49**	72.79	**1.14**	89.33	**1.40**	9.93	0.16	2.21	0.03
1000	27.61	36.82	**1.33**	16.88	0.61	20.71	0.75	2.30	0.08	0.51	0.02

[1] Risk Quotients are calculated using the following formula: EEC / NOAEC
[2] Chronic risk LOC = 1
[3] Based on mammalian chronic dose-based NOAEL: 35.9 mg/kg bw/day
[4] **Bolded** values exceed LOC

Potential risk to piscivorous mammals

The potential risk to piscivorous mammals considers exposure via consumption of fish contaminated with metrafenone total residues. None of the RQs exceed the chronic LOC for mammals. The following table provides estimated RQs from KABAM using the maximum application rate and the scenario yielding the highest aquatic EECs (New York Grapes scenario; metrafenone total residue and high runoff scenario).

Table 34. Chronic RQ Values for Mammals Consuming Fish Contaminated by Metrafenone (based on KABAM) [1]		
Wildlife Species	**Dose Based[2,3]**	**Dietary Based[2,3]**
fog/water shrew	0.103	0.019
rice rat/star-nosed mole	0.125	0.018
small mink	0.159	0.025
large mink	0.176	0.025
small river otter	0.189	0.025
large river otter	0.219	0.027

[1] NY Grapes scenario (at 0.3 lbs a.i./A with 6 applications/year)
[2] Based on mammalian chronic NOAEC = 35.9 mg/kg bw/day
[3] LOC for chronic risk = 1.0

b. Terrestrial Plants

Terrestrial plant risk quotients were not calculated on account of indeterminate endpoints generated in both the seedling emergence and vegetative vigor studies.

B. Risk Description

Based on the available ecotoxicity data and predicted environmental exposures, this ecological risk assessment supports the presumption of risk to mammals following chronic exposure.

1. Risk to Aquatic Animals and Plants

a. Aquatic Animals

1. *Risk following acute exposure*

Freshwater Fish and Aquatic-Phase Amphibians

Technical
The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12^{o}C. The acute rainbow trout study (MRID 47267443) indicated only 5% mortality at levels below (0.065 mg total a.i./L) and above (0.43 and 0.82 mg total a.i./L) the solubility limit, which implies the effect may not be dose related; 16% of fish were lethargic and 5% exhibited dark discoloration after 96 hours only at the highest concentration tested (0.82 mg total a.i./L). The acute bluegill sunfish study (MRID 47267442) indicated 15% mortality, 18% lethargic fish, and 12% virtually immobile but respiring fish at the highest concentration tested (0.87 mg total a.i./L) after 96 hours. Given that the test solution was not centrifuged, the amount of actual dissolved active ingredient potentially leading to the observed effects is unknown. Nevertheless, given these studies and assuming that metrafenone concentrations in the environment reach the solubility limit, the effect of the technical grade active ingredient on freshwater fish is likely to be low. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Therefore, acute risk to freshwater fish and aquatic-phase amphibians is not expected as a result of metrafenone use on grapes given the results from the studies using technical grade active ingredient.

Metabolites/Degradates
The limits of water solubility for metabolites of metrafenone were not reported in the available studies; exceedance of solubility limit for a given metabolite (Reg. No. 4084564) was assumed given observed undissolved test substance in all five treatment groups of one acute study (MRID 47267445). The acute rainbow trout study (MRID 47267444) was a limit test with no effects noted at 99 mg total a.i./L. Another acute

rainbow trout study (MRID 47267445) indicated 80% mortality at 20.3 mg total a.i./L and 100% mortality at 35.2 and 58.4 mg total a.i./L; sublethal effects were observed in the three highest concentrations: 20.3 mg total a.i./L (tottering, apathy, and distended abdomen), 35.2 mg total a.i./L (apathy and distended abdomen), and 58.4 mg total a.i./L (tottering and distended abdomen). Given that the test solution was not centrifuged, the amount of actual dissolved metabolite potentially leading to the observed effects is unknown. According to the model estimated EECs (0.00225 - 0.02 mg/L, which includes total metrafenone residue scenarios), levels of metrafenone metabolites at the tested levels are not expected to occur in the environment given the proposed grape use. Therefore, the effect of metrafenone metabolites on freshwater fish is likely to be low.

Formulations
The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12^{o}C. The acute study (MRID 47267605) using a formulation (BAS 560 00F, SF 10358, RLF 12359) indicated no effects on rainbow trout at concentrations above the solubility limit, from 3.0 to 23.3 mg total a.i./L. Given that the test solution was not centrifuged, the amount of actual dissolved active ingredient is unknown. Not unlike the conclusion drawn for the technical active ingredient, given this study and assuming that metrafenone concentrations in the environment reach the solubility limit, the effect of this particular formulation on freshwater fish is likely to be low. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Although this EU/UK formulation closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on freshwater fish and aquatic-phase amphibians is not known.

Freshwater Invertebrates

Technical
The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12^{o}C. The acute study (MRID 47267437), in which the three highest concentrations (0.22, 0.45, and 0.92 mg total a.i./L) were at or above the solubility limit, indicated no effects on daphnia. Given that the test solution was not centrifuged, the amount of actual dissolved active ingredient is unknown. Nevertheless, given this study and assuming that metrafenone concentrations in the environment reach the solubility limit, the effect of the technical grade active ingredient on freshwater invertebrates is likely to be low. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Therefore, acute risk to freshwater invertebrates is not expected as a result of metrafenone use on grapes given the results from the studies using technical grade active ingredient.

Metabolites/Degradates
The limits of water solubility for metabolites of metrafenone were not reported in the available studies. The acute daphnia study (MRID 47267438) with CL 375816 indicated no effects. Another acute daphnia study (MRID 47267439) indicated 5, 55, and 65% immobility at the three highest concentrations 23.2, 49.6, and 66.4 mg total a.i./L, respectively, after 48 hours (test termination). Given that the test solution was not centrifuged, the amount of actual dissolved metabolite potentially leading to the observed effects is unknown. According to the model estimated EECs (0.00225 - 0.02 mg/L, which includes total metrafenone residue scenarios), levels of metrafenone metabolites at the tested levels are not expected to occur in the environment given the proposed grape use. Therefore, the effect of metrafenone metabolites on freshwater invertebrates is likely to be low.

Formulations
The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12^{o}C. The acute study (MRID 47267604) using a formulation (BAS 560 00F, SF 10358, RLF 12359) indicated 65 and 90% mortality at the two highest concentrations 6.0 and 11.6 mg total a.i./L, respectively, at 48 hours (test termination); 5% immobility was observed in the negative control, 0.76 and 1.5 mg total a.i./L concentrations, 15% at 3.0 mg total a.i./L, and 25% at 6.0 mg total a.i./L. Given that the test solution was not centrifuged, the amount of actual dissolved active ingredient potentially leading to the observed effects is unknown. Not unlike the conclusion drawn for the technical active ingredient, given this study and assuming that metrafenone concentrations in the environment reach the solubility limit, the effect of this particular formulation on freshwater invertebrates is potentially low. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Although this EU/UK formulation closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on freshwater invertebrates is not known.

