Environmental Fate and Ecological Risk Assessment for the Registration of Prothioconazole

1 Executive Summary

1.1 Nature of Chemical Stressor

Bayer Cropscience is seeking registration for the use of the new chemical fungicide, prothioconazole (JAU6476; 2-(2-(1 **-chlorocylocpropyl)-3-(2-chlorophenyl)-2-hydroxy-propyl)- 2,4-dihydro(1,2,4)-triazol-3-thion),** and its end-use product PROLINEB 480SC (41 .O% a.i.). This is a national registration request for control of a wide variety of fungal diseases in barley, oil seed (except sunflower and safflower), dried shell pea and bean (except soybean), peanut, rice, and wheat. The proposed methods of application are ground and aerial sprays with maximum annual rates ranging from 0.268 lbs a.i./A to 0.712 Ibs a.i./A, depending on use.

This assessment utilizes environmental fate and toxicity data for prothioconazole and two primary degradates; the prothioconazole-desthio and prothioconazole-S-methyl. A total toxics residue approach was used to estimate exposure levels for aquatic organisms and to derive estimated concentrations in drinking water. Exposure was also estimated based on unbound residues alone, for characterization purposes. Given the uncertainties associated with accurately predicting the concentrations of parent or metabolites after application of prothioconazole, the lowest available toxicity endpoint, regardless of exposure chemical, was used in the assessment.

1.2 Potential Risks to Non-target Organisms

The results of this screening-level assessment indicate a potential for direct adverse acute effects to non-target fresh- and saltwater non-vascular plants, freshwater vascular plants, and saltwater invertebrates other than mollusks at the proposed application rates (Table 1.1). The results also indicate a potential for adverse effects associated with chronic exposures to mammals for all proposed uses of prothioconazole and a potential for adverse effects to semi-aquatic plant species (Table 1.1).

Table 1.1. Non-Listed species risks associated with direct effects due to applications of prothioconazole on wheat/barley, canola/oilseed, beans (incl. chickpea/lentil), peanuts, and rice at proposed label rates.

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^IThe model used to estimate rice aquatic exposure is highly conservative, especially for chronic exposure.

Overall, potential risks appear to be greatest for aquatic plant species since these organisms appear to be very sensitive. In addition, risk quotients were the highest for these organisms. Functionally, estimated risks may translate to reduced survival, reproduction, or growth in affected species with subsequent effects at higher levels of biological organization. Risk conclusions based on exposure estimated using unbound residues are the same as estimated in Table 1.1 (based on total residues). Also, while estimated environmental concentrations (EECs) are based on aerial applications at proposed label rates, for some usees ground application is also allowed. Aquatic exposure estimated assuming ground application is $8 - 27$ percent lower than when the pesticide is applied aerially. The exposure estimates associated with ground application would not alter overall risk conclusions although for some specific combination of crop and taxa, risk quotients may be below the LOC.

For listed species, acute risk levels of concern were exceeded for estuarine/marine invertebrates, semi-aquatic plants, aquatic plants, and freshwater fish. Listed species chronic risk levels of concern were exceeded for mammals. Overall, potential for adverse effects seems highest for aquatic plants followed by semi-aquatic plants. Because aquatic plant risk quotients are above non-endangered species level of concern, the Agency considers this to be indicative of a potential for adverse effects to those listed species that rely either on a specific plant species

(plant species obligate) or multiple plant species (plant dependant) for some important aspect of their life cycle.

The extent to which the proposed uses of prothioconazole will indirectly effect listed animal species will require further effort; specifically, clear delineation of action area, identification of listed species that co-occur in areas of prothioconazole use, species-specific life history information, and an evaluation of critical habit. Because of the national extent of the proposed uses of prothioconazole, there is a potential to affect some listed plant species and the species which depend upon listed or non-listed plant species. Indirect effects in this case may not be limited to aquatic species as terrestrial animals that rely on aquatic food items have potential to be affected indirectly.

1.3 Conclusions - **Exposure Characterization**

Prothioconazole degrades rapidly to prothioconazole-desthio via most degradation processes, and prothioconazole-desthio has a similar toxicological profile to that of its parent. Therefore, this assessment is conducted considering prothioconazole and prothioconazole-desthio jointly as the toxic moiety. Based on registrant-submitted environmental fate data, prothioconazole is expected to degrade quickly to prothioconazole-desthio, which is expected to be persistent with moderate mobility in the soil. Prothioconazole-desthio is stable to hydrolysis, very slowly degraded by aerobic soil metabolism, anaerobic aquatic metabolism and aqueous photolysis, and moderately degraded by aerobic aquatic metabolism. Transport to surface water of prothioconazole residues is predicted, and, in some soils, transport to groundwater is also predicted, particularly in areas with porous soil of low organic carbon content.

1.4 Conclusions - **Effects Characterization**

Prothioconazole and the prothioconazole-desthio are practically non-toxic to birds, mammals, and honeybees under acute exposure conditions and only slightly toxic to fish and freshwater aquatic invertebrates. Prothioconazole-desthio is highly toxic to aquatic non-vascular plants and to estuarine/marine invertebrates following acute exposure. Prothioconazole-desthio is slightly more toxic to birds and mammals under chronic exposure conditions compared to the parent compound based on the respective study-determined effect levels. In birds, chronic exposure to prothioconazole-desthio did not cause any significant effects in adults or offspring. In mammals, chronic effects of prothioconazole-desthio included decreased viability of offspring and decreased offspring body weights.

1.5 Uncertainties and Data Gaps

The major uncertainty in characterizing effects of prothioconazole and/or prothioconazoledesthio is associated with the toxicity of prothioconazole-desthio to estuarine/marine invertebrates, the lack of an acceptable sediment toxicity test, and the lack of data on the effects of the 1,2,4-triazole degradate. For estuarine/marine invertebrates, toxicity tests indicated for prothioconazole-desthio both the LC_{50} and the chronic NOAEC are approximately 60 ppb as for mysid shrimp. This suggests that chronic and acute thresholds are the same; a conclusion that is incongruent with typical toxicological patterns and logic. It appears that mysid sensitivity varies considerably and that repeating the chronic toxicity test may provide additional insights. However, since the original studies were classified as acceptable, new data or information may not alter current risk conclusions. Risks to sediment dwelling invertebrates could not be estimated since the submitted studies did not meet guideline requirements. It is recommended that guideline sediment toxicity tests be submitted. Lastly, the lack of toxicity data on the 1,2,4 triazole prevents an adequate estimation of potential risks associated with this degradate. Considering that the 1,2,4-triazole degradate can make up a significant percent of the total residues over time, these data are needed to adequately characterize potential risks of prothioconazole. Although a human health risk assessment is being conducted for the 1,2,4 triazole degradate (Drinking water assessment, D320682), the ecological risks associated with the 1,2,4-triazole degredate are not currently being addressed.

The environmental fate data submitted to the Agency are complete. However, because of the considerable uncertainty surrounding soil extraction procedures, the unextracted material in the aerobic soil, aerobic aquatic, and anaerobic aquatic metabolism studies was added to parent in calculation of half-lives used in environmental fate modeling and fate characterization. Therefore, the persistence and bioavailability of prothioconazole may be overestimated in this assessment; resulting in conservative estimated aquatic exposure. Importantly, Tier I1 modeling using the 90th percentile unextracted-material-incorporated half-lives did not change risk estimates for aquatic animals or aquatic plants relative to modeling using upper $90th$ percentile confidence bounds on the mean half-lives calculated without incorporating unextracted material. In neither case do aquatic concentrations approach levels-of-concern for aquatic animals; however, aquatic concentrations are sufficiently high to result in risk quotients that exceed levels-of-concern for aquatic plants. Additionally, there is uncertainty associated with the fate of the 1,2,4-triazole degradate, which is not detectable in studies using only the phenyl label *(i.e.,* hydrolysis, soil photolysis, anaerobic aquatic metabolism). Finally, the adsorption coefficient $(K_d \text{ or } K_{OC})$ of prothioconazole could not be calculated from submitted data because of the chemical's quick degradation in the systems. Therefore, conclusions about the mobility of prothioconazole combined residues of concern are drawn only from degradates. Similarly, bioaccumulation factors (BCF) of prothioconazole and prothioconazole-desthio could not be definitively calculated due to lack of clear accumulation plateaus. Therefore, there is also minimal uncertainty in the conclusion that neither chemical bioaccumulates.

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$\overline{2}$ **Problem Formulation**

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires that registered pesticide uses do not pose unreasonable adverse effects to the environment, and the Endangered Species Act requires that regulatory actions should not adversely affect Federally listed species or their habitats. The purpose of this assessment is to provide insight into the potential effects to the environment associated with the use of the fungicide, prothioconazole as part of the supporting information to determine the eligibility of prothioconazole for registration. This screening-level assessment follows methods detailed in the Overview Document (EPA, 2004). Briefly, the method involves comparing estimates of exposure (measures of exposure) with laboratory derived toxicity estimates (measures of effect). If measures of effect exceed specified levels of concern (LOCs) for a given measure of exposure, deleterious effects on wildlife are expected. Although screening-level methods are similar for all chemicals, the problem formulation section helps to focus attention on unique or important characteristics of a given chemical, thereby providing a sense of the potential environmental risks of that chemical.

Prothioconazole (JAU 6476; 2-[2-(1 **-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]- 1,2-dihydro-3H-l,2,4-triazole-3-thione;** CAS#: 178928-70-6; PC code: 1 13961) is a broadspectrum, systemic fungicide belonging to the conazole (triazolinthione) class of fungicides. This class of compounds is characterized structurally by inclusion of a nitrogen-containing five member ring (azole). Conazole fungicides act through disruption of normal fungal cell membrane structure and function primarily through interactions or inhibitions of ergosterol synthesis, the predominant membrane sterol component. Prothioconazole's specific mode of action is through the inhibition of demethylation of two precursors of sterols in fungi (lanosterol and 24-methylene dihydrolano-sterol). The manufacturer recommends that for maximum effectiveness in protection against fungal diseases, prothioconazole should be applied via ground or aerial foliar spray treatment prior to and during certain growth phases. Prothioconazole is intended for use on wheat, barley, lentils, canola, oilseed subgroup, chickpeas, dried peas and beans, peanuts and rice, with maximum applications amounts ranging from 0.134 to 0.178 lbs a.i./A for up to 4 applications.

Prothioconazole appears to degrade relatively quickly in the environment; however its degradates, primarily prothioconazole-desthio, are persistent and of similar toxicity as parent. This quick degradation, in concert with poor extraction methods in soil and sediment metabolism studies, leads to great uncertainty in composition and bioavailability of large amounts of unextracted material. Due to this uncertainty, biotic degradation rates cannot be calculated for prothioconazole alone. Therefore, a total toxic residues method evaluating the combined residues of concern, including unextracted material, needs to be utilized for environmental exposure estimate modeling. Combined residues of concern are prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl. There is evidence that the prothioconazole-desthio degradate is moderately mobile (K_d s of 4.13 to 13.38 mL/g in four soils) and may be a concern for groundwater contamination (stability to hydrolysis, long half-lives to other environmental degradation processes and multiple detections at 15-30 cm and one detection at 30-45 cm in terrestrial field dissipation studies). Prothioconazole-desthio is not expected to bioaccumulate due to its quick depuration.

Although a total toxic residues approach is used for estimation of prothioconazole exposure for aquatic species, the total mass of applied material is used to estimate exposure to terrestrial organisms. Tools are not currently available that would allow terrestrial exposure to be compartmentalized into the contributions of parent and metabolites.

In addition to the environmental fate characteristics of prothioconazole, the toxicity of the chemical plays an important role in evaluating potential environmental risks. A complete toxicity dataset was provided for the parent, prothioconazole. Numerous toxicity studies on the desthio-, and to a lesser extent, S-methyl metabolites were provided for review. The suite of toxicity studies provides the measures of effect, which are toxicity endpoints. These are intended to provide insight into the potential effects on assessment endpoints, defined as "explicit expressions of the actual environmental value to be protected" (US EPA, 1998). For most pesticides, including prothioconazole, the assessment endpoints relate to the sustainability of populations or the maintenance of community structure for plants and animals. While assessing risks at the population and community level are assessment endpoints useful for risk management, quantitative methods and data are lacking to address these endpoints with meaningful scientific rigor (ECOFRAM, 1999).

The toxicological profile for prothioconazole indicates that for most tested species, prothioconazole and its metabolites are not highly toxic with designations ranging from practically non-toxic (mammalian and avian species) to moderately toxic (some aquatic species). The prothioconazole degradate, prothioconazole-desthio, is highly toxic to some taxa; the most sensitive taxa appear to be aquatic and terrestrial plants followed by estuarine/marine invertebrates. Given the sensitivity of plant and algae species, it is possible that the most deleterious effects of prothioconazole may relate to the survival, growth, and reproduction of plants and algae. **Table 2.1** provides a list of the taxonomic groups, measures of effect and most sensitive test species evaluated for the toxicological effects of prothioconazole and/or prothioconazole degradates; these provide the toxicological basis for risk conclusions in this assessment.

Birds are used as surrogates for reptiles and terrestrial-phase amphibians (U.S. EPA, 2004). b Freshwater fish are used as surrogates for aquatic-phase amphibians (U.S. EPA, 2004).</sup>

For prothioconazole and pesticides in general, the ecosystems at greatest risk are those in close proximity to the proposed use areas. These would include agricultural fields (surrounding nonagricultural terrestrial habitats) and water bodies directly adjacent to treated fields that may receive chemical residues via drift, runoff or both. Within water bodies, the water column, sediments, and pore water are all compartments of concern. Organisms of concern include birds, mammals, reptiles, fish, and terrestrial and aquatic invertebrates, plants, and amphibians. The assessment endpoints are intended to reflect population sustainability and community structure within ecosystems and hence relate back to ecosystems at risk. If risks are expected for given species/taxa based on the screening-level assessment, then risks might be expected to translate to higher levels of biological organization. The following risk hypothesis provides a sense of the focus and intent of this risk assessment.

The risk hypothesis is that the use of prothioconazole in accordance with the label results in *adverse effects on strrvival, growth and/or reproduction ofnon-target terrestrial and/or aquatic animals; and that the use of prothioconazole in accordance with the label results in adverse effects on survival and/or growth of terrestrial, semi-aquatic, and aquatic plants.*

The conceptual model **(Figure 2.1)** depicts the potential pathways for ecological risk associated with prothioconazole use. This model is fairly generic and assumes that as a fungicide, prothioconazole can affect terrestrial and aquatic organisms if environmental concentrations are sufficiently elevated as a result of proposes label uses.

The conceptual model provides an overview of the expected exposure routes for organisms within the prothioconazole action area. For terrestrial organisms, the major route of exposure considered is the dietary route; consumption of food items such as plant leaves or insects that have prothioconazole residues as a result of spraying. For aquatic animal species, the major routes of exposure are considered to be via the respiratory surface (gills) or the integument. Direct contact and/or root uptake is the major route of exposure for terrestrial and wetland (riparian) plants, while aquatic plants may be exposed via direct uptake and adsorption. Estimated exposure concentrations for all organisms are not obtained from actual

prothioconazole experimental data, but are obtained through the use of several Agency exposure models.

To generate screening-level estimates of risk, the measures of exposure, (derived exclusively from models) are compared to the measures of effect (obtained from submitted toxicity studies). Functionally, the comparison involves dividing the measures of exposure (EECs) by the measures of effect for a given taxa. The resulting unitless value is the risk quotient (RQ). The RO is then compared to the Agency's levels of concern (LOC) for direct effects. The LOCs are the policy tool for analyzing potential risk to non-target organisms; if the RQ exceeds the LOC for a given taxa and exposure duration, there is a potential for adverse effects to non-target organisms. A more detailed description of the methods used to assess risks is provided in the next section (Section 3; Analysis).

The environmental fate and effects data submitted to the Agency are complete and overall indicate that prothioconazole and its metabolites, prothioconazole-desthio and prothioconazole-S-methyl, are most toxic to aquatic plants and are generally characterized together as persistent and having moderate to slight mobility (FA0 2000). However, there are some uncertainties worth noting. First, because of the considerable uncertainty surrounding soil extraction procedures, the unextracted material in the aerobic soil, aerobic aquatic, and anaerobic aquatic metabolism studies was added to the parent in calculating half-lives used in the environmental fate assessment. Therefore, the persistence of prothioconazole (and metabolites) may be overestimated in this assessment, which would yield fairly conservative estimated aquatic exposure concentrations. However, Tier I1 modeling using the unextracted-materialincorporated upper 90th percentile confidence bound on the mean half-lives did not change risk estimates for aquatic animals or aquatic plants relative to modeling using similar values calculated without incorporating unextracted material. In neither case do aquatic concentrations approach levels-of-concern for most aquatic animals. In both cases aquatic concentrations exceed levels-of-concern for aquatic plants. Secondly, the adsorption coefficient $(K_d \text{ or } K_{OC})$ of prothioconazole could not be calculated from submitted data because of the chemical's quick degradation in the systems. Therefore, conclusions about the mobility of prothioconazole combined residues of concern are drawn only from degradates. Similarly, bioaccumulation factors (BCF) of prothioconazole and prothioconazole-desthio could not be calculated due to lack of clear accumulation plateaus. Therefore, there is also minimal uncertainty in the conclusion that neither chemical bioaccumulates.

From an ecotoxicity perspective, most uncertainties surrounding prothioconazole are associated with aquatic invertebrate studies. First, the acute toxicity value ($LC_{50} = 60$ ppb) for estuarine/marine invertebrates is roughly equivalent to the chronic toxicity threshold (NOAEC $=$ 64 ppb). These results suggest that both the chronic no-observed effect concentration (NOAEC) and the acute median lethal concentration (LC_{50}) occur at similar concentrations. Evaluating the aquatic toxicity data as a whole suggests that the acute study is suspect despite classification as acceptable. Second, sediment toxicity studies on chironomids were submitted but did not follow Agency guidelines and, although they provide some insight into potential sediment toxicity, they are of limited utility for assessing risks. The uncertainties in the ecotoxicological dataset are not likely to significantly alter risk conclusions, however, repeating the studies mentioned would

reduce uncertainties associated with risks of prothioconazole to estuarine/marine and sedimentdwelling invertebrates.

Figure 2.1. Conceptual model of the fate/transport and effects of prothioconazole in the environment.

3 Analysis

3.1 Methods

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The primary method used to assess risk in this screening-level assessment is the deterministic risk quotient method and follows closely the approach outlined in the EPA Overview Document (US EPA, 2004). The RQ is a unitless value that is the result of comparing, by division, measures of exposure to measures of effect. Commonly used measures of exposure are the estimated exposure concentrations (EECs) and commonly used measures of effect include toxicity values such as the LD_{50} or the NOAEC. The assessment endpoints and the corresponding measures of exposure and measures of effect used to address risks to the assessment endpoints are outlined in **Table 3.1.**

The RQ that results from a given comparison of exposure and effect is compared to a specified level of concern; if the RQ exceeds the LOC, then adverse effects are considered likely. Although not a true estimate of risk since there is no estimated probability or magnitude of an

adverse effect, in general, the higher the RQ, the more concern for the likelihood of adverse effects (Table **3.2).**

Levels of concern are the policy tool for interpreting RQs in the context of risks to receptors as a result of direct pesticide effects. The magnitude of the LOC is set by the risk presumption for each endpoint (Tables **3.3-3.5).** Importantly, there are implicit components of the LOC, which include duration, frequency and spatial considerations. Duration is determined primarily by the assessment endpoint evaluated and falls typically within the context of acute (shorter) and chronic (longer) continuous exposures. The exact duration is taxa-specific and relates to the general characteristics of that taxa's life history. For example, many freshwater invertebrates have a relatively short life-span; hence chronic exposure estimates would be shorter than longerlived organisms such as fish. The frequency of potential risk is analyzed as the highest 21- or 60day average once every ten years for aquatic organisms and a single, maximum peak value for terrestrial animals. The spatial extent of the screening-level assessment is defined by the use area, and the areas downstream and areas potentially affected by spray drift. For aquatic assessments, exposure will vary with proximity to the treated field, and the runoff and erosion sediment generating characteristics of the soils in the watershed above the water body, and the local weather. Sites for assessment are selected so that they are expected to be more vulnerable than most sites with a use pattern, with a goal of a 90% site.

* Estimated Exposure Concentration (ppm) on avidmammalian food items

 $**$ mg/ft2 / LD50 (body-weight scaled)

*** mg of toxicant consumed/day / LD50 (body-weight scaled)

 $*$ EEC = (ppm or ppb) in water

 $*$ EEC = lbs. a.i./A

* $EEC = (ppm \text{ or } ppb)$ in water

The exposure estimates in this screening-level assessment are derived using maximum label rates and minimum application intervals for each use. Measures of effects are based on the lowest available toxicity endpoint for a given taxa and exposure duration.

3.2 **Use Characterization**

Prothioconazole is proposed for use as a pre- or post-infection fungicide on barley, canola, chickpea, dried shell peas and beans, lentils, oilseed crop, peanut, rice and numerous varieties of wheat. It is formulated as a flowable solution concentrate formulation, PROLINE@ 480SC fungicide/A $(41\% a.i.)$, and may be applied via ground spray or aerially. PROLINE® 480SC may be tank mixed but is not to be applied as chemigation. Based on current proposed labels, the maximum proposed single application rates across all uses range from 0.134 to 0.178 lbs a.i./A. Maximum seasonal applications vary predominantly based on the number of applications allowed. For example, the total yearly maximum proposed rate is 0.712 lbs a.i./A for use on peanuts and would result from 4 applications of 0.178 lbs a.i./A. Complete maximum use rates and management practices by crop according to proposed labels are presented in **Table** 3.6, based on the Petition for Tolerances submitted by Bayer Crop Science (March 31, 2004).

For purposes of this screening-level assessment, estimates of risk are based on maximum proposed label rates for a given use **(Table** 3.6).

Wheat **(Figure 3.1)** is grown nationally. Barley **(Figure 3.2)** is grown in the northwest of the United States. Canola **(Figure 3.3)** is grown in the north half of North Dakota, a portion of the Souris-Red-Rainy section of the Mississippi river basin. Dry edible peas and beans **(Figures 3.4 and 3.5)** are also grown in the north half of North Dakota, a portion of the Souris-Red-Rainy section of the Mississippi river basin and Pacific Northwest basin, west of the Western Divide. Peanuts **(Figure 3.6)** are grown in the Texas Gulf section of the Mississippi river basin and in the south of Virginia/north of North Carolina and southern Georgia and Alabama sections of the South Atlantic Gulf basin, east of the Eastern Divide. Rice is grown in Southwest Louisiana, Texas, and California and in the lower Mississippi river encompassing Arkansas and parts of Louisiana and Mississippi.

Figure 3.2. Barley grown in United States.

Figure 3.3. Canola grown in United States.

Figure 3.5. Dry edible beans grown in United States.

3.3 Exposure Characterization

3.3.1 Environmental Fate and Transport Characterization

Prothioconazole degrades rapidly to prothioconazole-desthio via most degradation processes, and prothioconazole-desthio has a similar toxicological profile to that of its parent. In instances where compounds degrade rapidly to another toxic compound, the degradate (in this case, prothioconazole-desthio) may in fact be the active ingredient. Examples of this type of circumstance include parents bromoxynil octanoate and 2,4-D-methyl hexyl ester, whose toxic degradates bromoxynil and 2,4-D, respectively, are considered the pesticidal compound. Additionally, prothioconazole degrades via metabolism to prothioconazole-S-methyl, which also has similar toxicity.

Degradation rates for prothioconazole alone can not be calculated from the available metabolism studies. Although prothioconazole disappears rapidly and a substantial fraction converts to prothioconazole-desthio and prothioconazole-S-methyl, a large portion of applied radioactivity is present in studies as unextracted material even at short periods of time after the initiation of the experiment. This may be a result of poor extraction methods, which did not use two different solvent systems and, therefore, were not sufficiently aggressive for removal and characterization. It cannot be determined which portions of the unextracted material are composed of potentially bioavailable parent, prothioconazole-desthio, prothioconazole-S-methyl or other degradates, and which are composed of legitimately unextractable, non-bioavailable material. Therefore, individual half-lives cannot be calculated. Biotic degradation rate kinetics are estimated over the sum of all four components (prothioconazole, prothioconazole-desthio, prothioconazole-Smethyl and unextracted material) and fate analysis is performed on prothioconazole combined residues of concern. For the purposes of risk assessment, this approach is supportable since prothioconazole-desthio or prothioconazole-S-methyl are as toxic as parent and may be the true active compound or compounds. To characterize uncertainty as a result of this approach, halflives were also calculated based on unbound residues alone and used to characterize exposure and risk.

Because prothioconazole degrades so quickly, in most instances the fate of prothioconazoledesthio and prothioconazole-S-methyl, where available, are characterized. Based on the submitted environmental fate data and reported physical-chemical properties, prothioconazole combined residues of concern are expected to be persistent (Goring 1975) and moderately mobile in the environment. There was no significant degradation of either prothioconazole or prothioconazole-desthio at any pH by hydrolysis, no significant degradation of prothioconazole combined residues of concern by anaerobic aquatic metabolism (stable) and very slow degradation via aerobic soil metabolism (prothioconazole combined residues of concern halflives of 533 to 1386 days). Aerobic aquatic metabolism rates were moderately persistent to persistent (prothioconazole combined residues of concern half-lives of 67 to 433 days). In the laboratory, prothioconazole degraded somewhat rapidly via aqueous photolysis (prothioconazole alone predicted environmental half-life of 9.7 days) to prothioconazole-desthio, which appears to be more resistant to photolysis, as the concentration was still increasing at end of study. The

joint **prothioconazole/prothioconazole-desthio** half-life was 101.9 days, corrected to represent natural sunlight at 40°N latitude. Prothioconazole-S-methyl was not formed via photolysis. This mode of degradation is only likely to be influential in clear, shallow water, under non-cloudy atmospheric conditions. In actual environmental systems, aqueous photolysis is likely to proceed at a slower rate due to attenuation of light with increasing depth of water bodies, light absorption by suspended solids, and natural obstruction of sunlight by plants and suspended sediments.

The predominant means of degradation of prothioconazole combined residues of concern in the environment is likely to be aerobic aquatic metabolism, with loss of prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl attributed to the formation of 1,2,4 triazole, prothioconazole-triazolinone, other minor unidentified degradates and $CO₂$. There is uncertainty in the rate of aerobic aquatic degradation of prothioconazole combined residues of concern associated with high amounts of poorly-extracted unextracted material, ranging from 1.3 to 8.2% of applied at time zero and 20.9 to 46.7% of applied at 59 to 121 days in two different sediment/water systems, using both phenyl and triazole radiolabels.

The soil-water partition coefficient of prothioconazole parent could not be determined due to instability (quick degradation) in the batch equilibrium test system and low resolution in the aged leaching column study. Qualitatively, prothioconazole parent may show very low potential for leaching as very low total radioactive residues were detected in the leachate and very little unchanged parent compound was translocated below the aged soil layer. This is likely due to its quick degradation. Prothioconazole-desthio, however, has soil-water partition coefficients **(I&)** in four soils ranging from $4.13-13.38$ mg/L which indicate a high to moderate mobility, and organic carbon-normalized soil-water partition coefficients (K_{OC}) in four soils ranging from 523.0-625.3 mL/g which indicate a moderate mobility (FA0 2000). Since prothioconazoledesthio has a K_d less than 5 in some soils and is persistent (hydrolysis half-life greater than 25 weeks, photolysis half-life greater than 1 week, aerobic soil metabolism half-life greater than 2-3 weeks), not volatile (Henry's Law constant less than 10^{-2} atm*m³/mol), and shows movement to 45 cm during field dissipation studies, this indicates potential for groundwater contamination (Cohen 1984). Prothioconazole-S-methyl has higher soil-water partition coefficient, K_d in four soils ranges from 15.6-64.1 mg/L, and K_{OC} in four soils ranges from 1973-2995 mL/g which indicate a slight mobility **(FA0** 2000) and less potential for groundwater contamination. Binding to organic carbon can not be evaluated for prothioconazole due to system instability (quick degradation), but there is a strong correlation between adsorption and organic carbon content for prothioconazole-desthio (r^2 = 0.981) and prothioconazole-S-methyl (r^2 = 0.984). The moderate to high water solubility of prothioconazole depends on pH (5, 300, and 2000 mg/L at pH 4, 8, and 9, respectively) and also suggests a high potential for run-off into surface water and leaching to groundwater. Despite prothioconazole's very hydrophobic octanol-water partition coefficient (unbuffered $\log K_{OW}$ of 4.05), significant bioaccumulation in aquatic organisms is not anticipated due to its quick degradation. The octanol-water partition coefficient for prothioconazole-desthio is unknown. Prothioconazole's and prothioconazole-desthio's bioconcentration factors (BCF) in fish can not be determined, since there was no clear accumulation plateau.

Table 3.7 summarizes the physical and chemical properties and environmental fate and transport characteristics of prothioconazole combined residues of concern, derived from information

submitted under product chemistry and fate guideline studies. All fate studies were conducted with prothioconazole, and some additional studies were conducted with prothioconazole-desthio (hydrolysis, batch equilibrium, bioconcentration in fish) and prothioconazole-S-methyl (batch equilibrium). More complete information on the environmental fate studies can be located in Appendix B. Although some registrant-submitted studies contain deficiencies and some are classified as supplemental, the studies as a whole provide sufficient information for assessing the environmental fate of prothioconazole combined residues of concern in this screening-level assessment.

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properties of prothioconazole combined residues of concern.							
PARAMETER	VALUE(S) (units)				SOURCE MRID	COMMENT	
Aerobic Aquatic Metabolism Half-life	Prothioconazole combined residues of concern: $t_{1/2}$ = 433.2 days (H, total system, p), 346.6 days $(H, total system, t)$, 106.6 days (A, total system, p), 67.3 days (A, total system, t). $t_{1/2}$ = 17.2 days (H, water layer, p), 16.2 days (H, water layer, t), 23.3 days (A, water layer, p), 21.7 days (A, water layer, t).					46246515	Two systems tested: (H) Honniger Weiher pond (loam/water) and (A) Anglerweiher lake (loamy sand/water). Both phenyl (p) and triazole (t) labels in each system. Half-lives are calculated via linear regression on log-transformed data, combining amounts of prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl per sampling interval. Non- extractable residues added in as parent.
Organic Carbon Partition	(mLy_{0C})	LS	SCL	SL	S		
Coefficient (K_{OC})	Prothioconazole					46246539 46246504	Parent mobility cannot be determined due to instability and low column resolution; very high sorption estimated, lower mobility than transformation products
	Prothioconazole- desthio	523	536	617	625	46246450	Conducted on prothioconazole- desthio as "parent." Used four soils: loamy sand (LS) at 0.79% OC, silty clay loam (SCL) at 1.66% OC, sandy loam (SL) at 2.02% OC, silt (S) at 2.14% OC.
	Prothioconazole- S-methyl	1973	2484	2772	2995	46246501	Conducted on prothioconazole- S-methyl as "parent." Used same soils as MRID: 46246450.
Soil Partition Coefficient (K_d)	(mL/g)	LS	SCL	SL	S		
	Prothioconazole	\overline{a}	٠.		--	46246539 46246504	Same as for K_{OC} .
	Prothioconazole- desthio	4.13	8.90	12.46	13.38	46246450	Same as for K_{OC} .
	Prothioconazole- S-methyl	15.6	41.2	56.0	64.1	46246501	Same as for Koc.

Table 3.7. Summary of physicaUchemica1 and environmental fate and transport

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Table 3.7. Summary of physicaVchemical and environmental fate and transport

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 DAT= days after treatment.

3.3.2 Degradates

The major transformation products (created in amounts greater than or equal to 10% of applied radioactivity) resulting from degradation processes of prothioconazole include:

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prothioconazole-desthio (SXX0665), **prothioconazole-S-methyl** (WAK786 1 -S-methyl), **prothioconazole-thiazocine** (JAU6476-thiazocine), and 1,2,4-triazole (Table **3.8).**

The identified minor transformation products (created in amounts less than 10% of applied radioactivity) resulting from degradation processes of prothioconazole include: **prothioconazole-sulfonic** acid (JAU6726), **prothioconazole-triazolinone** (WAK7860), prothioconazole-3,4,5, and 6-hydroxy-desthio (3,4, 5, and 6-HO-SXX0665), **2** chlorobenzoic acid, and JAU6476-triazolylketone (WAK4993) (Table 3.9).

Data are reported in single replicates.

Studies conducted with both phenyl and triazole radiolabels include: aerobic soil metabolism (MRID: 4624651 1 only), aerobic aquatic metabolism, and aqueous photolysis. All other studies are conducted with phenyl radiolabel only (ie., hydrolysis, soil photolysis, other aerobic soil metabolism (MRID: 46246512 only), anaerobic aquatic metabolism). The 1.2.4-triazole degradate is not able to be detected in studies using the phenyl radiolabel. (The 1,2,4-triazole degradate column for studies which did not use the triazole label are designated "Not able to be detected.")

Field studies not radiolabelled. The only degradates tracked in field studies are: prothioconazole-desthio, prothioconazole-S-methyl, **prothioconazole-thiazocine,** and 1,2,4-triazole. Maximum concentrations in the field studies are determined after the 6" (CA, NY) or Znd (GA) application. (DAT= days after 6th or 2nd treatment); 800 ug/kg, 400 ug/kg, and 1000 ug/kg total prothioconazole applied in CA, GA, and NY, respectively. Blank boxes represent degradates which are not detected above MDL.

Data are reported in single replicates.

Studies conducted with both phenyl and triazole radiolabels include: aerobic soil metabolism (MRID: 46246511 only), aerobic aquatic metabolism, and aqueous photolysis. All other studies are conducted with phenyl radiolabel only (ie., hydrolysis, soil photolysis, other aerobic soil metabolism (MRID: 46246512 only), anaerobic aquatic metabolism). The 1,2,4-triazole degradate is not able to be detected in studies using the phenyl radiolabel. (The 1,2,4-triazole degradate column for studies which did not use the triazole label are designated "Not able to be detected.")

Minor degradates were not tracked in field dissipation studies.

Prothioconazole-desthio (**Figure 3.1**) is formed quickly and in large amounts from all degradation processes evaluated (maximum of 5.7% of applied in hydrolysis, 55.7% in aqueous photolysis, 39.0% in soil photolysis, 49.4% in aerobic soil metabolism, and 54.6 % in aerobic aquatic metabolism total system). It is the result of desulfonation of prothioconazole parent. **~rothioconazole-S-methyl(~igure** 3.2) is also formed in large amounts from anaerobic aquatic metabolism (78.2% of applied in total system) and in lesser amounts from aerobic soil metabolism (14.6% of applied) and aerobic aquatic metabolism (12.7 % of applied in total system). It is the result of methylation of the sulfur of prothioconazole parent. Both of these identified major transformation products are expected to form in large concentrations in both terrestrial and aquatic environment compartments. In addition, based on submitted toxicity studies, prothioconazole-desthio and prothioconazole-S-methyl are expected to exhibit similar toxicity to parent prothioconazole. Therefore, since these degradates are expected to form at high concentrations and are considered toxic, both prothioconazole-desthio and prothioconazole-S-methyl are considered in this assessment, as part of a total toxic residue approach.

