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OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Rotenone: Draft Ecological Risk Assessment for Rotenone for Registration Review

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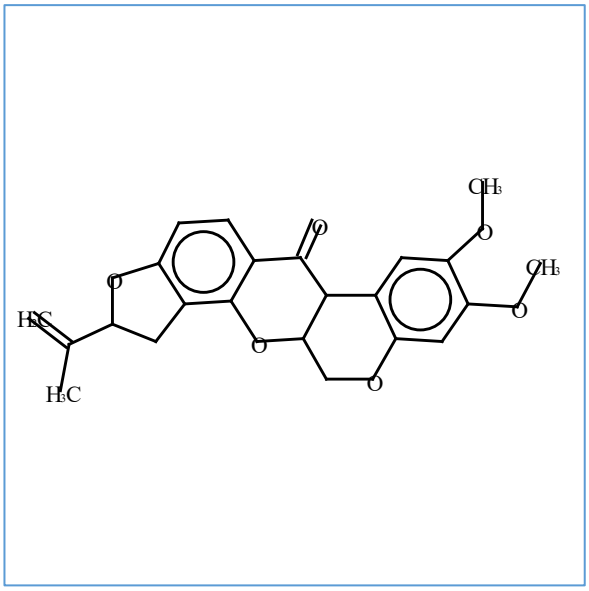
The Environmental Fate and Effects Division (EFED) has completed the draft environmental fate and ecological risk assessment (DRA) in support of the Registration Review of the piscicide rotenone (6R, 6aS, 12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromenyl[3,4-bfuro[2,3-h]chromen-6-one; PC Code 071003.; CAS No: 83-79-4). Given the minimal amount of new data (*i.e.*, acute oyster shell deposition study) received since the environmental fate and ecological risk assessment was written in support of the Reregistration Eligibility Decision (RED) for rotenone, the current assessment relies primarily on the earlier RED. Additionally, the label for this product is linked to relatively detailed standardized and technical operating procedures (SOP/TOP) which include specific conditions/requirements for addressing potential adverse effects to Federally-listed threatened/endangered species and their designated critical habitat; therefore, the labels are compliant with the Endangered Species Act. While historically labels have allowed for aerial applications of rotenone, this assessment is based on the understanding that the revised standard operating procedures no longer include aerial applications of rotenone and that rotenone must be deactivated in lotic environments; both of these revisions are intended to prevent movement of the compound outside of the targeted treatment area. To the extent to which labels do not reflect these restrictions, the assessment of potential risks to non-target organisms would not be protective.

Draft Ecological Risk Assessment for the Registration Review of Rotenone



Rotenone

6R, 6aS, 12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromenyl[3,4-bfuro[2,3-h]chromen-6-one

CAS Registry Number: 83-79-4

USEPA PC Code: 071003

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March 4, 2020

Contents

Executive Summary

This is a draft risk assessment (DRA) for rotenone (6R, 6aS, 12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromenyl[3,4-bfuro[2,3-h]chromen-6-one; PC Code 071003.; CAS No: 83-79-4), which is used to manage fish. This DRA relies on the past ecological risk assessment (USEPA 2006) written in support of the Reregistration Eligibility Decision (RED; USEPA, 2007) for the compound, but includes some updated information as discussed in the preliminary Problem Formulation conducted for Registration Review (RR PF; USEPA, 2015). Since the preliminary Problem Formulation, an acute toxicity study evaluating oyster shell deposition (MRID 506711-01) has been reviewed and included in this DRA.