Marine/Estuarine Fish

The study on the technical active ingredient (MRID 47267446) yielded an LC_{50} greater than the highest concentration tested because there were no mortalities and no sublethal effects; therefore, RQs were not reported. Comparison of the peak aquatic EECs (0.02 mg/L, taken from the total metrafenone residue scenario) with the highest concentration tested (0.65 mg a.i./L uncentrifuged; 0.35 mg a.i./L centrifuged) shows that the EECs were at least 17 times less than the highest concentrations tested in the studies. Therefore, acute risk to marine/estuarine fish is not expected as a result of metrafenone use on grapes.

Marine/Estuarine Invertebrates

The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12°C. The acute eastern oyster study (MRID 47267440) indicated effects on shell deposition, whereby relative to the negative control the mean percent reduction in shell growth starting with the negative solvent (then, 0.0522, 0.104, 0.203, 0.287 mg total a.i./L, and 0.33 mg dissolved a.i./L) is as follows: 17.4, 13.9, 26.3, 44.3, 84.2, and 100%, respectively. Therefore, should concentrations in the environment reach the solubility limit, the acute risk to marine/estuarine invertebrates may be expected. However, given that the test solution was not centrifuged for all but the highest test concentration, the amount of actual dissolved active ingredient potentially leading to the observed effects is unknown. Meaning that interpretation of effects from total concentrations cited here may underestimate potential risk. Similarly, the acute saltwater mysid study (MRID 47267441) indicated a dose related effect, this time on mortality at the four out of five highest concentrations: 5% at 0.129 and 0.240 mg total a.i./L, 15% at 0.416 mg total a.i./L, and 95% at 0.663 mg total a.i./L; in addition, erratic swimming was observed in the two highest concentrations by test termination. Despite the dose response, without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined and, again, interpretation of effects from total concentrations cited here may underestimate potential risk. However, according to the model estimated EECs (0.00153 - 0.02 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Given these studies and assuming that metrafenone concentrations in the environment are not likely to reach the solubility limit, the acute risk to marine/estuarine invertebrates is not expected.

2. *Risk following chronic exposure*

Freshwater Fish and Aquatic-Phase Amphibians

The metrafenone limit of water solubility is approximately 0.2-0.5 mg/L at 12°C. The chronic fathead minnow study (MRID 47267449) indicated post-hatch survival (28-days post-hatch) was statistically-reduced compared to the negative control at the three highest concentrations 0.227, 0.421, and 0.839 mg total a.i./L ($p \leq 0.05$). Post-hatch survival averaged 96% at the negative control through 0.118 mg total a.i./L levels, and 87, 86, and 11% at the 0.227, 0.421, and 0.839 mg total ai/L levels, respectively. Therefore, a dose response is evident and implies that should concentrations in the environment reach the solubility limit, the chronic risk to freshwater fish may be expected. No clinical signs of toxicity were observed during the study in any treatment group. Fish length was significantly reduced relative to the average negative control length at the two highest concentrations 0.421 and 0.839 mg total ai/L and wet and dry weight were significantly lower than the negative control weights at the highest concentration (0.839 mg total a.i./L level); however, significant impact on survival occurred at these levels. Given that the test solution was not centrifuged, the amount of actual dissolved active ingredient potentially leading to the observed effects is unknown. For example, the NOAEC value is low (0.118 mg total a.i./L) even though the measured concentration includes dissolved

and undissolved substance, but had the sample been centrifuged – including the dissolved compound only - the value would likely be lower. Therefore, conclusions drawn from these data would lead to an underestimation of potential risk. According to the model estimated EECs (0.00075 - 0.017 mg/L, which includes metrafenone and total metrafenone residue scenarios), levels of metrafenone at the solubility limit are not expected to occur in the environment given the proposed grape use. Assuming that metrafenone concentrations in the environment are not likely to reach the solubility limit or the concentrations used in this study[7], the chronic risk to freshwater fish and aquatic-phase amphibians is not expected.

Freshwater Invertebrates

No acceptable chronic ecotoxicity studies on freshwater invertebrates are available. Chronic risk to freshwater invertebrates cannot be precluded.

Marine/Estuarine Fish

No chronic ecotoxicity studies on estuarine/marine fish have been submitted to the Agency for review. Chronic risk to estuarine/marine fish cannot be precluded.

Marine/Estuarine Invertebrates

The chronic aquatic LOC was not exceeded for the proposed use of metrafenone for the technical active ingredient (MRID 47267448) tested on the marine/estuarine invertebrate (saltwater mysid); no data is available for metabolites or formulation studies on marine/estuarine invertebrates. Although reproduction (number of offspring per female per reproductive day) was the (most sensitive) affected endpoint in this study, yielding a definitive endpoint, the RQ calculations indicate that chronic risk to marine/estuarine invertebrates is not expected as a result of metrafenone use.

b. Aquatic Plants

For all aquatic plant studies, dissolved or soluble concentrations (*i.e.*, post-centrifugation) of test material were not determined. Without centrifugation, the amount of chemical that is freely dissolved and bioavailable cannot be determined. This is especially a problem as some of the testing concentrations were conducted above the solubility limit of the technical grade active ingredient (MRID 47267511, 47267512, 47267513, 47267514, 47267515, technical; MRID 47267607, formulation). In addition, the metabolite solubility limit is unknown which leads to uncertainty in interpreting the measured concentrations in the metabolite studies (MRID 47267516, 47267517). The lowest

[7] The acute eastern oyster study (MRID 47267440) using the technical grade active ingredient indicated a 42% reduction in concentration from uncentrifuged sample to centrifuged sample. If the percentage is applied to the NOAEC value from this study, 0.118 mg total a.i./L, it would reduce to 0.068 mg a.i./L, which is still above the highest model estimated EEC value (0.02 mg/L).

concentration tested among the vascular plants (MRID 47267511, *L. gibba*: 0.057 mg total a.i./L; or, 0.033 mg a.i./L assuming a 42% reduction after hypothetical centrifugation[8]) and non-vascular plants (MRID 47267513, *S. costatum*: 0.0509 mg total a.i./L; or 0.0295 mg a.i./L[9]) is higher than the highest peak aquatic EEC value of 0.02 mg/L (assuming the total metrafenone residue scenario). Therefore, had valid endpoints (*i.e.*, those based on dissolved concentrations) been determined using these studies, they would likely be greater than the highest model predicted concentration in the environment, which implies that risk to vascular and non-vascular species is not expected as a result of metrafenone use on grapes. Although the EU/UK formulation used in one of the studies closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on vascular and non-vascular aquatic plants is not known.