Figure 3.2. **Prothioconazole-S-methyl**

Prothioconazole-thiazocine (Figure 3.3) is formed at a maximum of 14.1% of applied radioactivity from aqueous photolysis only. This mode of degradation is only likely to be influential in clear, shallow water, under clear atmospheric conditions. In actual environmental systems, aqueous photolysis is likely to proceed at a much slower rate due to the attenuation of light due to increasing depth of water bodies, light adsorption by suspended solids, and natural obstruction of sunlight. Therefore, since it is likely to form only in very small amounts under very speciiic circumstances in the environment, and the Health Effects Division (HED) has very low concern regarding the hazard associated with this environmental metabolite, prothioconazole-thiazocine is not considered in this assessment. However, under specific conditions (for example, in shallow, clear waters in low order streams close to where the chemical is applied), total toxic residues are not conservative estimates for all of the potential degradates that may form. The 1,2,4-triazole degradate (Figure 3.4) was only tracked in aerobic soil metabolism (MRID: 4624651 1 only), aerobic aquatic metabolism, and aqueous photolysis studies. It formed in large amounts (maximum of 41.8% of applied radioactivity) as a result of aerobic aquatic metabolism, at medium amounts (maximum of 11.9% of applied radioactivity) as a result of aqueous photolysis and at very small, practically non-detectable amounts (maximum of <2.0% of applied radioactivity) as a result of aerobic soil metabolism. The 1,2,4-triazole degradate is being characterized in a separate, cumulative triazole assessment and is, therefore, not considered separately in this assessment.

Figure 3.3. **Prothioconazole-thiazocine**

None of the identified minor transformation products (prothioconazole-sulfonic acid, prothioconazole-triazolinone, prothioconazole-3,4,5, and 6-hydroxy-desthio, 2-chlorobenzoic acid, and JAU6476-triazolylketone) is expected to form at high concentrations in the environment (Table 3.9) nor are there sufficient data to demonstrate their toxicity and, therefore, they are not considered part of the total toxic residues in this assessment.

Unextracted residues were observed at maximums of 56.4% of applied radioactivity in an aerobic soil metabolism study (MRID 462465 1 1, Hofchen soil, triazole label, 365DAT), 46.7% of applied radioactivity in an aerobic aquatic metabolism study (MRID 46246515, Honniger Weiher Sediment-Water System, triazole label, 121DAT) and 26.5% in an anaerobic aquatic metabolism study (MRID 462465 16, Fuquay, Georgia Sediment-Water system, phenyl label, 360DAT). Residue extraction was attempted by extracting with a relatively mild acetonitrile/water solution (80:20, v:v for aerobic soil metabolism, 90:10, v:v for aerobic aquatic metabolism, 8020 or 70:30, v:v for anaerobic aquatic metabolism) on a mechanical shaker followed by hot extraction by reflux for all studies involving soil. Additionally, some aerobic aquatic metabolism study sediment samples were extracted further with acetone/concentrated HCl $(99:1, v:v)$. It is uncertain whether or not greater amounts of soil-bound material could have been extracted using harsher or more appropriate methods. For example, because prothioconazole and its major degradates are neutral organics, extracting with something neutral and organic (such as hexane) may have yielded less unextracted material. Because of this uncertainty associated with soil extraction, unextracted material was considered part of total toxic residues in this assessment.

3.3.3 Dissipation pathways (degradation and off-site movement)

Prothioconazole appears to degrade relatively quickly in the environment, primarily forming the persistent and toxic degradates prothioconazole-desthio and prothioconazole-S-methyl and, in biotic soil and sediment studies, large amounts of unextracted material. This quick degradation in concert with poor extraction methods leads to great uncertainty in composition and bioavailability of large amounts of unextracted material. Due to this uncertainty, biotic degradation rates can not be calculated for prothioconazole alone, and biotic prothioconazole degradation is characterized for prothioconazole, prothioconazole-desthio, prothioconazole-Smethyl and unextracted material together *(i.e.,* prothioconazole combined residues of concern). Prothioconazole combined residues of concern are persistent and likely to dissipate off site mainly via runoff of sediment-bound and dissolved residues from treated fields and possibly via leaching through soil. Deposition off-field or into surface water via spray drift may also play a role in the movement of prothioconazole combined residues of concern off-site. Additionally, prothioconazole combined residues of concern are expected to be subject to soil, water or air transport via soil-bound residues (adsorption-capable K_{OC} s and large amounts of unextracted

material). Prothioconazole combined residues of concern are not expected to undergo atmospheric transport via partitioning to air through spray drift or volatilization (low vapor pressure and small Henry's Law Constant).

3.3.3.1 Fate in soil

Despite high water solubility, given the soil adsorption capacity of prothioconazole combined residues of concern, they are likely to partition to the soil compartment in the environment in non-porous soils of higher organic carbon content, (Prothioconazole adsorption is unknown but it will likely quickly degrade to moderately mobile prothioconazole-desthio and slightly mobile prothioconazole-S-methyl in the soil (FA0 2000).) Therefore, prothioconazole combined residues of concern will likely be present in surface soil long enough to undergo slow degradation. This degradation may be attenuated by sediment sorption in some soils. Based on the available laboratory data, aerobic aquatic metabolism and, to a lesser extent, surface soil aerobic metabolism and anaerobic aquatic metabolism of prothioconazole to prothioconazoledesthio and prothioconazole-S-methyl, and then to 1,2,4-triazole, other minor degradates, and $CO₂$ are likely to be the major routes of degradation in the environment. Again, due to prothioconazole's quick degradation to prothioconazole-desthio and prothioconazole-S-methyl amidst large amounts of quickly-formed, unidentified, unextracted material, and similar toxicological profiles of prothioconazole and prothioconazole-desthio and prothioconazole-Smethyl, biotic degradation rates cannot be calculated for prothioconazole alone.

3.3.3.2 Fate in water

Prothioconazole will enter surface water through spray drift when applied using ground spray or aerial spray. Prothioconazole-desthio and prothioconazole-S-methyl will also reach surface water dissolved in runoff and through runoff of sediment-bound residues [erosion] from agricultural fields (where prothioconazole will degrade into prothioconazole-desthio and prothioconazole-S-methyl). The amount of prothioconazole combined residues of concern dissolved in runoff versus bound to sediment in runoff will depend upon type of soil, with sediment-binding increasing in soils of smaller particle size and greater organic carbon content. Even if there is a large temporal gap between pesticide application and rainfall, runoff concern will likely not be attenuated because of the very slow aerobic soil degradation rate of prothioconazole combined residues of concern. When it does reach surface water, prothioconazole combined residues of concern are expected to persist.

Given its mobility and persistence in soil and detections at 15-30 **cm** and 30-45 cm in terrestrial field dissipation studies, prothioconazole-desthio may leach to ground water, particularly in coarse, sandy soil types with less organic carbon content. If prothioconazole and prothioconazole-desthio reach anaerobic soil depths, however, degradation to prothioconazole-Smethyl will likely increase, and there will still be a chance of ground water contamination. Concentrations of prothioconazole combined residues of concern in ground water are anticipated to be higher in areas with a high water table (because there is less depth to travel before reaching groundwater) and during times when rainfall occurs soon after application because of preferential flow.

3.3.3.3 Fate in air

Based on its low vapor pressure (less than 4×10^{-7} Pa at 20° C) and small estimated Henry's Law constant (less than 2.96 x 10^{-10} atm*m^{3*}mol⁻¹ at 20 \textdegree C), prothioconazole is not likely to partition significantly to air. In addition, volatile products other than $CO₂$ were not detected in volatile traps in the laboratory studies; most of the applied radioactivity, except that of mineralized $CO₂$, was found in the soil or water compartments. At environmentally relevant pH ranges, prothioconazole is not likely to vaporize from soil or water. Therefore, long-range transport in air is not likely to be a dissipation route of concern for prothioconazole. No information regarding vapor pressure or Henry's Law constant are reported for prothioconazoledesthio or prothioconazole-S-methyl.

3.3.3.4 Prothioconazole field studies

The laboratory-predicted major route of dissipation cannot be completely confirmed from the three submitted U.S. terrestrial field dissipation guideline studies (MRIDs: 46246517, 46246518, 46246519) conducted in California, Georgia, and New York and the three submitted U.S. aquatic field dissipation guideline studies conducted in California and Arizona (MRIDs: 46246522, 46246523,46236524).

Even though the lab studies suggest that prothioconazole and prothioconazole-desthio are persistent, this classification results from treatment of unextracted radio-labeled material as parent, an assumption which cannot be replicated in non-radio-labeled field studies. Therefore, persistence predicted from terrestrial field dissipation studies is less than that predicted from the lab studies. These field data show that prothioconazole parent dissipates from the top layer of soil quickly (with a DT_{50} of less than 2 or 3 days), but that prothioconazole-desthio dissipates from the top layer of soil much more slowly (with extremely variable DT_{50} of 28-422 days). Moderate amounts of prothioconazole-S-methyl were detected above the level of quantitation (LOO) in 0-15 cm soil, and 1, 2, 4-triazole was detected at all soil depths, albeit not above the LOQ.

Persistence predicted from aquatic field dissipation studies, however, better corroborated lab studies, with long half-lives for prothioconazole or prothioconazole-desthio in sediment (203.9 days, 12 1.6 days, and 90.0 days in California, Arkansas, and Arkansas cropped aquatic fields, respectively). Dissipation half-lives in paddy water were extremely short (1.7 days, 0.9 days, and 0.6 days in California, Arkansas, and Arkansas cropped aquatic fields, respectively), likely more due to adsorption than degradation.

As expected, due to its quick degradation, prothioconazole was not detected below 15 cm in any of the three fields in California, Georgia or New York. Also, as expected, based on the labpredicted mobility of prothioconazole-desthio, some leaching was found in the terrestrial field studies. Prothioconazole-desthio was detected at levels above the LOQ down to 30 cm and at levels above the minimum detection limit (MDL) but below the LOQ down to 45 cm in one replicate in the California field study, and at levels below the LOQ at one to two sampling times in the Georgia and New York field studies. Prothioconazole-S-methyl was detected below 15

cm only in a single replicate (below LOQ) in the field study in California. Similarly, in the aquatic field dissipation studies, below 3 inches, prothioconazole was detected in only three sampling intervals below LOQ but above the MDL in sediment. Prothioconazole-desthio was detected at 3-6 inch deep sediment through 28 days after treatment (DAT) in the Arkansas flooded field and through 60 DAT in the Arkansas flooded and cropped field. Prothioconazole-S-methyl was detected below 3 inches in sediment only in three sampling intervals in the Arkansas flooded and cropped field.

Field dissipation studies cannot document to where and in what form prothioconazole and prothioconazole-desthio dissipate. Potentially, prothioconazole and prothioconazole-desthio could be degrading to $CO₂$ or degradates other than the additional three measured. Or, they could be moving off site through leaching downward through the soil, subsurface flow laterally, or surface runoff. Prothioconazole and prothioconazole-desthio are likely dissipating through runoff of sediment-bound and dissolved residues and are likely not volatilizing, but neither of these dissipation means can be empirically proved or disproved, as run-off of bound or unbound residues, and volatilization were not measured.

3.3.4 hleasures of Aquatic Exposure

Aquatic exposures estimated in this screening-level risk assessment are based on a set of standardized assumptions related to water body size, watershed size and proximity to the application area. These assumptions are intended to result in high-end, protective exposure estimates. Computer models that simulate the fate of pesticides in the environment are used to calculate estimated environmental concentrations (EECs) of prothioconazole total toxic residues in surface and ground water. This information is used to estimate exposure to fish, aquatic invertebrates and aquatic plants. The EECs are based on submitted fate data that describe how prothioconazole total toxic residues will degrade and move in the environment *(e.g.,* run off, leaching).

EFED used the Tier I1 pesticide aquatic ecological exposure assessment screening models, Pesticide Root Zone Model (PRZM, v3.12 beta) and Exposure Analysis Modeling System (EXAMS, v2.98.04), to calculate surface water EECs (Estimated Environmental Concentrations). The PRZM-EXAMS-calculated peak value represents a 1 -in- 10 year peak value and the maximum 4, 21, 60, and 90-day values represent the 1 -in-10 year maximum 4, 21, 60, and 90-day rolling mean, respectively. Additionally, the PRZM-EXAMS-calculated annual average represents the 1-in-10 year annual mean and the overall average represents the 30-year overall mean. A summary of the model input parameter values is presented in **Table 3.10.** Most input parameters were selected in accordance with EFED's "Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides," Version II (2-28-02).

Four crop scenarios were used to assess impacts of new uses of prothioconazole. Each scenario represents the most vulnerable of areas used to grow the respective crops. Application dates were chosen based on label information compared to scenario crop emergence, maturation, and harvest dates, as follows:

WHEAT (North Dakota):

From label: For fusarium head blight, apply from at least 75% of the wheat heads on the main stem are fully emerged to when 50% of the heads on the mainstem are in flower (ie., between about May 20 to July 1). Do not apply within 30 days of harvest (ie., before July 5). Therefore, June IS was chosen as the wheat scenario application date.

This scenario represents application to wheat (**Figure 3.1**) at a site that is vulnerable to runoff. The same scenario is used to represent barley (Figure 3.2). While a reasonable surrogate for small grains, barley growing areas are more spatially concentrated than wheat. Another minor difference is that the minimum application interval allowed for wheat is 5 days while that allowed for barley is 10 days.

CANOLA (North Dakota):

From label: Apply when canola crop is in 20-50% bloom stage, which is 4-8 days after the canola crop begins to flower. Best protection will be achieved if prothioconazole is applied prior to petals beginning to fall (i.e., about June 15 through August I).

Therefore, June 17 was chosen as the canola scenario application date.

This scenario represents application to canola (Figure 3.3) at a site that is vulnerable to runoff. The same scenario is used to represent oilseed subgroup composed of rapeseed, Indian rapeseed, Indian mustard, field mustard, black mustard, crambe and borage.

BEAN (Michigan):

Crop harvest date— September 4

From label: Apply at first sign of disease (*i.e.*, post emergence, post June 5). Do not apply within 7 days of harvest (*i.e.*, before August 28).

Therefore, June 29 was chosen as the bean scenario application date.

This scenario represents application to dried shelled peas (Figure 3.4) and beans (Figure 3.5) subgroup (except soybeans) at a site that is vulnerable to runoff. The same scenario is used to represent chickpeas and lentils. A minor difference is that the minimum application interval allowed for dried shelled peas and beans subgroup (except soybeans) is 5 days while that allowed for chickpeas and lentils is 10 days.

PEANUT **(North** Carolina):

From label: Preventative spray schedule—prothioconazole for sprays 3, 4, 5, and 6 of 7 spray application program, starting 30-40 days after planting *(i.e.,* Spray l= June 11, Spray 2= June 25, Spray 3= July 9). When using Leaf Sport Advisory Program, spray prothioconazole in the first advisory spray in July and continue at 14 day intervals.

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Therefore, July 9 was chosen as the peanut scenario application date.

This scenario represents application to peanuts **(Figure 3.6)** at a site that is vulnerable to runoff.

The application rate used in the surface water assessment represents the maximum single application rate on the proposed label for all crops (i.e., 5.7 fl. oz. PROLINE@ 480SC fungicide/A $(41\%$ a.i.), which translates into 0.2 kg a.i./ha or 0.178 lbs a.i./A). The total maximum application allowable for the annual growing season varied with crop type, according to the label (2 applications for wheat, barley, canola and oilseed subgroup, 3 applications for chickpeas, dried shelled peas and beans subgroup (except soybeans), and lentils, and 4 applications for peanuts). Minimum spray intervals between applications per crop specified on the label were used (7 days for wheat and barley, 5 days for canola and oilseed subgroup, chickpeas, dried shelled peas and beans subgroup (except soybeans), and lentils, and 14 days for peanuts).

As there were two major degradates (prothioconazole-desthio and prothioconazole-S-methyl) detected in major amounts in all fate laboratory studies (except hydrolysis, and aqueous and soil photolysis for prothioconazole-S-methyl), it is assumed that these degradates are likely to result in significant environmental concentrations. In addition, prothioconazole-desthio and prothioconazole-S-methyl are likely to exhibit similar toxicity to the parent prothioconazole, based on submitted toxicological studies on these degradates, and HED has concern regarding the mammalian hazard associated with these environmental metabolites. Therefore, estimated environmental concentrations are based on total toxic residues, *i.e.,* the parent prothioconazole compound plus prothioconazole-desthio and prothioconazole-S-methyl. Degradation rates are calculated by adding together these combined residues of concern per sampling interval.

For PRZM-EXAMS surface water modeling inputs, the organic carbon partition coefficient (K_{OC}) was used instead of the soil partition coefficient (K_d) because K_d was related to organic carbon for the four soils tested. Therefore, the partition coefficient corrected for organic carbon (K_{OC}) , was assumed to better represent partitioning in soil. The values from which the lowest non-sand K_{OC} was chosen are presented in Appendix C. Given the total toxic residue modeling approach, the K_{OC} of prothioconazole-desthio was chosen for use in modeling because it has the highest mobility of all compounds being assessed (prothioconazole, prothioconazole-desthio, and prothioconazole-S-methyl). The lowest non-sand prothioconazole-desthio K_{OC} (instead of the average K_{OC} specified in the Input Parameter Guidance) is used in surface water modeling as a conservative estimate of potential mobility, in order to account for uncertainties associated with the inability to calculate a K_{OC} for prothioconazole parent. A sensitivity analysis indicated that using Koc approximately four times larger (that of prothioconazole-S-methyl) resulted in only a minor EEC decrease which did not affect the risk conclusions. Thus, use of these two $K_{OC}s$ adequately covers the largest range of exposure possibilities.
Degradation half-lives were adjusted for use in the PRZM-EXAMS models according to the input-parameter guidelines. The aerobic soil metabolism half-lives and aerobic aquatic metabolism half-lives were calculated by linear regression on log-transformed data (prothioconazole total toxic residues plus unextracted residues), and then the $90th$ percentile confidence bound on the mean of those six and four values, respectively, was used in modeling. The values from which the aerobic soil metabolism and aerobic aquatic metabolism half-life input values were calculated are presented in Appendix C. The anaerobic aquatic metabolism half-life value, calculated by linear regression on log-transformed data (prothioconazole total toxic residues plus unextracted residues) was not significantly different than zero and was assumed to be stable for the purposes of modeling. The aqueous photolysis study was continuously illuminated and the aqueous photolysis half-life input value (calculated using prothioconazole total toxic residues) was adjusted to reflect photolysis in summer sunlight at 40 ⁰N latitude and corrected for degradation observed in the dark-controls.

While the proposed label allows for both ground and aerial application, aerial spray was modeled as the method of application in order to be protective of all application scenarios. In general, aerial application results in larger amounts of drift than ground applications. Therefore, when all other parameters remain the same, PRZM-EXAMS calculate higher surface water concentrations for aerial spray than for ground spray due to default drift assumptions.

Table 3.10. PRZM (v3.12 beta) and EXAMS (v2.98.04) input parameter values for prothioconazole use on wheat, canola, beans, and peanuts (total toxic residues)¹.

^I Parameters are selected as per Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides; Version I, February 28,2002.

The EECs calculated from PRZM-EXAMS modeling are presented in **Table 3.11.** The greater number of application for beans and peanuts resulted in proportionately higher estimated environmental concentrations than those calculated for wheat and canola. All concentrations are likely to be conservative, given conservative assumptions regarding the inclusion of unextracted material, low K_{oc} , aerial application, and shortest reapplication intervals. Not including unextracted material decreases EECs by 57-78%. Using the higher K_{α} value of prothioconazole-S-methyl decreases EECs by 0.3-29%. EECs resulting from ground application are 8-27% lower than those resulting from aerial application (see **Appendix C** for less conservative values).

Table 3.11. Maximum Tier I1 Estimated Environmental Concentrations (EECs) for surface water based on aerial application of prothioconazole (total toxic residues).

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3.3.4.1 Measures of Aquatic Exposure for Rice Use

Unlike the other crops for which prothioconazole use is proposed, rice is unique in that it is typically grown in several inches of water. Estimated exposure concentrations are based on applications of pesticides directly to the paddy water where rice is grown. The Environmental Fate and Effects Division's (EFED) Screening Rice Model (EPA, 2002) incorporates application rate and the K_d to obtain EECs; note that this model does not currently incorporate degradation into the EEC estimation. The K_d is related to the K_{oc} and is specific to the soil type present where the crop is grown. For rice, we assumed a soil carbon content of 2%, which is thought to be common to rice growing areas in Louisiana, for example. The proposed prothioconazole label specifies that Proline 480 SC should be applied to rice paddies when the rice crop is in the panicle differentiation to late boot stage. At this stage in the rice crop, there is likely to be roughly 70-90% coverage of the rice paddy water because the vegetative component of the rice crop is nearly full grown (Breithaupt, personal communication, 2006). A range of possible EECs for rice are provided in **Table** 3.12 to account for potential differences in rice crop coverage of the paddy water. A sample calculation is provided in Appendix G.

3.3.5 Measures of Terrestrial Exposure

Prothioconazole is proposed for use on food crops and will be applied by ground spray for most crops and in-furrow for peanuts. Measures of exposure for terrestrial organisms can be obtained from a variety of sources including monitoring data, field studies, GIs analysis, and exposure modeling. For this assessment, exposure modeling was used to generate EECs for both terrestrial animals and plants. Some refined methods may incorporate GIs analysis to evaluate species-specific exposures but these were not conducted for this analysis. Furthermore, other data sources such as monitoring data and field studies were not used to estimate prothioconazole exposure because these data were unavailable.

The screening-level assessment focuses on dietary exposure for terrestrial birds, mammals, reptiles, and amphibians that may come in contact with prothioconazole use areas. Although other routes of exposure may be important, for the most part dietary routes of exposure are considered to contribute most to total exposure and hence are the focus here. Moreover, suitable data are frequently unavailable to adequately assess other exposure routs such as dermal, inhalation, or incidental soil ingestion and, in addition, adequate tools for assessing these routes are not currently available.

3.3.5.1 Terrestrial Animal Species

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. EFED'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 maximum 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 although the frequency and duration of foraging events on the use site is not explicitly considered or specified.

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 prothioconazole use and sensitive ecological processes. However, it is worth noting that pesticides are frequently used from spring through fall, which are the 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. The majority of applied prothioconazole will likely be limited to the use area although some exposure may occur outside the use site via spray drift.

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 and important source of uncertainty in this assessment.

3.3.5.1.1 Terrestrial Animal Exposure Modeling

Estimated exposure concentrations for terrestrial receptors were determined using the standard screening-level exposure model, TREX (v1[1].2.3)(US EPA, 2004). Maximum exposure levels were calculated for spray applications of prothioconazole using maximum proposed application rates, maximum number of applications, and minimum reapplication intervals for all uses (Table 3.12). These 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. For 1.0 lb a.i. of pesticide per acre, the upper-bound, food item concentration in mg a.i./kg of diet (parts per million [ppm]) is: 240 for short grass, 110 for tall grass, 135 for broadleaf plants and small insects, and 15 for fruits pods, and large insects. 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.

TREX 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 EECs. TREX 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 concentrations 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 RQs.

The foliar dissipation half-life can be important in estimating exposure because it essentially determines how long the pesticide remains on food items after application. In many cases, an empirically determined foliar dissipation half-life value is not available, in which case the default value of 35 days is used (Willis and McDowell, 1987). For prothioconazole, there were three magnitude-of-residue studies that were used to generate and empirically determine a foliar dissipation half-life. The three studies (MRIDs $462461-33$, $462461-34$, $462461-35$) provided 14 estimates of foliar dissipation half life for both the parent and prothioconazole-desthio for three plants; wheat forage, turf forage, and peanut forage. Since a total-toxics residue approach is being used in this assessment to estimate aquatic EECs, the foliar dissipation half-life from the combined parent and prothioconazole-desthio was used. The $90th$ percentile foliar dissipation half-life is estimated to be 6.44 days calculated from the 14 estimates of foliar dissipation halflife assuming a normal distribution (Appendix G). The $90th$ percentile value was used to be consistent with the approach used for fate input data.

Table 3.12 lists EECs for birds, reptiles, terrestrial amphibians, and mammals obtained from TREX simulation for all proposed uses of prothioconazole at the maximum label rates.

* Label does not provide specific interval but states that Proline 480 SC may be applied from panicle differentiation to late boot and again as late as 70% panicle emergence; a 5-day interval was assumed.

3.3.5.2 Terrestrial Plant Species

 $\bar{\mathcal{A}}$

Exposure of naturally-occurring terrestrial and semi-aquatic (wetland) plant species is estimated using OPP's TerrPlant (v1 .O) model and is assumed to encompass areas outside the immediate

use site. For non-wetland and wetland areas, exposure calculations are based on the amount of pesticide present in soil as a function of runoff and drift. Loading to dry, non-target, adjacent areas is assumed to occur from one acre of treatment to one acre of the non-target area. In wetland areas, pesticide loading occurs from a larger area; 10 acres of treated area to one acre of non-treated area. Spray drift is also a source of pesticide loading to non-target areas. The default spray drift assumptions are 1% for ground applications and 5% for aerial, airblast, forced air, and chemigation applications. Drift is not considered for formulations of pesticides that are not spray-applied (e.g., granules); however, runoff is still considered and expressed on a percent of applied mass basis.

3.321 *Terrestrial* **and** *Semi-Aquatic Plant Exposure Modeling*

Prothioconazole exposure to terrestrial and semi-aquatic plants was estimated using OPP's TerrPlant (v1.0) model. The model generates EECs for plants residing near a use area that may be exposed via runoff and/or spray drift, as explained above. The EECs are generated from one application at the maximum rate for a particular use and compound-specific solubility information. Only a single application is considered because it is assumed that for plants, toxic effects are likely to manifest shortly after the initial exposure and that subsequent exposures do not contribute to the response. Hence, the model estimates EECs based on application rate, solubility factor and default assumptions of drift. The EECs for terrestrial and semi-aquatic plants for a single application of prothioconazole at the maximum label rate for proposed uses are presented in **Table 3.13.**

3.3.5.3 *Residue Studies*

Environmental residue studies can also provide useful information regarding the potential exposure of terrestrial wildlife and plant species in and around use areas. These data can be used to corroborate modeling results or to provide additional insights into chemical fate with respect

to exposure. For prothioconazole, no studies are available; all estimates of exposure are based on model outputs.

3.4 Ecological Effects Characterization

The ecological effects characterization for prothioconazole is based on registrant-submitted toxicity studies that provide data on the parent, prothioconazole, and major metabolites. Given the complexities associated with the fate, transport, and toxicity of prothioconazole in the environment, a total toxics residue approach is used to evaluate the potential risks of both parent and metabolites. Hence, the lowest available toxicity value for a taxa and duration (e.g., acute freshwater fish) will be used to calculate RQs. Generally, prothioconazole-desthio was the most toxic chemical tested and toxicity resulting from exposure to this degradate/metabolite were predominantly used for RQ calculations. A brief summary of available toxjcity data used to calculate RQs is provided below; a more detailed discussion of all available studies can be found in Appendix F.

Prothioconazole and metabolites (-desthio, and -S-methyl) are, for the most part, practically nontoxic to birds and honeybees under acute exposure conditions. The only exception is an acute dietary study in bobwhite quail involving exposure to prothioconazole-desthio where the compound is classified as slightly toxic to this test species. There were no significant effects of the parent or metabolites in several avian reproduction studies. For aquatic freshwater animals, prothioconazole and metabolites are moderately toxic. For estuarine/marine fish and invertebrates, the toxicity ranges from slightly to very highly toxic under acute exposure conditions. However, the very highly toxic designation is for estuarine/marine invertebrates and may be suspect as explained below. Although there were few toxicological effects of prothioconazole and metabolites on terrestrial plant species, aquatic plants appear particularly sensitive to both the parent, and especially prothioconazole-desthio. Importantly, results from submitted toxicity studies are not likely to capture the toxicity of prothioconazole and metabolites to all species of birds, mammals, plants, or aquatic organisms. Only a few surrogate species are used to represent all fish, birds, mammals, invertebrates, and plants. Furthermore, there are no currently required toxicity tests for amphibians or reptiles; birds are used as surrogates for reptiles and terrestrial-phase amphibians and freshwater fish are used as surrogates for aquatic-phase amphibians. In general, the representation of numerous species by a few commonly used laboratory species, which are often chosen for amenability to laboratory study, is a source of uncertainty.

In addition to the data submitted in support of registration and the information compiled through the Agency pesticide review process, the ECOTOX (ECOTOXicity) database was used to identify additional toxicity data from the open literature. The ECOTOX database is a userfriendly, publicly-available, quality-assured, comprehensive tool for locating toxicity data from the open literature and is maintained by EPA Mid-Atlantic Ecology Division. More information on ECOTOX can be found at: http://www.epa.gov/ecotox. Research papers are thoroughly screened using standard procedures before being accepted into ECOTOX thereby ensuring consistent, high quality information. For prothioconazole, two studies were identified by

ECOTOX, however, these studies were not used in the risk assessment since the endpoints were not relevant.

3.4.1 Aquatic Effects Characterization

3.4.1.1 Aquatic Animals

Toxicity values for aquatic animals are summarized in **Table 3.14.**

3.4.1.1.1 Fresh water **Fish**

Eight acute freshwater fish studies were submitted for review. The studies involved the parent, technical grade prothioconazole, the formulated end product (Proline 480 SC), and the major degradate, prothioconazole-desthio. In six of the eight studies, the data indicated that the compound tested is moderately toxic to freshwater fish. The most sensitive species is rainbow trout (*Oncorhynchus mykiss*) with median lethal concentrations (LC₅₀s) ranging from 1.69 mg a.i./L for the formulated product to 5.94 mg/L for prothioconazole-desthio.

Two freshwater fish early life-stage tests and one freshwater fish full life-cycle test were submitted for review. The two early life-stage tests are classified as invalid due to excessive control mortality. The remaining full life-cycle test is classified as supplemental but suitable for use in the risk assessment. In the life-cycle test, fathead minnows (Pimephales promelas) were exposed to prothioconazole-desthio; the test showed deleterious effects of the compound on larval/juvenile survival, spawning frequency, and growth. The no-observed-adverse-effect concentration (NOAEC) is 0.148 mg/L and the associated lowest-observed-adverse-effect concentration (LOAEC) is 0.296 mg/L.

3.4.1.1.2 Fresh water Invertebrates

Five invertebrate acute toxicity studies were submitted for review. Of these, four fulfilled guideline requirement and involved exposure of water fleas (Daphnia magna) to prothioconazole, the formulated product (Proline 480 SC), and the degradates prothioconazoledesthio, and prothioconazole-S-methyl. Daphnids are most sensitive to the parent, prothioconazole, with an median effect concentration (EC_{50}) of 1.20 mg a.i./L, which classifies prothioconazole as moderately toxic to freshwater invertebrates on an acute exposure basis.

Two freshwater invertebrate life-cycle toxicity studies were submitted for review. In one study, Daphnia magna were exposed to prothioconazole parent and in the other to prothioconazoledesthio. Daphnids were slightly more sensitive to prothioconazole-desthio with a NOAEC of 0.103 mg/L and an associated LOAEC of 0.206 mg/L. These effect levels are associated with reduced offspring production (offspring per parent and offspring per parent per reproductive day).

3.4.1.1.3 Estuarine/Marine Fish

One study on the acute toxicity of prothioconazole to an estuarine/marine fish was submitted for review. Sheepshead minnows (Cyprinidon variegates) were exposed to prothioconazole parent for 96-hours. The 96-h LC_{50} was greater than 10.3 mg a.i./L, which was the highest concentration tested. Based on these results, prothioconazole is classified as slightly toxic to estuarine/marine fish on an acute exposure basis.

3.4.1.1.4 EstuarineMarine Invertebrates

Three studies were reviewed on the acute toxicity of prothioconazole (and one metabolite) to estuarine and marine invertebrates and all were classified acceptable. A study of the toxicity of the parent, prothioconazole, on Eastern oysters (Crassostrea virginica) and mysid shrimp (*Americamysis bahia*) indicated that this compound is moderately toxic to these species on an acute exposure basis with EC_{50} and LC_{50} values of 3000 and 2400 μ g a.i./L, respectively. However, a study in which mysid shrimp were exposed to prothioconazole-desthio resulted in an LC_{50} of 60 µg a.i./L, which classifies prothioconazole as very highly toxic to this species under acute exposure conditions.

A life-cycle test involving mysid shrimp exposed to prothioconazole-desthio resulted in a NOAEC of 64 μ g a.i./L based on reductions in the number of offspring produced. This chronic NOAEC is actually slightly higher than the concentration that killed 50% of mysid shrimp following a 96-hr acute exposure LC_{50} estimate suggesting that lethal (LC_{50}) and sub-lethal (NOAEC) effects occur at more of less the same concentration for the same chemical. Followup acute studies produced LC_{50} 's in excess of 1000 ppb (Appendix 9 in the mysid life cycle test report MRID 462460-30), however, no explanation was provided for the differences in LC_{50} 's seen in the original acute test and the follow-up tests. Overall, these results suggest that there is considerable variation in the response of mysids to prothioconazole-desthio. Moreover, it is possible that the life cycle test, for whatever reason, did not adequately capture the potential sensitivity of mysids. To address this uncertainty, an acute-to-chronic ratio was used to estimate the chronic toxicity endpoint from the lowest available msyid LC_{50} . The acute-to-chronic ratio from freshwater daphnids is 11.7 (NOAEC/LC₅₀: 1200ppb/103ppb). Using this factor and adjusting the mysid LC_{50} of 60 ppb yields an estimated NOAEC of 5.2 ppb.

3.4.1.2 Aquatic Plants

Toxicity values for aquatic plants are summarized in **Table 3.15**

3.4.1.2.1 Freshwater Plants

Three studies were submitted on the acute toxicity of prothioconazole and related compounds on the aquatic vascular plant, *Lemna gibba.* This plant species is most sensitive aquatic vascular plant tested using prothioconazole-desthio with an EC_{50} of 35 μ g/L, based on decreased frond number in exposed plants. The corresponding NOAEC and EC_{05} are 5.8 and 3.9 μ g/L, respectively.

There were five studies submitted on the acute toxicity of prothioconazole and related compounds to aquatic non-vascular plants. Similar to the results for aquatic vascular plants, green algae *(Scenedesmus subspicatus)* are most sensitive to prothioconazole-desthio with an EC₅₀ of 0.07 :g/L. The corresponding NOAEC and EC₀₅ were less than 0.01 μ g/L and 0.01 μ g/L, respectively. Endpoints are based on decreased cell density.

3.4.1.2.2 EstuarineMarine Plants

Two 96-hr studies were submitted on the acute toxicity of prothioconazole and prothioconazoledesthio on nonvascular estuarine/marine diatoms *(Skeletonema costatum)*. The lowest toxicity endpoint is from the study involving exposure to prothioconazole-desthio. The 96-hr EC_{50} is 21 μ g/L. The associated NOAEC and EC₀₅ are 7.3 and 7.7 μ g/L, respectively.