Rotenone is a selective, non-specific botanical (isoflavone) pesticide obtained from extracts of roots, seeds, and leaves of tropical and sub-tropical plants in the genera *Lonchocarpus* and *Derris* and is used to kill fish (*i.e.*, as a piscicide). The compound acts by blocking electron transport in cell mitochondria. Similar to other piscicides, rotenone is classified as a Restricted Use Pesticide (RUP) that can be applied to both lentic (standing waterbodies such as ponds, lakes, and reservoirs) and lotic waters (flowing waterbodies such as streams and rivers) and is formulated as a dust, liquid, emulsifiable concentrate, and wettable powder (USEPA, 2015). Dust formulations must be mixed as a slurry to be applied to water surface by dragging equipment to release product behind a boat below the water surface. Applications can be made with backpack sprayer, drip can, or handheld or hand-directed nozzle to the water surface; although rotenone has also been applied by aircraft in the past, the registrants are no longer supporting the aerial application of rotenone (Finlayson and Skaar, 2017). In lotic environments such as streams and rivers, rotenone is applied through drip stations or sprayers where the moving water carries the rotenone downstream. In lentic environments such as lakes, reservoirs and ponds, rotenone is typically pumped through a hose into the propeller wash of an outboard motor to distribute the chemical uniformly through the water column. Rotenone use is not allowed in marine/estuarine environments.

Table 1 summarizes estimated risks across each of the taxonomic groups from the currently registered uses of rotenone. The previous risk assessment concluded that for exposure to the compound at the maximum treatment concentration (*i.e.*, 200 µg ai/L) and based on the most sensitive species, risk quotient (RQ) values exceed acute risk Levels of Concern (LOCs) by several orders of magnitude and that such treatments are expected to kill fish, aquatic-phase amphibians, and aquatic invertebrates in the immediate treatment area. While historically labels did not include deactivating the compound with a strong oxidizing agent (potassium permanganate; $KMnO_4$) to ensure that it does not move outside of the intended treatment area, revised standard operating procedures (Finlayson et al., 2010) indicate that rotenone must be deactivated in lotic environments, with limited exceptions. The likelihood of chronic exposure following the treatment of streams is considered low since rotenone is typically applied only once per year and the chemical is continually diluted via displacement by inflowing water and by abiotic degradation; however, in lentic systems where rotenone is not intentionally deactivated and where only a portion of the water body has been treated, there is potential for chronic exposure.

Since rotenone is applied directly to water, the risk of terrestrial animal acute mortality is considered low since there are not likely to be any rotenone residues on terrestrial animal forage items. Where application protocols require that dead and/or dying fish be collected and buried, there is reduced opportunity for either birds or mammals to consume fish that may contain rotenone residues. However, even if birds or mammals were to consume fish killed by rotenone, there would be insufficient quantities of rotenone in the carcasses to represent a risk of acute mortality in terrestrial animals. Since fish are either collected or shortly sink to the bottom of treated water and rapidly decompose, the likelihood of

chronic exposure through the diet of terrestrial animals is also considered to be low. While historically labels have allowed for aerial applications of rotenone, this assessment is based on the understanding that the revised standard operating procedures, which are linked to the label, no longer include aerial applications of rotenone. Also, as noted above, this assessment is based on the understanding that rotenone is deactivated in lotic environments; the elimination of aerial applications and the deactivation of rotenone in lotic environments are both intended to prevent movement of the compound outside of the targeted treatment area. To the extent to which labels do not reflect these restrictions, the assessment of potential risks to non-target organisms would not be protective.

Rotenone is practically non-toxic to adult honey bees (*Apis mellifera*) on an acute contact and oral exposure basis; however, no data are available with which to assess the acute toxicity to larval bees or the chronic toxicity of the compound to adult or larval bees. However, since the compound is a naturally occuring isoflavone in certain leguminous plant species, bees may be routinely exposed to such compounds with no apparent effect. Additionally, since rotenone is applied directly to water, exposure of bees to rotenone may be limited to ingestion of treated water. There are data examining the acute contact toxicity of rotenone to social non-*Apis* bees, (*i.e*., bumblebees; *Bombus terrestris*) and while at face value these data indicate that bumblebees are several orders of magnitude more sensitive to rotenone than honey bees, there is uncertainty regarding how the toxicity may have been influenced by the rotenone formulation tested.

Table 1. Summary of Risk Quotients (RQs) for Taxonomic Groups from the Piscicidal Use of Rotenone[3].