2. Risk to Terrestrial Animals and Plants

a. Terrestrial Animals

1. *Risk following acute exposure*

Birds

The acute oral and dietary endpoints are both greater than the highest concentrations tested (>2025 mg a.i./kg bw and >6070 mg a.i./kg diet, respectively). There were no mortalities or treatment related clinical signs of toxicity in the acute oral studies; one death in each dietary study was observed but was not considered treatment related. As a result, RQ values were not reported. Toxicity characterization implies that the technical is practically non-toxic. In addition, the highest concentration tested for the oral studies (2025 mg a.i./kg bw) is 7 times higher than the highest dose-based EEC (274.49 mg/kg) calculated from T-REX; meanwhile, the highest concentration tested for the dietary studies (6070 mg a.i./kg diet) is 25 times higher than the highest dietary-based EEC (241.01 mg/kg). Therefore, given that comparison, acute risk to birds is not expected as a result of metrafenone use.

Potential risk to piscivorous birds

Several characteristics of metrafenone indicate that it has the potential to accumulate in tissues of aquatic organisms. The log of the *n*-octanol/water partition coefficient for metrafenone is 4.3 (at 25^{o}C, pH 4). The lipid normalized BCF was determined to be between 140 and 530 (see MRID 47267450).

As stated in the risk estimation section, the acute oral and dietary endpoints are both greater than the highest concentrations tested (>2025 mg a.i./kg bw and >6070 mg a.i./kg diet, respectively). There were no mortalities or treatment related clinical signs of toxicity in the acute oral studies; one death in each dietary study was observed but was not considered treatment related. As a result, RQ values were not reported. The highest concentration tested for the oral studies (2025 mg a.i./kg bw) is 147 times higher than the

[8] See eastern oyster study (MRID 47267440)
[9] See eastern oyster study (MRID 47267440)

highest dose-based EEC (13.73 mg/kg bw/day) calculated from KABAM for birds consuming fish contaminated by metrafenone total residues; meanwhile, the highest concentration tested for the dietary studies (6070 mg a.i./kg diet) is 310 times higher than the highest dietary-based EEC (19.59 mg/kg). Therefore, given that comparison, acute risk to piscivorous birds is not expected due to consumption of fish contaminated with metrafenone.

Mammals

There were no mortalities in the acute oral rat study (MRID 47267609) on the formulation; one death was observed in the acute oral mouse study (MRID 47267522) on the technical active ingredient. Although the EU/UK formulation used in the formulation study closely matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on mammals is not known.

The acute endpoints for mammals are both greater than the highest concentrations tested (LD_{50}: >2000 mg technical/kg bw, mouse; >5000 mg form/kg bw, rat). Therefore, RQ values were not reported. In the mouse study, only 3 individuals/sex were tested with mortality in 1/6 animals (17%). With a larger test sample, the LD_{50} could be approached. The toxicity classification implies that the technical is practically non-toxic to mice. In addition, the highest concentration tested for the oral study (2000 mg a.i./kg bw) is 9 times higher than the highest dose-based EEC (229.78 mg/kg) calculated from T-REX. Therefore, given that comparison, acute risk to mammals is not expected as a result of metrafenone use.

Potential risk to piscivorous mammals

Risk to piscivorous mammals via consumption of fish contaminated with metrafenone was assessed because metrafenone has the potential to accumulate in tissues of aquatic organisms.

As stated in the risk estimation section, there were no mortalities in the acute oral rat study (MRID 47267609) on the formulation and there was only one death in the acute oral mouse study (MRID 47267522) on the technical. Although the EU/UK formulation used in the formulation study matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on mammals is not known.

The highest concentration tested for the oral studies (2000 mg a.i./kg bw) is 257 times higher than the highest dose-based EEC (7.795 mg/kg bw/day) calculated from KABAM for mammals consuming fish contaminated by metrafenone total residues. Therefore, given that comparison, acute risk to piscivorous mammals is not expected due to consumption of fish contaminated with metrafenone.

Terrestrial invertebrates

The 48-hour contact LD_{50} is >24.4 µg a.i./bee [>100 µg form/bee] with a NOAEC of <24.4 µg a.i./bee and a LOAEC of 24.4 µg a.i./bee based on mortality. Based on this toxicity data, metrafenone is classified as 'practically non-toxic' to honeybees on an acute contact basis. Thus, risk to honeybees is not expected as a result of direct contact with metrafenone. Similarly, the non-guideline earthworm studies consider metrafenone, its degradate, and a formulation to be non-lethal to earthworms up to a concentration of 1000 mg a.i./kg dry soil, 1000 mg/kg dry weight of soil, and 1000 mg formulation/kg dry soil, respectively. *E. fetida* is found in topsoil at depths of approximately 5-20cm. Given a soil depth of 20 cm and assuming a maximum application rate per season (1.8 lbs a.i./A, which assumes a hypothetical maximum active ingredient loading rate since the seasonal rate would not be applied all at once and likely degrade if applied in the recommended 6 applications of at most 0.3 lbs a.i./A), the EEC is much lower (0.77 mg/kg soil) than the concentrations generated in these studies. At shallower depths (5 cm), the EEC is higher (3.08 mg/kg soil), but still lower than the study concentration (1000 mg a.i./kg soil). Therefore, risk to earthworms is not expected as a result of metrafenone use on grapes. Although the EU/UK formulation used in the formulation studies for the bee and earthworm matches the U.S. formulation (*i.e.*, BAS 560 03F) it is still not its equivalent. Therefore, the effect of the U.S. formulated product on bees (and earthworms) is not known.

2. *Risk following chronic exposure*

Birds

The chronic avian dietary-based RQ does not exceed the chronic LOC for birds for any food catogory. The study (MRID 47267507) was done on mallard duck using the technical grade and yielded a definite endpoint based on egg production (eggs laid per female per week) and hatchability (% dead-in-shell of fertile eggs). Due to the fact that the RQ is only based on one bird species, there is an uncertainty associated with the estimated risk for all avian species. Nevertheless, given that LOCs were not exceeded, chronic risk to birds is not expected as a result of metrafenone use on grapes.

Potential risk to piscivorous birds

As previously stated, risk to piscivorous birds via consumption of fish contaminated with metrafenone was assessed because metrafenone has the potential to accumulate in tissues of aquatic organisms. As stated in the risk estimation section, none of the RQs exceed the chronic LOC for birds. Therefore, chronic risk to piscivorous birds is not expected due to consumption of fish contaminated with metrafenone total residues.

Mammals

The chronic LOC is exceeded on a dose basis for mammals in all size classes eating short grass, for the 15 and 35 gram size classes eating tall grass and broadleaf plants / small insects. The chronic LOC on a dietary basis is not exceeded for any of the food categories. The study (MRID 46415729, 46415728) was done on the rat using the technical grade and yielded a definite endpoint based on decreased body weights and body weight gain in F_1 males as well as body weights in F_1 and F_2 females. Due to the fact that the RQ is only based on one mammalian species, there is an uncertainty associated with the estimated risk for all mammals. Nevertheless, given the LOC exceedance chronic risk to mammals is expected as a result of metrafenone use. To reduce chronic risk to mammals, several components of the application protocol would have to change. For example, in order to have no chronic LOC exceedances (*i.e.*, all RQs < 1) for mammals, the minimum single application rate (0.2 lbs a.i./A) would have to be cut by 25% (*i.e.*, to 0.15 lbs a.i./A) yet considered the maximum instead, the application interval would have to nearly double (from 14 days up to 26 days), and the maximum allowed number of applications would have to be cut from 6 to 5. Alternatively, in order to have no chronic LOC exceedances for mammals, the minimum single application rate (0.2 lbs a.i./A) can still be applied yet considered the maximum instead, but the application interval would have to nearly double (from 14 days up to 26 days), and the maximum number of applications would have to be cut by 50% (*i.e.*, from 6 to 3). Furthermore, in order to have no chronic LOC exceedances in nearly all cases for mammals –the one exception being an exceedance for the 15g size glass consuming short grass where the calculated RQ is 1.02 – the minimum single application rate (0.2 lbs a.i./A) would be changed to the maximum single application rate and the application interval (14 days) could remain as currently prescribed by the label, but the maximum number of applications would have to be reduced from 6 to 2.