3.4.2 Terrestrial Effects Characterization

3.4.2.1 Terrestrial Animals

Toxicity values for terrestrial animals are summarized in **Table 3.16.**

3.4.2.1.1 Birds

Two acute oral and three acute dietary studies were conducted to determine the acute toxicity of prothioconazole and its metabolites to avian species. Results from the acute oral studies indicated that prothioconazole and prothioconazole-desthio are practically non-toxic to bobwhite quail (Colinus virginianus) under acute oral exposure conditions. In both studies, the median lethal dose (LD₅₀) exceeded the highest dose level tested, i.e., > 2000 mg/kg body weight. The subacute dietary studies indicated that prothioconazole-desthio is slightly toxic to bobwhite quail while the parent is practically non-toxic to both bobwhite quail and mallard ducks (*Anas* platyrhychos). Mortality of bobwhite quail occurred mostly at the highest exposure level of 5,215 mg/kg diet where there was 70% mortality. The LC_{50} is 4,252 mg/kg diet. Sub-lethal effects observed in both the acute oral and acute dietary studies were related to decreased food consumption and/or body weight in exposed birds.

Four avian reproduction studies were submitted for review; bobwhite quail and mallard duck studies were conducted for both prothioconazole and prothioconazole-desthio. In the study in which mallard ducks were exposed to prothioconazole-desthio, there were stability problems with the chemical, which lead to a supplemental classification of this study. In all four studies, there were no significant effects of either chemical on any adult or reproductive parameters. The toxicity estimate is therefore based on the highest measured concentration; the true effect level cannot be determined from these data. The NOAEC (449mg/kg diet) is based on mallard ducks exposed to prothioconazole-desthio.

3.4.2.1.2 Mammals

In an acute oral study on rats, SPF-bred Wistar rats *(Rattus* norvegicus) of the strain Bor:WISW (SPF-Cpb), 5/sex/group were given a single oral dose of prothioconazole-desthio of 100, 500 (males only), 1000 (females only), 2000, 2500 (males only), 3150 and 4000 mg/kg bw (MRID

462462-31). The lowest LD_{50} is for female rats $(LD_{50}=2,506 \text{ mg/kg bw})$.

A chronic, 2-generation reproduction study (MRID 462463-33) was conducted using 30 Sprague-Dawley rats/sex/dose. Dietary exposure levels of prothioconazole-desthio were 0, 40, 160, and 640 mg/kg diet, which corresponded to approximately 0, 2.5, 9.5, and 40 mg/kg bwlday, respectively. The LOAEL for reproductive effects is 640 ppm and resulted in decreased pup viability and decreased pup body weight. Specifically, day 4 pup viability showed the greatest effect (33% decrease compared to control); this estimate compares the number of pups alive on post-partum day 4 compared to the number of pups born. The corresponding NOAEL is 160 mg/kg diet which is equivalent to a dose of 9.5 mg/kg bw/day in female rats. The study is classified as ACCEPTABLE and satisfies guideline requirements.

3.4.2.1.3 Insects

Two acute oral studies were submitted to evaluate the toxicity of prothioconazole and the formulated end product (Proline 480 SC) on insects. Results indicated that for both studies, the compounds were practically non-toxic to honeybees, Apis mellfera, under acute oral exposure conditions. The lowest LD_{50} is greater than the highest dose tested, i.e., $LD_{50} > 71$:g/bee. Results are similar for acute contact studies of honeybees in which the LC_{50} is greater than 200 :g/bee.

Several non-guideline toxicity tests on soil-dwelling terrestrial invertebrates were submitted. These studies were not formally reviewed and were taken at face value to provide a sense of the potential effects of prothioconazole on soil-dwelling terrestrial invertebrates; EFED does not calculate RQs to assess risks to terrestrial invertebrates at this time. The studies on terrestrial invertebrates included exposures of both earthworms (Eisenia fetida) and springtails (Folsomia candida) to prothioconazole and the major metabolites, prothioconazole-desthio and prothioconazole-s-methyl. From an acute toxicity basis, prothioconazole and degradates did not appear to be toxic to terrestrial invertebrates since all LC_{50} s were greater than the highest tested concentration. Under long-term exposures, prothioconazole-destho appeared to be the most toxic of the chemicals and produced soil NOAECs of 1.0 and 62.5 mg/kg soil for earthworms and springtails, respectively. In the earthworm study, the number of offspring produced by 56 days was lowered by 26% compared to controls in the 3.2 mg/kg soil treatment, which was the LOAEC. In springtails, the 28 day day LOAEC was 125 mg/kg soild and was associated with a 24% reduction in the number of juveniles compared to the controls.

3.4.21.4 Terrestrial Plants

Tier I plant studies were conducted with 10 species of plants; 5 monocots and 5 dicots were exposed to 0.272 Ibs a.i./A, which is greater than any proposed single application rate of 0.178 lbs a.i./A. For most species, effects did not exceed a *25%* inhibition compared to controls. However, for cucumber plants, there was a greater than 25% effect on shoot length and dry weight in the seedling emergence study. Although effects in cucumbers did not exceed 25% in the vegetative vigor study, the percent inhibition for this species was generally among the highest. The results from the Tier I study indicated that a Tier I1 study is required for cucumber.

The Tier I1 study on cucumber plants involved exposure at several concentrations to prothioconazole, not just the highest level used in the Tier I study. Interestingly, there were no effects that exceeded a *25%* inhibition compared to control for the Tier I1 study. However, there were significant effects on both shoot height and dry weight with the lowest NOAEC associated with shoot height. The NOAEC and EC_{05} for shoot height are equivalent to application rates of 0.03 and 0.08 lbs ai/A, respectively; the NOAEC is used to calculate RQs. Toxicity values for terrestrial plants are summarized in **Table 3.17.**

4 Risk Characterization

Risk characterization is the integration of exposure and effects to estimate the ecological risk from the use of prothioconazole and the potential for effects on aquatic life, wildlife, and plants, based on a number of pesticide use scenarios. The goal of risk characterization is to provide an estimate and description of potential adverse effects and specifically to articulate risk assessment assumptions, limitation, and uncertainties; synthesize an overall risk conclusion; and provide risk managers with sufficient information to support regulatory decisions.

4.1 Risk Estimation - **Integration of Exposure and Effects Data**

Toxicity data and exposure estimates are used to evaluate the potential for adverse ecological effects on non-target species. For this screening-level assessment of prothioconazole, the deterministic risk quotient method is used to provide a metric of potential risks. The RQ is a comparison of exposure estimates to toxicity endpoints; estimated exposure concentrations are divided by acute and chronic toxicity values. The resulting RQs are compared to the Agency's LOCs, which are the Agency's interpretive policy such that when LOCs are exceeded, the need for regulatory action should be considered. These criteria are used to indicate when the use of a pesticide, as directed on the label, has the potential to cause adverse effects on non-target organisms.

4.1.1 Non-target Aquatic Organisms

Surface water concentrations resulting from prothioconazole application to agricultural crops are estimated with Tier I1 models PRZMIEXAMS. Four scenarios are evaluated and consist of aerial or ground spray applications of prothioconazole to wheat (ND), canola (ND), bean (MI), and peanut (NC).

One-in-10 year peak EECs were compared to acute toxicity endpoints to derive acute RQs and 1in- 10 year 2 1 -day average EECs are compared to chronic toxicity endpoints to derive chronic RQs. Acute and chronic RQs for fresh- and saltwater organisms are summarized in Tables 4.1 **and 4.2,** respectively.

For aquatic vascular and non-vascular plants, I-in-10 year peak EECs are compared to acute EC_{50} values to derive acute non-listed species RQs. In addition, peak EECs are also compared to NOAEC or EC_{05} values for aquatic plants to derive listed species RQs. All RQs for aquatic plants are presented in **Tables 4.3 and 4.4.**

4.1.1.1 Fresh water Fish and In vertebrates

Table 4.1 lists the RQs for freshwater fish, aquatic-phase amphibians, and freshwater invertebrates potentially exposed to prothioconazole for the modeled uses on wheat, bean, canola, and peanut. For all four scenarios, the RQs did not exceed non-listed or listed species acute or chronic risk LOCs. The highest EECs are associated with the peanut scenario. Table 4.2 lists the RQs for freshwater fish, aquatic-phase amphibians, and freshwater invertebrates potentially exposed to prothioconazole associated with the proposed use on rice. Risk quotients exceed the acute risk LOC (RQ \geq 0.05) for the proposed use on rice when there was no simulated interception of the applied chemical. Also, the chronic RQ exceeds the chronic risk LOC $(RQ₂1.0)$ for freshwater invertebrates if no interception of the applied chemical occurs.

4.1.1.2 EstuarineMarine Fish and Invertebrates

Table 4.3 lists the ROs for estuarine/marine fish and invertebrates potentially exposed to prothioconazole for modeled uses on wheat, canola, bean, and peanuts. The RQs for estuarine/marine fish do not exceed any acute risk LOCs with all RQs less than 0.01; chronic toxicity data were not available for a representative fish species so RQs could not be calculated. The RQs for estuarine/marine mollusks similarly did not exceed any acute risk LOCs with all RQs<O.Ol. Based on mysid shrimp toxicity data, the listed species acute risk LOC of *0.05* is exceeded for estuarine/marine invertebrates for all modeled scenarios while the acute non-listed species LOC of *0.5* is exceeded for use on beans and peanuts (RQs = *0.55* & 0.57, respectively).

Chronic RQ values were only calculated for non-molluskan invertebrates since this was the only taxa with chronic toxicity data. The listed and non-listed species chronic risk LOC ($RQ>1.0$) for estuarine marine invertebrates is exceeded for all modeled uses with RQs ranging from 2.4 to 6.4.

*Exceeds the listed species acute risk LOC ($RQ \ge 0.05$)

**Exceeds the non-listed species acute risk LOC (RQ>0.50) and the listed species acute risk LOC

Bolded chronic RQs exceed the listed and non-listed species chronic risk LOC ($RQ \ge 1.0$)

Table 4.4 lists the RQs for estuarine/marine fish and invertebrates exposed to prothioconazole applied to rice according to the proposed label. Acute and chronic RQs exceed the acute and chronic risk LOCs for non-molluskan invertebrates; RQs do not exceed acute risk LOCs for estuarine/marine fish or mollusks.

**Exceeds the non-listed species acute risk LOC (RQ>0.50) and the listed species acute risk LOC (RQ≥0.05)

**Exceeds the non-listed and listed species chronic risk LOC ($RQ \ge 1.0$)

4.1.1.3 Aquatic Plants

Table 4.5 lists the RQs for aquatic vascular and non-vascular plants potentially exposed to prothioconazole. For both vascular and non-vascular plants, the listed species acute risk LOC of 1.0 is exceeded for all modeled scenarios ($RQs = 2.20-3127$). In addition, only RQs for nonvascular plants exceed the non-listed species acute risk LOC for all modeled scenarios ($RQs =$ 173-465); no non-listed species acute risk LOCs are exceeded for vascular plants except for use on rice. **Table 4.7** lists the RQS for freshwater aquatic plants potentially exposed to prothioconazole for use on rice. All acute and listed species acute risk LOCs are exceeded except for the scenario in which there is a 90% interception rate in which the acute risk LOCs are not exceeded for freshwater vascular and saltwater non-vascular plants.

Table 4.6 lists the RQs for estuarine/marine non-vascular plants potentially exposed to prothioconazole. These RQs exceed the listed species acute risk LOC for all modeled uses (1.75-4.71) and exceed the non-listed species acute risk LOC at the proposed application rates to beans and peanuts ($RQs = 1.58 \& 1.64$, respectively).

Aquatic plant acute risk LOC ($RQ \ge 1.0$); applies to non-listed and listed species

Aquatic plant acute risk LOC (RQ \geq 1.0); applies to non-listed and listed species

4.1.2 Non-target Terrestrial Organisms

The EEC values for estimated exposure to terrestrial animals for spray applications of prothioconazole were derived using the Kenaga nomogram, as modified by Fletcher (Fletcher et al., 1994). Exposure estimates were generated for all proposed label uses with single application rates ranging from 0.134 to 0.178 lbs a.i./A with 2-4 applications depending on the specific use. The application rates and number of applications represent the maximum as specified by the proposed label. The RQs are based on these maximum exposure estimates and the lowest available toxicity endpoints for a given taxa and exposure duration *(e.g.* acute avian). Specifically for this assessment, the lowest LD_{50} and NOAEC values were used for birds and mammals. Note again that data from avian toxicity studies were used to represent reptiles and terrestrial-phase amphibians.

Acute and chronic RQs for birds, reptiles, and terrestrial-phase amphibians are presented in **Tables** 4.5 and **4.6,** respectively, acute and chronic RQs for mammals are summarized in **Tables 4.7, 4.8-4.9,** respectively.

4.1.2.1 Birds

Table 4.5 lists the avian dose-based acute RQs for proposed uses of prothioconazole. No RQs exceed non-listed or listed species acute risk LOCs with RQs ranging from <0.01 to 0.05.

Acute Risk LOCs; non-listed species RQ20.5 and listed species RQ2O. **¹**

Table 4.6 lists the acute and chronic dietary-based avian RQs for proposed uses of prothioconazole. No acute or chronic LOCs are exceeded for any proposed uses. Acute dietarybased RQs range from <0.01 to 0.01 and dietary-based chronic RQs ranged from 0.01 to 0.18.

Acute Risk LOCs; non-listed species $RQ \geq 0.5$ and listed species $RQ \geq 0.1$

Chronic Risk LOC; RQ> 1.0 for non-listed and listed species

4.1.2.2 Mammals

Table 4.7 lists the dose-based acute mammalian RQs for proposes uses of prothioconazole. No acute risk LOCs are exceeded with RQs ranging from <0.01 to 0.01.

Acute Risk LOCs; non-listed species $RQ \ge 0.5$ and listed species $RQ \ge 0.1$

Table 4.8 lists the dose-based chronic mammalian RQs for proposed uses of prothioconazole. The non-listed and listed species chronic risk LOC (RQ>1.0) is exceeded for all proposed uses of prothioconazole. However, LOC exceedances are specific to food item with no exceedances associated with mammals that consume seeds or fruits/pods/large insects with RQs ranging from 0.01-0.23. For all other food items, the chronic risk LOCs are exceeded, particularly for smaller mammals. The highest RQs are for mammals that consume short grass with RQs ranging from 0.99-3.75 followed by mammals that consume broadleaf plants/small insects $(RQs = 0.56-2.11)$ and tall grass $(RQs = 0.45-1.72)$. In addition, RQs are higher for smaller mammals for all uses and food items due to an increased food ingestion rate associated with the higher metabolic rate of smaller mammals.

Bolded values exceed the chronic risk LOC (RQ \geq 1.0) for non-listed and listed mammalian species

Table 4.9 lists chronic dietary-based mammalian RQs for proposed uses of prothioconazole. These RQs are based on effects levels associated with chemical concentrations in feed. The RQs do not exceed the chronic risk LOCs for any proposed uses of prothioconazole with RQs ranging from 0.02-0.5 1.

The chronic risk LOC for non-listed and listed mammalian species is $RQ \ge 1.0$

4.1.2.3 Terrestrial Plants

Table 4.10 lists the terrestrial and semi-aquatic plant RQs for proposed uses of prothioconazole. Risk quotients exceed the acute plant risk LOC ($RQ>1.0$) for semi-aquatic listed plants; no other LOCs are exceeded. Risk quotients for non-listed terrestrial and semi-aquatic plant species are not calculated because an EC_{25} (>0.272 lbs a.i./A, highest test level) could not be estimated from the Tier I1 plant study.

Bolded values exceed listed plant acute risk LOC (RO >1.0)

4.1.3 RQs Based on Mean Kenaga Residues

Table 4.11 lists dose-based chronic mammalian RQs using mean Kenaga residue values instead of the upper-bound values. Dietary-based chronic mammalian RQs using mean Kenaga values are presented in **Table 4.12.** Dose-based chronic mammalian RQs are the only RQs to exceed chronic risk LOCs using the upper-bound Kenaga values. These RQs do not form the basis of risk conclusions for birds and mammals but are provided for comparison purposes. Using the mean Kenaga residue values for RQ calculation would not sufficiently protect mammals that consume food items that have residues on the higher end of the residue distribution. In effect, risk decisions based on the mean Kenaga values would not account for up to 37% of the higherend residues. Importantly, using the upper-bound Kenaga residue values does not represent a highest-possible-concentration; up to 13% of higher-end residues are not accommodated. The implications and utility of these values are described further in the Risk Description section.

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4.2 Risk Description

The available data on the fate and effects of prothioconazole are sufficient to address the risk hypothesis for all taxa as specified in the Overview Document (EPA, 2004). The results of this screening-level risk assessment indicate partial acceptance of the risk hypothesis; there is potential for direct adverse acute effects for freshwater non-vascular plants, estuarinelmarine non-vascular plants, and estuarine/marine invertebrates and direct chronic effects for mammals and estuarine/marine invertebrates associated with proposed uses of prothioconazole. These results are based on modeled spray application rates ranging from 0.268 to 0.712 lbs a.i./A per year, which represent most of the proposed uses of prothioconazole applied at the maximum label rate.

4.2.1 Risk to Aquatic Organisms

The risk assessment for aquatic species is based on data from prothioconazole and prothioconazole degradates (-desthio, and -s-methyl). This is based on the likely behavior of the parent and degradates in the environment; the parent is expected to degrade rapidly. Aquatic EECs were based on a total toxics residue approach to account for the simultaneous presence of the parent and degradates.

4.2.1.1 Fresh water Fish

Acute and chronic RQs for freshwater fish ranged fiom 0.01-0.02 and 0.08-0.22, respectively, indicating that a potential for direct effects to freshwater fish is low for non-rice uses of prothioconazole. Since the proposed label specifies that prothioconazole should be applied to rice from panicle differentiation to late boot, it is likely that the rice crop will intercept some applied prothioconazole before it gets to the paddy water. Assuming a 70-90% interception rate (equivalent to 70-90% crop cover) reduces RQs to below the LOCs.

The freshwater fish toxicity data indicate that prothioconazole and degradates are slightly to moderately toxic to tested species, which partly explains the low RQ values. Extrapolation to other freshwater fish is uncertain. In all likelihood, more sensitive species are likely to exist but given the low RQs for tested species, the likelihood of adverse effects on freshwater fish or aquatic-phase amphibians is believed to be low.

4.2.1.2 Freshwater Invertebrates

Acute and chronic RQs for freshwater invertebrates ranged from 0.0 1-0.03 and 0.12-0.32, respectively, indicating that a potential for direct adverse effects to freshwater invertebrates is low for non-rice uses of prothioconazole. For rice, results were similar to those of freshwater fish; the chronic RO, exceeds the chronic risk LOC ($RO = 1.28$) when 0% interception is assumed. Assuming even moderate (50%) interception, however, would lower EECs to the point where RQs would not exceed LOCs. Given that rice plants must be fairly mature before prothioconazole can be applied, it is likely that up to 90% of the paddy area may be covered by vegetative material which would effectively intercept applied chemical. However, given that at least prothioconazole-desthio is somewhat persistent, it is possible that the majority of this chemical will remain in the paddy eventually ending up in the water. The persistence of the desthio metabolite may also have implications for crawfish which are sometimes raised in paddies that were previously used for rice or may be raised in paddies adjacent to rice paddies. In the former case, residual prothioconazole-desthio may be a concern. In the latter case, crawfish ponds may receive prothioconazole as a result from drift associated with application to adjacent rice paddies or through flooding with water released from adjacent or nearby rice paddies. Although these exposure scenarios are possible, it seems unlikely, given the RQs, the apparent toxicity of prothioconazole-desthio to freshwater invertebrates, and the conservative nature of the model that exposure levels would be high enough to result in significant toxicological effects on crawfish. However, the possibility cannot be excluded. Uncertainties could be reduced with a toxicity study on crawfish and improved understanding of the behavior of prothioconazole and metabolites in rice paddies.

The freshwater invertebrates toxicity data indicate that prothioconazole and degradates are moderately toxic to tested species, which partly explains the low RQ values. Extrapolation to other fieshwater invertebrates is uncertain. In all likelihood, more sensitive species are likely to exist but given the low RQs for tested species, likelihood of adverse effects on freshwater invertebrates is expected to be low.

In addition to toxicity studies on fieshwater fish and commonly used freshwater invertebrates (Daphnia), a non-guideline 28-day (chronic) toxicity study on sediment-dwelling larvae of black flies, *Chironomus riparius*, was available for review (MRID 462461-32. The study was classified as SUPPLEMENTAL because it did not follow guideline requirements and not all exposure levels were analytically verified; the study provides limited insight into the potential effects of prothioconazole on sediment dwelling invertebrates. However, to provide some perspective on the potential for adverse effects to sediment-dwelling invertebrates, a range of RQs was calculated based on the NOAEC, which could only be estimated as between 99 and 1010 ppb because not all exposure levels were verified. The highest EEC based on PRZM/EXAMS model runs was for the proposed use of prothioconazole on beans and is 31.33 ppb; using this EEC results in a RQ range of 0.32-0.031, which is below the chronic risk LOC.

For rice, assuming a 70% cover, the EEC is 39.6 ppb which results in RQs ranging from 0.04 to 0.4, the latter of which exceeds the acute risk LOC for aquatic invertebrates ($RQ \ge 0.05$). Hence, if the actual NOAEL is closer to 99 than 1010 ppb for sediment dwelling invertebrates, some risks may be expected associated with the use of prothioconazole on rice.

4.2.1.3 Saltwater **Fish**

The likelihood of acute mortality for estuarine/marine fish is low, based on the results of the screening assessment; acute RQs for modeled scenarios (including rice) are 0.01 or lower. No chronic estuarine/marine fish toxicity data were submitted for prothioconazole or its degradates, however, given the low potential for acute adverse effects and the low potential for chronic effects to freshwater fish species, the likelihood of chronic effects in saltwater fish species would be low.

4.2.1.4 Saltwater Invertebrates

Based on this screening-level analysis, the estuarine/marine invertebrate RQ values for the proposed application to rice, beans and peanuts exceeds the acute risk LOC (RQs=0.55 and 2.2). In addition estuarine/marine invertebrate chronic risk LOCs are exceeded for the proposed use of prothioconazole on rice, even when the % interception (equivalent to crop cover) is 90%. Therefore, there is a potential for chronic effects to saltwater invertebrates associated with the use of prothioconazole on rice.

Although the screening-level analysis indicates a potential for acute and chronic risks to saltwater invertebrates, there is some uncertainty in this conclusion based on the toxicity data. The acute LC_{50} for mysid shrimp is 60 ppb and is actually lower than the experimentallydetermined chronic NOAEC of 64 ppb. These results are incongruent with typical toxicological patterns and logic; it is highly unlikely that the sub-lethal effects threshold (NOAEC) would equal or be less than the concentration that causes 50% mortality under shorter-term exposures $(LC₅₀)$. Importantly, both the EPA and the Canadian Pest Management Regulatory Agency (PMRA) reviewers classified both the acute and chronic studies as ACCEPTABLE. Follow-up acute toxicity study with mysids by the same laboratory that conducted the original acute test indicated that the LC_{50} may be greater than 1000 ppb. However, given that the original acute test was classified as acceptable, these results taken as a whole, suggest that there is considerable variability in the mysid population or husbandry conditions. Furthermore, given this backdrop of potential mysid test system variability, the life cycle test may not have captured mysids in their most sensitive state. Therefore, an acute-to-chronic ration was used to estimate a NOAEC from the mysid LC_{so} of 60 ppb; the estimated NOAEC is 5.2 ppb based on daphnid acute-to-chronic ratio. Although repeating the mysid life cycle test may reduce uncertainty associated with the NOAEC, any new results would not negate the results of the original mysid acute test, which is the basis for risk conclusions concerning saltwater invertebrates.

If risk conclusions concerning estuarine/marine invertebrates are, in fact, accurate, it is important to acknowledge that invertebrates are vitally important components of estuarine and marine environments providing food for a wide variety of species, including listed species. In addition

to a potential reduction in biomass, mortality of invertebrates could lead to a shift in the invertebrate community structure towards less sensitive species, which may or may not result in a change in ecosystem function (see Relyea, 2005). However, exposure to estuarinelmarine invertebrates is likely overestimated in this assessment. Because estuarine/marine environments are characterized by large and dynamic volumes of water, it is highly likely that any amount of chemical that reaches the estuarine environment will dissipate quickly.

4.2.1.5 Aquatic Plants

Based on predicted EECs for the modeled prothioconazole uses and available toxicity data, LOCs are exceeded for non-listed, non-vascular aquatic plants. For aquatic vascular plants, the acute risk non-listed species acute risk LOC is exceeded for rice uses unless there is approximately 90% interception of applied chemical. The relatively high RQs for these species are largely due to the high toxicity of mostly prothioconazole-desthio. In comparison to the parent, prothioconazole-desthio was about twice as toxic to vascular plants and it was about 10- 45 times more toxic to non-vascular plants. This comparison for non-vascular plants, however, is not based on the same species and hence must take that into consideration. Regardless, these data suggest that non-vascular plants are particularly sensitive.

The LOCs for non-listed non-vascular plants are exceeded for all modeled uses $(RQs = 173$ -3127). Peak aquatic EECs would have to be as low as 0.01 ppb to reduce all RQ values for nonvascular aquatic plants to below the LOCs. Even using toxicity data from the parent, prothioconazole, all RQs would range from 14.5-107.5, which exceed all acute risk LOCs for non-vascular plants.

The non-listed species acute risk LOCs are not exceeded for any modeled use other than rice. Similar to non-vascular plants, data from prothioconazole-desthio study was used as toxicity endpoints because it had the lowest EC_{50} . However, the study on the parent compound actually yielded a lower NOAEC/EC₀₅. Using these data would have generated RQs ranging from 8.0-21.5, which are qualitatively similar to the results presented above. Similarly, the endpoint chosen for the vascular aquatic plant study, frond number, had the lowest EC_{50} but not the lowest $EC_{0.5}$, which was associated with effects on dry weight ($EC_{0.5}$ was 1.7 ppb vs. 3.9 ppb for frond number); using the lower EC_{05} for dry mass would have yielded higher acute listed species RQs but would not have altered the risk conclusions.

Aquatic plants are key components to all aquatic ecosystems and provide a multitude of ecological functions. They provide food and shelter for a wide variety of aquatic animal species and help maintain water quality through temperature modulation, filtration, and oxygen supply. Any effects on aquatic plants as a result of prothioconazole use would be expected to result in significant ecosystem-level effects. Most notably, there would likely be a near instant decrease in water quality associated with plant decay and depletion of oxygen. In turn, sedimentation would likely increase due to decay and a loss in filtering capacity. The depletion of oxygen and increased siltation could result in widespread fish and invertebrate mortality. The cascade of effects due to effects on aquatic plants would pose a risk to any aquatic listed species near the use area as well as terrestrial species that rely on aquatic organisms as food items such as

piscivorous birds, mammals, or reptiles (see Relyea, 2005 for an example of pesticide-induced effects on aquatic communities).

4.2.2 **Risks to Terrestrial Organisms**

4.2.2.1 Birds

No avian acute or chronic risk LOCs are exceeded for any uses of prothioconazole indicating that the likelihood of adverse effects on birds is low. Toxicity studies on prothioconazole and prothioconazole-desthio indicated that the compound and degradates are not very toxic to birds. The parent and prothioconazole-desthio were both classified as practically non-toxic to birds based on acute oral toxicity studies. For the subacute dietary study, prothioconazole-desthio is classified as slightly toxic with an LC_{50} of 4252 mg/kg feed. Results from the chronic study yielded a NOAEC for mallard ducks exposed to the desthio metabolite of 449 mg/kg feed, which was the highest exposure level tested. Taken as a whole, the risk estimation results and the toxicity data indicate a low potential for adverse effects to avian species associated with proposed uses of prothioconazole.

4.2.2.2 Mammals

Acute risks to wild mammals were evaluated using a common laboratory rat LD_{50} , which is greater than the highest tested concentration $(>2000 \text{ mg/kg bw})$. The rats were exposed to prothioconazole-desthio and the data indicate that prothioconazole-desthio is practically nontoxic to mammals on an acute exposure basis. Calculated dose-based RQs for all proposed uses of prothioconazole are $\leq 0.01 - 0.01$. The low apparent acute toxicity of prothioconazole and the low RQs indicate a low potential for adverse effects to mammals associated with all proposed uses of prothioconazole.

In contrast to the acute risk conclusions for mammals, an evaluation of chronic risks showed that the dose-based chronic risk LOCs are exceeded for all uses of prothioconazole for at least some combinations of mammal body size and food item type. Generally, RQs are higher for smaller mammals that consume short grass, followed by consumers of broadleaf plants/small insects, and finally, tall grass; for these food items, $ROs = 0.52-3.75$. No chronic risk LOCs are exceeded for mammals (35-1000g) that consume seeds or broadleaf fruits/pods/large insects. In order to reduce dose-based chronic RQs below the LOC for chronic mammalian risk for all uses of prothioconazole, the application rate would have to be below 0.10 Ib a.i./A for a single application. The use on dried shell peas and beans results in the highest RQ; to reduce RQs to below the chronic risk LOCs would require an application rate below 0.05 Ibs a.i./A applying 3 times at 5-day intervals.

To provide bounds on the estimate of RQs associated with food item residues, RQs associated with mean Kenaga residue values are also provided. For maximum application rates of prothioconazole, risk from chronic exposure is likely with ROs ranging form \leq LOC -1.09 **(Table** 4.11). Chronic risk in this exposure scenario, however, is primarily limited to the smallest mammals that consume short grass and for the higher application rates of

prothioconazole associated with some uses. Using the data summarized in Fletcher *et* al. (1 994) for input values, distributions were generated that describe residue levels on the various food items, assuming a log-normal distribution. In this case, the mean Kenaga residue estimates typically fell within the 62-87 percentiles, indicating that about 38-13% of the higher-end residue estimates were not captured in estimating exposure. In contrast, for the upper-end Kenaga residue estimates, about 3-13% of the upper-end residue estimates were not captured. This highlights the fact that the upper-end Kenaga values are not a maximum exposure level.

The dose-based estimate of risk as opposed to dietary-based risk quotients is the basis of risk conclusions because it addresses differential feed consumption and is body-weight specific. The dose-based estimate of risk is derived fiom data on rat body weight, food consumption, and concentration of compound in feed. These data are available for prothioconazole. Risk quotients based on the dietary estimates of toxicity are provided for comparison purposes. In general, RQs based on dietary exposures are lower; for prothioconazole, dietary-based RQs did not exceed the chronic risk LOC (\overline{R} Os = 0.02-0.42). However, the uncertainties associated with dietary-based RQs reduce the confidence in these estimates. The dose-based approach considers the uptake and absorption kinetics of an oral toxicity study to approximate exposure associated with uptake from a dietary matrix. Toxic response is a function of duration and intensity of exposure. For many compounds an oral gavage dose represents a very short-term high intensity exposure. Although the dose-based estimates may not reflect reality in that animals do not receive a gavage while feeding, it is possible that a short-duration, high-intensity exposure could occur associated with feeding on a agricultural field if food items are readily available. While the dietary-based estimates may suggest greater "realism", they too suffer from some uncertainties. Primarily, the dietary-based approach assumes that animals in the field are consuming food at a rate similar to that of confined laboratory animals despite the fact that energy content in food items differs between the field and the laboratory as does the energy requirements of wild and captive animals. Generally, dose-based estimates of toxicity are taken as the EFED default although the dietary-based estimates can provide further insight into potential risks of some compounds. Additionally, if dietary-based RQ values are adjusted for differential feeding rates for various sized animals, the values would be roughly similar to the oral-based RQ values.

Chronic risk to wild mammals was evaluated using a laboratory rat NOAEL of 9.5 mg/kg bw/day and a corresponding NOAEC of 160 mg/kg-feed, based on reduced pup viability and body weight and increased developmental defects in offspring. Although use of the NOAEL is standard for estimating chronic RQs, there is considerable uncertainty associated with this toxicity value because the actual no effect level is likely between the study determined NOAEL and the LOAEL. In the case here, a LOAEL was established at the highest test concentration which corresponded to 40 mg/kg bw. In reality, a no or minimal effect level likely lies between the NOAEL (9.5 mg/kg bw) and the LOAEL (40 mg/kg bw). A curve fitting approach was used to estimate a minimal effect level for F2 pup viability to explore the possible impacts on RQs. The Benchmark Dose Approach (BMD; US EPA, 2006) was used to fit toxicity data and then interpolate to 5, 10, and 20% effect levels, which represent a range of effect levels that likely overlap where ecologically significant effects may be expected to occur (Appendix G). The endpoint chosen for this analysis was second generation (F2) viability index which showed the largest effect level compared to control; there was a 33% decrease in the day 4 viability index in

pups at the highest exposure (40 mg/kg bw) and no decrease in the next lowest dose (9.5 mg/kg) bw). The BMD software allows the choice of several models; in this case the polynomial function had the best fit and appeared to model the data adequately. The polynomial function generated a 5, 10 and 20% effect levels of 20.8, 25.7, and 33.0 mgkg bw, respectively. Using these values to estimate risks result in a range of RQs for 20g mammals from 1.1 (20% effect level) to 1.7 (5% effect level) for dried shell peas and beans for small mammals that consume short grass. The RQs for dried shell peas and beans represent the highest RQs associated with proposed uses of prothioconazole. Alternatively, the same exercise conducted for the proposed use on barley (leaf and stem disease), which results in the lowest estimated RQs, produces a range of RQs for 20g mammals from 0.62 (20% effect level) to 1.0 (5% effect level). Taken as a whole, the results from this exercise indicate that even using effect levels that may be considered closer to the threshold of ecological relevance, some RQs still exceed the LOC. In particular, for smaller mammals that consume short grasses, RQs exceed chronic risk LOCs for most proposed uses of prothioconazole, especially at the 10 and 20% effect levels. The results from this exercise would be more robust if an acceptable/unacceptable effect level were known or established for day 4 pup viability. Other parameters for which there are significant differences show a less drastic reduction compared to control. For example, pup body weights in the 40 $mg/kg-d$ treatment are only about 8-10% lower than controls for F1 and F2 litters suggesting that for this effect, ecologically relevant effect levels are likely to be fairly close to the experimental LOAEL. The results from this analysis and the observed reproductive effects of prothioconazole-desthio suggests that species of mammals that rely on a high reproductive rate (r-selected) for population sustainability may be most susceptible to the effects of prothioconazole.

4.2.2.3 Potential Risk to Birds and Mammals: BCF Analysis

A fish bioconcentration study was submitted for both prothioconazole and prothioconazoledesthio. Prothioconazole-desthio is considered persistent in the environment and therefore, bioconcentration of this chemical is possible. The highest BCF for prothioconazole-desthio was estimated to be 94.3 for whole bluegill sunfish. Although prothioconazole-desthio was shown to depurate quickly with an approximate half-life of less than a day, because it is persistent, it is possible for fish to maintain prothioconazole-desthio tissue levels that correspond to water concentrations. The accumulation of prothioconazole-desthio in fish species may present an exposure route to piscivorous birds and mammals. Risk quotients for birds were calculated by comparing estimated prothioconazole+degradate concentrations in fish to the dietary-based NOAEC. For mammals, RQs were calculated using estimates of food ingestion rate (US EPA, 1993; Appendix G) and comparing a daily dose to dose-based NOAEL. Prothioconazole-desthio in fish was assumed to be 94.3 times the highest aquatic EEC, which was 0.139 mg/l representing a maximum EEC for rice. This exercise indicates that the potential adverse chronic effects to avian and mammalian piscivorous species, is limited since chronic RQs ranged from 0.80 (mammals) to 0.02 (birds). Since the acute toxicity values were significantly higher than chronic endpoints, there is also limited potential for acute risks. Hence this analysis indicates that risks to piscivorous birds and mammals associated with proposed uses of prothioconazole are unlikely. This corroborates conclusions drawn from the fish BCF studies in which results indicated that prothioconazole-desthio does not bioaccumulate.