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species	Additional Information/Lines of Evidence
Freshwater fish	Acute	103	**Yes**	–
	Chronic	NC	--	Likelihood of chronic exposure considered low since maximum application rate is considered likely to kill all fish in the treatment area. However, chronic exposure may occur if treatment concentrations are less than maximum and less sensitive fish are present.
Estuarine/ marine fish	Acute	No Data		–
	Chronic	No Data		–
Freshwater	Acute	54	**Yes**	–

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species	Additional Information/Lines of Evidence
invertebrates	Chronic	NC	--	Likelihood of chronic exposure considered low since maximum application rate is considered likely to kill all aquatic invertebrates in the treatment area. However, chronic exposure may occur if treatment concentrations are less than maximum and less sensitive invertebrates are present.
Estuarine/ marine invertebrates	Acute	16.7	**Yes**	Rotenone is not registered for use in estuarine/marine environments and is deactivated in lotic environments; therefore, the likelihood of exposure is considered low.
	Chronic	No Data		
Mammals	Acute		No	Direct application to water; likelihood of exposure considered low. Ingestion of contaminated fish does not represent acute risk.
	Chronic dietary-based Chronic dose-based	NC	No	–
Birds	Dose-based Acute	NC	No	Direct application to water; likelihood of exposure considered low. Ingestion of contaminated fish does not represent acute risk.
	Dietary-based Acute	NC	No	
	Dietary-based Chronic	No Data		–
Terrestrial invertebrates[2]	Acute Adult	NC	--	Direct application to water; likelihood of exposure considered low.
	Chronic Adult	No data		–
	Acute Larval	No data		
	Chronic Larval	No data		
Aquatic plants	N/A	No data		–
		No data		–
Terrestrial plants	N/A	No data		–

NC=not calculated.
Level of Concern (LOC) Definitions:
Terrestrial Animals: Acute risk LOC=0.5; Chronic risk LOC=1.0; Terrestrial invertebrates Acute risk LOC=0.4
Aquatic Animals: Acute risk LOC=0.5; Chronic risk LOC=1.0
Plants risk LOC= 1.0
[1] RQs reflect exposure estimates for parent and maximum treatment concentration (200 µg ai/L) allowed on labels.
[2] RQs for terrestrial invertebrates are applicable to honey bees (*Apis mellifera*), which are also a surrogate for other species of bees. Risks to other terrestrial invertebrates (*e.g.*, earthworms, beneficial arthropods) are only characterized when toxicity data are available.

[3] Risk estimates are based on the understanding that aerial applications of rotenone are no longer permitted, and that rotenone is deactivated in lotic environments to prevent movement of the compound outside of the targeted treatment area. To the extent to which labels do not reflect these restrictions, the assessment of potential risks to non-target organisms may not be applicable.

Introduction

This Draft Risk Assessment is written in support of Registration Review of the piscicide rotenone and summarizes the environmental fate, ecological effects and potential ecological risks to non-target organisms that are not listed as Federally threatened/endangered species. For more information on assessing risk to Federally listed species, see **Appendix B**. The DRA uses the best available scientific information on the use, environmental fate and transport, and ecological effects of rotenone. The general risk assessment methodology is described in the *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs* ("Overview Document") (USEPA, 2004). Additionally, the process is consistent with other guidance produced by the Environmental Fate and Effects Division (EFED).

Problem Formulation

The purpose of problem formulation is to provide the foundation for the environmental fate and ecological risk assessment being conducted for the labeled use of rotenone. The problem formulation identifies the objectives for the risk assessment and provides a plan for analyzing the data and characterizing the risk. Although the Registration Review (RR) process typically includes a detailed preliminary Problem Formulation for a DRA, minimal data have been submitted following the previous assessment of rotenone (USEPA 2006); therefore, the following sections summarize the key points of the previous risk assessment.