Potential risk to piscivorous mammals

As previously stated, risk to piscivorous mammals via consumption of fish contaminated with metrafenone was assessed because metrafenone has the potential to accumulate in tissues of aquatic organisms. As stated in the risk estimation section, none of the RQs exceed the chronic LOC for mammals. Therefore, chronic risk to piscivorous mammals is not expected due to consumption of fish contaminated with metrafenone.

b. Terrestrial Plants

As stated in the risk estimation section, terrestrial plant risk quotients were not calculated on account of indeterminate endpoints generated in both the seedling emergence and vegetative vigor studies. The lower application rates in the studies (0.091 and 0.288 lbs a.i./A for the seedling emergence study; and, 0.091 and 0.285 lbs a.i./A for all but soybean [0.099 and 0.283 lbs a.i./A] for the vegetative vigor study) relative to the label application rate (0.3 lbs a.i./A) leads to uncertainty in the risk characterization especially as effects were noted. However, the effects in both studies are not considered significant and the NOAEC and EC_{05} are undefined (seedling emergence: NOAEC 0.288 lbs a.i./A; EC_{05}, EC_{25} > 0.288 lbs a.i./A; vegetative vigor: NOAEC 0.285 lbs a.i./A; EC_{05}, EC_{25} >

0.285 lbs a.i./A[10]). Consequently, the most sensitive monocot and dicot species could not be determined from either study. Hypothetically, had the NOAEC been determined to be 0.091 lbs a.i./A (the lower concentration tested) in both the vegetative vigor and seedling emergence studies and given the maximum single application rate (0.3 lbs a.i./A), the risk would not have triggered concern for either the dicot or monocot species. In addition, the EECs based on the maximum seasonal application rate (1.8 lbs a.i./A) range from 0.018 – 0.198 lbs a.i./A, which are below the concentrations tested in these studies. Since effects were not significant at the test concentrations, which are higher than the calculated EECs, risk to terrestrial plants is not expected as a result of use of the EU/UK formulation. Both studies were conducted on the EU/UK formulation (BAS 560 00F); therefore, uncertainty exists in determining risk to the U.S. formulation (BAS 560 03F). Tier II tests are requested to better define the toxicity endpoints, to have a study available that is based on the U.S. formulation, and, subsequently, reduce uncertainty in risk characterization.

3. Review of Incident Data

With the proposed use on grapes, metrafenone will be applied in the United States for the first time. Therefore, no incident data are available at this time.

4. Endocrine Effects

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

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

[10] Applicable to all dicot (and monocot) species except for the soybean for which the endpoints are NOAEC 0.283 lbs a.i./A and EC_{05}, EC_{25} > 0.283 lbs a.i./A in the vegetative vigor study only.

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

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

5. Federally Threatened and Endangered (Listed) Species

Section 7 of the Endangered Species Act, 16 U.S.C. Section 1536(a)(2), requires all federal agencies to consult with the National Marine Fisheries Service (NMFS) for marine and anadromous listed species, or the United States Fish and Wildlife Services (FWS) for listed wildlife and freshwater organisms, if they are proposing an "action" that may affect listed species or their designated habitat. Each federal agency is required under the Act to insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. To jeopardize the continued existence of a listed species means "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of the species" (50 C.F.R. § 402.02).

To facilitate compliance with the requirements of the Endangered Species Act (subsection (a)(2)), the Office of Pesticide Programs has established procedures to evaluate whether a proposed registration action may directly or indirectly reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of any listed species (USEPA, 2004). After the Agency's screening level risk assessment is conducted, if any of the Agency's listed species LOCs are exceeded for either direct or indirect effects, an analysis is conducted to determine if any listed or candidate species may co-occur in the area of the proposed pesticide use or areas downstream or downwind that could be contaminated from drift or runoff/erosion. If listed or candidate species may be present in the proposed action areas, further biological assessment is undertaken. The extent to which listed species may be at risk then determines the need for the development of a more comprehensive consultation package as required by the Endangered Species Act.

Both acute endangered species and chronic risk LOCs are considered in the screening-level risk assessment of pesticide risks to listed species. Endangered species acute LOCs are a fraction of the non-endangered species LOCs or, in the case of endangered plants, RQs are derived using lower toxicity endpoints than non-endangered plants. Therefore, concerns regarding listed species within a taxonomic group are triggered in exposure situations where restricted use or acute risk LOCs are triggered for the same taxonomic group. The risk assessment also includes an evaluation of the potential probability of individual effects for exposures that may occur at the established endangered species

LOC both in the risk characterization and the endangered species sections. This probability is calculated using the established dose/response relationship and assumes a probit (probability unit) dose/response relationship.

a. Action Area

For listed species assessments, the action area is considered to be the area affected directly or indirectly by the Federal action and not merely the immediate area where metrafenone is applied. At the initial Level 1 screening assessment, broadly described taxonomic groups are considered, and thus, conservatively assumes that listed species within those broad groups are co-located with the pesticide treatment area. This means that terrestrial plants and wildlife are assumed to be located on or adjacent to the treated site and aquatic organisms are assumed to be located in a surface water body adjacent to the treated site. The assessment also assumes that listed species are located within the area of highest exposure to the pesticide, and that exposure will decrease with increasing distance from the treated area.

If the assumptions associated with the screening-level action area result in RQs that are below the listed species LOCs, a "no effect" determination conclusion is made with respect to listed species in that taxa, and no further refinement of the action area is necessary. Furthermore, RQs below the listed species LOCs for a given taxonomic group indicate no concern for indirect effects upon listed species that depend upon the taxonomic group covered by the RQ as a resource. However, in situations where the screening assumptions lead to RQs in excess of the listed species LOCs for a given taxonomic group, a potential for a "may affect" conclusion exists and may be associated with direct effects on listed species belonging to that taxonomic group or may extend to indirect effects upon listed species that depend upon that taxonomic group as a resource. In such cases, additional information on the biology of listed species, the locations of these species, and the locations of use sites could be considered along with available information on the fate and transport properties of the pesticide to determine the extent to which screening assumptions regarding an action area apply to a particular listed organism. These subsequent refinement steps could consider how this information would impact the action area for a particular listed organism and may potentially include areas of exposure that are downwind and downstream of the pesticide use site.

b. Taxonomic Groups Potentially at Risk

The preliminary risk assessment for endangered species indicates that the proposed use and application rate for metrafenone either exceeds the Endangered Species LOCs for the following taxonomic groups (underlined [chronic exposure to mammals]) or those for which risk cannot be precluded (also underlined [chronic exposure to freshwater fish and invertebrates, acute exposure to marine/estuarine invertebrate, chronic exposure to marine/estuarine fish]).