4.2.2.4 Plants

Tier I plant studies demonstrate the potential for prothioconazole to affect some terrestrial plants, specifically cucumbers showed effects of greater than 25% compared to control at an exposure equivalent to an application rate of 0.272 Ibs a.i./A. Cucumber is the only plant species out of a total of 10 species tested for which an effect of greater than 25% was observed. A Tier I1 study was conducted on cucumber given the Tier I results. In the Tier I1 study, cucumber did not show effects greater than 25% compared to control up to the highest tested concentration, so an EC_{25} could not be calculated. However, both an EC_{05} and a NOAEC could be determined based on the effects of prothioconazole on growth. Only RQs for listed plant species were presented since there were no effects greater than 25% in the Tier I1 study on cucumber (and no effects >25% on other species). However, to provide perspective on potential effects to non-listed species, RQs were calculated assuming the $EC_{25} = 0.272$ lbs a.i./A and ranged from 0.03-0.36, further corroborating that the likelihood of adverse effects on plants is low. Taken as a whole, the results from the toxicity study and the RQs do not provide compelling evidence that non-listed terrestrial plants are likely to suffer adverse effects associated with the proposed uses of prothioconazole.

As with any toxicity test, there are uncertainties regarding whether test species adequately represent the range of possible sensitivities in native organisms. Plants tested are crop plants, typically subjected to hundreds of years of human selection. It is likely that some native species would be more sensitive than commonly used crop species given the tremendous variation and number of native plant species. Tests using wild-type species may help reduce this uncertainty, but a critical review paper McKelvey *et* al. (2002) suggests that, in general, crop testing may be sufficiently protective of most plants.

There are several uncertainties regarding risk to plants. One is whether the default assumption of 5% spray drift (from aerial application) in TerrPlant is sufficiently protective. Estimates made from actual drift assessments range to higher than 20% for fine sprays, which could indicate that risk to plants is underestimated. To gain a better understanding of the potential for spray drift to affect terrestrial plants, Tier I AgDRIFT modeling (v. 2.01) was used to determine how far offfield prothioconazole levels would remain above the NOAEC (0.03 ppb). AgDRIFT[®] utilizes empirical data to estimate off-site deposition of aerial and ground applied pesticides, and acts as a tool for evaluating the potential of buffer zones to protect sensitive habitats from undesired exposures. Assuming the maximum single application rate of 0.178 lbs a.i./A, fine to medium droplet size, 10 mph winds, and 10 ft application height, plants 50 ft or closer to the treated use area may be exposed to levels of prothioconazole above the NOAEC. If droplet size were reduced to very fine to fine, plants 150 ft and closer to the treated area may be exposed to levels of prothioconazole above 0.03 ppb. The proposed prothioconazole label specifies not to spray if wind speeds are greater and 15 mph, which is higher than the AgDrift Tier I default of 10 mph. Higher wind speeds would result in greater potential distribution of droplets thereby increasing

the distance from the treated area where prothioconazole exposure levels may be above the NOAEC. However, Tier I modeling with AgDrift does not allow changing most parameters. For semi-aquatic plants, results from AgDRIFT do not significantly alter exposure compared to TerrPlant estimates. There are a number of factors that contribute to the actual extent of spray drift including aircraft type, nozzle number and placement, atmospheric conditions, speed of operation, and swath specifics. The Tier I AgDRIFT model incorporates assumptions for these factors based on typically encountered scenarios. Nonetheless, Tier I model results provide some perspective on the impact of spray drift on risk estimates for terrestrial and semi-aquatic plants. More details concerning the specifics and uncertainties of AgDRIFT are available online at www.agdrift.com. Although Tier I modeling suggests that plants may be exposed to levels of prothioconazole above the NOAEC, many agricultural areas have been widely cultivated and water bodies are not typically natural so it may be unlikely for listed plant species to occupy areas within 50 ft of an agricultural field. However, a more detailed assessment of species location and prothioconazole use areas is needed to determine, with confidence, the potential for risks to listed plant species. This was not conducted for this assessment.

Another uncertainty associated with estimating risks to plants is that current assessment methods account for only a single application of the chemical since it is assumed that effects to plants would likely manifest after a single application and that toxicity is less dependent on subsequent exposures. It may be difficult to confidently apply this reasoning to all plants under all circumstances and hence remains a source of some uncertainty. Alternatively, results from submitted plant studies address inhibition of growth, which, in many cases may become less important as exposure decreases through time as the result of chemical degradation or dissipation. Since prothioconazole is a fungicide intended to protect plant species from fungal diseases, it is possible that any prothioconazole-induced effects would not exert permanent damage.

4.2.2.5 Non-Target Terrestrial In vertebrates

EFED currently does not estimate risk quotients for terrestrial non-target insects. However, an appropriate label statement is required to protect foraging honeybees when the LD_{50} is < 11 μ g/bee. Based on the acute contact toxicity study to honeybees, the LD₅₀ for prothioconazole is $>$ 200 µg/bee. This classifies prothioconazole as practically non-toxic to honeybees.

Although EFED does not currently assess risks to terrestrial invertebrates, several studies submitted on the toxicity of prothioconazole and it's degradates on soil-dwelling invertebrates allows a sense of the potential effects. The acute LC_{so} for soil-dwelling invertebrates were higher than the highest tested concentration, which was 1000 mgkg soil. Potential prothioconazole soil concentrations can be estimated using a simple and conservative approach whereby the total chemical added to a use are is divided by the volume of soil present assuming a given depth (and soil density of 2.6 $g/cm³$). The highest yearly application rate for prothioconazole is 0.712 lbs/A. Assuming that prothioconazole is mixed down to only 1 cm soil depth, the soil concentration of prothioconazole would be approximately 3 mg/kg soil, which is at least 100 times lower than any acute toxicity value. Conversely, if we look at the lowest chronic NOAEC, which was 1.0 mg/kg soil for reproductive effects in earthworms, if
prothioconazole was mixed into the top 3 cm of soil (or less), the potential exposure would equal or exceed the NOAEC but up to a factor of 3. However, given the mobility of prothioconazole and it's degradates and the fact that the NOAEC was based on results from a 56 day study, it's likely that prothioconazole would not be confined to the top 3 cm of soil.

4.2.3 Federally Threatened and Endangered (Listed) Species of Concern

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. *5* 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 (U.S. EPA 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.

The federal action addressed herein is the proposed registration of pesticide products that contain the active ingredient prothioconazole. Crops for which prothioconazole uses are proposed for reregistration are identified in Section **1** .O. Growing areas for these crops encompasses most of the United States.

4.2.3.1 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by prothioconazole use and not merely the immediate area where prothioconazole is applied. At the initial screening-level, the risk assessment considers broadly described taxonomic groups and so 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 the listed species are located within an assumed area, which has the relatively highest potential exposure to the pesticide, and that exposures are likely to decrease with distance from the treatment area. Section 1.0 of this risk assessment presents the pesticide use sites that are used to establish initial co-location of species with treatment areas.

4.2.3.2 Taxonomic Groups Potentially at Risk

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 on listed species that depend upon the taxonomic group for which the RQ was calculated. 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 are considered to determine the extent to which screening assumptions regarding an action area apply to a particular listed organism. These subsequent refinement steps will consider how this information would impact the action area for a particular listed organism and potentially include areas of exposure that are downwind and downstream of the pesticide use site.

Assessment endpoints, exposure pathways, and the conceptual model addressing proposed prothioconazole uses, and the associated exposure and effects analyses conducted for the prothioconazole screening-level risk assessment are in Sections 2 to 3. The assessment endpoints used in the screening-level risk assessment include those defined operationally as reduced survival and reproductive impairment for both aquatic and terrestrial animal species and survival, reproduction, and growth of aquatic and terrestrial plant species from both direct acute and chronic exposures. These assessment endpoints address the standard set forth in the Endangered Species Act requiring federal agencies to ensure that any action they authorize does not 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. Risk estimates, RQs, integrating exposure and effects are calculated for broad based taxa groups for the screeninglevel risk assessment are presented in Section 4.1.

Both acute endangered species and chronic risk LOCs are considered in the screening-level risk assessment to identify direct and indirect effects to taxa of listed species. 'This section identifies direct effect concerns, by taxa, triggered by exceeding endangered LOCs in the screening level risk assessment with an evaluation of the potential probability of individual effects for exposures that may occur at the established endangered species LOC. Data on exposure and effects collected under field conditions are evaluated to make determinations on the predictive utility of the direct effect screening assessment findings to listed species. Additionally, the results of a

screen for indirect effects to listed species, using direct effect acute and chronic LOCs for each taxonomic group, is presented and evaluated.

A description of the potential direct effects associated with exposure to prothioconazole for each of the taxonomic groups is provided below. **Table 4.13** provides a summary of the direct effects for non-listed and Federally listed species, including the range of RQ values.

'Associated Taxa refers to those taxa for which there are direct effects that may indirectly affect a listed species taxa.

4.2.3.2.1 Listed Species Risk Quotients

Fresh water Fish and Amphibians

The listed species acute LOC is exceeded for fish and aquatic amphibians exposed to prothioconazole for the proposed use on rice with and RQ of 0.08. For all other uses, the listed species acute and chronic LOCs are not exceeded with acute RQs for these taxa ranging from $\leq 0.01 - 0.03$ and chronic RQs ranging from 0.08-0.32.

Freshwater Invertebrates

For rice, results were similar to those of freshwater fish; the acute and chronic RQs, exceed the listed species acute risk LOC (RQ = 0.11) and the chronic risk LOC (RQ = 1.28) when 0% interception is assumed.

Estuarine/Marine Fish

The listed species acute risk LOCs for estuarine/marine fish are not exceeded for any uses of prothioconazole when applied at the maximum label rates. Acute RQs for estuarine marine fish were all ≤ 0.01 ; chronic ROs were not generated since there was no life-cycle estuarine/marine fish toxicity test.

Estuarine/Marine Invertebrates

The listed species acute risk LOCs are exceeded for non-molluskan, estuarine/marine invertebrates for all modeled uses of prothioconazole ($RQ \ge 0.05$). In contrast, the corresponding chronic risk LOCs are not exceeded for all uses except rice; RQs associated with the proposed rice use range was 26.4. Uncertainties associated with the risk conclusions for acute risks to estuarine/marine invertebrates are discussed under section 4.1.2.4. Briefly, several acute mysid tests indicate a large degree of variability in sensitivity to prothioconazole-desthio with toxicity endpoints varying by several orders of magnitude between different tests. The mysid life-cycle test, however, does not reflect the more sensitive mysids seen in the acute studies. In addition, there are no estuarine/marine invertebrates currently listed as threatened or endangered.

Aquatic Plants

The listed species acute risk LOCs are exceeded for vascular and non-vascular aquatic plants for all modeled uses of prothioconazole ($RQ = 1.75-3127$, calculated as NOAEC or EC_{05}/EEC). This includes estuarine/marine non-vascular plants.

For aquatic vascular plants, the acute listed species LOC is exceeded for all modeled uses and the non-listed species acute risk LOC is exceeded for rice uses unless there is approximately 90% interception of applied chemical. The relatively high RQs for these species are largely due to the high toxicity of mostly prothioconazole-desthio. In comparison to the parent, prothioconazole-desthio was about twice as toxic to vascular plants and it was about 10-45 times more toxic to non-vascular plants. This comparison for non-vascular plants, however, is not based on the same species and hence must take that into consideration. Regardless, these data suggest that non-vascular plants are particularly sensitive.

The LOCs for listed non-vascular plants are exceeded for all modeled uses $(ROs = 173-3127)$. Peak aquatic EECs would have to be as low as 0.01 ppb to reduce all RQ values for non-vascular aquatic plants to below the LOCs. Even using toxicity data from the parent, prothioconazole, all RQs would range from 14.5-107.5, which exceed all acute risk LOCs for non-vascular plants.

The listed species acute risk LOCs for aquatic vascular plants are exceeded with ROs = 2.2-5.9.

The non-listed species acute risk LOCs are not exceeded for any modeled use other than rice. Similar to non-vascular plants, data from prothioconazole-desthio study was used as toxicity endpoints because it had the lowest EC_{50} . However, the study on the parent compound actually yielded a lower NOAEC/EC₀₅. Using these data would have generated RQs ranging from 8.0-2 1.5, which are qualitatively similar to the results presented above. Similarly, the endpoint chosen for the vascular aquatic plant study, frond number, had the lowest EC_{50} but not the lowest EC_{05} , which was associated with effects on dry weight (EC_{05} was 1.7 ppb vs. 3.9 ppb for frond number); using the lower EC_{05} for dry mass would have yielded higher acute listed species RQs but would not have altered the risk conclusions.

Birds

The listed species acute and chronic risk LOCs for birds, reptiles, and terrestrial-phase amphibians are not exceeded for any uses of prothioconazole applied at the maximum label rates. Acute RQs ranged from $\leq 0.01 - 0.06$ and chronic RQs ranged from $\leq 0.01 - 0.18$.

Mammals

Listed species acute risk LOCs ($RQ \ge 0.1$) for direct effects of prothioconazole on mammals are not exceeded for all uses of prothioconazole. Alternatively, listed species chronic risk LOCs $(RQ \ge 1.0$; range of $RQs = 0.01-3.75$) are exceeded for all uses of prothioconazole for at least some mammals that consume short grass, tall grass, broadleaf plants, and small insects. In particular, RQs for smaller mammals are generally higher compared to those of larger mammals. For mammals that consume fruits/pods/large insects, or seeds, the chronic risk LOCs are not exceeded.

Terrestrial Plants

Listed species acute risk LOCs $(RQ>1.0)$ for direct effects of prothioconazole on semi-aquatic plants are exceeded for a single application for all uses of prothioconazole with RQs = 2.28- 3.03). Listed species acute risk LOCs are not exceeded for terrestrial plants adjacent to treated areas.

4.2.3.2.2 Probit Dose-Response Analysis

Aquatic Listed Species Probability of Effects on Individuals

The probability of individual effects at the acute endangered species LOC ($RQ = 0.05$ which is equivalent to $1/20$ of the LC₅₀ or EC₅₀) for each major listed species' taxonomic group and the probability of individual effects at estimated acute RQs above the endangered species acute risk LOC is provided here. In addition, extrapolation of low probability events such as those occurring at the LOC, are associated with a high degree of uncertainty. To address this

uncertainty, analyses of individual effects are also conducted at the upper and lower 95% confidence interval of the probit slope for each taxon. The probit slopes used in this analysis were obtained from dose-response relationships for toxicity endpoints used in calculating RQs.

For freshwater fish, a probit dose-response slope could not be estimated from the rainbow trout data and therefore the probit dose-response analysis is based on a default slope of 4.5. Should exposure to listed freshwater fish occur at the acute listed species LOC, the probability of one individual being affected is 1 in 4.2 x **08.** Analyses of the probability of individual effects occurring at exposures that occur at the EECs for these organisms indicate that for fish, the probability of individual effects is 1 in 2.5 x 10⁶ (for rice) to 1 in 1.0 x 10¹⁶; low probability events.

The probability of individual effects to listed freshwater invertebrates should exposure occur at the LOC is 1 in 1 .OE+16. The probit dose-response slope used for freshwater invertebrates is 13.7 (95% C.I.-9.1 -18.2) based on a 48-h acute study of water fleas. At the lower and upper 95% confidence limits the probability of individual effects is still 1 in 1.0 x 10^{16} . For freshwater invertebrates that might be exposed to levels of prothioconazole corresponding to the cropspecific EECs, the probability of individual effects is1 in 1.0×10^{16} . The probability of individual effects to mollusks is not calculated since the estimated EC_{50} is based on effects of growth, a continuous endpoint.

For estuarine/marine non-molluskan invertebrates, the probability of individual effects is I .O in $8.1E2E+08$. The analysis of the probability of effects to individual listed estuarine/marine invertebrates is based on a probit dose-response slope of 3.62. The lower and upper 95% confidence limits were -1.9 and 9.2, respectively which correspond to probabilities of individual effects ranging from 1 in 1.01 to 1 in 1.00 x 10^{16} . For exposures that occur at EECs for which LOCs are exceeded, the probabilities of individual effects ranged from 1.0 in 1.12 from use on rice to 1.0 in 531 for use of peanuts.

Terrestrial Listed Species Probability of Effects on Individuals

Since available data indicate that prothioconazole is practically non-toxic to birds, mammals, and terrestrial invertebrates, the probability of individual effects at the listed species LOC is not calculated. There is no evidence available that suggests potential acute risks to birds, mammals, and terrestrial invertebrates associated with proposed uses of prothioconazole. However, it is important to note that there are potential chronic risks to mammals associated with proposed uses of prothioconazole, although the probit dose response analysis does not apply to chronic risk.

For plants, a probit dose-response analysis is not conducted since the Tier I1 plant tests do not evaluate mortality (LC_{50}) and instead measures the inhibitory effects of a chemical; therefore it is difficult to estimate the probability that an individual will be affected.

Uncertainties and Assumptions of the Probit Dose-Response Analysis

Estimates of the probability of affecting individual organisms are based on extrapolation of very low probability events and are associated with considerable uncertainty in the resulting estimates. To provide a sense of possible probability values, the analysis is also conducted using the lower and upper 95% confidence limits of the probit dose-response slope, when available. In addition to noting the relatively large uncertainty bounds around the probabilities, care should be employed in interpreting these probabilities beyond their intended purpose as an indication of a margin of safety, or lack there of, for the endangered species risk estimates based on the deterministic risk quotient model.

4.2.3.2.3 Indirect Effects

Pesticides have the potential to cause indirect effects to endangered or threatened species by, for example, perturbing forage or prey availability, altering the extent of nesting habitat, etc. The potential for indirect effects is determined by comparing RQs to the listed species LOCs. If the RQ exceeds the listed species LOC then there is the potential for indirect effects to listed species dependent on those taxa for which the RQ exceeded the listed species LOC.

For aquatic species potentially exposed to prothioconazole, RQs exceeded the listed species acute risk LOCs to varying degrees for estuarine/marine invertebrates, aquatic vascular and nonvascular plants and terrestrial (semi-aquatic) plants. Aquatic non-vascular plants had the highest RQs and for all uses of prothioconazole, ranging from 1 164-3 127. The probability of individual effects for saltwater invertebrates did not exceed 1 in 141 for all uses of prothioconazole.

Given the sensitivities of the aquatic plant taxa to prothioconazole, indirect effects to listed species would be expected most for species dependent on fresh- and saltwater non-vascular (and perhaps vascular) plants and saltwater invertebrates based on the results of the above analysis. Given that both aquatic plants and invertebrates are important components of any aquatic ecosystem, indirect effects on a number of aquatic (and terrestrial) listed species is possible. The most obvious indirect effects would likely relate directly to reductions in food availability or habitat alterations associated with reduced aquatic plant and invertebrate biomass. Other, less obvious, indirect effects might include disruptions of listed species life cycles if certain life-cycle components are dependent on particular plant or invertebrate species.

For terrestrial species, the screening-level analysis indicated that, for most uses, prothioconazole has the potential to cause deleterious effects in exposed mammal populations (chronic LOCs are exceeded for mammals) (Section 4.1). This suggests potential concern for indirect effects on listed organisms dependant upon mammalian species as prey items or as potential pollinators. A potential drop in vertebrate biomass associated with prothioconazole use may reduce a significant portion of the prey base. While it is likely that fields can be repopulated by immigrants and living breeders after the use of pesticides, if the prey base is removed at a critical life-cycle juncture, over a large area or of if it is removed for long enough duration, some species may have difficulty meeting energy needs. Also, some species may be particularly sensitive during reproductive or developmental periods. A starting point for evaluating the potential risk of such a scenario would be to first identify listed species likely to occur in the proposed

prothioconazole use areas, compare life histories of listed species in known prothioconazole use areas and determine if use is likely to overlap with a sensitive life-cycle component.

The information presented on indirect effects serves as a guide to establish the need for and extent of additional analyses 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 the 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).

4.2.3.2.4 Listed Species Occurrence Associated with Prothioconazole Uses

A preliminary analysis of the co-occurrence of listed species and proposed re-registration of prothioconazole uses was conducted using EFED's LOCATES database (Version 2.10). The objective is to provide insight into the potential for exposure of listed species and to identify those areas, crop uses, and listed species that warrant further attention. A tabulation of the number of unique listed species in each state associated with proposed uses of prothioconazole is provided in Table 4.16.

By this tabulation there are possibly a total of 1,147 listed species in counties associated with counties where prothioconazole may potentially be used. A total of 50 states have listed species associated with crops on which prothioconazole may be used. Hawaii has the highest number (302) of listed species that may co-occur with proposed prothioconazole use areas. California is the second highest with 278 total species followed by Alabama with 87, Tennessee with 85, Florida with 81, and Texas with 61.

In general, for all proposed uses of prothioconazole there is at least one, and usually more, listed species that may potentially occur in or near a proposed use area. Appendix H lists the occurrence in each state of counties that have a listed species of specified taxa and the total list of endangered species that may co-occur with proposed uses of prothioconazole and a comprehensive list of species in counties where prothioconazole may be used. This preliminary analysis indicates that there is a potential for prothioconazole use to overlap with listed species and that a more refined assessment is warranted. The more refined assessment should involve clear delineation of the action area associated with proposed uses of prothioconazole and best available information on the temporal and spatial co-location of listed species with respect to the action area. This analysis has not been conducted for this assessment.

Table 4.16. Tabulation by State and Taxonomic Group of Listed Species that Occur in Prothioconazole Use Areas for All Proposed Uses

4.3 Description of Assumptions, Limitations, and Data Gaps

4.3.1 Assumptions and Limitations Related to Exposure for All Taxa

This screening-level risk assessment relies on proposed labeled statements of the maximum rate of prothioconazole application, the maximum number of applications, and the shortest interval between applications. The frequency at which actual uses approach these maxima is dependant on agricultural conditions (presence of fungi) and market forces. Moreover, conditions can change from year to year as fungicide resistance changes through time. It is important to realize that while a certain use pattern may prevail at present; these patterns can change as a result of changing conditions. In addition, rates of application less than the maximum rate are also considered for characterization.

4.3.2 Assumptions and Limitations of Aquatic Exposure Estimates

Although there are uncertainties associated with using the standard PRZM/EXAMS runoff scenario (10-ha field draining into a 20,000- $m³$ pond with no outlet) for an aquatic exposure assessment, it is designed to represent pesticide exposure from an agricultural watershed impacting a vulnerable aquatic environment. Extrapolating the risk conclusions from this standard pond scenario may either underestimate or overestimate the potential risks.

Major uncertainties associated with the standard runoff scenario include the physical construct of the watershed and representation of vulnerable aquatic environments for different geographic regions. The physicochemical properties (pH, redox conditions, etc.) of the standard farm pond are based on a Georgia farm pond. These properties are likely to be regionally specific because of local hydrogeological conditions. Any alteration in water quality parameters may impact the environmental behavior of a pesticide. The farm pond represents a well mixed, static water body. Because the farm pond is a static water body (no flow through), it does not account for pesticide removal through flow through or water releases. The lack of flow through the farm pond provides an environmental condition for accumulation of persistent pesticides. The assumption of uniform mixing does not account for stratification due to thermoclines $(e.g.,)$ seasonal stratification in deep water bodies). Additionally, the dimensions of the standard runoff scenario assumes a watershed area to water body volume ratio of 10 ha: $20,000m^3$. This ratio is recommended to maintain a sustainable constructed pond in the Southeastern United States. Different ratios will result in different relative loadings to the pond. Higher watershed area to water body volume ratios (as recommended for sustainable ponds in drier regions of the United States) may lead to higher pesticide concentrations when compared to the standard watershed area to water body volume ratio. However, larger watershed become increasingly likely to have multiple land uses and thus, no longer may be reasonably assumed to be 100% cropped with one crop and all treated with the pesticide.

The standard runoff scenario assumes uniform soils and agronomic management practices across the standard 10 hectare field. Soils can vary substantially across even small areas; this variation is not reflected in the model simulations. Additionally, the impact of unique soil characteristics and soil management practices (e.g., tile drainage) are not considered in the standard runoff scenario. The assumption of uniform site and management conditions is not expected to represent some site-specific conditions. Extrapolating the risk conclusions from the standard

pond scenario to other aquatic habitats (e.g., marshes, streams, creeks, and shallow rivers, intermittent aquatic areas) may either underestimate or overestimate the potential risks in those habitats.

Estimated environmental concentrations as a result of the proposed rice use of prothioconazole were determined using EFED's rice model. The model estimates EECs by applying the total annual application to the paddy and partitioning the pesticide between the water and the paddy sediment according to a linear or K_d partitioning model. The resulting EEC represents the dissolved concentration occurring in the water column and the concentration in water released from the paddy. Importantly, for ecological risk assessment purposes, exposure to aquatic species is assumed to occur once the paddy water is released. The EECs are expected to exceed the true values found in the environment the great majority of the time because the model does not consider degradation or dilution processes. The EECs estimated in this assessment resulted in RQs that exceed the acute risk LOC for freshwater fish and invertebrates and the chronic risk LOC for freshwater invertebrates. These RQs were based on the maximum possible level of prothioconazole present in paddy water. However, the label specifies that the chemical should be applied when the rice plant is nearly mature and is likely to cover much of the paddy water; one estimate indicated 70-90% cover at this stage in the rice plant development (J. Breithaupt, pers comm., 2006). However, prothioconazole-desthio is somewhat persistent and may lay intact on plants or in water eventually resulting in EECs higher than those calculated with 70-90% cover. Foliar dissipation half-lives were estimated to be on the order of 6 days, which suggests that prothioconazole and degradates are unlikely to remain on rice plants although they may remain in the paddy since degradation rates cannot be estimated from foliar dissipation rates. To reduce uncertainties associated with rice paddy EECs would require further study into the longevity of prothioconazole and metabolites on plants and in rice paddy water. Lastly, it is important to acknowledge that some rice paddies are used to raise crawfish as well and consideration should be given concerning the possible exposure of crawfish to residual prothioconazole and/or prothioconazole metabolites. However, given the apparently moderate toxicity to freshwater invertebrates and the conservative nature of rice paddy EECs, it is unlikely that the use of prothioconazole on rice would pose significant risk to crawfish, although the possibility cannot be completed excluded.

4.3.3 Assumptions and Limitations of Terrestrial Exposure Estimates

4.3.3.1 Location of wildlife species

For screening terrestrial risk assessments for listed species, a generic bird or mammal is assumed to occupy either the treated field or adjacent areas receiving pesticide at a rate similar to the proposed treatment rate on the field. This assumption leads to an overestimation of exposure to species that do not occupy the treated field. For screening risk assessment purposes, the actual habitat requirements of any particular terrestrial species are not considered, and it assumed that species occupy, exclusively and permanently, the treated area being modeled. This assumption leads to an overestimate of exposure in the risk estimates for a proportion of individuals of the exposed population. Although this estimate represents higher levels of exposure, it is within the range of possibility as some species may occupy habitats near the proposed use site and utilize

the site to forage. Gorging can be a common opportunistic behavior in some animals whereby food items are consumed in excess of the daily requirement due to availability. This example is more likely to support an acute exposure scenario. Chronic exposure is more difficult to ascertain since it occurs over a longer duration providing more opportunity for animals to move and seek forage elsewhere. Nonetheless, many animals do forage over a range that would be included in agricultural fields; all prey items for these species may come from agricultural use areas.

4.3.3.2 Routes of exposure

Screening-level risk assessments for spray applications of pesticides consider dietary exposure alone. Other routes of exposure, not considered in this assessment, are discussed below:

(a) Incidental soil ingestion exposure

This risk assessment does not consider incidental soil ingestion. Available data suggests that up to 15% of the diet can consist of incidentally ingested soil depending on the species and feeding strategy (Beyer *et al.,* 1994). Given the low acute toxicity of prothioconazole to birds and mammals, incidental soil ingestion is unlikely to pose additional acute risks to these species.

Alternatively, for estimates of chronic exposure to mammalian species, the effect of a1 5% incidental soil ingestions is estimated from the following:

Assuming a maximum application rate of 0.178 lb prothioconazole/A (0.2 kg/ha) to a bare, very *low density soil* (1 g/cm³) incorporated to 1-cm depth (actual incorporation depths may range from 5 to 20 cm), the following soil concentrations can be calculated for a depth of 1 cm:

soil concentration = $\{((0.2 \text{ kg/ha})(1,000,000 \text{ mg/kg}) / (100,000,000 \text{ cm}^3/\text{ha}))\}$ x $(1 \text{ cm}^3/0.001 \text{ kg}) = 2 \text{ mg/kg}$

Including this concentration into the standard screening-level method and assumptions for food item pesticide residues (e.g., 82 ppm residue assumption for short grass) shows that ingestion of soil at an incidental rate of up to 15% of the diet would not significantly increase dietary exposure. For example a 15 g mammal consumes approximately 7.2 g food daily. The amount of prothioconazole ingested from short grass is approximately 0.6 mg (7.2 g food consumed x 82 ppm in food). The amount of prothioconazole ingested as a result of incidental soil ingestion is approximately 0.002 mg (0.15 x 7.2 g x 2 mg/kg), which represents about **3%** of the total exposure due to eating prothioconazole-contaminated food-items. Basically, this brief analysis indicates that incorporating incidental soil ingestion as an exposure route is not likely to alter risk conclusions associated with chronic exposures to mammals.

(b) Inhalation exposure

The screening risk assessment does not consider inhalation exposure. Such exposure may occur through three potential sources: (1) spray material in droplet form at the time of application (2) vapor phase pesticide volatilizing from treated surfaces, and **(3)** airborne particulate (soil, vegetative material, and pesticide dusts).

Available data suggest that inhalation exposure at the time of application is not an appreciable route of exposure for birds. According to research on mallards and bobwhite quail, respirable particle size in birds (particles reaching the lung) is limited to a maximum diameter of 2 to 5 microns (EPA, 1990). The spray droplet spectra covering the majority of pesticide application situations (AgDrift model scenarios for very-fine to coarse droplet applications) suggests that less than 1% of the applied material is within the respirable particle size. However, the particles still may be ingested, and the model does not address this.

Theoretically, inhalation of pesticide active ingredient in the vapor phase may be another source of exposure for some pesticides under some exposure situations. Considering prothioconazole's low vapor pressure, it is unlikely that prothioconazole will exist in the gaseous phase at any appreciable amount to cause adverse effects via inhalation.

The impact from exposure to dusts contaminated with the pesticide cannot be assessed generically as partitioning issues related to application site soils and chemical properties render the exposure potential from this route highly situation specific.

(c) Dermal exposure

The screening assessment does not consider dermal exposure, except as it is indirectly included in calculations of RQs based on lethal doses per unit of pesticide treated area. Dermal exposure may occur through three potential sources: (1) direct application of spray to terrestrial wildlife in the treated area or within the drift footprint, (2) incidental contact with contaminated vegetation, or (3) contact with contaminated water or soil.

The available measured data related to wildlife dermal contact with pesticides are extremely limited. The Agency is actively pursuing modeling techniques to account for dermal exposure via direct application of spray and by incidental contact with vegetation.

4.3.3.3 Incidental Pesticide Releases Associated with Use

This risk assessment is based on the assumption that the entire treatment area is subject to prothioconazole application at the proposed application rates. In reality, there is the potential for uneven application of prothioconazole through such plausible incidents as 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 used *(e.g.,* increased application at turnabouts when using older ground application equipment).

4.3.3.4 Residue Level Selection

As discussed earlier in the exposure section of this document, the Agency relies on the work of Hoerger and Kenaga (1972) as modified by Fletcher *et al.* (1994) for setting the assumed pesticide residues in wildlife dietary items. The Agency believes that these residue assumptions reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to accurately 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 reflect residues averaged over 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. In addition, the data that represent residue levels on insects are not based on insect-specific data; plant residue data are used to represent insect residue data and is a source of uncertainty regarding insect residue levels.

4.3.3.5 Dietary Intake-The Dl;fference Between Laboratory and Field Conditions

The acute and chronic dietary-based characterizations of risk rely on comparisons of wildlife dietary residues with LC_{50} or NOAEC values expressed in concentrations of pesticides in laboratory feed. These comparisons assume that ingestion of food items in the field occurs at rates similar to those in the laboratory. Although the screening assessment process adjusts dryweight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy and assimilative efficiency differences between wildlife food items and laboratory feed.

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.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and its dietary matrix, bird assimilation of wild diet energy ranges from 23 - 80%, and mammal assimilation of diet ranges from 41 - 85% (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. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

Finally, 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.

In contrast, there 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. However, reduced food intake, particularly over an extended period, could result in reduced survival or reproductive output.

4.3.4 Effects Assessment Assumptions and Limitation

4.3.4.1 Age Class and sensitivity of effects thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The screening risk assessment acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes ($e.g.,$ first instar for daphnids, second instar for amphipods, stoneflies and mayflies, and third instar for midges). Similarly, acute dietary testing with birds is also performed on juveniles, with mallard being 5-10 days old and quail 10-14 days old. The screening risk assessment has no current provisions for a generally applied method that accounts for uncertainty associated with study organism age. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, the risk assessment uses the most sensitive life-stage information as the screening endpoint.

4.3.4.2 Lack of Effects Data for Amphibians and Reptiles

Currently, toxicity studies on amphibians and reptiles are not required for pesticide registration. Since these data are lacking, the Agency uses fish as surrogates for aquatic phase amphibians and birds as surrogates for terrestrial phase amphibians and reptiles. These surrogates are thought to be reflective of or protective (more sensitive) of herpetofauna. Amphibians are characterized by a permeable skin. The most important route of exposure for aquatic amphibians would likely be the dermal route. Using freshwater fish may be suitable surrogates since exposure would likely be surface area dependent and the gill surface of many fish is a fairly large surface area. Also, both fish and amphibians are ectothermic so metabolic rates and demands would likely be similar. For terrestrial species, however, the difference between amphibians and birds and reptiles and birds is quite large. Terrestrial amphibians and reptiles are both ectothermic while birds are endothermic; birds have a higher basal metabolic rate required to maintain constant body temperature. The higher metabolic demands of birds may predispose birds to higher relative exposures. However, this does not address any potential differences in toxicity. To date, there are few controlled studies on reptile species that could be used to compare to similar studies on birds. *A* **priori,** there is no strong reason suggesting that one taxon is more or less sensitive than another. Further research is required to determine whether reptiles and terrestrialphase amphibians are suitably represented by bird species in assessing risks.

4.3.4.3 Use of the Most Sensitive Species Tested

Although the screening risk assessment relies on a selected toxicity endpoint from the most sensitive species tested, it does not necessarily mean that the selected toxicity endpoints reflect sensitivity of the most sensitive species existing 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. The relationship between the sensitivity of the most tested species versus wild species (including listed species) is unknown and a source of significant uncertainty. The use of laboratory species has historically been driven by availability and ease of maintenance. A widespread comparison of species is lacking, however, even variation within a species can be quite high. For example, in this assessment, acute studies on water fleas yielded three different values. Granted these were within an order of magnitude but examples exist where differences have been more extreme.