The Food Quality Protection Act (FQPA) requires EPA to screen pesticide chemicals for their potential to produce effects similar to those produced by estrogen in humans and gives EPA the authority to screen certain other chemicals and to include other endocrine effects. In response, EPA developed the Endocrine Disruptor Screening Program (EDSP). Additional information on the EDSP is available in **Appendix C.**

Rotenone has been used extensively as a piscicide throughout the United States. By the mid-1980s, its greatest use was in the management of warm water fisheries in the Southeast and cool water fisheries of the Midwest and Mountain states (McClay 2005). According to a survey conducted by the American Fisheries Society, during the period of 1988 to 2002 rotenone was used for fisheries management in 38 states and five Canadian provinces; eleven states and one Canadian province accounted for 89% of rotenone applications to surface waters in North America (McClay 2005). Approximately 97% of rotenone applications during this period were made to standing waters (ponds and lakes). Reported uses were grouped into the following five categories, reflecting different fisheries management objectives: (1) obtain a sample of representative fish species and sizes to characterize fish populations (*i.e.*, sampling; 34%); (2) manipulate fish populations to maintain desirable fish species for sport fisheries (*e.g.*, reducing the density of planktivorous fish) (27%); (3) treat rearing ponds (17%); (4) remove exotic species (10%); and (5) restore threatened and endangered species (7%). Formulated products used in lentic environments were roughly equally split between rotenone powder (53%) and liquid (47%) (McClay 2005).

Similar to the classification of other piscicides, rotenone is a Restricted Use product (RUP) and the chemical has relatively detailed standard operating procedures (SOPs) developed by the American Fisheries Society and associated with its use to renovate/restore fish populations (Finlayson *et al.*, 2010).

Since the SOPs are linked to (*i.e.,* specified on) the federal label, they are considered part of the label and must be followed by the end user. The current SOPs (Finlayson *et al*., 2010) state that, "*The flow of a stream or outflow of a treated lake beyond the treatment area now requires chemical deactivation with potassium permanganate unless it is demonstrated to be unnecessary.*" An example of a situation in which deactivation would not be conducted is when it is deemed unfeasible, such as when the stream flow goes into a canyon (or other physical limitation) or "*if there is no discharge from the Treatment Area or the discharge goes dry in a distance shorter than 2 miles or 2 hours travel-time (maximum distance/time between drip stations recommended on label) from the lowest drip station*." For the first scenario, "*the Certified Applicator through bioassay or analytical testing assures that the discharge is no longer toxic at 30 minutes travel time downstream of where the stream emerges at an accessible location and deactivation could be accomplished*." Examples of the latter scenario include ponds or lakes with no discharge or a stream that goes dry within two miles of the treatment area. An additional scenario in which deactivation is not required is when dilution of untreated waters renders "*undetectable concentrations"* (*i.e*., <2 µg a.i./L) in discharge waters. Regardless of whether the compound is deactivated, bioassays are typically conducted and cages containing sentinel species (typically the target fish species) are placed downstream of treated waters to ensure that the chemical does not substantially move outside of the targeted treatment area.

Since the previous assessments, major changes to the SOPs include:

- Maximum Rotenone Treatment Levels—The maximum treatment level in standing waters was reduced from 250 to 200 $\mu g\ a.i./L$ rotenone, and the maximum treatment level in flowing water was increased to 200 $\mu g\ a.i./L$ rotenone. For all applications, the selected treatment rate is based on response of target fish (or surrogate species) in a bioassay with site water (or in similar water) within the maximum level on the label (SOP 5 in AFS 2018).

- Chemical Deactivation of Treated Flowing Water—The flow of a stream or outflow of a treated lake beyond the treatment area now requires chemical deactivation with potassium permanganate unless it is demonstrated to be unnecessary (SOP 7 in AFS 2018). The deactivation zone and other areas affected by the treatment are included in the definition of a project area (SOP 6 in AFS 2018).

- Elimination of aerial uses.

Included in the sections below are the risk conclusions, and basic information on the parent compound and its environmental fate and ecological effects, as well as a discussion of uncertainties. More complete information on the usage, environmental fate, and ecological effects of rotenone may be found in the RR PF (USEPA 2015).

Other than the acute oyster toxicity data noted previously, no new data have been submitted since the time the RED (USEPA 2007) and the RR PF (USEPA 2015) were written. Additionally, since specific treatment concentrations are used directly as the Estimated Environmental Concentrations (EEC) in lieu of model estimates, any changes since the RED to EFED's aquatic exposure models do not influence EPA's understanding of exposure resulting from the use of rotenone. Therefore, despite a now lower (20%) maximum treatment rate which results in lower RQs, EFED's risk conclusions from the previous assessments, which were reiterated in the RR PF (USEPA, 2013), have not changed. A complete discussion

of the available study data and other information which led to these conclusions may be found in the RR PF (USEPA 2015).