- Chronic exposure to mammals
- Data was not submitted or was deemed invalid for the following: chronic exposure to freshwater invertebrates and marine/estuarine fish. A passerine bird study is also not available at this time. Lack of data does not preclude risk.
- Data was supplemental, but inadequate for risk quotient calculations which would have helped to determine risk to endangered species for the following: acute exposure to freshwater fish and invertebrates, acute exposure to marine/estuarine invertebrates, chronic exposure to freshwater fish; and, aquatic plants. Therefore, there is uncertainty in determining risk to these taxonomic groups. However, the total concentration based endpoint (EC_{50}: 0.22 mg total a.i./L) for the acute marine/estuarine invertebrate (eastern oyster) study (MRID 47267440) with an effect on shell deposition is 11x greater than the highest estimated EEC (0.02 mg/L), hence risk to federally listed marine/estuarine invertebrates cannot be precluded. The total concentration based endpoint (NOAEC: 0.118 mg total a.i./L) for the chronic freshwater fish (fathead minnow) study (MRID 47267449) with an effect on post-hatch survival is approximately 7x greater than the highest estimated EEC (0.016 mg/L), hence risk to federally listed freshwater fish cannot be precluded. Applying similar calculations to the acute freshwater fish and invertebrate data as well as data for aquatic plants assumes low risk to federally listed species.
- Data was acceptable, but inadequate for risk quotient calculations for the following: acute exposure to marine/estuarine fish. Therefore, there is uncertainty in determining risk to these taxonomic groups. The highest concentration tested in the acute marine/estuarine fish (sheepshead minnow) study (MRID 47267446) was 0.35 mg dissolved a.i./L which is 17.5x greater than the highest estimated EEC (0.02 mg/L) but there were no effects on mortality or sublethal effects, hence risk to federally listed marine/estuarine fish is assumed to be low. Acceptable data for acute exposure to birds yields non-definitive endpoints with no effects; therefore, acute risk to federally listed birds is expected to be low. For similar reasons, acute risk to federally listed piscivorous birds, mammals, and piscivorous mammals is also expected to be low.

Concerns For Federally Listed as Endangered and/or Threatened Species

Table 35. Listed Species Risks Associated with Direct or Indirect Effects from Metrafenone use on Grapes at the Maximum Proposed Application Rate (0.3 lbs a.i./A, Assuming 6 Applications/Year)

Listed Taxon	Direct Effects	Indirect Effects
Terrestrial and semi-aquatic plants - monocots	No	Yes from effects to mammals
Terrestrial and semi-aquatic plants – dicots	No	Yes from effects to mammals
Terrestrial invertebrates	No	Yes from effects to mammals
Birds	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Terrestrial-phase amphibians	No	Yes from effects to mammals
Reptiles	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Mammals	Yes for chronic[1]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic non-vascular plants	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic vascular plants	No	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Freshwater (FW) fish	Yes for chronic[2]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Aquatic-phase amphibians	Yes for chronic[3]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Freshwater (FW) invertebrates	Yes for chronic[4]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Marine/estuarine (M/E) fish	Yes for chronic[4]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)
Marine/estuarine (M/E) invertebrates (mollusk)	Yes for acute[5], No for chronic[6]	Yes from effects to mammals, FW fish, FW inverts, M/E fish, M/E inverts (mollusks)

[1] The chronic LOC is exceeded on a dose basis for mammals in all size classes eating short grass, for the 15 and 35 gram size classes eating tall grass and broadleaf plants / small insects. The chronic LOC on a dietary basis is not exceeded for any of the food categories.
[2] The total concentration based endpoint (NOAEC: 0.118 mg total a.i./L) for the chronic freshwater fish (fathead minnow) study (MRID 47267449) with an effect on post-hatch survival is approximately 7x greater than the highest estimated EEC (0.016 mg/L), hence risk to federally listed freshwater fish cannot be precluded.
[3] Results from freshwater fish used as surrogate for assessing risk to aquatic-phase amphibians
[4] Studies not submitted or invalid for which risk cannot be precluded.
[5] Mollusk (Eastern oyster); [6] Saltwater mysid

1. *Discussion of risk quotients*

The Agency's LOCs for mammals (chronic) are exceeded for the use of metrafenone on grapes as outlined in previous sections. The risk to the remaining federally listed taxonomic groups (freshwater fish and invertebrates (chronic), marine/estuarine invertebrate (acute), marine/estuarine fish (chronic)) cannot be precluded on the basis of toxicity data and estimated exposures. Should estimated exposure levels occur in proximity to listed resources, the available screening level information suggests a potential concern for direct effects on listed species within the taxonomic groups listed above associated with the uses of metrafenone as described in Section III.A. The registrant must provide information on the proximity of federally listed mammals, freshwater fish and invertebrates, as well as marine/estuarine fish and invertebrates to the metrafenone use sites. This requirement may be satisfied in one of three ways: 1) having membership in the FIFRA Endangered Species Task Force (Pesticide Registration [PR] Notice 2000-2); 2) citing FIFRA Endangered Species Task Force data; or 3) independently producing these data, provided the information is of sufficient quality to meet FIFRA requirements. The information will be used by the OPP Endangered Species Protection Program to develop recommendations to avoid adverse effects to listed species.

2. *Probit dose response relationship*

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to aquatic and terrestrial animals (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to metrafenone on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. Studies with good probit fit characteristics (i.e., statistically appropriate for the data set) are associated with a high degree of confidence. Conversely, a low degree of confidence is associated with data from studies that do not statistically support a probit dose response relationship. In addition, confidence in the data set may be reduced by high variance in the slope (i.e., large 95% confidence intervals), despite good probit fit characteristics. In the event that dose response information is not available to estimate a slope, a default slope assumption of 4.5 (95% C.I.: 2 to 9) (Urban and Cook, 1986) is used.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IEC v1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold. However, on account of either non-definitive acute endpoints or endpoints based on total concentrations (dissolved + undissolved test compound) instead of dissolved concentrations only, the acute studies which would otherwise be useful for calculating individual effect probabilities cannot be used.

3. *Data related to under-represented taxa*

Effects data on under-represented taxonomic groups were not submitted by the Registrant. Effects data from other analyzed sources were either not obtained (ECOTOX Database, PAN Database) or were not available (publicly available ECOTOX) for this screening risk assessment.