4.3.4.4 Data Gaps

The dataset for prothioconazole is mostly complete although there are several uncertainties that could potentially be reduced by conducting or repeating toxicity studies. For example, there is some uncertainty associated with the life-cycle toxicity study on an estuarine/marine invertebrate (mysid shrimp). The acute study, which was conducted using prothioconazole-desthio, was classified as ACCEPTABLE with no major deviations. However, when comparing the results from the acute study to the chronic study (also conducted using prothioconazole-desthio and classified ACCEPTABLE), the LC_{50} is more or less equivalent to the chronic NOAEC suggesting that mortality and sub-lethal effects occur at or near the same concentration. It appears that there is considerable variability in the mysid population concerning the response to prothioconazole-desthio and that for whatever reason; the acute tests have captured some of this variability. Since there is no reason to exclude the results from the submitted acute toxicity test (again, classified ACCEPTABLE), an acute-to-chronic ratio using daphnid acute and chronic data is used to estimate a chronic mysid toxicity endpoint from the LC_{50} . Although repeating the mysid life-cycle test may reduce some uncertainty associated with the toxicity value, unless the value from a repeated test is lower than the current value, it is unlikely to alter the current approach or conclusions since there is no reason to discount the results from the acute mysid test.

In addition, the sediment toxicity study using chironomids was classified as SUPPLEMENTAL because it did not follow Agency guidelines and because not all exposure levels were analytically verified. Repeating the study following Agency guidelines in which the chemical is first added to the sediment would reduce some uncertainty associated with assessing risks to sediment dwelling invertebrates.

On source of considerable uncertainty in this assessment (and all conazole assessments) is the lack of toxicity and fate data for the common conazole degradate, 1,2,4-triazole. The triazole degradate has been shown to be a major degradate $(>10\%$ of degradates) in an aerobic aquatic metabolism study (MRID# 462465 15) where concentrations rose above 10% of the total degradate mixture between days 29 and 59 and by day 121 comprised 41% of the total degradate mixture. Furthermore, the study indicated that 1,2,4-triazole is mostly in the aquatic phase. These data indicate that the triazole degradate may reach fairly high concentrations associated with use of prothioconazole. Although this appears to be an important degradate, especially

considering that it is a degradate in common with other conazole fungicides, there are basically no fate or toxicity data available to generate a robust assessment of the potential risks. However, data may be forthcoming in support of a cumulative 1,2,4-triazole risk assessment currently assigned to EFED; to date, little progress has been made on this assessment. To provide some perspective on the potential risks to aquatic species associated with the 1,2,4-triazole degradate, a structure activity relationship (SAR) was used to estimate acute and chronic toxicity values for aquatic species. EPA's SAR program, ECOSAR

(http://www.epa.gov/oppt/newchems/tools/2 **1** ecosar.htm), was used in estimating aquatic toxicity data. ECOSAR uses SARs to predict aquatic toxicity data for a given chemical based on the structural similarity of that chemical to other chemicals for which toxicity data are available. For 1,2,4-triazole, the only inputs needed were the octanol/water partitioning coefficient (K_{ow} = -0.29), the molecular weight (69.07), and the Simplified Molecular Input Line Entry System notation (SMILES) that is a description of the chemical structure. ECOSAR generates aquatic toxicity estimates for fish, invertebrates, and algae. The most sensitive taxa, based on acute toxicity estimates were freshwater invertebrates (daphnia) with an estimated EC_{50} of 645 mg/L (645000 μ g/L). Comparing this value to the highest estimated aquatic EEC, which was 139 μ g/L (rice), yields an RQ well below 0.01 indicating that the potential for adverse effects to freshwater invertebrates associated with proposed prothioconazole uses is low. Moreover, since the endpoint for freshwater invertebrates was the lowest, this analysis indicates a low potential for adverse effects for fish and green algae as well. Similarly, the chronic values were 648, 125, and 97 mg/L for fish, invertebrates and green algae; risk estimates generated using these toxicity endpoints resulted in RQs that were all well below 0.01, indicating a low potential for adverse effects. Although the risk estimates based on ECOSAR outputs for the 1,2,4-triazole degradate suggest that risks to aquatic species are unlikely, there is still considerable uncertainty regarding the actual toxicity of the triazole degradate. To conduct a thorough, robust risk assessment on 1,2,4-triazole, a complete data set (fate and toxicity) would be optimal.

4.3.5 Assumptions Associated with the Acute LOCs

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 taxon. 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 taxon is unknown. In some cases, a probit dose-response relationship cannot be calculated; as was the case with mammals in this assessment (data was unavailable). Here, event probabilities for mammalian species were calculated based on a default slope assumption of 4.5 with upper and lower confidence intervals of 2 and 9 (Urban and Cook, 1986). Given the large uncertainty

associated with the probability estimates, it is not possible to accurately predict the chance of an individual mortality event for listed mammalian species

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APPENDIX A. Preliminary Data Screen.

iTED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460 **OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES**

> DP Barcodes: 303488, 303495 PC Code: 11 3961 Date: November 9, 2004

MEMORANDUM:

- SUBJECT: EFED Preliminary Screen of Environmental Fate and Ecological Effect Studies of Prothioconazole
- To: Bob Tomerlin, Product Manager Registration Division
- FROM: John Ravenscroft, Biologist Roxolana Kashuba, Environmental Scientist Christopher Salice, Biologist Environmental Fate and Effects Division (7507C)
- THRU: Kevin Costello, Geologist, RAPL Elizabeth Behl, Branch Chief Environmental Risk Branch IV Environmental Fate and Effects Division (7507C)

The Environmental Fate and Effects Division (EFED) has completed its review of the preliminary screen of both environmental fate and ecological effect studies on prothioconazole. There were a total of 33 environmental fate studies, including eight non-guideline supplementary studies, (Table A) and 51 ecological effect studies (Table B) submitted for prothioconazole. Both tables list each of the studies and whether there were any issues associated with the study that may limit its utility in ecological risk assessment. In general, the studies appeared to contain sufficient information on the fate and effects of prothioconazole for EFED to complete data evaluation records and an ecological risk assessment of the chemical; however, there were some gross deficiencies that call in to question the validity of a few of the submitted studies.

Environmental fate studies submitted to the Agency in support of the new chemical registration of prothioconazole are summarized in Table A and include the required degradation, metabolism, and mobility tests using technical grade active

ingredient (JAU6476) and terrestrial field dissipation tests using the formulated end product 250 EC and 250 SC (-250 g a.i./L). There is one required environmental fate guideline study which was not submitted: Accumulation in Laboratory Fish (165-4). There are two submitted studies which do not appear to be scientifically valid and/or do not meet data requirements. The parent hydrolysis study did not sample long enough to establish the degradation pattern of the parent (less than 10% degradation in 7 days), despite the additional submitted 30-day hydrolysis study of one of degradates. The terrestrial field dissipation study in Saskatchewan reported 6 out of 7 recoveries \geq 110% (with an average of 113.1%) for JAU6476-S-methyl and 3 out of 7 recoveries \geq 110% for JAU- thiazocine (with an average of 109.3%) during method validation at 10 ug/kg fortification level. Recoveries in experimental soil cores were at 115% for JAU6476 desthio, 1 14% for JAU6476-S-methyl, and 1 12-1 13% for JAU6476-thiazocine. The repeated recovery of excess mass calls the validity of the data into question. However, the terrestrial field dissipation data requirement is met, due to the submission of three additional terrestrial field dissipation studies conducted in the United States.

There were several other important guideline deviations which merit closer scrutiny during full review of these studies. The first aerobic soil metabolism study did not identify diffuse radioactivity measured at 11.8%, 12.4%, and 16.3%, while the last aerobic soil metabolism study did not identify degradates at 11% and 27%. Three aerobic metabolism studies sampled for less than the required 365 days (120, 120, and 125 days, respectively). The Manitoba terrestrial field dissipation study pretreatment samples and deepest samples contained concentrations of one degradate above the minimum detection level. Other minor guideline deviations are noted, such as no LOQ/LOD reporting.

Ecological effect studies submitted to the Agency in support of the new use registration of prothioconazole are summarized in Table **6** and included the required acute and chronic toxicity tests using the technical grade active ingredient (JAU6476) and formulation 480SC, plus three degradates, SXX0665, S-methyl, and desthio. Not all combinations of parent, formulation, and degradates were tested in each toxicity category; however, almost all guideline studies were tested with the parent and at least one of the degradates. Common problems with the studies included insufficient organism size in the fish toxicity studies, solubility problems with the chemical and issues with verifying actual test concentrations in the aquatic studies.

The array of studies provided by the registrant in support of this chemical's registration, including two chironomus life cycle tests with the parent and the SXX0665 degradate, is impressive and commendable.

Table A. Summary of preliminary screen on environmental fate studies for prothioconazole. (N/A = not applicable).

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Table B. Summary ~nazole. kf preliminary screen for ecological effects tests on

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APPENDIX B. Environmental Fate Data.

Abiotic Degradation

Hydrolysis

Prothioconazole is **stable** to hydrolysis at environmentally relevant pH's and temperatures. In a study conducted in darkness for 7 days at 50°C, [phenyl-UL-¹⁴C]prothioconazole (at 3.6-3.9 mg a.i./L) did not hydrolyze in sterile pH 7 and 9 aqueous buffer solutions (degradation rate was not statistically different from zero), and hydrolyzed with a half-life of 120 days in sterile pH 4 aqueous buffer solution (MRID: 46246505). The half-life of prothioconaole at $25 \square C$ at pH 4 was extrapolated by the study author from the 50C data to between 679 days and >10 years. No major transformation products were detected in any pH solution. The minor transformation products were JAU6476-desthio (SXX0665; 2-[2-(1-chlorocyclopropyl)-3-(2-chloropheny1)-2 **hydroxypropyl]-l,2-dihydro-3H-l,2,4-triazole),** formed at a maximum of 5.3%, 2.7%, and 2.4% of the applied at pH 4, pH 7, and pH9, respectively, and an unidentified transformation product (M1) formed at a maximum of 2.5%, 1.9%, and 1.9% of the applied at pH 4, pH 7, and pH9, respectively. The submitted study was classified as acceptable and provides adequate data for the risk assessment.

Prothioconazole-desthio is **stable** to hydrolysis at environmentally relevant pH's and temperatures. In a study conducted in darkness for 30 days at 25^oC, [phenyl-UL-
¹⁴ Clprothioconazole-desthio (at 5.00 mg/L (pH 5), 3.67 mg/L (pH 7) and 3.72 mg/L (pH 9)) did not hydrolyze in sterile pH 5, 7 and 9 aqueous buffer solutions (MRID: 46246506). The submitted study was classified as supplemental because multiple replicates were not analyzed via the same method (either TLC or HPLC) for each sampling interval, not allowing for knowledge of the precision of the data. However, this study provides useful data for the risk assessment.

Aqueous Photolysis

Prothioconazole is rapidly photodegraded to prothioconazole-desthio in water under favorable light conditions, however, prothioconazole-desthio persists under further irradiation. In the guideline study submitted, [phenyl-UL-¹⁴C] and [triazole-3,5-¹⁴C]prothioconazole, at 4.09 - 4.47 mg a.i./L, photodegrades with a half-life of approximately 9.7 days (corrected for continuous irradiation conditions and natural sunlight; uncorrected laboratory-measured half-live of 1.9 days) in sterile pH 7 aqueous buffered solution maintained at 25° C and irradiated with a xenon lamp for 18 days (the equivalent of 93 days of summer sunlight at 40^oN latitude) (MRID 46246507). Prothioconazole and prothioconazole-desthio together, however, photodegrade with a half-life of **101.9 days** (corrected for continuous irradiation conditions and natural sunlight; uncorrected laboratory-measured half-live of 19.9 days). In addition to prothioconazole-desthio, there are two other major degradates identified: prothioconazole-thiazocine and 1,2,4-triazole. The three major degradates have maximum concentrations of 55.7%, 14.1%, and 1 1.9% of the applied observed on the 11^{th} , 5^{th} , and 18^{th} day of incubation from the phenyl, phenyl, and triazole labelled parent, respectively. The six minor transformation products in the irradiated samples were characterized but not identified and were formed at a maximum of 4.6-7.0% of the applied

amount in both phenyl and triazole labelled treatments. From seven to nine additional unassigned peaks were detected in both labelled treatments, together representing 13 .O-19.2% of the applied radioactivity on day 18, with the largest single peak at 3.1% of the applied radioactivity (2.9% HPLC peak area ratio) in either treatment. At test termination, in the irradiated samples, the evolved $CO₂$ and volatile organic compounds amounted to 3.0% and 1.7% of the applied radioactivity, respectively, for the phenyl label, and 0.5% and 0.1 % of the applied radioactivity, respectively, for the triazole label. Based on the results of the study, photodegradation is expected to be a potential route of dissipation for prothioconaozle and prothioconazole-desthio together in the environment when the compounds are present in clear, shallow surface water. This aqueous photodegradation study was classified as supplemental because single replicates from a bulk sample were used (multiple replicates per sampling interval from separate vessels are prefered), all dark control data were not reported, and 13.0- 19.2% of applied radioactivity ("unassigned metabolites") were not separately reported. However, this study still provides useful data for the risk assessment.

Soil Ph otolysis

Prothioconazole photodegradation on soil is insignificant compared to metabolism, as evidenced by similar degradation rates in both irradiated and dark samples. In the guideline study submitted, $[phenyl-UL-¹⁴C]$ prothioconazole *(applied at 1.4 mg a.i./kg soil)* together with prothioconazole-desthio are considered **stable** to photodegradation on loamy sand soil maintained at 20° C and 75% of $1/3$ bar moisture content, and continuously irradiated with a xenon lamp for 15 days (the equivalent of 77 days of summer sunlight at 40 **O** N latitude) (MRID 462465 10). Correction for soil metabolism lead to a negative (impossible) soil photolysis halflife because dark samples actually degraded slightly faster than irradiated samples (experimental half-life of 8.6 days for irradiated samples compared to 7.8 days for dark controls) and, therefore, degradation could not have been due to photolysis. Correction for natural sunlight was, then, not necessary. The major transformation product detected in both dark and irradiated samples is prothioconazole-desthio, which is formed quickly and in large amounts (maximum of 38.5% of the applied radioactivity on day 7). Due to this and to the fact that it has similar toxicity to that of parent prothioconazole, prothioconazole-desthio is added to parent in half-life calculations, resulting in the same stable to soil photolysis conclusion (experimental half-life of 33.6 days for irradiated samples compared to 21.8 days for dark controls). The minor transformation products in the irradiated samples are JAU6476-triazolinone, JAU6476-sulfonic acid, and two unidentified products (Unknown 1 and Unknown 3) detected at a maximum of 2.5%, 3.0%, 1.4% and 3.1 % of the applied radioactivity, respectively. The minor transformation products in the dark samples are JAU6476-triazolinone and one unidentified products (Unknown 2) detected at a maximum of 3.2% and 1.1% of the applied radioactivity, respectively. Unknown2 was detected only in a dark sample, and not detected in irradiated sample. JAU6476-sulfonic acid, Unknown1 and Unknown3 were detected only in irradiated samples (albeit in small amounts). It appears that no significant transformation products are specifically generated by phototransformation on a soil surface. In the irradiated samples, non-extracted \int_0^{14} C residues increased from 8.4% of the applied radioactivity at day 0 to 25.5% of the applied radioactivity by study termination. Nonextracted \int_0^{14} C residues in the dark samples increased from 8.4% of the applied radioactivity at day 0 to a maximum of 36.5% of the applied radioactivity on day 7 and decreased to 26.4% of

the applied radioactivity at study termination. A maximum of 9% (irradiated soils) and 12% (dark controls) of the applied radioactivity was unidentified in the study (origin and diffuse radioactivity). Based on the results of the study, photodegradation on soil is not expected to be a potential route of dissipation for prothioconaozle and prothioconazole-desthio together in the environment. This study was classified as acceptable and provides adequate data for the risk assessment.

Metabolism

Aerobic Soil Metabolism

Two aerobic soil metabolism studies conducted on four soils total are submitted and useable for quantitative estimates of aerobic biotic degradation of prothioconazole and prothioconazoledesthio together. Prothioconazole rapidly dissipates from aerobic soil systems (down to 7.9-52.1 % applied by 1 day post-treatment and <2.0-23.2 % of applied by 7 days post-treatment). However, high amounts of prothioconazole-desthio (11.7-39.8% of applied at 1 day posttreatment and 19.2-4 1.3 % of applied at 3 days post-treatment) and unextracted material (20.6- 30.7% of applied at 1 day post-treatment and 29.7-39.5 % of applied at 3 days post-treatment) are reported simultaneously. Both chemicals are similarly toxic and unextracted material is poorly extracted, so these three components do not have to be separated in estimates of aerobic soil degradation. Half-lives and empirical $DT₅₀S$ and $DT₉₀S$ of reported parent and prothioconazole-desthio alone may substantially underestimate degradation rates. Unextracted residues are, therefore, assumed to consist of toxic material which has just not been extracted harshly enough (ie., may become bioavailable in the environment), and their amounts are added to parent concentration in half-life calculation. In silt, loamy sand, silty loam, and silty clay loam soils, calculated aerobic biotic degradation of both compounds together was very slow (MRID 46246511: $t_{1/2} = 578$ days (silt, phenyl label), $t_{1/2} = 1155$ days (silt, triazole label), $t_{1/2} =$ **1155 days** (loamy sand, phenyl label), $t_{1/2} = 1386$ days (loamy sand, triazole label); MRID 46246512: $t_{1/2}$ = 990 days (sandy loam, phenyl label), $t_{1/2}$ = 533 days (silty clay loam, phenyl label)). When incubated in darkness for up to one year at 20 $^{\circ}$ C, maintained at 75% of 1/3 bar moisture, prothioconazole degraded to prothioconazole-desthio, prothioconazole-S-methyl, 1,2,4-triazole, prothioconazole-sulfonic acid, prothioconazole-triazolinone, prothioconazole-3,4, 5, and 6-hydroxy-desthio, 2-chlorobenzoic acid, and C02. Non-extractable residues comprised 2.7-10.9% of applied at time zero and 20.6-52.7 % of applied from 1 DAT through study termination in all soils with both labels.

High uncertainty surrounds the estimate of the aerobic soil metabolism half-life given the large assumption about the unextracted residues. Studies using more extensive and appropriate extraction procedures would help reduce this uncertainty. Because the aerobic soil metabolism half-lives are calculated in this conservative fashion, the persistence of prothioconazole and prothioconazole-desthio together may be slightly overestimated in this assessment. Additionally, these half-life values are likely imprecise due to extrapolation beyond the time limits of the sampling period (120-day and 365-day study durations). These studies were classified as acceptable.

Aerobic Aquatic Metabolism

As prothioconazole degraded relatively quickly to prothioconazole-desthio, unextracted material is poorly extracted and both chemicals are similarly toxic, aerobic aquatic metabolism cannot be quantitatively evaluated for prothioconazole alone. Prothioconazole and prothioconazole-desthio together degraded at a moderate pace in two aerobic water/sediment systems incubated in the dark at 20^oC for 121 days (total system: $t_{1/2} = 75.3$ days, pond/loam, phenyl label; $t_{1/2} = 66.6$ days, pond/loam, triazole label; $t_{1/2} = 44.4$ days, lake/loamy sand, phenyl label; $t_{1/2} = 41.0$ days, lake/loamy sand, triazole label; water layer: $t_{1/2} = 17.4$ days, pond/loam, phenyl label; $t_{1/2} = 16.3$ days, pond/loam, triazole label; $t_{1/2} = 23.7$ days, lake/loamy sand, phenyl label; $t_{1/2} = 21.9$ days, lake/loamy sand, triazole label; MRID: 46246515). Prothioconazole degraded to prothioconazole-desthio, prothioconazole-S-methyl, 1,2,4-triazole (triazole label only), prothioconazole-triazolinone, prothioconazole-triazolylketone, and C02. Prothioconazoledesthio appears to degrade more quickly in aerobic water/sediment systems than in aerobic soil alone. Similar to but slightly less than in aerobic soil degradation studies, non-extractable residues compose 19.6-19.9% of applied at 1 DAT and 21.1-46.7% of applied from 3 DAT through study termination in one (pond/loam) aerobic water/sediment system with both labels, and 6.7-7.3% of applied at 1 DAT and 9.6-30.3% of applied from 3 DAT through study termination in the other (lake/loamy sand) aerobic water/sediment system with both labels. These data suggest the formation of a degradate and the subsequent sorption of the degradate to sediment. Due to this large amount of claimed unextracted material and the mild extraction procedures utilized in this study, unextracted residues are assumed to consist of parent which had just not been extracted harshly enough and may actually be bioavailable in the environment). Therefore, unextracted amounts were included with parent residues in half-life calculations.

Uncertainty surrounds the estimate of the aerobic aquatic metabolism half-life given the assumption regarding unextracted residues. Studies using more extensive and appropriate extraction procedures could help reduce this uncertainty. Since the anaerobic aquatic metabolism half-lives were calculated in this conservative fashion, the persistence of prothioconazole and prothioconazole-desthio may be overestimated in this assessment. This study is classified as supplemental because multiple samples were not used per interval, exclusively foreign soils were used without reporting taxonomic classifications, and water/sediment characterization of actual samples used in the study was lacking.

Anaerobic Aquatic Metabolism

Prothioconazole degraded to prothioconazole-desthio while in storage and, therefore, reported amounts of prothioconazole-desthio are considered parent. Prothioconazole and prothioconazole-desthio together degraded slowly ($t_{1/2}$ = 231 days, total system; $t_{1/2}$ = 61.9 days, water layer; MRID: 46246516) in an anaerobic pond and flooded sandy clay loam sediment system incubated in the dark at 20.3 0 C for 360 days. Prothioconazole degraded extensively to prothioconazole-S-methyl (maximum of 78.2% of applied radioactivity in total system). The 1,2,4-triazole degradate was not tracked as the study was conducted using only the phenyl label. Non-extractable residues compose only 3.4-3.5% of applied at 1 DAT and 3.0-26.5% of applied from 3 DAT through study termination. However, due to the mild extraction procedures

implemented in this study, unextracted residues were assumed to consist of parent which had just not been extracted harshly enough (ie., may become bioavailable in the environment), and their amounts were added to parent concentration in half-life calculation.

Mobility and Persistence

Adsorption/Desorption

Prothioconazole adsorption coefficients could not be calculated due to instability (rapid degradation) in the test system and lack of resolution in column, as characterized by two column leaching studies on prothioconazole (MRIDs: 46246539 and 46246504). Prothioconazoledesthio is expected to have some mobility in most soils, but may be expected to bind to some benthic sediments in the aquatic environments based on laboratory sorption coefficients derived from a batch equilibrium study on prothioconazole-desthio on four types of soil (Kd of **4.13- 13.38 mg/L, Gc** of **523-678 mL/goc;** MRID: 46246450). The soil binding is strongly correlated to organic carbon content of soil (r^2 = 0.996). An additional degradate prothioconazole-S-methyl is also expected to have low to slight mobility, predicted from high to very high laboratory sorption coefficients derived from a batch equilibrium study on four types of soil (Kd of **15.6-64.1 mg/L, Gc** of **1973-2995 mL,/goc;** MRID: 46246501). Mobility may vary slightly relative to the rate of drainage of soil, increasing in coarse-grained, well-drained soils and decreasing in fine-grained, poorly-drained soils. However, in general, prothioconazoledesthio may be mobile in some soils where agriculture is typically conducted. Two of these studies (MRIDs: 46246504 and 46246450) are classified as acceptable; one study (MRID: 46246539) is classified as supplemental and does not satisfy Subdivision N Guideline § 163-1 data requirements for a mobility study using unaged soil because the soil columns were leached with an insufficient volume of $0.01M$ CaCl₂ solution.

Terrestrial Field Dissipation

There were three submitted U.S. terrestrial field dissipation guideline studies (MRIDs: 46246517, 46246518, 46246519). Prothioconazole was applied in total concentrations [in soil] of 800 ug/kg, 400 ug/kg, and 1000 ug/kg in terrestrial California, Georgia, and New York fields, respectively, over the course of 6,2, and 6 applications, respectively. Prothioconazole and degradates prothioconazole-desthio, prothioconazole-S-methyl, prothioconazole-thiazocine, and 1,2,4-triazole were measured.

Prothioconazole alone dissipated from the top layer of soil with a DT₅₀ of less than 2 or 3 days, but prothioconazole-desthio dissipated from the top layer of soil with extremely variable DT_{50} of **28-422** days. Moderate amounts of prothioconazole-S-methyl were detected above the level of quantitation (LOQ) in 0-15 cm soil, and 1,2,4-triazole was detected at all soil depths, albeit not above the LOQ. Prothioconazole-thiazocine was not detected above the MDL.

Prothioconazole was not detected below 15 cm in any of the three fields in California, Georgia or New York. Prothioconazole-desthio was detected at levels above the LOQ down to 30 cm and at levels above the minimum detection limit [MDL] but below the LOQ down to 45 cm in one

replicate in the California field study, and at levels below the LOQ at one to two sampling times in the Georgia and New York field studies. Prothioconazole-S-methyl was detected below 15 cm only in a single replicate (below LOQ) in the field study in California.

Uncertainties in the terrestrial field studies include uneven application, temporally variable concentrations, and questionably adequate sampling schedules. In the California, Georgia, and New York fields, there was a range of 17.2-44.7 ug/kg, 56.9-67.7 ug/kg, and 57.6-78.2 ug/kg soil of parent prothioconazole measured at time zero (post sixth, second, and sixth application), respectively, and a 64.8-100.4%, 63-1 13%, and 47.3-12 1.1% recovery, respectively, in application rate verification procedures across all six applications. These terrestrial field dissipation studies are classified supplemental because prothioconazole was not stable in frozen storage.

Aquatic Field Dissipation

There were three submitted U.S. aquatic field dissipation guideline studies (MRIDs: 46246522, 46246523,46246524). Prothioconazole was applied at rates of 220.7 g a.i./ha (two applications in California), 220.7 g a.i./ha and 287.0 g a.i./ha (two applications in Arizona) and 287.0 g a.i./ha (one application in Arizonia-cropped) in aquatic fields. Prothioconazole and degradates prothioconazole-desthio, prothioconazole-S-methyl, prothioconazole-thiazocine, and 1,2,4 triazole were measured.

Prothioconazole and prothiconazole-desthio dissipated with long half-lives in sediment (203.9 **days,** 121.6 **days, and** 90.0 **days** in California, Arkansas, and Arkansas cropped aquatic fields, respectively). Dissipation half-lives in paddy water were extremely short **(1.7 days,** 0.9 **days, and** 0.6 **days** in California, Arkansas, and Arkansas cropped aquatic fields, respectively), likely more due to adsorption than degradation.

Prothioconazole was detected in only three sampling intervals below LOQ but above the MDL in sediment below 3 inches. Prothioconazole-desthio was detected at 3-6 inch deep sediment through 28 DAT in the Arkansas flooded field and through 60 DAT in the Arkansas flooded and cropped field. Prothioconazole-S-methyl was detected below 3 inches in sediment only in three sampling intervals in the Arkansas flooded and cropped field.

Uncertainties in the aquatic field studies include instability of prothioconazole and some degradates in storage. Recovery of prothioconazole in soil and water ranged from 9.9-39.0% after 650-822 days in storage in all three studies. These aquatic field dissipation studies are classified supplemental because prothioconazole was not stable in frozen storage, and the laboratory storage stability study was inadequate to demonstrate stability of any of the analytes during storage because samples were not analyzed at time 0, and sampling intervals were inadequate to determine stability of the analytes over time.

APPENDIX C. Aquatic Exposure Model and Results.

Table C.1. Soil partition coefficients used to calculate PRZM/EXAMS input parameter.

Table C.2. Aerobic soil metabolism half-lives used to calculate PRZMJEXAMS input parameter.

Soil (label)	MRID	t_{12} (days) with unextracted	$t_{1/2}$ (days) without unextracted
Hofchen silt (phenyl)	46246511	533.2	123.8
Hofchen silt (triazole)	46246511	866.4	157.5
Byromville loamy sand (phenyl)	46246511	990.2	346.6
Byromville loamy sand (triazole)	46246511	1386.3	364.8
Laacher hof sandy loam (phenyl)	46246512	866.4	231.0
Stanley silty clay loam (phenyl)	46246512	462.1	462.1

Table C.3. Aerobic aquatic metabolism half-lives used to calculate PRZM/EXAMS input **parameter.**

Table C4. Surface water concentration differences.

Baseline Prothioconazole total tox surface water (ppb)-- aerial, low K_{oc}, with unextracted

Prothioconazole total tox benthic pore water (ppb)-- aerial, low K_{oc}, with unextracted

Prothioconazole total tox surface water (ppb)-- aerial, high Koc, with unextracted

Prothioconazole total tox surface water (ppb)-- aerial, low K_{oc}, without unextracted

Prothioconazole total tox surface water (ppb)-- ground, low K_{oc}, with unextracted

Percent decrease from baseline with high Koc.

Percent decrease from baseline without unextracted.

Percent decrease from baseline with ground application

North Dakota Wheat

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stored as WEcoA.out Chemical: Prothioconazole PRZM environment: NDwheatC.txt modified Satday, 12 October 2002 at 16:15:08 EXAMS environment: pond298.exv modified Thuday, 29 August 2002 at 16:33:30 Metfile: w14914.dvf modified Wedday, 3 July 2002 at 09:05:52

Sorted results

Benthic segment concentrations (gpb)

Sorted results
Prob. Peak 96 hr 21 Day 60 Day 90 Day Yearly 0.032258064516129 19.32 19.32 19.31 19.31 19.29 18.76 0.064516129032258 19.26 19.26 19.24 19.19 19.14 18.48

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 $\sim 10^7$

Data used for this run: Output File: WEcoA
Metfile: w14 w14914.dvf PRZM scenario: NDwheatC.txt EXAMS environment file: pond298.exv Chemical Name: Prothioconazole
Description Variable Name Value Units Variable Name Value Units Comments
ht $mwt = 344.264 g/mol$ Molecular weight mwt 344.264 g
Henry's Law Const. henry 2.96E-10 Henry's Law Const. henry $2.96E-10$ atm-m^3/mol
Vapor Pressure vapr $3E-9$ torr Vapor Pressure vapr 3E-9
Solubility sol 300 Solubility sol 300 mg/L
Kd Kd mg/L Kd Kd mg/L
Koc Koc 523 mg/L mg/L Photolysis half-life kdp 101.9 days Half-life Aerobic Aquatic Metabolism kbacw 385.2 days Halfife Anaerobic Aquatic Metabolism kbacs 0 days Halfife Aerobic Soil Metabolism asm 1052.2 da

Hydrolysis: pH 4 0 days Half-life Hydrolysis: pH 4 0 days Half-life Hydrolysis: pH 7 0 days Half-life
Hydrolysis: pH 9 0 days Half-life Hydrolysis:
Method: CAM 2 integer See PRZM manual Incorporation Depth: DEPI 0 cm
Application Rate: TAPP 0.2 kg Application Rate: TAPP 0.2 kg/ha
Application Efficiency: APPEFF 0.95 Application Efficiency: APPEFF 0.95 fraction Spray Drift DRFT 0.05 fraction of application rate applied to pond
Application Date Date 18-06 dd/mm or dd/mmm or dd-mm or dd-mmm Application Date Date 18-06 dd/mm or dd/mm or dd-mm or dd-mmm
Interval 1 interval 7 days Set to 0 or delete line for interval 1 days Set to 0 or delete line for single app.