The SOPs, which are associated with the product labels for rotenone and are therefore considered as part of the label, contain procedures specifying conditions/requirements for use. In situations where there may be risks to Federally listed threatened/endangered species and/or their designated critical habitat from the use of rotenone, there are SOPs for addressing such risks. Since the SOPs are linked to the label, users are required to follow these procedures. Therefore, the labels are compliant with the Endangered Species Act.

Environmental Fate

The physical and chemical properties that determine the fate and transport of rotenone are detailed in **Table 2**. The environmental fate data set for rotenone is incomplete, as most of the studies submitted previously do not meet the current standards for environmental fate guideline data requirements (OCSPP Series 835). Based on available data, rotenone has a relatively low potential to bioconcentrate in aquatic organisms (BCF <30 L/kg). Based on its estimated low vapor pressure and estimated low Henry's Law constant, rotenone is not expected to volatilize.

When applied to water, rotenone is expected to hydrolyze, but guideline data are not available to accurately determine the rates at which this will occur at various environmental pH levels, or to characterize the transformation products. Based on information submitted as part of a data waiver request (USEPA 2017), hydrolysis is considered a major route of environmental degradation leading to the formation of rotenolone; rotenone dissipation (DT_{50}) rates (ostensibly due to hydrolysis and possibly some photolysis) have been shown to increase with water pH and temperatures, and rotenolone concentrations increase as the parent compound decreases. Based on information available in the literature (Draper, 2002), aqueous photolysis may also occur, but acceptable guideline data have not been submitted to address this degradation route. The extent of degradation through biotic mechanisms is unknown since no acceptable guideline metabolism data are available to assess this potential route of degradation.

Rotenone is expected to partition somewhat to the sediment phase of an aquatic environment, although the extent to which that will occur is unclear, as the submitted adsorption/desorption data do not fully meet guideline requirements and the data waiver requested for this guideline was granted. Based on a limited amount of provisionally useful data from the submitted study, the parent compound is expected to be moderately mobile to slightly mobile in the environment, indicating that some partitioning to sediment may occur. While abiotic degradation may limit the amount of rotenone available for adsorption, it is unknown whether or to what extent the degradate rotenolone may sorb to sediment.

Although an acceptable guideline aquatic field dissipation study has not been submitted, supplemental information is available through submitted studies on the aquatic dissipation of rotenone (at 250 µg a.i./L, the former maximum treatment rate) in two experimental ponds in Wisconsin that were maintained at two different temperatures (5°C and 23–27°C). Rotenone dissipated from the water column with half-lives of 23 hours in a cold-water pond and 10.6 hours in a warm-water pond (Acc. # 00157851/TRID 4701520-10). Rotenone degraded from the entire system (water + sediment) with half-lives of approximately 20 days in the cold-water pond and 1.5 days in the warm-water pond. Only the parent was monitored in the study. As would be expected, all the resident fish in the ponds died shortly after the initial application.

After 7 days in the cold-water pond and 1 day in the warm-water pond, fat-head minnows (*Pimephales promelas*) were placed in the pond in cages until 9 of 10 survived for 24 hours. This occurred after 4 days in the warm pond and 30 days in the cold-water pond. Notably, the pH near 8.5 in both ponds would have been expected to result in fairly rapid hydrolysis of rotenone based on available information. While this occurred in the warm-water pond, it did not in the cold-water pond, suggesting that rotenone degradation may slow substantially in cooler environments. As discussed more extensively in the PF, in a 1997 study of rotenone dissipation for 25 days following treatment at 45 $\mu g \cdot L^{-1}$ of California's Lake Davis, a reservoir, rotenone dissipated with a half-life of 10.3 days in cold (9°C) water. The degradate rotenolone, which formed at 55% of the parent application rate, dissipated with a half-life of 5.5 days. Some portion of the dissipation of both rotenone and rotenolone would have been due to discharge from the lake; the volume of discharge was not reported.