4. *Implications of sublethal effects*

For the sublethal effects discussed below, it is noted that EFED cannot quantitatively assess the relationship between any of the observed sublethal effects and potential reduction in survival or reproductive impairment at this time. Instead, the concentrations at which sublethal effects were observed in laboratory studies are discussed in relation to the concentrations at which mortality and/or reproductive effects were observed in the same laboratory studies and compared to aquatic and terrestrial EECs and assessed as to whether or not they may be expected under field conditions. The EU/UK formulation (BAS 560 00F), which is used in all formulation studies, closely matches the U.S. formulation (*i.e.*, BAS 560 03F) but is not equivalent. The formulation studies are cited and evaluated in this assessment. However, at this time, the effect of the U.S. formulation on any given taxa is not known.

Acute Studies

Aquatic Organisms

The greatest amount of uncertainty in the assessment stems from aquatic studies which were largely based on total concentrations (both dissolved and undissolved) instead of the soluble concentrations of the test compound.

Given the freshwater fish acute toxicity data the bluegill sunfish (*Lepomis macrochirus*) appears to be more sensitive than the rainbow trout (*Oncorhynchus mykiss*) on the technical grade active ingredient. Sublethal effects (lethargic and motionless fish) were observed in the bluegill study at the highest concentration tested (0.87 mg total a.i./L)

which is greater than the peak aquatic EEC (0.02 mg a.i./L). Similarly, the lowest tested metabolite concentration at which sublethal effects were observed for the trout study was at 20.3 mg total a.i./L, which is also below the total metrafenone residue peak EEC. The numbers imply that sublethal effects on freshwater fish due to the parent or metabolite of metrafenone are not expected under field conditions.

The freshwater invertebrate study on daphnia using the technical grade active ingredient and a metabolite (CL 375816) indicated no effects. On the other hand, another metabolite study (using CL 4084564) indicated immobility at 23.2 mg total a.i./L, which is also above peak EEC levels. The numbers imply that sublethal effects on freshwater invertebrates due to the parent or metabolite of metrafenone are not expected under field conditions.

No effects were observed for the marine/estuarine fish technical grade active ingredient study. Erratic swimming was observed in the saltwater mysid (*Americamysis bahia*) study at 0.416 mg total a.i./L and mortality at 0.129 mg total a.i./L, which are again above peak EEC levels. Therefore, the values imply that sublethal effects on marine/estuarine fish and invertebrates due to the parent metrafenone are not expected under field conditions.

Terrestrial Organisms

The acute oral avian studies indicated no effects; the acute dietary avian studies indicated a significant change in body weight that was not associated with a dose-response pattern. In the mammal study on the technical grade active ingredient, only mortality was reported. Therefore, no implications with sublethal effects can be made.

Chronic Studies

Aquatic Organisms

The freshwater fish chronic toxicity endpoint (NOAEC 0.118 mg total a.i./L) based on post-hatch is greater than the highest chronic EEC (0.017 mg a.i./L). Similarly, the marine/estuarine invertebrate chronic toxicity endpoint (NOAEC 0.022 mg a.i./L) based on reproduction is greater than the highest chronic EEC (0.018 mg a.i./L). The values imply that chronic effects on freshwater fish and marine/estuarine invertebrates are not expected under field conditions.

No acceptable chronic freshwater invertebrate studies are available. No chronic marine/estuarine fish studies were submitted for review.

Terrestrial Organisms

The avian chronic toxicity endpoint (NOAEC 848 mg a.i./kg diet) based on egg production and hatchability did not yield chronic LOC exceedances. The most sensitive chronic mammalian endpoint (NOAEL 35.9 mg/kg bw/day) based on decreased body

weights and body weight gain in F_1 males as well as body weights in F_1 and F_2 females, however, exceeded the chronic LOC, which implies that sublethal chronic effects on mammals are expected under field conditions.

c. Indirect Effects Analysis

In conducting a screen for indirect effects, direct effects LOCs for each taxonomic group are used to make inferences concerning the potential for indirect effects upon listed species. The listed species rely upon non-listed organisms in these taxonomic groups as resources critical to their life cycle. Pesticide-use scenarios, resulting in RQs that are below all direct effect listed species LOCs for all taxonomic groups assessed are considered of no concern for risks to listed species either by direct or indirect effects. However, there may be situations where a taxonomic group is not quantitatively assessed (e.g., terrestrial insects), but other lines of evidence are sufficiently supportive of concerns for indirect effects on listed organisms that are dependant upon that taxonomic group.

- **Where One or More Animal Taxonomic Group RQs Exceed the LOC for Listed Species**

The Level I screening indirect effects analysis documents those types of dependencies upon non-listed organisms that could be important sources of indirect effects to listed organisms should effective levels of the pesticide coincide with locations of listed species and the biologically based resources upon which they depend. In cases where screening-level acute RQs for a given animal group equal or exceed the endangered species acute LOC, the Agency uses the dose response relationship from the toxicity study used for calculating the RQ to estimate the probability of acute effects associated with an exposure equivalent to the EEC. This information serves as a guide to establish the need for and extent of additional analysis that may be performed using Services-provided "species profiles" as well as evaluations of the geographical and temporal nature of the exposure to ascertain if a not likely to adversely affect determination can be made. The degree to which additional analyses are performed is commensurate with the predicted probability of adverse effects from the comparison of dose response information with the EECs. The greater the probability that exposures will produce effects on a taxa, the greater the concern for potential indirect effects for listed species dependant upon that taxa, and therefore, the more intensive the analysis on the potential listed species of concern, their locations relative to the use site, and information regarding the use scenario (e.g., timing, frequency, and geographical extent of pesticide application). The greatest concerns would exist when exposure is associated with a risk higher than the effects probability associated with the non-endangered LOC for a pesticide with an average slope of 4.5.

For metrafenone, risks to listed species are predicted within the following taxa: mammals, freshwater fish and invertebrates, marine/estuarine invertebrates, and marine/estuarine fish. Changes in fish and aquatic invertebrate populations could indirectly affect other fish and aquatic invertebrates, aquatic plants, birds, reptiles and

mammals. The chronic endpoint for mammalian species is based on decreases in body weight and/or body weight gain in both the parents and pups. If body size following chronic metrafenone exposure is reduced to the extent that it has an impact on mammalian populations, reduction in mammalian populations that are used as a resource for listed species may be of concern. Given that the chronic LOC is exceeded for mammals, indirect effects to listed species (e.g., other mammals, birds, amphibians, reptiles, plants (pollination) and terrestrial invertebrates) that rely on mammals as a primary food source, or on mammal burrows for shelter or breeding habitat, may be of concern.

d. Critical Habitat

In the evaluation of pesticide effects on designated critical habitat, consideration is given to the physical and biological features (constituent elements) of a critical habitat identified by the U.S Fish and Wildlife and National Marine Fisheries Services as essential to the conservation of a listed species and which may require special management considerations or protection. The evaluation of impacts for a screening level pesticide risk assessment focuses on the biological features that are constituent elements and is accomplished using the screening-level taxonomic analysis (risk quotients, RQs) and listed species levels of concern (LOCs) that are used to evaluate direct and indirect effects to listed organisms.