Res. Run 1R Pond Flag for Index Res. Run IR
Flag for runoff calc. RUNOFF none none, monthly or total(average of entire run)
North Dakota Canola

stored as CEcoA.out Chemical: Prothioconazole PRZM environment: NDcanolaC.txt modified Tueday, 24 September 2002 at 07:20:06 EXAMS environment: pond298.exv modified Thuday, 29 August 2002 at 16:33:30 Metfile: w24013.dvf modified Wedday, 3 July 2002 at 09:05:54

0.206896551724138 11.73 11.66 11.44 11.33 11.3 10.77 0.241379310344828 11.57 11.53 11.4 11.2 11.07 10.7 0.275862068965517 11.56 11.49 11.37 11.09 11.04 10.7 0.310344827586207 11.54 11.49 11.3 11.08 10.93 10.5 0.344827586206897 11.49 11.45 11.29 10.93 10.92 10.27 0.379310344827586 11.38 11.34 11.15 10.85 10.67 9.97 0.413793103448276 10.91 10.88 10.79 10.65 10.51 9.711 0.448275862068966 10.66 10.63 10.48 10.31 10.28 9.651 0.482758620689655 10.35 10.31 10.22 10.12 10.06 9.39 0.517241379310345 10.11 10.07 9.93 9.693 9.638 9.335 0.551724137931034 10.07 10.03 9.886 9.673 9.576 9.222 0.586206896551724 9.926 9.888 9.735 9.611 9.448 9.008 0.620689655172414 9.835 9.794 9.628 9.358 9.249 8.914 0.655172413793103 8.54 8.481 8.306 7.962 7.8 6.985 0.689655172413793 7.501 7.466 7.334 7.072 6.911 6.405 0.724137931034483 7.061 7.021 6.885 6.664 6.559 5.875 0.758620689655172 6.272 6.243 6.124 5.914 5.795 5.471 0.793103448275862 5.822 5.799 5.715 5.537 5.553 5.235 0.827586206896552 5.684 5.633 5.499 5.39 5.336 4.949 0.862068965517241 5.637 5.608 5.46 5.223 5.101 3.727 0.896551724137931 3.217 3.19 3.069 2.981 2.912 2.137 0.931034482758621 1.954 1.929 1.89 1.823 1.749 1.119 0.96551724137931 0.9686 0.9477 0.872 0.7564 0.6998 0.3313 **Prob. Peak 96 hr 21 Day 60 Day 90 Day Yearly 0.1 12.778 12.706 12.474 12.012 11.758 11.012 Average of yearly averages: 8.05161785714286**

Benthic segment concentrations (ggb)

Inputs generated by pe4.pl - 8-August-2003 Data used for this run: Output File: CEcoA
Metfile: w24 w24013.dvf PRZM scenario: NDcanolaC.txt EXAMS environment file: pond298.exv Chemical Name: Prothioconazole
Description Variable Name Variable Name Value Units Comments
tt mwt 344.264 g/mol Molecular weight mwt 344.264 g
Henry's Law Const. henry 2.96E-10 Henry's Law Const. henry 2.96E-10 atm-m^3/mol
Vapor Pressure vapr 3E-9 torr Vapor Pressure vapr 3E-9 torr
Solubility sol 300 mg/L Solubility
Kd Kd Kd Kd mg/L
Koc Koc 523 mg/L mg/L
kdp Photolysis half-life kdp 101.9 days Half-life Aerobic Aquatic Metabolism kbacw 385.2 days Halfife Anaerobic Aquatic Metabolism kbacs 0 days Halfife
Aerobic Soil Metabolism asm 1052.2 days Halfife Aerobic Soil Metabolism asm 1052.2 da

Hydrolysis: pH 4 0 days Half-life Hydrolysis: pH 4 0 days Half-life Hydrolysis: pH 7 0 days Half-life Hydrolysis: pH 9 0 days
Method: CAM 2 integer See integer See PRZM manual Incorporation Depth: DEPI 0 cm
Application Rate: TAPP 0.2 kg/ha Application Rate: TAPP 0.2 kg/ha
Application Efficiency: APPEFF 0.95 Application Efficiency: APPEFF 0.95 fraction
Spray Drift DRFT 0.05 fraction of application Spray Drift DRFT 0.05 fraction of application rate applied to pond
Application Date Date 17-06 dd/mm or dd/mmm or dd-mm or dd-mmm Application Date Date 17-06 dd/mm or dd/mmm or dd-mm or dd-mmm
Interval 1 interval 5 days Set to 0 or delete line for Interval 1 interval 5 days Set to 0 or delete line for single app.
Record 17: FILTRA IPSCND 1 UPTKF Record 17: FILTRA IPSCND 1 UPTKF
Record 18: PLVKRT PLDKRT FEXTRC 0.5 PLVKRT PLDKRT FEX
Res. Run TR Flag for Index Res. Run 11 R Pond
Flag for runoff calc. RUNOFF none none none, monthly or total(average of entire run)

Michigan Bean

stored as BEcoA.out Chemical: Prothioconazole PRZM environment: MIbeansC.txt modified Monday, 10 May 2004 at 06:24:24 EXAMS environment: pond298.exv modified Thuday, 29 August 2002 at 16:33:30 Metfile: w14826.dvf modified Wedday, 3 July 2002 at 09:05:38 **Water segment concentrations (ggb)** Year Peak 96 hr 21 Day 60 Day 90 Day Yearly 1961 6.823 6.713 6.381 6.046 5.883 2.341 1962 7.224 7.179 7.09 7.013 6.991 5.988 1963 8.661 8.619 8.537 8.47 8.375 7.471 1964 12.39 12.31 12 11.69 11.65 9.757 1965 15.5 15.39 15.01 14.76 14.52 12.54 1966 14.7 14.64 14.45 14.29 14.27 13.97 1967 16.1 16.08 15.99 15.92 15.81 14.78 1968 24.37 24.18 23.46 22.36 21.84 18 1969 24.8 24.68 24.37 23.71 23.34 21.59 1970 25.84 25.72 25.52 25.37 25.28 23.42 1971 26.69 26.59 26.34 25.74 25.48 24.71 1972 29.08 28.93 28.38 27.9 27.81 26 1973 27.33 27.32 27.25 27.11 27 26.3 1974 27 26.97 26.88 26.8 26.74 25.89 1975 34.32 34.19 33.7 32.72 32.07 27.16 1976 30.19 30.11 30.03 29.89 29.79 29.21 1977 29.03 29.01 28.95 28.84 28.79 28.02 1978 27.5 27.44 27.25 27.14 27.08 26.56
1979 27.83 27.72 27.34 27.03 26.75 26.03 1979 27.83 27.72 27.34 27.03 26.75 26.03 1980 28.88 28.75 28.45 28.03 27.96 26.39 1981 29.69 29.62 29.36 28.95 28.79 27.31 1982 28.36 28.26 28.1 27.98 27.91 27.44 1983 28.34 28.26 28.01 27.87 27.77 27.03 1984 28.19 28.09 27.84 27.53 27.35 26.86 31.14 30.71 27.48 1986 34.51 34.37 34 33.3 32.98 29.94 1987 33.18 33.07 32.74 32.17 31.93 31.05 1988 31.64 31.53 31.28 31.14 31.04 30.6 1989 33.19 33.08 32.71 32.09 31.88 30.54 1990 31.15 31.14 31.07 30.95 30.87 30.12 Sorted results Prob. Peak 96 hr 21 Day 60 Day 90 Day Yearly 0.032258064516129 34.51 34.37 34 33.3 32.98 31.05 0.064516129032258 34.32 34.19 33.7 32.72 32.07 30.6 0.096774193548387 33.19 33.08 32.74 32.17 31.93 30.54 0.129032258064516 33.18 33.07 32.71 32.09 31.88 30.12 0.161290322580645 32.8 32.62 32 31.14 31.04 29.94

0.193548387096774 31.64 31.53 31.28 31.14 30.87 29.21 0.225806451612903 31.15 31.14 31.07 30.95 30.71 28.02 0.258064516129032 30.19 30.11 30.03 29.89 29.79 27.48 0.290322580645161 29.69 29.62 29.36 28.95 28.79 27.44 0.32258064516129 29.08 29.01 28.95 28.84 28.79 27.31 0.354838709677419 29.03 28.93 28.45 28.03 27.96 27.16 0.387096774193548 28.88 28.75 28.38 27.98 27.91 27.03 0.419354838709677 28.36 28.26 28.1 27.9 27.81 26.86 0.451612903225806 28.34 28.26 28.01 27.87 27.77 26.56 0.483870967741936 28.19 28.09 27.84 27.53 27.35 26.39

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Benthic segment concentrations (ggb)

Sorted results
Prob. Peak 96 hr 21 Day 60 Day 90 Day Yearly 0.032258064516129 31.72 31.72 31.71 31.66 31.6 31.08 0.064516129032258 31.72 31.72 31.7 31.58 31.33 30.77

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North Carolina Peanut

stored as PEcoA.out Chemical: Prothioconazole PRZM environment: NCpeanutC.txt modified Satday, 12 October 2002 at 16:12:46 EXAMS environment: pond298.exv modified Thuday, 29 August 2002 at 16:33:30 Metfile: w13737.dvf modified Wedday, 3 July 2002 at 09:06:30

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Sorted results

Benthic segment concentrations (ggb)

Sorted results Prob. Peak 96 hr 21 Day 60 Day 90 Day Yearly 0.032258064516129 30.73 30.73 30.72 30.68 30.63 29.8 0.064516129032258 30.59 30.59 30.58 30.53 30.38 29.42 0.096774193548387 30.58 30.57 30.55 30.47 30.38 29.12 0.129032258064516 30.49 30.47 30.41 30.24 30.11 28.62 0.161290322580645 30.34 30.32 30.27 30.13 30.02 28.4

APPENDIX D: Example T-Rex Input and Output for Prothioconazole.

T-Rex can be found at:

http://www.epa.gov/oppefedl/models/terrestriaVtrex~usersguide.htm

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ote To provide risk management with the maximum possible information,
To recommended that both the dose-based and concentration-based
Qs be calculated when data are available

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{2\pi} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\$

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2}d\mu\,d\mu\,.$

 $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\int_{0}^{\pi} \frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2} \frac{1}{\sqrt{2\pi}}\int_{0}^{\pi}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2} \frac{1}{\sqrt{2\pi}}\int_{0}^{\pi}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac$

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Note. To provide risk management with the maximum possible information,
I is recommended that both the dose-based and concentration-based
ROs be calculated when data are available

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APPENDIX E: Example Terrplant (v. 1.2.1) Input and Output for Prothioconazole.

APPENDIX F: Ecological Effects Assessment.

Toxicity to Terrestrial Animals

Acute and Subacute Toxicity to Birds

JAU6476 techn. a.i.: Acute Oral Toxicity to Bobwhite Quail (MRID 462460-36)

The acute oral toxicity of JAU6476 Technical (98.4% prothioconozole) to 39-week old Northern Bobwhite quail was assessed over 14 days (MRID 462460-36). The compound was administered to birds via gelatin capsule at nominal concentrations of 0 (vehicle control), 200,650, and 2000 mg a.i./kg bw. The 14-day LD50 was > 2000 mg a.i./kg bw since no mortality was observed. There were no significant effects on body weights although there was an apparent treatment related effect on feed consumption in birds from the 2000 mg a.i./kg bw treatment between days 0-3; the NOEL for food consumption was 650 mg a.i./kg bw. No treatment related abnormalities were observed at necropsy. Based on these results, prothioconozole can be classified as practically non-toxic to Northern Bobwhite quail under acute oral exposure conditions. This study is classified as ACCEPTABLE and satisfies Guideline 71-1 for Avian Oral LD50.

SXX 0665 techn. a.i. (prothioconazole - **desthio): Acute Oral Toxicity to Bobwhite Quail (MRID 462460-37)**

The acute oral toxicity of SXX 0665 Technical (93.7% prothioconazole - desthio) to 30- to 35-week old Northern Bobwhite quail (Colinus virginianus) was assessed over 14 days (MRID 462460-37). SXX 0665 Technical was administered to the birds via gelatin capsule at nominal concentrations of 0 (negative control), 125, 250, 500, 1000, and 2000 mg a.i./kg bw. Mortality was 30% (one male and two females) at the 2000 mg a.i./kg bw level. The mortality observed was delayed, with two birds found dead on Day 6, and one bird found dead on Day 12. The 14 -day LD_{50} was >2000 mg a.i./kg bw, which categorizes SXX 0665 Technical (prothioconazole - desthio) as practically non-toxic to Northern Bobwhite quail on an acute oral basis. The NOAEL for body weight data was 250 mg a.i/kg bw, based on reductions in weight gain for the females during the exposure period; body weight differences between control and the 500 mg a.i.lkg bw treatment were on the order of 5%. This study is classified as ACCEPTABLE and satisfies Guideline 71-1 for Avian Oral LD_{50} .

Table **F1.** Summary of avian acute toxicity test on Bobwhite Quail (Colinus virginianus) Exposed to prothioconozole and the desthio metabolite

JAU6476 techn: 5-Day-Dietary LC₅₀ for Bobwhite Quail (MRID 462460-38)

The acute dietary toxicity of JAU6476 Technical (98.4% prothioconozole) to 10-day old Northern Bobwhite quail was assessed over 8 days (MRID 462460-38). The compound was administered to the chicks via the diet at nominal concentrations of 0 (negative control), 313, 625, 1250, 2500, and 5000 ppm which corresponded to n/a (control not reported), 299, 622, 1215, 2380, and 4983 ppm a.i., respectively

(MRID 46246038). There were no apparent treatment related effects on survival, clinical signs, body weight, or feed consumption. The 8-day acute dietary LC_{50} was > 4983 ppm a.i., which was the highest concentration tested. There was, however, a concentration-dependent increase in intestinal inflammation observed in some birds at necropsy; the NOEC and LOEC were 622 and 1215, respectively. Based on these results, JAU6476 (prothioconozole) can be categorized as practically non-toxic to Northern Bobwhite quail when administered via the diet. This study is classified as ACCEPTABLE and satisfies Guideline 71-2a for Avian Dietary LC_{50} .

SXX 0665 techn. a.i. (prothioconazole-desthio): 5-Day Dietary LC₅₀ for Bobwhite Quail (MRID 462460-39)

The acute dietary toxicity of JAU 6476 - Desthio (96.8% prothioconazole - desthio) to 10-day old Northern Bobwhite quail (Colinus virginianus) was assessed over 8 days (MRID 462460-39). JAU 6476 - Desthio was administered to the birds in the diet at nominal concentrations of 0 (negative control), 3 13, 625, 1250,2500, and 5000 ppm. Mean-measured toxicant concentrations were 321,639, 1243,2577, and 5215 ppm a.i., respectively (control results not provided). Cumulative mortality was 0% in the control and #1243 ppm a.i. groups, 10% in the 2577 ppm a.i. group, and 70% in the 5215 ppm a.i. group. The 8-day LC₅₀ was 4252 ppm (3161-6501 ppm), which categorizes JAU 6476 - Desthio (prothioconazole - desthio) as slightly toxic to the Northern Bobwhite quail on an acute dietary basis. In addition to mortality, treatment-related effects were observed in clinical effects (reduced vigilance), body weight, and food consumption parameters at the 2577 and 5215 pprn a.i. test levels. Calculated mean test substance intakes were 3.0, 5.3, 10.4, 10.7, and 15.2 mg/bird/day for the nominal 313, 625, 1250, 2500, and 5000 ppm a.i. treatment groups, respectively. This study is scientifically sound and fulfills the guideline requirements for an avian dietary toxicity study using JAU 6476 - Desthio and the Northern Bobwhite quail (\$71-2a). This study is classified as ACCEPTABLE.

JAU 6476 techn.: 5-Day Dietary LC50 to Mallard Duck (MRID 4624960-40)

Ten-day-old mallard ducks were used to study the acute dietary toxicity of JAU 6476 Technical (98.7% prothioconazole; MRID 462460-40). Birds were exposed for 8 days to prothioconozole in feed at the following measured concentrations $0, 256, 555, 1180, 2532,$ and 5567 ppm a.i. There were no treatment related effects of dietary exposure to prothioconazole in Mallards under these exposure conditions. The LC50 was >5567 ppm a.i., the highest concentration tested which classifies JAU6476 technical as practically nontoxic to Mallards on an acute dietary exposure basis. The NOEC and LOEC were 5567 and >5567 ppm a.i., respectively. The study is classified as CORE and meets the requirements for an avian dietary toxicity study using the Mallard duck (71 -2b).

Table F2. Summary of avian subacute dietary toxicity test on Bobwhite Quail (Colinus virginianus)

Exposed to prothioconozole and the desthio metabolite

Supervisory

Species | Supervisory

Species | Supervisory

Species |

Chronic Toxicity to Birds

JAU6476 techn: Reproductive Toxicity in Bobwhite Quail (MRID 462460-42)

The one-generation reproductive toxicity of JAU 6476 Technical (prothioconazole) to groups (16 pensltreatment level) of 1 male and 1 female of 24-week-old Northern Bobwhite quail was assessed over approximately 22 weeks (MRID 462460-42). JAU 6476 Technical was administered to the birds in the diet at mean-measured concentrations of <LOD (negative control), 60, 251, and 982 ppm a.i. diet. Nominal concentrations were 0, 60, 245, and 1000 pprn diet. There were no significant treatment-related effects on any adult or offspring parameter. The NOAEC and LOAEC levels were 982 and >982 pprn a.i. diet, respectively. This toxicity study is scientifically sound. The maximum expected field residue level was not provided, however, the highest level tested was at an appropriate level to approximate field exposure for this species based on currently proposed uses. This study fulfills guideline requirements for an avian reproduction study using the Northern Bobwhite quail (\$71-4a) and is classified as ACCEPTABLE.

SXX0665 techn (prothioconazole-desthio): Reproductive Toxicity in Bobwhite Quail (MRID 462460-43)

The one-generation reproductive toxicity of JAU 6476 - Desthio (prothioconazole - desthio) to groups (20 pens/treatment level) of 1 male and 1 female of > 16-week old Northern Bobwhite quail (exact age not known) was assessed over approximately 22 weeks (MRID 462460-43). JAU 6476 - Desthio was administered to the birds in the diet at nominal concentrations of 0 (negative control), 60, 173, and 500 ppm. Mean-measured concentrations were 59.5, 173.0, and 506.7 pprn a:i.(control feed was not analyzed for a.i. content).There were no significant treatment-related effects on any adult parameter. In addition, no treatment-related effects were observed on egg production or quality, fertility, embryonic development, hatchability, or clinical effects, food consumption, or body weights of chlcks during the 14 day observation period. Although study-author reported significant effects on some reproductive endpoints, reviewer-conducted statistical analyses indicated that there were no significant, treatmentrelated effects on any reproductive endpoints. The difference in conclusions between the reviewer and the original study author is likely the result of different statistical tests, as described below. The reviewer determined NOAEC was 506. 7 ppm a.i. This study is scientifically sound, fulfills guideline requirements for the reproductive toxicity of JAU 6476 - Desthio (prothioconazole - desthio) to Northern Bobwhite quail (\$71-4a), and is classified as ACCEPTABLE.

JAU6476 techn: Reproductive Toxicity in Mallard Duck (MRID 462460-44)

The one-generation reproductive toxicity of JAU 6476 Technical (prothioconazole) to groups (16 pensltreatment level) of 1 male and 1 female of 7-month old Mallard duck was assessed over approximately 21 weeks (MRID 462460-44). JAU 6476 Technical was administered to the birds in the diet at mean-measured concentrations of <LOD (negative control), 248, 698, and 1978 ppm a.i. diet. Nominal concentrations were 0, 245, 700, and 2000 ppm diet.

There were no significant treatment-related effects on any adult parameter. In addition, no treatmentrelated effects were observed on egg production or quality, fertility, early embryonic development, hatching success, or clinical effects or body weights of chicks during the 14-day observation period. Although study-author reported results showed significant effects on chick survival, reviewer calculated statistics indicate no significant effects of JAU 6476 on hatchling survival at any treatment level. This study is scientifically sound, fulfills guideline requirements for the reproductive toxicity of JAU 6476 Technical (prothioconazole) to Mallard duck (\$71 -4b), and is classified as ACCEPTABLE.

SXX0665 techn (prothioconazole-desthio): Reproductive Toxicity in Mallard Duck (nRID 462460-45)

The one-generation reproductive toxicity of **JAU** 6476 - Desthio (prothioconazole - desthio) to groups (1 6 pensltreatment level) of 1 male and 1 female, 20-week-old Mallard duck was assessed over approximately 20 weeks (MRID 462460-45). **JAU** 6476 - Desthio was administered to the birds in the diet nominal concentrations of 0 (solvent control), 60, 120, and 500 ppm. Mean-measured concentrations were <6.0 (<LOD, control), 56.7, 120, and 499 ppm a.i., respectively. However, stability experiments indicated that the test substance likely degraded slightly (approximately 20-25%) during the duration of open feeder storage (7 days). Taking into account this degradation, the modified, mean-measured concentrations would be 50.2, 105, and 449 ppm a.i. There were no significant treatment-related effects on any adult or offspring parameter. As a result, the NOAEC and LOAEC levels were 449 and >449 pprn a.i., respectively.

This toxicity study is scientifically sound. However, due to the apparent degredation of **JAU** 6476 - Desthio in feed, this study does not satisfy the guideline requirements for the reproductive toxicity of **JAU** 6476 - Desthio (prothioconazole - desthio) to Mallard duck (\$71-4b). The study is, therefore, classified as SUPPLEMENTAL although it does not need to be repeated and the values may be useful for risk assessment purposes. Since the test substance likely degraded during open feeder storage, this study is not upgradable.

Table F3. Summary of avian chronic toxicity studies on prothioconazole and metabolites

Acute toxicity to mammals

JAU6476 techn.: Acute oral toxicity in the laboratory rat (MRID 462462-30)

In an acute oral toxicity study (MRID 46246230), three male and three female SPF-bred Wistar rats of the strain HsdCpb:Wu (Age: 7 weeks male; 10 weeks female; Weight: 186-194 g males; 178-184 g females; Source: Harlan Winkelmann GmbH, Borchen, District of Paderborn, Germany) were administered 5000 mglkg b.w. of **JAU** 6476 (Prothioconazole; Purity 99.8%) in a single dose by stomach tube. The test substance was formulated in water with the aid of Cremophor EL 2% (v/v) before administration. The applied formulations were well mixed by stirring on a magnetic mixer before and during administration, and by pumping the syringe several times. Individual animal body weights were recorded on day 1 before dosing and again on Days 8 and 15 after dosing. Clinical signs of toxicity were made several times on the day of dosing and at least once daily thereafter. **A** gross necropsy examination was performed on all animals. The oral LD50 for males, females and combined \Rightarrow 6200 mg/kg bw. All animals survived and gained weight during the study. Clinical signs observed included decreased motility and diarrhea. The observed clinical signs occurred within 1 and 2 hours after dosing and lasted up to 6 hours. No gross pathological findings were observed. This acute oral study is classified as Acceptable.

SXX0665 techn. (prothioconazole-desthio): Acute oral toxicity in laboratory rats (MRID 462462-31)

In an acute oral toxicity study (MRID 4624623 1), groups of SPF-bred Wistar rats of the strain Bor:WISW (SPF-Cpb), 5/sex/group (Age: 7 weeks males; 10 weeks female; Weight: 167-184 g males; 167-1 84 g female; Source: Winkelmann Experimental Animal Breeders, Borchen, Kreis Paderborn, Germany) were given a single oral dose of SXX 0665 (Purity: 93.7%) at doses of 100, 500 (males only), 1000 (females only), 2000, 2500 (males only), 3150 and 4000 mg/kg b.w. The test material was formulated with 1% v/v Cremophor EL in deionized water immediately (less than one hour) prior to treatment. Body weights were obtained before dosing, on days 4, 8 and 15 and in cases where the recovery period was extended to 21 days, on day 22. Animals were observed for clinical signs of toxicity and mortality several times on the day of dosing and at least once daily thereafter. A gross necropsy examination was performed on all animals at scheduled euthanasia. The study was performed in accordance with OECD - Guideline for Testing of Chemicals, Section 4: Health Effects, No. 401, "Acute Oral Toxicity", adopted February 24, 1987.

Mortality was observed in the 2000, 2500, 3150 and 4000 mg/kg b.w. treatments. Results for these doses are provided here.

2500 mglkg b.w. (males only) - Two animals died on day 9 and 10, respectively. Decreased body weights were observed in males at the 4 and 8 day weighing intervals. Final weights of the surviving animals exceeded initial body weights. Observed clinical signs included piloerection, pallor, emaciation, apathy, decreased motility, poor reflexes, staggering and spastic gait, atony, labored breathing, increased excretion of urine, soft feces, lightly colored feces, narrowed palpebral fissure and bloody snout.

Gross pathological findings included patchy, distended lungs, liver slightly enlarged and somewhat swollen, partly with scattered or multiple light zones, light cut surface, dark spleen, kidneys slightly pale and red mucid contents in stomach and small intestine.

3 150 mglkg b.w. - Three males died between days 9-10 and four females died between days 9-13. Body weights were decreased in males and females. The surviving male's final weight exceeded their initial weight but the one surviving female's final weight was less than its initial body weight. Observed clinical signs included piloerection, pallor, emaciation, apathy, decreased motility, poor reflexes, staggering and spastic gait, atony, labored breathing, increased excretion of urine, narrowed palpebral fissure and bloody snout.

Gross pathological findings included slightly patchy lungs, distended lungs, patchy spleen and kidneys, glandular stomach reddened, stomach and intestinal tract distended and stomach filled with food.

4000 mg/kg b.w. - All male animals were dead by day 9 and all females by day 12. All animals had decreased body weights at the time of death. Observed clinical signs included piloerection, pallor, emaciation, apathy, decreased motility, poor reflexes, staggering and spastic gait, atony, labored breathing, increased salivation, lacrimation, narrowed palpebral fissure, redly incrusted eyelids, lying on side and bloody snout.

Gross pathological findings included lungs patchy to dark red, distended, slightly pale liver, lobulated, with multiple light zones, forestomach engorged with food, glandular stomach severely reddened, empty, intestinal tract empty and pale spleen.

Oral LD_{50} Males = 2806 mg/kg b.w. (approximate) Oral LD_{50} Females = 2506 mg/kg b.w. (approximate) This acute oral study is classified as Acceptable.

JAU6476 480SC (formulation): Acute oral toxicity in the laboratory rat (MRID 462462-32)

In an acute oral toxicity study (MRID 462462-32)-Up and Down Method, female Wistar rats (Wt. 122- 164 g, Source Charles River Labs, Raleigh, NC) were treated with Proline 480 SC (a.i. Prothioconazole 40.6%, Batch No. 02GJS038) at doses of 175,550,2000 and 5000 mg/kg body weight. Evaluation parameters included signs of gross toxicity and mortality for a subsequent period of 14 days. Initial and weekly body weights and necropsy findings were recorded on all animals. All animals died at 5000 mg/kg. Clinical signs (2000-5000 mg/kg) were loose stools, greenish stain perianal area, decreased activity, lying on the side, labored respiration, yellow wet urinogenital and perianal area, thinning of hair, and laceration at hind quarters. Oral LD_{50} for female rats was > 2000 and < 5000 mg/kg bw. All but one surviving animals gained weight during the study. This acute oral study is classified as Acceptable.

Species	Study Type	% active ingredient	LD50 mg/kgbw	MRID No.	Toxicity Category.	Fulfills Guideline Requirement
Laboratory rat Rattus norvegicus	Oral acute	99.8 JAU6476	>6200	462462-30	Practically non- toxic	Yes
Laboratory rat Rattus norvegicus	Oral acute	93.7 SXX0665	2506	462462-31	Practically non- toxic	Yes
Laboratory rat Rattus norvegicus	Oral acute	40.6 a.1 formulation	>2000	462462-32	Practically non- toxic	Yes

Table F4. Summary of mammalian acute toxicity studies

Chronic toxicity to mammals

JAU6476 techn.: Multigeneration reproduction study in rats (MRID 462463-34)

In a 2-generation reproduction study (MRID 462463-34) prothioconazole was administered by gavage to 30 Wistar rats/sex/dose in aqueous 0.5% methylcellulose/0.4% Tween 80 suspension. Doses were 0 (control), 10, 100, and 750 mg/kg bwlday in a dosing volurn of 10 mg/kg. There were no treatment-ralted mortalities or clinical finding throughout the study in any generation in either adults or pups. The parental LOAEL is 750 mg/kg bwlday based on decreased body weights, body weight gains and increased food consumption, increased liver weights and kidney weights. The NOAEL is 100 mg/kg bw/day. For reproductive effects, the LOAEL is 750 mg/kg bwlday based on decrased number of estrous cycles in both generation and increased duration of estrous cycle in the P generation. The corresponding NOAEL is 100 mg/kg bw/day. The LOAEL for offspring effects is 750 mg/kg bw/day based on decreased body weight and reduced spleen weight. The NOAEL is 100 mg/kg bw/day. For ecological risk assessment purposes, effects on body weight can be most clearly linked to effects on higher levels of biological organization (population-level effects). Regardless, the NOAEL for all effects, including reduced body weight, was 100 mg/kg bw/day. The study is scientifically sound and is classified ACCEPTABLE.

SXX0665 techn. (prothioconazole-desthio): Multigeneration reproduction study in rats (MRID 462463-33)

In a 2-generation reproduction study, SXX0665 (prothioconazole-desthio) was adrmnistered to 30 Sprague-Dawley rats/sex/dose in the diet. Exposure levels were 0 (control), 40, 160, and 640 mg/kg diet.

In the parental animals, one control and one high-dose female died due to dystocia; three high-dose females were euthanized prematurely due to signs of dystocia; one high-dose female was euthanized prematurely due to complete litter loss on lactation day 1. In F1 parental animals, three high-dose females died due to dystocia, one mid-dose female and one high-dose female were euthanized prematurely due to complete litter loss on lactation day 2. The increased incidence of dystocia was considered treatment-related. The LOAEL for parental effects is 640 pprn (equivalent to 40-46 or 41-73 mg/kg bw/day $[M/F]$) based on increased liver weight, liver histopathology and decrased food consumption during lactation (females only). The NOAEL is 160 ppm $(9.5-11 \text{ or } 10-19 \text{ mg/kg})$ bw/day [M/F]). For reproductive effects, the LOAEL is 640 ppm based on increased incidence of dystocia, decrased viability and decreased pup weight. The NOAEL is 160 ppm $(9.5-11)$ or 10-19 mg/kg bw/day [M/F]). The LOAEL for offspring effects is 640 pprn based on decreased pup body weight. The corresponding NOAEL is 160 ppm $(9.5-11 \text{ or } 10-19 \text{ mg/kg}$ bw/day [M/F]). For ecological risk assessment purposes, the reproductive effects relate directly to assessment endpoints for mammals. This study is scientifically sound and classified ACCEPTABLE.

Species	Study Type	% active ingredient	NOAEC mg/kgbw/day	MRID No. Author Year	Effects	Fulfills Guideline Requirement
Laboratory rat Rattus norvegicus	Multigeneration reproduction	98.1-98.4	100.	462463-34	Reduced body weight	Yes
Laboratory rat Rattus norvegicus	Multigeneration reproduction	92.8-95.6 SXX0665	9.5	462463-33	Reduced pup viability	Yes

Table F5. Summary of mammalian chronic toxicity studies

Toxicity to Insects (Terrestrial Invertebrates)

JAU6476 a.i.: Acute Effects on the Honeybee *Apis mellifea* **(MRID 462460-48)**

The honeybee, **Apis** *rnellzjera* L., was exposed to prothioconazole for 48 hours in both an oral and a contact test (MRID 462460-48). Negative and solvent (vehicle) controls were used in both tests and the nominal concentrations of prothioconozole for both tests were 12.5, 25, 50, 100, and 200 µg a.i./bee. The actual intake of prothioconozole in the oral study was $107, 22.0, 31.9, 47.3$, and 71.0μ g a.i./bee. By 48 hours in the oral test there was nor mortality in the negative control, 7% mortality in the vehicle control, 3% mortality in the 31.9 and 71.0 pg a.i./bee treatment groups and no mortality in the other treatment groups. One bee was observed lying on its back in 71.0 **pg** a.i./bee treatment group. By 48 hours in the contact test, there was 10% in negative control, 7% in vehicle (acetone) control, and 23, 30, 30, 13, and 27% mortality in the 12.5, 25, 50, 100, and 200 μ g a.i./bee treatment groups, respectively. The corresponding solvent-control corrected mortality was 17,25,25,6, and 22% in the 12.5,25,50, 100, and 200 µg a.i./bee treatment groups, respectively. The estimated LC50 for the oral test was > 71.0 µg a.i./bee. The estimated LD50 for the contact test was $> 200 \mu$ g a.i./bee. Given that less than 50% mortality occurred at highest exposure levels in both studies, prothioconozole is classified as practically non-toxic to honeybees via both oral and contact routes of exposure. These studies are scientifically sounds and satisfy the guideline requirements; the contact study classified as ACCEPTABLE and the oral study classified as SUPPLEMENTAL.

JAU6476 480 SC a.i. (formulation): Acute Effects on Honeybee, *Apis mellifera* **(4624690-46)**

The honey bee, Apis *rnellifera* L., was exposed to Prothioconazole formulation for 48 hours in the oral and the contact test (MRID 462460-46). The oral and contact nominal concentrations were 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee. The actual intake concentrations of Prothioconazole formulation in the oral

toxicity test were 3.6, 6.6, 18.1, 44.0, 97.9, and 232.0 μ g/bee. By 48 hours in the oral test, 3.3% mortality was observed in the 3.6, 6.6, 44.0, and 232.0 µg a.i./bee treatment groups. No mortality was observed in the control, 18.1, or 97.9 μ g/bee treatment groups. By 48 hours in the contact test, 3.3% mortality was observed in the control, and no mortalities were observed in the treatment groups. No sublethal effects were observed in any treatment group during the test. The LC_{50} value for the oral test was estimated as $>$ 232.0 µg/bee, the highest concentration of intake. The LD₅₀ value for the contact test was $>$ 200.0 ug/bee. As a result, JAU6476 480 SC formulation is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis. The NOAELs for the oral and contact tests were 232.0 and 200.0 ug/bee, respectively.

Table F6. Summary of terrestrial invertebrate acute toxicity tests with the honeybee

Toxicity to Soil Invertebrates (Non-Guideline Studies)

JAU6476 techn.: Acute toxicity to Earthworms (Eisenia fetida) (MRID 462461-22)

Adult earthworms, Eisenia fetida (4 x 10 animal per concentration) were exposed to nominal concentrations of 0 (control), 100, 178, 316, 562, and 1000 mg prothioconazole/kd soil (dry) in artificial soil for 14 days. There was 3% mortality in both the control and 1000 mg/kg soil treatment, which resulted in an $LC50 > 1000$ mg/kg soil, the highest treatment. There was, however, a significant reduction in earthworm weights in the highest treatment compared to the control; controls had about a 7% decrease in weight whereas worms in the 1000 mg/kg treatment showed a 16% decrease in weight. This study has not been formally reviewed by EFED but are used for risk characterization purposes only; RQs for soil-dwelling terrestrial invertebrates are not calculated.

JAU6476 S-methyl (prothioconazole-S-methyl): Acute toxicity to Earthworms (Eisenia fetida) (MRID 462461-26)

Adult earthworms, Eisenia fetida (4 x 10 animal per concentration) were exposed to nominal concentrations of 0 (control), 10, 32, 100, 316, and 1000 mg prothioconazole-S-methyl/kd soil (dry) in artificial soil for 14 days. There was 3% mortality in the control and the 10, 32, and 316 mg/kg soil treatments and 5% mortality in the 1000 mg/kg soil treatment, which resulted in an LC50 > 1000 mg/kg soil, the highest treatment. There was, however, a significant reduction in earthworm weights in the two highest treatments compared to the control; controls had about a 6% decrease in weight whereas worms in the 3 16 and I000 mg/kg treatment showed 12% and 39% decrease in weight, respectively. This study has not been formally reviewed by EFED.

JAU6476 480 SC (formulation): Acute toxicity to earthworms (Eisenia fetida) (MRID 462461-24)

Adult earthworms, Eisenia fetida (4 x 10 animal per concentration) were exposed to nominal concentrations of 0 (control), 100, 178, 316, 562, and 1000 mg prothioconazole 480SC/kd soil (dry) in artificial soil for 14 days. There was no mortality in controls or treatments, which resulted in an LC50 > 1000 mg/kg soil, the highest treatment. There was also no reduction in earthworm weights in controls and treatment groups. This study has not been formally reviewed by EFED.

SXX0665 (prothioconazole-desthio): Acute toxicity to earthworms (Eisenia fetida) (MRID 462461 xx)

JAU6476-S-methyl (prothioconazole-S-methyl): Acute and reproduction toxicity to collembolan (Folsomia candida) (MRID 462461-21)

Ten to twelve-day old springtails (Folsomia candida) were exposed to nominal concentrations of 0,0.32, 1.0, 3.16, 10, and 31.6 mg prothioconazole-S-methyl/kg soil (dry). There were 5 replicates of 10 individuals for each treatment and controls. Springtails were fed yeast at the beginning and at 14 days. Mortality and reproduction were determined after 28 days. Mortality across controls and all treatments ranged from 12 (1.0 mg/kg soil) to 20% (10 and 3 1.6 mg/kg soil) with no significant differences between any treatments and the control. There was a significant reduction (27%) in the number of juvenile produced in the 10 mg/kg soil treatment compared to controls; no effects on juvenile production were significant. The authors concluded that the NOAEC = 31.6 mg/kg soil, the highest tested concentration. This study was not formally reviewed by EFED.

SXX0665 (prothioconazole-desthio): Acute and reproduction toxicity to collembolan (Folsomia candida) (MRID 462461-19)

Ten to twelve-day old springtails (Folsomia candida) were exposed to nominal concentrations of 0,62.5, 125,250, 500, and 1000 mg prothioconazole-desthiokg soil (dry). There were 5 replicates of 10 individuals for each treatment and controls. Springtails were fed granulated yeast weekly. Mortality and reproduction were determined after 28 days. Mortality ranged from 2 to 8%; no statistical analyses were conducted, however, the author-reported LC50 was > 1000 mg/kg soil. There was a significant reduction in the number of juvenile produced in the 1000, 500, and 125 mg/kg soil treatments compared to controls; although the reduction in juveniles observed in the 250 mg/kg soil treatment was not statistically significant, the study authors considered in biologically significant. The study-author reported NOAEC was 62.5 mg/kg soil. This study was not formally reviewed by EFED.

SXX0665 (prothioconazole-desthio): Reproduction toxicity study in earthworms (Eisenia fetida) (MRID 462461-28)

Adult earthworms, Eisenia fetida, (4 x 10 animals per treatment) were exposed to nominal soil concentrations of $0, 0.1, 0.32, 1.0, 3.2, 10,$ and 32 mg prothioconazole-desthio/kg dry weight soil. After 28 days, the number of surviving animal and their weight alteration was determined. They were then removed from the artificial soil and after a further 28 days, the number of offspring were determined. There was little adult mortality in the experiment, however, there were significant effects of prothioconazole-desthio on the number of offspring produced. There was significant reduction in the number of offspring produced compared to the controls in the nominal 3.2, 10.0 and 32.0 mg **pothioconazole-desthiolkg** soil. The study author reported NOAEC is nominal 1.0 mg/kg dry soil based on reduced number of offspring in the 3.2 mg/kg soil treatment group. This study was not formally reviewed by EFED.