Much of the concentration data related to the application of rotenone to California's Lake Davis to control northern pike (Esox lucius) was collected in 1997 (California Department of Fish and Game, 1999). Lake Davis, which is in the Plumas National Forest, has a surface area of 4030 acres and a normal capacity of 84,370 acre-feet. The dataset can be used to estimate the dissipation rate of rotenone from Lake Davis after the initial application, which was made on October 17, 1997 (rather late in the season for rotenone application) when the water was fairly cold (USEPA 2008). Measurements were made at nine sites on each of six time periods extending out to 25 days post-application. The mean concentration of rotenone in the lake on the day of application was 45 µg/L. A control sample taken prior to application showed that no rotenone or rotenolone was present.

Table 2. Physical-Chemical Properties and Environmental Fate Parameters of Rotenone.

Chemical/Fate Parameter	Value	Description/Comments	Source (MRID)
Selected Physical/Chemical Parameters			
Chemical name	(6R, 6aS, 12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromenyl[3,4-bfuro[2,3-h]chromen-6-one	-	-
Rotenone CAS Number	83-79-4	-	-
SMILES Notation	COc5cc4OCC3Oc2c1CC(Oc1ccc2C(=O)C3c4cc5OC)C(C)=C	-	-
Molecular formula	$C_{23}H_{22}O_6$	-	-
Molecular mass	394.4 g mol^{-1}	-	Tomlin, 1994
Vapor pressure (25°C)	6.9 x 10^{-10} Torr	Estimated value. Not likely to volatilize.	EPISuite™ v4.11
Water solubility (20°C)	0.2 mg/L	-	Augustijn-Beckers, 1994
Octanol-water partition coefficient (Log K_{OW})	4.10	-	Hansch *et al*., 1995
Henry's Law Constant (25°C)	1.1x10^{-13} atm-m^3 mol^{-1}	Estimated value. Not likely to volatilize from moist soil or water.	EPISuite™ v4.11

Chemical/Fate Parameter	Value	Description/Comments	Source (MRID)
Persistence			
Hydrolysis half-life (days; 25°C)	No data.	-	-
Aqueous photolysis half-life (25°C)	No data.	-	-
Soil photolysis half-life (25°C)	No data.	Not required for aquatic use.	-
Aerobic soil metabolism half-life (25°C)	No data.	-	-
Anaerobic soil metabolism half-life (25°C)	No data.	Not required for aquatic use.	-
Aerobic aquatic metabolism half-life (25°C)	No data.	-	-
Anaerobic Aquatic half-life (25°C)	No data.	-	-
Mobility			
Soil-water partition coefficients (K_d);	No data.	-	-
Organic carbon-normalized soil partition coefficients (K_{FOC})	No data.	-	-
Field Dissipation			
Aquatic field dissipation half-life	Experimental Ponds (250 ppb) 1.5 days (5 °C) 20 days (23-27 °C) Lake Davis (45 ppb; reservoir, 9°C) 10.3 days (parent) 5.5 days (rotenolone)	Non-guideline, supplemental data.	Acc. # 00157851 (TRID 470152-010) MRID 42217701
Fish Bioconcentration			
Fish bioconcentration factors (steady-state; L/kg)	10.8 (viscera) 27.9 (head) 27.6 (carcass)	No whole fish tissue value available.	USEPA 2008 (Acc. #'s 146183, 143252)

Residues of Concern for Ecological Risk

The major degradate of rotenone is 6αß, 12 αß-rotenolone, which is formed by hydrolysis (and possibly photolysis) is structurally similar to the parent compound and is considered a Residue of Concern (ROC)

along with the parent compound. It is noted, however, that environmental degradation data are not available, and that aquatic exposure is based on the treatment rate of the parent compound in the water body prior to any deactivation.

Ecological Effects

The most sensitive aquatic and terrestrial toxicity data for rotenone are summarized in **Tables 3** and **4**, respectively. **Appendix A** contains a more detailed summary of the ecological effects data for rotenone. Within each of these very broad taxonomic groups, an acute and/or chronic endpoint is selected from the available test data and is used as a denominator in ratios comparing point estimates of exposure to point estimates of effect.