The screening-level risk assessment has identified potential concerns for indirect effects on listed species for those organisms dependant upon mammals, freshwater fish and invertebrates, marine/estuarine invertebrates, and marine/estuarine fish. In light of the potential for indirect effects, the next step for EPA and the Service(s) is to identify which listed species and critical habitat are potentially implicated. Analytically, the identification of such species and critical habitat can occur in either of two ways. First, the agencies could determine whether the action area overlaps critical habitat or the occupied range of any listed species. If so, EPA would examine whether the pesticide's potential impacts on non-endangered species would affect the listed species indirectly or directly affect a constituent element of the critical habitat. Alternatively, the agencies could determine which listed species depend on biological resources, or have constituent elements, that fall into the taxa that may be directly or indirectly impacted by the pesticide. Then EPA would determine whether use of the pesticide overlaps the critical habitat or the occupied range of those listed species. At present, the information reviewed by EPA does not permit use of either analytical approach to make a definitive identification of species that are potentially impacted indirectly or critical habitat that is potentially impacted directly by the use of the pesticide. EPA and the Service(s) are working together to conduct the necessary analysis.

This screening-level risk assessment for critical habitat provides a listing of potential biological features that, if they are constituent elements of one or more critical habitats, would be of potential concern. These correspond to the taxonomic groups identified above as being of potential concern for indirect effects (i.e., mammals, freshwater fish and invertebrates, marine/estuarine invertebrates, and marine/estuarine fish). This should

serve as an initial step in problem formulation for further assessment of critical habitat impacts outlined above, should additional work be necessary.

e. Co-occurrence Analysis

The goal of the analysis for co-location is to determine whether sites of pesticide use are geographically associated with known locations of listed species. At the screening level, this analysis is accomplished using the LOCATES v. 2.10.4 database. The database uses location information for listed species at the county level and compares it to agricultural census data for crop production at the same county level of resolution. The product is a listing of federally listed species that are located within counties known to produce the crop upon which the pesticide will be used.

Tables 36 and **37** below report the number of states and counties in which endangered species reside that have the proposed metrafenone use. The 'grape' category was selected in LOCATES. The data suggest that there is considerable potential for exposure to a variety of endangered species from metrafenone use. For additional LOCATES output refer to **Appendix E**.

Species Counts by State for Indicated Crops

No species were excluded.
Minimum of 1 Acre.
All Medium Types Reported

grapes

AL, AK, AZ, AR, CA, CO, CT, DE, DC, FL, GA, HI, ID, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, OR, PA, PR, RI, SC, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY

Table 36. Number of Endangered Species Potentially Exposed to Metrafenone with the Proposed Uses

	Mammals	Amphibians	Birds	Reptiles	Arachnids	Insects	Conf/Cyc	Dicot	Ferns	Lichen	Monocots
Counties	744	93	595	231	12	153	6	493	28	14	272
States	47	11	43	24	4	28	3	41	8	4	37
Species	129	21	163	74	13	73	4	607	20	4	109

Table 37. Number of Endangered Species Potentially Exposed to Metrafenone with the Proposed Uses

	Bivalve	Crustacean	Fish	Gastropod	Marine Mammal
Counties	290	56	449	44	50
States	27	13	38	16	7
Species	205	21	197	32	10

C. Description of Assumptions, Limitations, Uncertainties, Strengths, and Data Gaps

1. Assumptions, Limitations, and Uncertainties Related to Exposure for all Taxa

a. Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependant on fungicide resistance, timing of applications, cultural practices, and market forces.

2. Assumptions, Limitations, and Uncertainties Related to Exposure for Aquatic Species

a. Environmental Fate Studies

All of the environmental fate studies for the parent compound were determined to be scientifically valid and therefore results from all of the studies can be used to characterize the mobility and rates of transformation of metrafenone. However, many of the metabolism studies have major uncertainties in the identification and pattern of formation and decline of transformation products. In all of the aquatic metabolism studies, between 57% and 65% of the applied radioactivity remains unidentified with incomplete characterization, and in two aerobic soil metabolism studies, 15% and 44% of the applied radioactivity is unidentified. This includes at least four major degradates that individually reach levels of 11% to 35% of the applied. Other transformation products appear as groups of up to 15 components, in some cases characterized as each being <5% of the applied radioactivity, but in other cases, some individual components make up 9% to 10% of the applied. Even when individual components can all be classified as minor degradates, these groups represent such a large portion of the applied radioactivity overall that the possibility that they may have some impact as a group cannot be precluded despite their lower individual levels. This is especially true given that the degradation pathways suggest that groups of degradates may have a high degree of structural similarity and so may have similar fate and effects behavior. Without information to adequately characterize the degradates, it may be necessary to assume that they are of equal toxicity to the parents in order to quantify risks.

b. Aquatic Exposure Modeling

The lack of complete characterization and identification of degradation products prompted additional aquatic exposure modeling on total metrafenone residues. Although this modeling approach is conservative, it is reasonable modeling approach to address uncertainties in degradation product identification.

c. Bioaccumulation Modeling

Bioaccumulation modeling was conducted because metrafenone has a log $K_{ow} > 4$. The bioaccumulation modeling was conducted using guidance for the KABAM model. It is recommended to report the sediment pore water and water column concentrations at the appropriate time when the pesticide concentration reaches steady-state. An evaluation of the time series for metrafenone showed no clear plateau in metrafenone concentrations. Therefore, the appropriate averaging time was selected at 21 days to serve as a conservative exposure concentration for bioaccumulation modeling.

3. Assumptions, Limitations, and Uncertainties Related to Exposure for Terrestrial Species

a. Location of Wildlife Species

For this screening-level terrestrial risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving metrafenone at the treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

b. Routes of Exposure

This screening-level assessment for ground (liquid) applications of metrafenone only considered dietary exposure. Other routes of exposure that were not considered in the assessment are incidental soil ingestion exposure, inhalation exposure, dermal exposure, and drinking water exposure.

c. Dietary Intake and Other Limitations of Oral Studies in Terrestrial Species

The avian acute oral study and the avian subacute dietary study each have limitations for estimating the risk to wild species exposed to pesticides in the environment. Both studies have a fixed exposure period and do not allow for differences in the responses of individuals to different durations of exposure. With the acute oral study, the chemical is administered in a single dose. This does not mimic wild bird exposure through multiple feedings. Also, it does not account for the effect of different environmental matrices on absorption rate into the gastrointestinal tract of the animal. With the acute dietary study, the endpoint is reported as the concentration mixed with food that produces a response rather than as the dose ingested. Although food consumption sometimes allows for estimation of a dose, calculations of the mg/kg/day are confounded by undocumented spillage of feed and how consumption is measured over the duration of the test. Usually, if measured at all, food consumption is estimated once at the end of the five-day exposure

period. Group housing of birds undergoing testing allows for a measure of only the average consumption per day for a group, and consumption estimates can be further confounded if birds die within a treatment group. In addition, the dietary study utilizes young birds. The exponential growth of young birds complicates the estimate of the dose; controls often nearly double in size over the duration of the test. Since weights are only taken at the initiation and at the end of the exposure period, the dose per body weight (mg/kg) is difficult to estimate with any precision. The interpretation of this test can be further confounded by dietary consumption. Estimation of the acute LC_{50} value is not only a function of the intrinsic toxicity of the pesticide, but also the willingness of the birds to consume treated food.