JAU6476-S-methyl (prothioconazole-S-methyl): Reproduction toxicity study in earthworms (Eisenia fetida) (MRID 462461-29)

Adult earthworms, Eisenia fetida, (4 x 10 animals per treatment) were exposed to nominal soil concentrations of 0, 10, 32, 100, 316, and 1000 mg prothioconazole-S-methyl/kg dry weight soil. After 28 days, the number of surviving animal and their weight alteration was determined. They were then removed from the artificial soil and after a further 28 days, the number of offspring were determined. There was little adult mortality in the experiment, however, there were significant effects of prothioconazole-S-methyl on weight increase with less weight gained in the nominal 3 16 and 1000 mg prothioconazole-S-methyl treatments compared to control. In addition, there was a significant reduction in the number of offspring produced in the 1000 mg/kg soil treatment compared to the control. The study author reported NOAEC is nominal 316 mg/kg dry soil based on reduced number of offspring in the 1000 mg/kg soil treatment group and 100 mg/kg dry soil based on reduced weight gain in the 316 mg/kg soil treatment group. This study was not formally reviewed by EFED.

JAU6476 techn.: Reproduction toxicity study in the collembolan, Folsomia candida (MRID 462461- 18)

Nine to twelve-day-old collembolans, *Folsomia candida*, were exposed to nominal concentrations of 0 (solven and negative control), 0.125, 0.25, 0.5, 1.0, 2.0,4.0, 8.0, 16.0, 32.0, and 64.0 mg prothoconazole/kg dry soil in artificial soil. Five replicates of each treatment were used and mortality and reproduction was assessed at the end of the study (28 days). There were no statistically significant effects of prothioconazole on mortality or reproduction so the study-author reported NOAEC is 64 mg/kg soil. This study was not formally reviewed by EFED.

SXX0665 (prothioconazole-desthio): Reproduction toxicity study in the collembolan, Folsomia candida (MRID 462461-20)

Ten to twelve-day-old collembolans, Folsomia candida, were exposed to nominal concentrations of 0 (solven and negative control), 0.125, 0.25, 0.5, 1 .O, 2.0,4.0, 8.0, 16.0, 32.0, and 64.0 mg prothioconazole-desthio/kg dry soil in artificial soil. Five replicates of each treatment were used and mortality and reproduction was assessed at the end of the study (28 days). There were no statistically significant effects of prothioconazole on mortality or reproduction so the study-author reported NOAEC is 64 mg/kg soil. This study was not formally reviewed by EFED.

Species	Study Type	% active ingredient	$LC_{\rm M}$ mg/kg soil	NOAEC Mg/kg soil	MRID No. Author Year	Fulfills Guideline Requirement
Eisenia fetida	Soil - acute	JAU 6476 Techn.98.6	>1000	N/A	462461-22	No.
Eisenia fetida	Soil acute	JAU 6476 S- methyl	>1000	N/A	462461-26	No.
Eisenia fetida	Soil - acute	JAU 6476 480 SC.	>1000	N/A	462461-24	No.
Eisenia fetida	Soil - acute	JAU 6476 - desthio	>1000	N/A	No MRID assigned	No.
Folsomia candida	$Soil - acute$, reproductive	JAU 6476-S- methyl	>31.6	31.6	462461-21	N ₀
Folsomia candida	Soil – acute. reproductive	JAU 6476- desthio	>1000	62.5	462461-19	No.
Eisenia fetida	Soil- reproduction	JAU 6476- desthio	N/A	1.0	462461-28	No.
Eisenia fetida	$Soil -$ reproduction	JAU 6476-S- methyl	N/A	100	462461-29	N _o
Folsomia candida	Soil- reproduction	JAU 6476 Techn.	N/A	64	462461-18	N ₀

Table F7. Summary of Terrestrial Soil-Invertebrate Toxicity Studies

Toxicity to Terrestrial Plants

JAU6476 480 SC a.i. (formulation): Tier I plant study (MRID 462460-49)

Seedling emergence and vegetative vigor were studied on 10 plant species after application of Prothioconazole formulation at 0.272 1bs ai/A. Test species included buckwheat, corn, cucumber, soybean, sunflower, tomato, onion, ryegrass, tumip, and wheat. By 21 days, cucumber was the only test species in the seedling emergence test which experienced inhibition greater than 25%; shoot height and dry weight were reduced 26% and 31%. While inhibition did not exceed 25%, sunflower emergence, turnip height, and soybean dry weight exhbited significant reductions from control, as well. By 21 days, none of the test species in the vegetative vigor test experienced $>25\%$ inhibition for any of the endpoints. However, there were significant reductions in cucumber height (22%) and tomato dry weight (11%). The phytoxicity percentage rating ranged from 0-1.3%. Based on cucumber shoot height and dry weight, the EC_{25} was <0.272 lbs ai/A in the seedling emergence test; the NOAEC was <0.272 lbs ai/A for sunflower emergence, cucumber and tumip height, and cucumber and soybean weight, and was 0.272 lbs ai/A for all other species and endpoints. The EC_2 for all test species in the vegetative vigor test was ≥ 0.272 lbs ai/A; the NOAEC for cucumber and tomato was <0.272 lbs ai/A and 0.272 lbs ai/A for all other species and endpoints. This study was classified as acceptable.

JAU6476 480 SC a.i. (formulation): Tier I1 plant study (MRID 462460-50)

Seedling emergence was studied on cucumber after application of JAU 6476 480SC typical end use formulation (Prothioconazole) at 19, 38, 76, 153, and 305 g a.i./ha. By 21 days, the percent inhibitions for emergence were $0, 0, 2, 0, 0$, and 2% for the pooled control, 19, 38, 76, 153, and 305 g a.i./ha treatment groups, respectively. Minor phytotoxic effects of distortion andlor stunting were observed at all treatment levels, as well as in the pooled control group. No parameter showed sensitivity (i.e., inhibition equal to or exceeding 25%), but shoot height and dry weight were significantly reduced as a result of treatment. The NOAEC for shoot height and dry weight were 0.03 and 0.272 lbs ai/A. The EC_{25} for all parameters was >0.272 lbs ai/A. This study is classified as acceptable. The single species tested in this study was the only species to display sensitivity in a previously conducted Tier I study (MRID 46246049) This study is scientifically sound and it fulfills the guideline requirements for a seedling emergence study (Subdivision J, $\S 123-1$ (TIER II)).

Toxicity to Freshwater Aquatic Animals

Freshwater Fish, Acute

JAU 6476 techn.: Acute toxicity (96 hours) to Rainbow Trout (Oncorhynchus mykiss) in a Static Test (MRID 462460-18)

To assess the acute toxicity of prothioconazole to freshwater fish, Rainbow trout were exposed to prothioconazole (JAU 6476) under static conditions for 96-hours (MRID 462460-18). The measured exposure concentrations were $(0.13 ppm a.i., $<$ LOD), 0.99, 1.70, 3.08, 5.26, and 8.02 ppm a.i. There$ was no mortality in the control or the 0.99 ppm a.i. treatment, however, % mortality was 40, 100, 100, and 100% in the 1.70, 3.08, 5.26, and 8.02 ppm a.i. treatments. The 96-h LC50 was 1.83 (C.I. 0.99-3.08), which categorizes prothioconazole as moderately toxic to rainbow trout under acute exposure conditions. No sub-lethal effects were observed in the control or the lowest exposure (0.99 pprn a.i.), however, by 96-hours surviving fish in the 1.70 ppm a.i. treatment were darkened and quiescent. The NOEC and LOECs were 0.99 and 1.70 pprn a.i., respectively. This study is classified as ACCEPTABLE and satisfies requirements for an acute freshwater fish study, guideline 72-lc.

JAU 6476 techn.:-Acute toxicity (96 hours) to Bluegill Sunfish (Lepomis macrochirus) in a Static Test (MRID 462460-22)

The 96-hour acute toxicity of JAU 6476 (Prothioconazole) to Bluegill (Lepomis macrochirus) was studied under static conditions (MRID 462460-22). Fish were exposed to JAU 6476 (Prothioconazole) at meanmeasured concentrations of <0.11 (<LOQ, controls), 1.69,2.81,4.81, 6.65, and 8.88 pprn a.i. After 96 hours of exposure, there was 5,45, 100, and 100% mortality in the mean-measured 2.81, 4.81, 6.65, and 8.88 pprn a.i. treament groups, respectively. There were no mortalities in the controls, or in the 1.69 pprn a.i. treatment group. The calculated 96-hour LC₅₀ (with 95% C.I.) was 4.59 (4.02-5.09) ppm a.i., which categorizes JAU 6476 (Prothioconazole) as moderately toxic to Bluegill (Lepomis macrochirus) on an acute toxicity basis. Sub-lethal effects observed during the exposure period included loss of equilibrium, quiescent, lying on the aquarium bottom, and/or vertical oriented. After 96 hours of exposure, sub-lethal effects were observed in surviving fish from the mean-measured 2.81 and 4.81 ppm a.i. treatment groups. The NOAEC and LOAEC values for mortality and sub-lethal effects were 1.69 and 2.81 ppm a.i.,

respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish $(\S 72-1)$. This study is classified as ACCEPTABLE.

JAU 6476 techn.: Acute toxicity (96 hours) to Common Carp(Cyprinus carpio) in a Static Test (MRID 462460-25)

In a 96-hour acute toxicity study, Common Carp (Cyprinus carpio) were exposed to JAU6476 (Prothioconazole) at mean-measured concentrations of <0.02 (<LOQ; pooled control), 0.91, 1.83, 3.66, 7.38, and 16.6 ppm a.i (MRID 462460-25). After 96 hours of exposure, mortality was 0% in the pooled control and the 0.91, and 1.83 ppm a.i. treatment groups, and 10, 60, and 100% in the 3.66, 7.38, and 16.6 ppm a.i. treatment groups, respectively. The LC_{50} was 6.42 ppm a.i., which categorizes JAU6476 (Prothioconazole) as moderately toxic to juvenile Common Carp (Cyprinus carpio) on an acute toxicity basis. The NOAEC and LOAEC values for mortality were 3.66 and 7.38 pprn a.i., respectively. Sublethal effects were observed in surviving fish from the mean-measured 1.83, 3.66, and 7.38 pprn a.i. treatment groups and included quiescence and fish lying on the bottom. No sub-lethal effects were observed in the pooled control or the 0.91 ppm a.i. treatment group. The NOAEC and LOAEC values for sub-lethal effects were 0.91 and 1.83 ppm a.i., respectively. This study is scientifically sound, but does not fulfill U.S. EPA guideline §72-1a because the test species, Common Carp (Cyprinus carpio) is not one of the preferred US EPA species. Consequently this study is classified as SUPPLEMENTAL.

JAU 6476 480 SC (formulation): Acute toxicity (96 hours) to Rainbow Trout (Oncorhynchus mykiss) in a Static Test (MRID 462460-19)

The 96-hour acute toxicity of JAU 6476 480 SC (Prothioconazole formulation) to Rainbow trout (Oncorhynchus mykiss) was studied under static conditions (MRID $462460-19$). Fish were exposed to Prothioconazole at mean-measured concentrations of <0.03 (<LOQ, controls), 0.32, 0.64, 1.29,2.56, and 5.77 pprn a.i. After 96 hours of exposure, there was 10, 100, and 100% mortality in the mean-measured 1.29, 2.56, and 5.77 pprn a.i. treament groups, respectively. There were no mortalities in the controls, or in the 0.32 and 0.64 ppm a.i. treatment groups. The 96-hour LC₅₀ (with 95% C.I.) was 1.69 (1.29 to 2.56) pprn a.i., which categorizes JAU 6476 480 SC (Prothioconazole formulation) as moderately toxic to Rainbow trout (Oncorhynchus mykiss) on an acute toxicity basis. The NOAEC and LOAEC values based on mortality and sub-lethal effects were 1.29 and 2.56 pprn a.i., respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1c). This study is classified ACCEPTABLE and provides information that may be useful for future risk assessment purposes.

JAU 6476 480 SC (formulation): Acute toxicity (96 hours) to Bluegill Sunfish (Lepomis macrochirus) in a Static Test (MRID 462460-23)

The 96-hour acute toxicity of JAU 6476 480 SC (Prothioconazole Formulation) to Bluegill (Lepomis macrochirus) was studied under static-renewal conditions (MRID 462460-23). Fish were exposed to prothioconazole at mean-measured concentrations of <0.03 (<LOQ, controls), 0.33, 0.68, 1.41, 2.81, and 5.80 pprn a.i. After 96 hours of exposure, there was 55% mortality in the 5.80 pprn a.i. treament group. There were no mortalities in the controls, or in the 0.33, 0.68, 1.41, and 2.81 ppm a.i. treatment groups. The 96-hour LC_{s0} (with 95% C.I.) was 5.53 (2.81 \rightarrow 5.80) ppm a.i., which categorizes JAU 6476 480 SC (Prothioconazole Formulation) as moderately toxic to Bluegill (Lepomis macrochirus) on an acute toxicity basis. The sub-lethal effects included fish at the surface and on bottom of test vessel in surviving fish from the 5.80 ppm a.i. treatment group. No sub-lethal effects were observed in the controls or the 0.33 through 2.81 ppm a.i. The NOAEC and LOAEC values for mortality and sub-lethal effects were 2.81 and 5.80 pprn a.i., respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish [\$72-11. This study is classified as ACCEPTABLE.

SXX 0665 (prothioconazole -desthio): Acute toxicity (96 hours) to Rainbow Trout (Oncorhynchus mykiss) in a Static Test (MRID 462460-20)

The 96-hour acute toxicity of SXX 0665 Technical (JAU6476-desthio) to Rainbow trout (Oncorhynchus mykiss) was studied under static conditions (MRID 462460-20). Fish were exposed to SXX 0665 Technical at mean-measured concentrations of 2.22, 4.20, 8.40, and 14.9 ppm a.i. for the nominal 2.34, 4.69, 9.37, and 18.7 pprn a.i. treatment groups, respectively. After 96 hours of exposure, there was 100% mortality in the 9.37 and 18.74 ppm a.i. treament groups, compared to 10% mortality in the control. There were no mortalities in the 1.17, 2.34, and 4.69 ppm a.i. treatment groups. The calculated 96-hour LC_{50} (with 95% C.I.) was 5.94 (4.20-8.40) ppm a.i., which categorizes SXX 0665 Technical as moderately toxic to Rainbow trout (Oncorhynchus mykiss) on an acute toxicity basis. The NOAEC and LOAEC values based on sub-lethal effects (on bottom, convulsions, lying on side/back, tumbling) were 2.22 and 4.20 pprn a.i., respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with Rainbow trout $\lceil \frac{8}{2} \cdot 2 - 1(c) \rceil$. This study is classified as ACCEPTABLE.

SXX 0665 (prtothioconazole-desthio): Acute toxicity (96 hours) to Golden Orfe (Leuciscus idas melanotus) in a Static Test (MRID 462460-24)

In a 96-hour acute toxicity study, Golden Orfe (Leuciscus idus melanotus) were exposed to SXX 0665 (JAU6476-desthio) at mean-measured treatment concentrations of <0.01 (<LOQ; negative controls), 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i for Part 1 of the experiment and 17.2 ppm a.i. for the nominal 18.9 ppm a.i. treatment concentration in Part 2 of the experiment (MRID 462460-24). The nominal 37.8, and 75.6 pprn a.i. (Part 2) treatment concentrations were not analytically verified because their respective biological results were identical to those of the nominal 18.9 pprn a.i. treatment group. By 96-hours, no mortalities were observed in either the control or the mean-measured 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i. treatment groups (Part 1). By 4 hours, 100% mortality was observed in the mean-measured 17.2 pprn a.i. and nominal 37.8 and 75.6 pprn a.i. all treatment groups (Part 2). By 96-hours, 100% of surviving fish in the 3.45 ppm a.i. treatment group were swimming irregularly, while surviving fish from the 6.80 were observed swimming at the bottom, apathetic, lying on their side or back, and tumbling. No sublethal effects were observed in either control group (Parts 1 & 2) or in the mean-measured 0.44 through 2.02 pprn a.i. treatment groups. This study is not scientifically sound and does not fulfill U.S. EPA guideline \$72-la because the reported results aud toxicity values are based on a combination of two separate experiments performed approximately three months apart with no overlap in test concentrations. Consequently, this study is classified as INVALID.

SXX 0665 (prothioconazole-desthio)-Acute toxicity (96 hours) to Fathead Minnow (Pimephales promelas) in a Static Test (MRID 462460-26)

In a 96-hour acute toxicity study, Fathead Minnow (Pimephales promelas) were exposed under staticrenewal conditions (renewed at 48 hours) to JAU6476-Desthio at mean-measured concentrations of <0.08 $(SLOQ, controls), 0.96, 2.06, 3.85, 7.99, and 16.3 ppm a.$ (MRID 462460-26). After 96 hours of exposure, mortality was 0% in the controls and mean-measured 0.96 through 2.06 and 7.99 ppm a.i. treatment groups. Mortality was 5 and 100% in the 3.85 and 16.3 ppm a.i. treatment groups, respectively. The 96-hour LC₅₀ (with 95% C.I.) was 11.41 (7.99-16.30) ppm a.i., which classifies JAU6476-Desthio as slightly toxic to Fathead Minnow (Pimephales promelas) on an acute toxicity basis. The NOAEC and LOAEC, based on mortality, were 7.99 and 16.3 ppm a.i., respectively. Sub-lethal effects observed

during the exposure period included fish at the surface and on the bottom of the test vessel, loss of equilibrium, quiescence, darkened coloration, and erratic behavior. Treatment related effects were observed in 15% of surviving fish from the mean-measured 7.99 ppm a.i. treatment group. No sub-lethal effects were observed in the controls or the 0.96 through 3.85 ppm a.i. treatment groups. The NOAEC and LOAEC, based on sub-lethal effects, were 3.85 and 7.99 ppm a.i., respectively. This study is scientifically sound, and satisfies the guideline requirements for an acute toxicity study with freshwater fish, warm water species (\$72-la). The study is classified as ACCEPTABLE.

Species	Study Type	% active ingredient	LC_{40} mglL (95% C.I.)	MRID No.	Toxicity Category	Fulfills Guideline Requirement
Onchorynchus $m\nu k$ iss	Acute	JAU 6476 98.4	1.83 $(0.99 - 3.08)$	462460-18	Moderately Toxic	Yes
Lepomis macrochirus	Acute	JAU 6476 98.4	4.59 $(4.02 - 5.09)$	46240-22	Moderately Toxic	Yes
Cyprinus Carpio	Acute	JAU 6476 98.4	6.42 $(4.79 - 8.91)$	46240-25	Moderately Toxic	No
Onchorynchus mykiss	Acute	JAU 6476 480 SC 41.4	1.69 $(1.29 - 2.56)$	462460-19	Moderately Toxic	Yes
Lepomis macrochirus	Acute	JAU 6476 480 SC 41.4	5.53 $(2.81 - 5.8)$	462460-23	Moderately Toxic	Yes
Onchorynchus mykiss	Acute	SXX 0665 93.7	5.94 $(4.20 - 8.40)$	462460-20	Moderately Toxic	Yes
Leuciscus idus melanotus	Acute	SXX 0665 93.7	N/A (Invalid)	46240-24	N/A	No.
Pimephales promelas	Acute	SXX 0665 96.5	11.41 $(7.99-16.3)$	46240-26	Slightly Toxic	Yes

Table F10. Summary of freshwater fish acute toxicity studies

Freshwater Fish, Chronic

JAU 6476 techn.: Toxicity to Early Life Stage Rainbow Trout (Oncorhynchus mykiss) (MRID: 462460-3 1)

The chronic toxicity of JAU 6476 Technical (98.3% prothioconazole) to the early life-stage of Rainbow Trout (Oncorhynchus mykiss) was studied under flow-through conditions for 97 days (37-day hatching period and 60-day post-hatch period). Fertilized embryos (140/treatment), <24 hours old, were exposed to JAU 6476 Technical at nominal concentrations of 0 (negative and solvent controls), 36.9, 73.7, 147, 295, and 590 ppb (adjusted for purity). Mean-measured concentrations were <6.37 (<LOQ, controls), 35.6, 74.9, 140,308, and 553 ppb a.i. (94 to 104% of adjusted nominal concentrations); however, excessive analytical variability was observed at all toxicant levels (high-low ratios $\exists 1.5$). No treatmentrelated effects on hatchlng success or time-to-hatch were observed. Hatching commenced on Day 34 and continued until Day 40 in all treatment and control levels. Mean percent hatch ranged from 30 to 42% for all treatment and control groups. This study is not scientifically sound. Hatching success was below 50% in both control groups, and test concentrations were highly variable in the test media at all toxicant levels. This study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (\$72-4a). Consequently, this study is classified as INVALID.

SXX 0665 (prothioconazole-desthio) -Toxicity to Early Life Stage Rainbow Trout (Oncorhynchus mykiss) (MRID: 462460-32)

The chronic toxicity of JAU 6476 - Desthio (96.8% prothioconazole - desthio) to the early life-stage of Rainbow Trout (Oncorhynchus mykiss) was studied under flow-through conditions for 96 days (35-day hatching period and 61-day post-hatch period; MRID 462460-32). Fertilized embryos (400/treatment), \leq 24 hours old, were exposed to JAU 6476 - Desthio at mean-measured concentrations of \leq 1.83 (\leq LOQ, control), 1.90, 3.34, 7.52, 14.1,27.5, and 53.0 ppb a.i. (85.5-98.2% of nominal concentrations). No treatment-related effects on time to first hatch, time-to-completion of hatch, or hatching success (on Day 35) were observed. Hatching commenced at all test levels within 3 days of each other, with the first alevins observed on Day 26 at the 3.34 ppb a.i. level. Mean percent hatch on Day 35 ranged from 28 to 36% for all treatment and control groups. This study is not scientifically sound because hatching success was below 50% in the control group, and a high level of (hatching success) variability was observed between the two control replicates. Consequently, this study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (\$72-4a). This study is classified as INVALID.

SXX0665 (prothioconazole-desthio): Fish Life cycle toxicity test on fathead minnows (Pimephales promelas) (MRID 462460-33)

The chronic toxicity of Desthio JAU 6476-Desthio (Prothioconazole metabolite) to the full life stage of Fathead Minnow (Pimephales promelas) was studied under flow-through conditions for approximately 9 months (MRID 462460-33). Fertilized eggs (200 embryos/treatment, <24 hours old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 19, 38, 75, 150, and 300 ppb. Mean-measured concentrations of the parent generation were <2.00 (<LOQ, controls), 19, 37, 74, 148, and 296 ppb a.i. Mean-measured concentrations of the second-generation were ≤ 2.00 (\leq LOO, controls), 19, 37, 75, 150, and 295 ppb a.i. Reproduction of fathead minnow was assessed by spawning frequency and the mean number of eggs produced per female per reproductive day. There were statistical differences from the pooled control at the 19, 37, 148, and 296 ppb a.i. treatment levels. The study authors and reviewer concluded that the lack of spawning in the 296 ppb a.i. group was biologically significant. Consequently, the NOAEC for spawning frequency was 148 ppb a.i. Egg production was not significantly reduced in any treatment group compared to pooled control. The NOAEC for egg production was 148 ppb a.i., the highest treatment level at which eggs were produced. The NOAEC for first-generation (P) growth was 148 ppb a.i. Based on adverse effects on first-generation larval/juvenile survival, juvenile (8-weeks post-hatch) and male growth, spawning frequency, and morphological deformities, the NOAEC and LOAEC are 148 and 296 ppb a.i., respectively. This study did not fulfill the guideline requirements for a fish life-cycle toxicity test because the F_1 generation was only maintained for 4 weeks post-hatch rather than 8 weeks as required. This study is classified SUPPLEMENTAL and although results do not meet guideline requirements; the information may be useful for future risk assessment purposes.

Freshwater Invertebrates, *Acute*

JAU 6476 techn.: Acute Toxicity to Waterileas (Daphnia magna) Under Static Exposure

(MRID 462460-09)

A 48-hour study on the acute toxicity of prothioconazole (JAU 6476 (tech.)) to waterfleas, Daphnia magna, was conducted under static exposure conditions (MRID 462460-09). Daphnids were exposed to control $(< 0.13$ ppm a.i., $<$ LOD), 0.48, 0.93, 1.63, 2.99, 5.12, and 9.24 ppm a.i mean-measured concentrations. There was no mortality in the control or the 0.48 pprn a.i. treatment group; % mortality was 7, 97, 100, 100, and 100% for the 0.93, 1.63, 2.99, 5.12, and 9.24 ppm a.i. levels, respectively. The 48-hour LC₅₀ was 1.20 (C.I. 1.09-1.32) ppm a.i. which categorizes prothioconazole as moderately toxic to Daphnia magna on an acute toxicity basis. The corresponding NOEC for mortality was 0.93 ppm a.i. No sub-lethal effects were observed. The study is classified as ACCEPTABLE and satisfies the requirements for an acute toxicity study with freshwater invertebrates, guideline 72-2.

JAU 6476 480 SC (formulation): Acute Toxicity to Waterfleas (Daphnia magna) Under Static Exposure (MRID 462460-10)

The 48-hour acute toxicity of JAU 6476 480 SC (Prothioconazole Formulation) to the water flea, Daphnia magna, was studied under static renewal conditions (MRID 462460-10). Daphnids were exposed to the test material mean-measured concentrations of <0.03 (<LOO, controls), 0.14, 0.34, 0.81, 2.20, and 5.47 pprn a.i. After 48-hours of exposure, mortality was 0% in the negative and formulation controls and mean-measured 0.34, 0.81, and 2.20 ppm a.i. treatment groups; and 10 and 80% in the 0.14 and 5.47 ppm a.i. treatment groups, respectively. The 10% mortality observed in the 0.14 ppm a.i. treatment group was not considered to be treatment related by the study authors or the reviewer. The 48 hour LC₅₀ (with 95% C.I.) was 4.1 (2.2-5.47) ppm a.i., which categorizes JAU 6476 480 SC (Prothioconazole Formulation) as moderately toxic to the water flea (Daphnia magna) on an acute toxicity basis. Surviving daphnids from the 2.20 (45%) and 5.47 (80%) pprn a.i. treatment groups were reported to be lying on the bottom test vessels and /or lethargic. The NOAEC and LOAEC values based on sub-lethal effects were 0.81 and 2.20 pprn a.i., respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as ACCEPTABLE.

SXX 0665 (prothioconazole-desthio): Acute Toxicity to Waterfleas (Daphnia magna) Under Static Exposure (MRID 462460-11)

The 48-hour acute toxicity of SXX0665 Technical (JAU6476-desthio) to the water flea, Daphnia magna, was studied under static conditions (MRID 462460-11). Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 32 ppm a.i. Due to the solubility of the chemical, higher concentrations could not be tested. Measured zero-hour concentrations were 0.96, 1.8, 3.2, 5.5, 10, and 25 pprn a.i. The nominal 3.2 pprn a.i. test solutions was the only treatment concentration to be analytically verified at 48-hours (test termination) and had a 100% of nominal recovery. After 48-hours of exposure, mortality was 0% in the negative control and nominal 3.2, and 5.6 ppm a.i. treatment groups; and 3, 3, 7, and 27% in the nominal 1.0, 1.8, 10, and 32 ppm a.i. treatment groups, respectively. Surviving daphnids from the 10 and 32 ppm a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. The EC_{50} is > 5.5 ppm based on mortality and sub-lethal effects. This study is not scientifically sound and does not fulfill U.S. EPA guideline \$72-2a because not all treatment levels were analytically verified at test termination. However, measurement of the day-4 3.2 ppm nominal treatment level showed 100% recovery. The NOAEC is based on mortality and observations of sublethal effects. This study is classified as SUPPLEMENTAL.

JAU 6476-S-methyl (prothioconazole-S-methyl): Acute Toxicity to Waterfieas (Daphnia magna) Under Static Exposure (MRID 462460-12)

The 48-hour acute toxicity of the metabolite JAU6476-S-Methyl (Prothioconazole) to the water flea, Daphnia magna, was studied under static conditions (MRID 462460-12). Daphnids were exposed to the test material at mean-measured concentrations of <0.0504 (<LOQ, controls), 0.7, 1 .l, 2.3, 3.9, and 7.0 ppm a.i. After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 0.7 ppm a.i. treatment group; and $3, 3, 10, 90$, and 100% in the solvent control, 1.1, 2.3, 3.9 and 7.0 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.:) was 2.7 (2.4-3.2) ppm a.i., which categorizes the JAU6476-S-Methyl (Prothioconazole) as moderately toxic to the water flea (Daphnia magna) on an acute toxicity basis. All surviving daphnids from the negative and solvent controls and mean-measured 0.7 ppm a.i. treatment level were reported to be normal. Surviving daphnids from the1.1, 2.3, and 3.9 pprn a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified in the study report. The NOAEC and LOAEC values based on the reported sub-lethal effects were 0.7 and 1.1 ppm a.i., respectively. This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (\$72-2a). This study is classified as ACCEPTABLE.

SXX0665 techn. (prothioconazole-desthio): Acute toxicity to crayfish, Procambarus clarkia, under static renewal conditions (MRID 462460-13)

The 96-hour acute toxicity of JAU6476-Desthio to the Crayfish, *Procambarus clarkii*, was studied under static-renewal conditions renewed at 48 hours (MRID 462460-13). Crayfish were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 1.6, 3.1,6.3, 13, and 25 pprn a.i. Mean-measured concentrations were <0.61-0.68 (LOQ, negative and solvent controls), 1.9, 3.3, 6.8, 13, and 26 pprn a.i. This study is not scientifically sound due to excessive mortality in controls and issues with molting and cannibalization. As a consequence, this study is classified as INVALID. In addition, the test species, Crayfish (Procambarus clarkii), is not a US EPA-recommended species for an acute toxicity test with freshwater invertebrates (\S 72-2).

Species	Study Type	% active ingredient	EC ₅₀ /LC ₅₀ mp/L (95% C.I.)	MRID No.	Toxicity Category	Fulfills Guideline Requirement
Daphnia magna	Acute	JAU 6476 98.4	1.20 $(1.09-1.32)$	462460-09	Moderately Toxic	Yes
Daphnia magna	Acute	JAU 6476 480 SC 41.4	4.1 $(2.2 - 5.47)$	462460-10	Moderately Toxic	Yes
Daphnia magna	Acute	SXX 0665 93.7	EC_{50} > 5.5	462460-11	Moderately Toxic	Yes
Daphnia magna	Acute	JAU 6476 S-methyl 98.6	2.7 $(2.4-3.2)$	462460-12	Moderately Toxic	Yes
Procambarus clakii	Acute	SXX 0665 98.5	N/A	462460-13	N/A	N _o

Table F12. Summary of freshwater invertebrate acute toxicity studies

Freshwater Invertebrates, Chronic

Jau6476 techn.: Chronic Toxicity to Water Fleas (Daphnia magna) (MRID 462460-28)

A 21 -day chronic toxicity study in Daphnia magna was conducted using 98.8% pure prothioconozole in a static-renewal design (MRID 462460-28). Ten daphnids per treatment were exposed to nominal concentrations of 0 (negative and solvent controls), 0.56 , 1.0 , 1.8 , 3.2 , 5.6 , 10.0 and 18.0 ppm prothioconozole. Eight media renewals were performed. Newly prepared media was sampled from all treatment levels a day 0 , 9 and 19 while 72-hour old media was sampled from the control and $0,56, 1.8$ and 5.6 ppm on day 2, 12 and 21. Not all 72-hour old solutions were analyzed which deviates from EPA guidelines, however, all measurements were near nominal suggesting that solutions were prepared carefully and that the compound was stable in solution. Mortality after 21 days was 0% in both controls, 10% at 1.0 ppm, 60% at 1.8 ppm and 100% at 3.2 ppm and above. The LC_{50} and associated 95% confidence interval was 1.59 (1.25-2.02) and the NOEC for mortality was 1 .O ppm. Length of surviving daphnids was significantly affected by prothioconozole. Average length of surviving daphnids was 4.13, 4.06, 3.99, 3.81, and 3.70 **mm** for the negative control, solvent control, 0.56, 1 .O, and 1.8 pprn treatment groups; the corresponding NOEC was 0.56 ppm.

No offspring were produced at levels above 3.2 ppm. The sum of offspring per parent averaged 73.8, 80.3, 67.2, 64.3, and 51.3 for the negative control, solvent control, 0.56, 1.0, 1.8, and 3.2 pprn treatment groups, respectively. The number of offspring per parent and reproduction day averaged 5.84, 6.70, 5.62, 4.67, 3.99, and 0.96 for the negative control, solvent control, 0.56, 1 .O, 1.8, and 3.2 pprn treatment groups, respectively. The mean first day of reproduction was 9.3, 10.0, 10.1, 9.6,9.9, and 11.0 for the negative control, solvent control, nominal 0.56, 1.0, 1.8, and 3.2 treatment groups, respectively. For all three endpoints, differences from control were significant at concentration *2* 1.0 pprn with a corresponding NOEC of 0.56 ppm.

The study is scientifically sound and fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna* (\$72-4b). Although not all "old" treatment solutions were analytically verified in this static-renewal design during the exposure period, analyzed treatment solutions indicated compound stability. Furthermore, the solvent was neither identified or quantified in the test media although there were no statistical effects of the solvent. Consequently, this study is classified as ACCEPTABLE.

SXX 0665 (prothioconazole-desthio): Chronic Toxicity to Water Fleas *(Daphnia magna)* **(MRID 462460-29)**

The 21-day-chronic toxicity of SXX 0665 (JAU 6476 – Desthio: 96.5% Prothioconazole - desthio) to *Daphnia magna* was studied under static renewal conditions (MRID 462460-29). Mean-measured concentrations were 0 (controls), 0.025, 0.052, 0.103, 0.206, 0.408, and 0.830 ppm. Eight media renewals were performed. "New" test media was sampled from all test levels at 0, 9, and 19 Days, and "old" test media (after 48 or 72 hours) was sampled from the negative control, 0.025 , 0.103 , and 0.830 ppm levels on Days 2, 12, and 21. Recoveries for all samples ranged from 96 to 109% of nominal concentrations indicating precision and stability. Since "old" treatment solutions were not sampled for all toxicant levels and recoveries were high for those "new and old" treatment solutions sampled, meanmeasured concentrations were based on all available data. After 21 days of exposure, mortality was #10% for all test and control groups. The 21-day LC_{50} was >0.830 ppm, and the NOAEC for mortality was 0.830 ppm.

The mean first day of reproduction (time to first brood release) was 9.9, 10.0, 9.6, 9.8, 10.1, 10.3, 10.4, and 11.4 for the negative control, solvent control, 0.025, 0.052, 0.103,0.206, 0.408, and 0.830 ppm test groups, respectively, and the NOAEC was 0.408 ppm. Offspring production was adversely affected by treatment at the $\exists 0.2$ ppm test levels. The sum of offspring per parent and the number of

offspring per parent per reproduction day averaged were significantly different from the solvent control at concentrations 30.206 ppm. The subsequent NOAEC was 0.103 ppm. A statistically-significant reduction compared to the solvent control in terminal lengths was not observed.