In addition to the uncertainties associated with the two toxicity studies utilized for estimating acute risk to birds, other factors, not normally taken into account in a screening level risk assessment may narrow the differences between the dose-based and dietary-based acute RQs for birds. The factors include differences in gross energy and assimilative efficiency of laboratory feed versus food items in the field, basic maintenance metabolic rates between wild birds and captive birds, seasonal free living dietary requirements for wild birds (including gorging behavior) and specific food avoidance behavior. These uncertainties may either overestimate or underestimate the risk in a screening level assessment.

Gross Energy and Assimilative Efficiency. This screening level risk assessment does not allow for gross energy and assimilative efficiency differences between wildlife food items and laboratory feed. For example, a typical laboratory avian feed, as used, contains approximately 2750 kcal/ kg. The Agency's Wildlife Exposure Factors Handbook (U.S. Environmental Protection Agency, 1993) presents the following dry-weight and fresh weight caloric contents for selected wildlife food items:

Food Item	Energy Dry (kcal/kg)	Energy Fresh (kcal/kg)
grasses	4200	1300
broadleaf forage	4200	2200
seeds	5100	4700
fruits	2000	1100
insects	5600	1600

On gross energy content alone, direct comparison of a laboratory dietary concentration-based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 - 2.5 for most food items. Only for seeds would the direct comparison of dietary threshold to residue estimate lead to an overestimate of exposure.

Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 - 80%, and mammal's assimilation ranges from 41 - 85% (U.S. EPA, 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (e.g., a value of 85%), a potential for underestimation of exposure may exist by assuming that

consumption of food in the wild is comparable with consumption during laboratory testing.

Metabolic Rates. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption. For example, the Wildlife Exposure Factors Handbook (U.S. EPA, 1993) includes allometric models for estimating both existing metabolic rate (EMR) and free living metabolic rate (FMR). EMR is the metabolic rate necessary for animal maintenance in captivity without body weight loss, a condition similar to caged test animals. FMR is the energy requirement for an organism in the wild. For passerine birds these relationships are as follows:

$$\text{EMR (kcal/day)} = 1.572\ (\text{body weight g})^{0.6210}$$
$$\text{FMR (kcal/day)} = 2.123\ (\text{body weight g})^{0.749}$$

Using a weight range for passerines of 10 - 150 g, the EMR predictions range from 6.6 to 35.3, and the FMR ranges from 11.9 to 90.5 kcal/day. Thus, it appears that not accounting for increased energy demands of organisms in the wild when comparing dietary residues to dietary toxicity thresholds represents about a two-fold underestimation in exposure potential.

Free Living Metabolic Requirements. The screening procedure does not account for situations where the feeding rate may be above or below requirements to meet free living metabolic requirements. Gorging behavior is a possibility under some specific wildlife scenarios (e.g., bird migration) where the food intake rate may be greatly increased. Kirkwood (1983) has suggested that an upper-bound limit to this behavior might be the typical intake rate multiplied by a factor of 5.

Avoidance. In contrast is the potential for avoidance, operationally defined as animals responding to the presence of noxious chemicals in their food by reducing consumption of treated dietary elements. This response is seen in nature where herbivores avoid plant secondary compounds. For agrochemicals, Dolbeer *et al.* (1994) reported that the use of methiocarb on fruit crops reduced depredation by birds. Of course, chemical treatment of food sources and any subsequent avoidance of those food sources by a species may, in itself, result in detrimental effects on the energetics of the species.

d. Incidental Releases Associated with Use

This risk assessment was based on the assumption that the entire treatment area is subject to pesticide application at the rates specified on the label. Uneven application of the pesticide through changes in calibration of application equipment, spillage, and localized releases at specific areas of the treated field that are associated with specifics of the type of application equipment were not accounted for in this assessment.

e. Residue Levels Selection

The Agency relies on the work of Fletcher et al. (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflects residues averaged over the entire above ground plants in the case of grass and forage sampling. Depending upon a specific wildlife species' foraging habits, whole aboveground plant samples may either underestimate or overestimate actual exposure.

f. TerrPlant Model

At this time, the TerrPlant model cannot accurately estimate terrestrial exposure levels with pesticides applied with multiple applications or application intervals. The technology is not yet available for these types of estimations. The label states that a maximum of 1.8 lbs a.i./A may be applied per season, with a maximum of six applications the highest single application rate is 0.3 lbs a.i./A. In modeling the terrestrial EECs, it was assumed that there was one application per year. If assuming one application of 1.8 lbs a.i./A the RQ values may be considered an overestimate of risk, but assuming one application of 0.3 lbs a.i./A the RQ values may be considered an underestimate of risk. Therefore, the model was used to bracket the potential risk on terrestrial plants as a result of metrafenone use.

4. Assumptions, Limitations, and Uncertainties Related to Effects Assessment

a. Sublethal Effects

For an acute risk assessment, the screening risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the assessment is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints.

b. Age Class and Sensitivity of Effects Thresholds

Testing of juvenile organisms may overestimate toxicity at older age classes for pesticidal active ingredients that act directly (without metabolic transformation) because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. However, the influence of age may not be uniform for all compounds, and compounds

requiring metabolic activation may be more toxic in older age classes. The risk assessment uses the most sensitive life-stage information as the conservative screening endpoint.

c. Use of Most Sensitive Species Tested

Screening risk assessment relies on a selected toxicity endpoint from the most sensitive species tested; however, the selected toxicity endpoints do not necessarily reflect sensitivity of the most sensitive species in a given environment. The relative position of the most sensitive species tested in the distribution of all possible species is a function of the overall variability among species to a particular chemical. Toxicity thresholds may vary up to four orders of magnitude across species for some chemicals[11]. Therefore, risk conclusions may under- or overestimate actual ecological risk for a given species.

5. Assumptions, Limitations, Uncertainties, Strengths, and Data Gaps Related to the Acute and Chronic LOC's

The risk characterization section of the assessment document includes an evaluation of the potential for individual effects to listed species at an exposure level equivalent to the LOC. This evaluation is based on the median lethal dose estimate and dose/response relationship established for the effects study corresponding to each taxonomic group for which the LOCs are exceeded. The slope of the probit-dose response is used to generate a probability of individual effects near the low end tail of the curve. Predictions based on low probability events are by nature highly uncertain. Moreover, for this assessment the dose-response curve representing a given taxa is generated from one study using one species. It is likely that the resulting dose-response relationship does not represent the response of all species within a taxa. Calculating the probability of individual effects at the lower and upper bounds of the slope is designed to address this source of uncertainty but the extent to which this captures the variability within a taxa is unknown. In some cases, a probit dose-response relationship cannot be calculated. In these instances, event probabilities are calculated based on a default slope assumption of 4.5 (Urban and Cook, 1986).

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VI. Appendices

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