This study fulfills the guideline requirements for an aquatic invertebrate life-cycle study using *Daphnia* magna (§72-4b). This study is classified as ACCEPTABLE.

Species	Study Type	% active ingredient	NOAEC mg/L (95% C.I.)	MRID No.	Endpoint	Fulfills Guideline Requirement
Daphnia magna	Chronic. life-cycle	JAU 6476 98.4	0.56	462460-28	Reproductive effects, adult length	Yes
Daphnia magna	Chronic, life-cycle	SXX 0665 96.5	0.103	462460-29	Reproductive Effects	Yes

Table F13. Summary of freshwater invertebrate chronic toxicity studies

Toxicity to Estuarine and Marine Animals

Estuarine/Marine Fish, Acute

JAU 6476 techn.: Acute toxicity (96 hours) to Sheepshead Minnow (Cyprinidon variegatus) in a Static Test (MRID 462460-27)

The 96-hour acute toxicity of JAU 6476 Technical (Prothioconazole) to Sheepshead minnow (Cyprinodon variegatus) was studied under static-renewal conditions (MRID 462460-27). Fish were exposed at meanmeasured concentrations of <0.075 (LOQ, controls), 0.69, 1.34, 2.51, 5.42, and 10.30 ppm a.i. This study was performed at the practical limit of solubility (0.3 g/L in distilled water at 20EC and approximately pH 8.0; practical limit in saltwater approximately 12 mg a.i./L) and no undissolved test material was observed in any of the test vessels. After 96 hours of exposure, there were no mortalities or sub-lethal effects in the controls or treatment groups. The 96-hour LC_{50} was estimated as >10.3 ppm a.i. and the NOAEC and LOAEC values were 10.3 and >10.3 ppm a.i., respectively. Based on the results of this study (LC₅₀: >10.3 pprn a.i.), JAU 6476 Technical (Prothioconazole) is classified as slightly toxic to the Sheepshead Minnow (Cyprinodon variegatus) on an acute toxicity basis. This study is scientifically sound, and satisfies the guideline requirements for an acute toxicity study with an estuarine/marine fish [§72-3a]. This study is classified ACCEPTABLE.

EstuarineMarine Invertebrates, Acute

JAU 6476 techn.: A 96-Hour Shell Deposition Test with the Eastern Oyster (Crassostrea viginica) (MRID 462460-14)

The Eastern Oyster (Crassostrea virginica) was exposed to JAU 6476 Technical (Prothioconazole) at mean measured concentrations of <0.100 (<LOD, controls), 0.37, 0.76, 1.4, 2,85, and 5.4 ppm a.i under flow-through conditions to establish the 96-hour acute toxicity (MRID 462460-14). At 96 hours, there were no mortalities but shell deposition was reduced 10,22,29,47, and 98% in the 0.37,0.76, 1.4,2,85, and 5,4 pprn a.i. treatment groups, respectively when compared to pooled controls. The NOEC and LOEC for reduced shell deposition were 0.37 and 0.76 ppm a.i., respectively; the corresponding EC_{50} was 3.0 (C.I. 2.6-3.5) pprn a.i. This study is classified as CORE and satisfies the requirements for an acute toxicity study with estuarine/marine mollusks (72-3b).

JAU 6476 techn.: A 96-Hour Flow Through Acute Toxicity Test with the Saltwater Mysid (Americamysis bahia, formerly Mysidopsis bahia) (MRID 462460-16)

Saltwater mysid shrimp, Americamysis bahia (formerly Mysidopsis bahia), were exposed to JAU 6476 Technical (prothioconazole) for 96-hours under flow-through conditions (MRID 462460-16). Mysids were exposed to mean measured concentrations of prothioconazole of <0.100 (LOQ, negative and DMF controls), 0.25, 0.51, 0.99, 2.0, and 4.0 ppm a.i. Mortality at 96 hours was 5, 25, and 100% in the 0.25, 2.0, and 4.1 ppm a.i. treatments; there were no mortalities in the other treatments. Erratic swimming and lethargy were also observed in only the 0.25, 2.0 and 4.1 ppm a.i. treatments. The 96-hour LC50 was 2.4 (C.I. 2.0-4.1) ppm a.i., which categorizes JAU 6476 Technical as moderately toxic to the saltwater mysid on an acute toxicity basis. The NOEC and LOEC for mortality and sub-lethal effects were 0.99 and 2.0 pprn a.i., respectively. This study is classified as CORE and satisfies the requirements for an acute toxicity test with an estuarine/marine invertebrate, guideline 72-3c.

SXX0665 (prothioconazole-desthio): A 96-Hour Flow Through Acute Toxicity Test with the Saltwater Mysid (Americamysis bahia, formerly Mysidopsis bahia) (MRID: 462460-17)

The 96-hour acute toxicity of JAU 6476-Desthio to the saltwater mysid, Americamysis bahia, was studied under flow-through conditions (MRID 462460-17). Mysids were exposed to the test material at meanmeasured concentrations of ≤ 0.0025 (LOQ; controls), 0.013, 0.026, 0.050, 0.099, and 0.20 ppm a.i. After 96 hours, mortality was 10, 5, 15, 95, and 100% in the 0.013, 0.026, 0.050, 0.099, and 0.20 ppm a.i. treatment levels, respectively. No mortalities were observed in the controls. Erratic swimming and/or lethargy were observed in surviving mysids from the solvent control (1 mysid; only at 72-hours), and the 0.013 (1 mysid; 24-48 hours), 0.050 (1-3 mysids; 24-72 hours), 0.099, and 0.20 pprn a.i. treatment levels during the test. No sub-lethal effects were observed in the negative control and 0.026 ppm a.i. treatment level. The **96-hour LC₅₀ value (with 95% C.I.) was 0.060 (0.046-0.079) ppm a.i.,** which categorizes JAU 6476-Desthio (Prothioconazole) as **very highly toxic** to the saltwater mysid, Americamysis bahia, on an acute toxicity basis. Based on mortality and sub-lethal effects, the **NOAEC and LOAEC values were 0.026 and 0.050 pprn a.i.,** respectively. This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, $\S72-3(c)$ [mysid] shrimp]). This study is classified as **ACCEPTABLE.**

Estuarine/Marine Invertebrates, Chronic

SXX0665 (prothioconazole-desthio): A Flow Through Life-cycle Toxicity Test with the Saltwater Mysid (Americamysis bahia, formerly Mysidopsis bahia) (MRID: 462460-30)

In a 29-day life cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to JAU 6476 - Desthio (prothioconazole - desthio) at nominal concentrations of 0 (negative and solvent controls), 16, 32, 63, 125, and 250 ppb (MRID 462460-30). Mean-measured concentrations were ≤ 10 (<LOQ, controls), 16,32,64, 128, and 252 ppb a.i., respectively. Prior to sexual maturity and pairing, there were 60 mysids/level. At Day 14, up to 20 pair/level were isolated for individual matings. Firstgeneration mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Data endpoints included percent survival of first-generation mysids on Days 14 (at pairing) and 29 (study termination; combined sexes), the number of offspring per reproductive day, and total length and dry weight of surviving first-generation mysids (Day 29; combined sexes). No treatment-related effect on survival was observed, either prior to or after pairing for reproduction. The day of first brood release was Day 17, and the median time of the first brood release for the negative and solvent controls was Day 22. There were no significant effects on young produced per reproductive day. The mean number of young produced per reproductive day averaged 0.592 and 0.573 for the negative and solvent control groups, respectively, and 0.527, 0.610, 0.615, 0.398, and 0.407 for the 16, 32, 64, 128, and 252 ppb a.i. test levels, respectively. Consequently, the **NOAEC** for reproduction was **64 ppb a.i.** since there was an apparent 30% reduction in the number of offspring produced per reproductive day at **128 ppb a.i,** the **LOAEC.** Although not statistically significant, a reproductive effect of this magnitude is likely to be biologically significant. No treatment-related effects on growth were observed.

This study fulfills the guideline requirements for an aquatic invertebrate life-cycle toxicity test using *Americamysis bahia* (\$72-4c), and is classified as ACCEPTABLE.

Species	Study Type	% active ingredient	NOAEC/LOAEC $m\omega L$	MRID No.	Toxicity Endpoints	Fulfills Guideline Requirement
Americamysis bahia	Life cycle	SXX0665 96.5	0.064	462460-30	Number young/reproductive aav	Yes

Table F16. Summary of estuarine/marine invertebrate chronic toxicity studies

Toxicity to Aquatic Plants

JAU6476 techn.: Toxicity to Duckweed (Lemna gibba G3) Under Static-Renewal Conditions (MRID 462461-01)

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lernna gibba* G3, were exposed to Prothioconazole at nominal concentrations of 0 (negative and solvent controls), 0.97, 3.24, 10.8, 36.0, 120, and 400 ppb a.i. under static renewal conditions (MRID 462461-01). The measured concentrations were #0.5 (<LOQ, negative and solvent controls), 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i.. Frond number was the most sensitive endpoint tested; the percent inhibitions for mean frond numbers were O,0, 10, 39,64, and 71% in the 1.01, 3.34, 10.4, 35.1, 106.4, and 404.0 ppb a.i. treatment groups, respectively, compared to the pooled control. The EC_{50} for frond number was 73 ppb a.i.; the NOAEC and EC_{05} were 3.34 and 1.6 ppb a.i.. This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, \$123-2 for an aquatic vascular plant study with *Lemna gibba.* As *a* result, this study is classified as ACCEPTABLE.
JAU 6476 480 SC (formulation): Toxicity to Duckweed (Lemna gibba G3) Under Static-Renewal Conditions (MRID 462461-02)

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to the formulation JAU 6476 480 SC (42.1 % Prothioconazole) at nominal concentrations of 0 (negative and formulation blank controls), 2.0, 6.7, 22.2, 74.1, 247, and 823 ppb a.i. under static renewal conditions (MRID 462461-02). The measured concentrations were $\#0.98$ (<LOD, negative and formulation blank controls), 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i.. The percent inhibitions for mean live frond numbers were -5, 1, 19, 62, 60, and 67% in the 2.3, 7.5, 28.8, 88.6, 289, and 963 ppb a.i. treatment groups, respectively, compared to the formulation blank control. The NOAEC for dry weight could not be determined (i.e., \leq 2.3 ppb a.i.); the NOAEC for all other endpoints was 7.5 ppb a.i.. Frond number was the most sensitive endpoint tested with an EC_{50} of 110 ppb a.i.. This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, \$123-2 for an aquatic vascular plant study with *Lemna gibba.* As a result, this study is classified as Acceptable.

SXX0665 (prothioconazole-desthio: Toxicity to Duckweed (Lemna gibba G3) Under Static-Renewal Conditions (MRID 46241-04)

In a 7-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to prothioconazole metabolite, JAU 6476-desthio, at nominal concentrations of 0 (negative and solvent controls), 2.56, 6.4, 16.0,40.0, and 100 ppb under static renewal conditions (MRID 462461-04). The measured concentrations were #0.5 (<LOQ, negative and solvent controls), 2.42, 5.78, 14.3, 35.6, and 89.77 ppb. The percent inhibitions for mean frond numbers were -4,0, 14, 52, and 73% in the 2.42, 5.78. 14.30,65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for dry weights were 4, 7,26, 57, and 61% in the 2.42, 5.78, 14.30,65.60, and 89.77 ppb treatment groups, respectively, compared to the pooled control. The percent inhibitions for growth rates were -2, 0, 6, 30, and 53% in the 2.42, 5.78, 14.30, 65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The percent inhibitions for areas under the growth curve were -9, -3, 9,41, and 62% in the 2.42,5.78, 14.30,65.60, and 89.77 ppb treatment groups, respectively, compared to the solvent control. The NOAEC for all endpoints was 5.8 ppb. Frond number was the most sensitive endpoint tested, with an EC_{50} of 35 ppb. This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, \$123-2 for an aquatic vascular plant study with *Lemna gibba.* As a result, this study is classified as Acceptable.

JAU6476 techn.: Influence on the Growth of the Green Alga, Selenastrum capricornutum (MRID 462461-05)

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0.098, 0.197, 0.393, 0.786, 1.57, and 3.15 ppm a.i.(MRID 462461-05). The 0-hour measured concentrations were 0.086, 0.182, 0.371, 0.747, 1.52, and 3.03 ppm a.i.. The 96-hour cell density percent inhibitions were -24.80, -18.09, -3.78, 35.03, 81.21, and 97.02% in the 0.086, 0.182, 0.371,0.747, I .52, and 3.03 ppma.i. treatment groups, respectively. The NOAEC was 0.371 ppm a.i. for cell density, area under the growth curve, and growth rate. Cell density was the most sensitive endpoint tested; the 96-hour EC_{50} was 0.88 ppm a.i.. The study is scientifically sound and satisfies the U.S. EPA Guideline \$123-2 for an aquatic nonvascular plant study with *Selenastrum capricornutum.* This study is classified as ACCEPTABLE.

JAU6476 techn.: Toxicity to the Blue-green Alga Anabaena flos-aquae (MRID 462461-03)

In a 96-hour acute toxicity study, cultures of *Anabaena Jlos-aquae* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0 (negative and solvent controls), 0.02, 0.08, 0.27, 0.90, 3.0, and 10.0 ppm a.i. (MRID 462461-03). The 0-hour measured concentrations were c0.5 (< LOQ, controls), 0.02, 0.08,0.22,0.82,2.97, and 9.12 pprn a.i.; the 0-hour measured concentrations were used for toxicity estimates. The 96-hour cell density percent inhibitions were -6.2, - 18.5, -17.3, 5.8, 35.8,and91.4%inthe0.02, 0.08,0.22, 0.82, 2.97, and9.12ppma.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 9.1, 10.1, -21.3, 17.5, 41.5, and 83.2% in the 0.02,0.08, 0.22, 0.82,2.97, and 9.12 pprn a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were -1.6, -3.9, -3.6, 1.1, 10.0, and 60.2% in the 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 ppm a.i. treatment groups, respectively. The NOAEC was 0.82 ppm a.i. for cell density and the NOAEC for growth rate and biomass was 2.97 pprn a.i.. Cell density and biomass (area under the growth curve) both had an EC_{50} of 3.5 ppm a.i.. The study is scientifically sound and satisfies the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Anabaena flosaquae.* This study is classified as ACCEPTABLE.

JAU6476 480 SC (formulation): Toxicity to the Green Alga Pseudokirchneriella subcapitata (aka Selenastrum capricornutum) (MRID 462461-06)

In a 96-hour acute toxicity study, cultures of *Pseudokirchneriella subcapitata* were exposed to Prothioconazole formulation (JAU 6476 480 SC) under static conditions at nominal concentrations of 0 (negative and formulation blank controls), 0.063, 0.125, 0.25, 0.5, and 1.0 and 2.0 pprn a.i. (MRID 462461-06). The 0-hour measured concentrations were <0.021 (< LOQ, controls), 0.053,0.111,0.24, 0.48, 0.92, and 1.97 pprn a.i.; the 0-hour measured concentrations were used for toxicity estimates. The NOAEC for cell density, biomass (area under the growth curve), and growth rate was 0.48 ppm a.i. Biomass (area under the growth curve) was the most sensitive endpoint tested with an EC_{50} of 0.92 ppm a.i. The area under the growth curve (0 to 96 hours) percent inhibitions were 0,2, 16, 10, 52, and 95% in the 0.053 , 0.11 , 0.24 , 0.48 , 0.92 , and 1.97 ppm a.i. treatment groups, respectively. The study is scientifically sound and satisfies the U.S. EPA Guideline \$123-2 for an aquatic nonvascular plant study with *Pseudokirchneriella subcapitata.* This study is classified as ACCEPTABLE.

JAU6476-S-Methyl (prothioconazole-S-methyl): Influence on the Growth of the Green Alga, Selenastrum capricornutum (MRID 462461-07)

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Prothioconazole metabolite (JAU 6476-S-Methyl) under static conditions at nominal concentrations of 1.54, 3.09,6.16, 12.3,24.7,49.3, and 98.6 mg a.s./L (MRId 46246 1-07). The 0-hour measured concentrations were 1.03, 1.60, 2.84,4.91, 8.74, 15.4, and 55.5 mg a.s./L; the 0-hour measured concentrations were <70% of nominal for all test concentrations and the poor recovery of the test material was not discussed. The NOAEC was \leq 1.03 mg a.s./L for cell density, biomass (area under the growth curve), and growth rate. Cell density was the most sensitive endpoint tested (and the only endpoint for which replicate data were provided), based on an EC_{50} of 2.8 mg a.s./L. The 96-hour cell density percent inhibitions were 18.91, 31.09, 52.01, 70.80, 79.23, 81.09, and 87.56% in the 1.03, 1.60, 2.84,4.91, 8.74, 15.4, and 55.5 mg a.s./L treatment groups, respectively. The study is scientifically sound, but it does not satisfy the U.S. EPA Guideline \$ 123-2 for an aquatic nonvascular plant study with *Selenastrum capricornutum* because the analytical recovery of the test material at test initiation was less than 70% of nominal for all test levels and this issue was not addressed in the study report. As a result, this study is classified as Supplemental. Results from this study may be useful for future risk assessments.

SXX 0665 techn. (prothioconazole-desthio): Growth Inhibition of Green Algae (Scenedesmus subspicatus) (MRID 462461-08)

In a 96-hour acute toxicity study, cultures of *Scenedesmus subspicatus* were exposed to JAU6476-desthio (SXX 0665 Technical) under static conditions at nominal concentrations of 0 (negative control), 0.0094, 0.030, 0.052, 0.094, 0.17, 0.30, 0.52, 0.94, 1.7, and 3.0 ppm a.i. (corresponding to 0.01, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L) (MRID 462461-08). The 0-hour measured concentrations were not detected (control), 0.01 1, 0.030, 0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 pprn a.i. Cell density was the most sensitive endpoint tested, based on an EC_{50} of 0.074 ppm a.i.; the NOAEC was ≤ 0.011 ppm a.i. and the EC₀₅ was 0.011 ppm a.i. The 96-hour cell density percent inhibitions were 15, 8, 13, 52, 82, 97, 96,98, 97, and 98% in the 0.01 1, 0.030,0.050, 0.085, 0.17, 0.28, 0.48, 0.87, 1.6, and 2.9 pprn a.i. treatment groups, respectively. The study is scientifically sound and satisfies the U.S. EPA Guideline 123-2 for an aquatic nonvascular plant study with *Scenedesmus subspicatus.* This study is classified as ACCEPTABLE.

Toxicity to Estuarine and Marine Plants

JAU6476 techn.: Toxicity to the Saltwater Algae, Navicula pelliculosa **(MRID 462461-09)**

In a 96-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0 (negative and solvent controls), 26.0, 64.0, 160.0,400.0, and 1000.0 ppb a.i. (MRID 462461-09). The 0-hour measured concentrations were <2.6 (< LOQ, controls), 23.5, 56.6, 146.3,356.4, and 889.5 ppb a.i.; the 0-hour measured concentrations were used for toxicity estimates. The NOAEC was <23.5 ppb a.i., the lowest test concentration, for cell density and biomass. Biomass (area under the growth curve) was the most sensitive endpoint tested, with an EC_{50} of 180 ppb a.i. The biomass (area under the growth curve) (0 to 96 hours) percent inhibitions were 16, 17,43, 83, and 101% in the 23.5,56.6, 146.3,356.4, and 889.5 ppb a.i. treatment groups, respectively. The study is scientifically sound and satisfies the U.S. EPA Guideline 5123-2 for an aquatic nonvascular plant study with *Navicula pelliculosa.* This study is classified as Acceptable.

JAU6476 techn.: Toxicity to the Saltwater Diatom, *Skeletonema costatum* **(MRID 462461-09)**

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Prothioconazole $(JAU 6476 Technical)$ under static conditions at nominal concentrations of 0 (negative and solvent controls), 3.10, 7.70, 19.2, 48.0, and 120 μ g/L (MRId 462461-09). The 0-hour measured concentrations were ≤ 0.5 (\leq LOQ, controls), 3.00, 7.30, 17.5, 46.8, and 117 ppb a.i. Biomass (area under the growth curve) was the most sensitive endpoint tested, based on an EC_{50} of 21 ppb a.i.; the NOAEC was 7.3 ppb a.i. The biomass (area under the growth curve) (0 to 96 hours) percent inhibitions were 4, 1,40, 92, and 100% in the 3.0, 7.3, 17.5, 46.8, and 1 17.0 ppb a.i. treatment groups, respectively. The study is scientifically sound and satisfies the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Skeletonema costatum.* This study is classified as Acceptable.

Species	Study Type	% active ingredient	EC_{50} μ g/L	NOAEC/EC _{ts} μ g/L	MRID No. Author Year	Most sensitive endpoint	Fulfills Guideline Requirement
Navicula pelliculosa	JAU 6476	97.5	180	$<$ 23.5/60	462461-09 Ker and Lam. 2004	Biomass	Yes Acceptable
Skeletonema Costatum	JAU 6476	98.2	21	7.3/7.7	462461-10 Kern and DeHaan, 2004	Biomass	Yes Acceptable

Table F18. Summary of saltwater aquatic plant toxicity tests.

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APPENDIX G: Additional Calculations (4 sections)

G.1. Example calculation for EECs associated with use on rice

Estimating prothioconazole water concentration in flooded rice paddy: Maximum seasonal application of prothioconazole to rice paddies = 0.286 lbs ai/A

 $\text{EEC} = 10^6 * M_T / (V_T + m_{\text{sed}} * K_d)$

 M_T = total mass of pesticide in kg/ha (0.321 kg/ha from 0.286 lbs ai/A) V_T = water volume assuming 4 inches deep = 1.067 x 10⁶ L ha⁻¹ K_d prothioconazole = 10.46 $m_{\text{sed}} = \text{mass}$ sediment in top 1 cm = 130000 kg ha⁻¹ 10^6 – conversion from kg to mg fro estimate of ppm

EEC = $10^6 * 0.286$ kg/ha / (1.067 x 106 + 130000 kg ha⁻¹ * 10.46) = 132 ppm

APPENDIX G Continued:

G.2. Estimating foliar dissipation half-life from magnitude of residue studies

Requirements:

 $\bar{\lambda}$

1 time zero sample

2 at least 3 samples at 3 different times

3 HED approved

4 whole plant or leaves

WHEAT FORAGE - **Detailed**

Slopes significantly different from zero.

Requirements fulfilled for use in foliar dissipation half-life calculation?

Parent, 61 18 SUMMARY OUTPUT

ANOVA

APPENDIX G Continued: G.3. Benchmark Dose Calculation

Polynomial Model. Revision: 2.2 Date: 9/12/2002 Input Data File: C:\BMDS\UNSAVEDl .(d) Gnuplot Plotting File: C:\BMDS\UNSAVED 1 .plt Wed May 10 08:49:04 2006

.. --------_-----__-__---

BMDS MODEL RUN

The form of the response function is:

--N----NNNNN--N-N-N------------------------------------,-----------

 $Y[dose] = beta_0 + beta_1 * dose + beta_2 * dose^2 + ...$

Dependent variable = MEAN Independent variable = COLUMN1 rho is set to 0 Signs of the polynomial coefficients are not restricted A constant variance model is fit

Total number of dose groups = 4 Total number of records with missing values $= 0$ Maximum number of iterations = 250 Relative Function Convergence has been set to: 1 e-008 Parameter Convergence has been set to: 1 e-008

> Default Initial Parameter Values alpha = 314.649 $rho = 0$ Specified beta $0 = 98.0949$ $beta_1 = 0.381018$ beta $2 = -0.029586$

> > Parameter Estimates

95 .O% Wald Confidence Interval

Asymptotic Correlation Matrix of Parameter Estimates

Table of Data and Estimated Values of Interest

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$ $Var{e(ij)} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$ $Var{e(ij)} = Sigma(i)^2$

Model R: $\text{Yi} = \text{Mu} + \text{e(i)}$ $Var{e(i)} = Sigma^2$

Likelihoods of Interest

Test 1: Does response and/or variances differ among dose levels

(A2 vs. R)

Test 2: Are Variances Homogeneous (A1 vs A2)

 $\mathcal{A}^{\mathcal{A}}$

Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test -2*log(Likelihood Ratio) Test df p-value

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is less than .05. Consider running a non-homogeneous variance model

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation Specified effect = $\qquad \qquad 0.1$

Risk Type $=$ Relative risk

Confidence level $=$ 0.95

 $BMD = 25.7505$

 $BMDL = 12.4238$

156

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APPENDIX G Continued: G.4. Analysis of Prothiconazole BCF

Calculations for determining Risk Quotients based on bioconcentration of prothioconazole in fish.

The maximum BCF, for all residues, as determined in a prothioconazole-desthio bioconcentration study is 94.3. An analysis was conducted to evaluate whether piscivorous birds and mammals may be at risk if they consume fish from waters contaminated with prothioconazole-desthio. The basic approach involved determining prothioconazole-desthio levels in fish by applying the BCF to aquatic EECs. Avian and mammalian food ingestion rate was determined using allometric equations found in U.S. EPA (1993, Exposure Factors Handbook). A sample calculation for mammals is provided below.

 $BCF = 94.3$ Concentration of total toxic residues = 33.32 ppb = 0.033 ppm (mg/l) (NC peanuts)

Conc. of prothioconazole+degradates in fish (mg/kg) = $94.3 * 0.033 = 3.11$ mg/kg

Mammalian Food Ingestion Rate (FIR; g dry weight/d) = $0.235 * BW^0.822$ $BW = body weight(g)$, assume 35 g for this exercise Assume fish are comprised of 75% water

FIR (100 g bird) = $0.235 * (35)^0.822 = 4.37g$ dry In wet weight 4.37 g is equivalent to $4.37 / (1 - 0.75) = 17.48$ g

Total **prothioconazole+degradates** consumed = BCF * prothio in water * FIR

 $= 3.11$ mg/kg * (17.48 g /1000g/kg) = 0.054 mg prothio consumed

Dose (mg/kg bw) = 0.054 mg / $(35$ g * 1 kg/1000 g) = 1.43 mg/kg bw

Chronic RQ = 1.43 mg/kg $/ 9.5$ mg/kg = 0.15

For birds the calculation is simpler since the NOAEC is dietary based. For prothioconazole this entails comparing the levels in feed (3.11 mg/kg) to the toxicity value 449 mg /kg feed.

 $RQ = 3.11$ mg/kg food / 449 mg/kg food = <0.01

APPENDIX H: Federally Listed Species Associated with Prothioconazole Use Areas

Species Listing by State

barley for grain, canola, flaxseed, mustard seed, rapeseed (see text), rice, crambe, wheat for grain, all, dry edible peas, dry lima beans, dry southern peas (cowpeas), lentils, peanuts for nuts, green lima beans, dry edible beans, excluding limas, guar, guar (irrigated), dry edible beans, excluding limas (irrigated), dry edible peas (irrigated), dry lima beans (irrigated), dry southern peas (cowpeas) (irrigated), lentils (irrigated), mungbeans for beans, peas, green southern (cowpeas) - *blackeyed, crowder, etc. (see text), peas, green (excluding southern)*

No species were excluded Minimum of 1 Acre.

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 $\frac{1}{2}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\sim 10^7$

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2\alpha} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{\alpha} \frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}$

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 $\mathcal{L}(\mathcal{A})$ and $\mathcal{L}(\mathcal{A})$

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 $\sim 10^6$

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 $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}})) \leq \mathcal{L}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}}))$

 $\mathcal{L}^{\text{max}}_{\text{max}}$, $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$, where $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$

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 $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$, where $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\label{eq:2.1} \frac{1}{2} \int_{\mathbb{R}^3} \left| \frac{d\mu}{d\mu} \right|^2 \, d\mu \, d\mu$

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 $\sim 10^{11}$ km s $^{-1}$

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 $\mathcal{L}^{\text{max}}_{\text{max}}$, $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\sim 10^{-1}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\frac{1}{2} \left(\frac{1}{2} \right)$

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 $\sim 10^{-1}$

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 $\label{eq:2} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2}d\mu_{\rm{eff}}\,d\mu_{\rm{eff}}$

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 $\mathcal{L}^{\text{max}}_{\text{max}}$, $\mathcal{L}^{\text{max}}_{\text{max}}$

Fleabane, Zuni (Erigeron rhizomatus)	Threatened	Dicot	No
Ipomopsis, Holy Ghost (Ipomopsis sancti-spiritus)	Endangered	Dicot	No
Milk-vetch, Mancos (Astragalus humillimus)	Endangered	Dicot	No
Pennyroyal, Todsen's (Hedeoma todsenii)	Endangered	Dicot	Yes
Sunflower, Pecos (Helianthus paradoxus)	Threatened	Dicot	No.
Wild-buckwheat, Gypsum (Eriogonum gypsophilum)	Threatened	Dicot	Yes
Bat, Lesser (=Sanborn's) Long-nosed (Leptonycteris curasoae yerbabuenae)	Endangered	Mammal	No
Bat, Mexican Long-nosed (Leptonycteris nivalis)	Endangered	Mammal	No
Ferret, Black-footed (Mustela nigripes)	Endangered	Mammal	No
Jaguar (Panthera onca)	Endangered	Mammal	No
Wolf, Gray (Canis lupus)	Endangered	Mammal	Yes
New York (7) species affected		Taxa	Critical Habitat
Amaranth, Seabeach (Amaranthus pumilus)	Threatened	Dicot	No
Gerardia, Sandplain (Agalinis acuta)	Endangered	Dicot	No
Monkshood, Northern Wild (Aconitum noveboracense)	Threatened	Dicot	No
Roseroot, Leedy's (Sedum integrifolium ssp. leedyi)	Threatened	Dicot	No
Bat, Indiana (Myotis sodalis)	Endangered	Mammal	Yes
Whale, northern right (Eubalaena glacialis (incl. australis))	Endangered	Mammal	Yes
Pogonia, Small Whorled (Isotria medeoloides)	Threatened	Monocot	No

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 $\mathcal{A}^{\text{max}}_{\text{max}}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\frac{1}{2} \sum_{i=1}^{n} \frac{1}{2} \sum_{j=1}^{n} \frac{1}{2} \sum_{j=1}^{n$

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 $\sim 10^{11}$ km s $^{-1}$

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 $\sim 10^{-1}$

 $\sim 10^{-1}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

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 $\sim 10^{-1}$

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 $\sim 10^{-10}$

 $\label{eq:2} \mathcal{L} = \mathcal{L} \left(\mathcal{L} \right) \left(\mathcal{L} \right) \left(\mathcal{L} \right)$

Sunflower, Pecos (Helianthus paradoxus)	Threatened	Dicot	No
Wild-buckwheat, Gypsum (Eriogonum gypsophilum)	Threatened	Dicot	Yes
Bat, Mexican Long-nosed (Leptonycteris nivalis)	Endangered	Mammal	No
Bear, Louisiana Black (Ursus americanus luteolus)	Threatened	Mammal	Yes
Jaquarundi, Gulf Coast (Herpailurus (=Felis) yagouaroundi cacomitli)	Endangered	Mammal	No
Jaguarundi, Sinaloan (Herpailurus (=Felis) yagouaroundi tolteca)	Endangered	Mammal	No
Ocelot	Endangered	Mammal	No
(Leopardus (=Felis) pardalis) Ladies'-tresses, Navasota (Spiranthes parksii)	Endangered	Monocot	No
Pondweed, Little Aguja Creek (Potamogeton clystocarpus)	Endangered	Monocot	No
Wild-rice, Texas	Endangered	Monocot	Yes
(Zizania texana)			
Utah (23) species affected		<u>Taxa</u>	Critical Habitat
Bear-poppy, Dwarf (Arctomecon humilis)	Endangered	Dicot	No
Cactus, San Rafael (Pediocactus despainii)	Endangered	Dicot	No
Cactus, Siler Pincushion (Pediocactus (=Echinocactus,=Utahia) sileri)	Threatened	Dicot	No
Cactus, Uinta Basin Hookless (Sclerocactus glaucus)	Threatened	Dicot	No
Cactus, Winkler (Pediocactus winkleri)	Threatened	Dicot	No
Cactus, Wright Fishhook (Sclerocactus wrightiae)	Endangered	Dicot	No
Cycladenia, Jones (Cycladenia jonesii (=humilis))	Threatened	Dicot	No

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Squirrel, Virginia Northern Flying (Glaucomys sabrinus fuscus)	Endangered	Mammal	No
Whale, northern right (Eubalaena glacialis (incl. australis))	Endangered	Mammal	Yes
Bulrush, Northeastern (=Barbed Bristle) (Scirpus ancistrochaetus)	Endangered	Monocot	No
Orchid, Eastern Prairie Fringed (Platanthera leucophaea)	Threatened	Monocot	No
Pink, Swamp (Helonias bullata)	Threatened	Monocot	No
Pogonia, Small Whorled (Isotria medeoloides)	Threatened	Monocot	No
(12) species affected Washington		<u>Taxa</u>	Critical Habitat
Catchfly, Spalding's (Silene spaldingii)	Threatened	Dicot	No
Checker-mallow, Nelson's (Sidalcea nelsoniana)	Threatened	Dicot	No
Checker-mallow, Wenatchee Mountains (Sidalcea oregana var. calva)	Endangered	Dicot	Yes
Howellia, Water (Howellia aquatilis)	Threatened	Dicot	No
Lupine, Kincaid's (Lupinus sulphureus (=oreganus) ssp. kincaidii (=var. kincaidii))	Threatened	Dicot	Yes
Paintbrush, Golden (Castilleja levisecta)	Threatened	Dicot	No
Stickseed, Showy (Hackelia venusta)	Endangered	Dicot	No
Bear, Grizzly (Ursus arctos horribilis)	Threatened	Mammal	No
Caribou, Woodland (Rangifer tarandus caribou)	Endangered	Mammal	No
Deer, Columbian White-tailed (Odocoileus virginianus leucurus)	Endangered	Mammal	No
Rabbit, Pygmy (Brachylagus idahoensis)	Endangered	Mammal	No
Wolf, Gray (Canis Iupus)	Endangered	Mammal	Yes

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 $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^2\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac$

No species were excluded.

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APPENDIX I. Data Requirements.

Table 1-1. Status of environmental fate data adequacy for prothioconazole.

Table 1-2. Status of ecological effects data adequacy for prothioconazole.

	Guideline	Description	MRID	Title	Substrate	Study Classification
$71-1$	850,2100	Avian acute oral	462460-36 462460-37	Acute oral toxicity study with the Bobwhite	TGAL SXX0665.	Acceptable Acceptable
$71 - 2$	OECD 205	Avian acute dietary	462460-38 462460-39	A dietary LC50 study with the Northern Bobwhite	TGAL. Desthio	Acceptable
$71-2$	OECD 205	Avian acute dietary	462460-40	A dietary LC50 study with the Mallard	TGAI.	Acceptable
$71-4a$	$- -$	Avian repro	462460-42 462460-43	Avian reproduction study in Northern Bobwhite	TGAI. Desthio. No.	Acceptable Acceptable
$71-4b$	٠.	Avian repro	462460-44 462460-45	Avian reproduction study in Mallard	TGAI. No. Desthio. No.	Acceptable Supplemental

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