Since the last assessment, acute data have been provided on an estuarine/marine invertebrate, *i.e*., the Eastern oyster (*Crassostrea virginica*) for the rotenone formulated end-use product Prentox™ Cube Resign (39.1% active ingredient; MRID 506711-01). The study indicated that with a 96-hr IC_{50} of 12 µg ai/L the formulated product is very highly toxic to estuarine/marine invertebrates on an acute exposure basis. The study is classified as supplemental since it was conducted using formulated end-product rather than technical grade active ingredient.

Two additional studies were identified through a review of the ECOTOXicology (ECOTOX) Knowledgebase (https://cfpub.epa.gov/ecotox/). These studies included a research examining the toxicity of rotenone to bumblebees (*Bombus terrestris*; Marletto *et al*. 2003) and a study summarizing the toxicity of rotenone to fish in standardized laboratory tests (Marking and Bills 1976). While the bumblebee study provided a more sensitive contact toxicity endpoint (72-hr LD_{50}=0.68 µg/bee) than what is reported for honey bees (honey bee contact LD_{50}>60 µg/bee), the study reports using formulated rotenone; however, it does not report the percent of active ingredient in the study and it is uncertain whether the LD_{50} represents µg of product or µg of active ingredient per bee. With respect to the Marking and Bills paper, the study provides 96-hr LC_{50} values for a wide range of freshwater fish at different temperatures, pH values and water hardness levels; however, none of the values are more sensitive that what is reported for rainbow trout (LC_{50}=1.94 µg ai/L) in **Table 3**.

Table 3. Rotenone toxicity values for aquatic organisms.

Species	Toxicity Value (µg a.i./L)	Effects Endpoint	Exposure Duration	Toxicity Classification	Reference (classification)
Acute toxicity					
Freshwater Fish Rainbow Trout (*Oncorhynchus mykiss*)	LC_{50} = 1.94 98% a.i.	Survival	96-hour	Very highly toxic	MRID 439751-02 (Acceptable)
Freshwater Invertebrates Daphnid (*Daphnia magna*)	EC_{50} = 3.7 96.5% a.i.	Survival	48-hour	Very highly toxic	MRID 400633-03 (Supplemental)
Estuarine/Marine Fish	No data				
Estuarine/Marine Invertebrates	IC_{50}=12 39.1% a.i.	Shell Growth	96-hr	Very highly toxic	MRID 506711-01 (Supplemental)
Aquatic Plants	No data				
Chronic Toxicity					
Freshwater Fish Rainbow Trout (*Oncorhynchus mykiss*)	NOAEC = 1.01 96.5% a.i.	Growth	32-day	NR	MRID 400633-02 (Supplemental)
Freshwater Invertebrates Daphnid (*Daphnia magna*)	NOAEC = 1.25 96.5% a.i.	Reproduction	21-day	NR	MRID 400633-03 (Supplemental)
Estuarine/Marine Fish	No data				
Estuarine/Marine Invertebrates	No data				

NR = not relevant; EFED has not established toxicity classifications for chronic endpoints.

Table 4. Rotenone toxicity values for terrestrial organisms.

Species	% a.i.	Toxicity Value	Toxicity Category	Reference	Study Classification
Mammalian Acute Oral Toxicity					
Rat (*Rattus norvegicus*)	99.2%	LD_{50}: 102 mg a.i./kg (Males) 39.5 mg a.i./kg (Females)	Highly toxic	Acc. 00145496	Acceptable
Mammalian Chronic Toxicity					

Species	% a.i.	Toxicity Value	Toxicity Category	Reference	Study Classification
Rat (*Rattus norvegicus*) Two-generation reproductive study	97.9%	NOAEL = 7.5 mg/kg (0.5 and 0.6 mg/kg/day for males and females, respectively) based on decreased body weight and body weight gain	NA	Acc. 00141408	Acceptable
Avian Acute Oral Studies					
Ring-necked pheasant (*Phasianus colchicus*)	32.4	LD_{50} = 1680 mg/kg bw	Slightly toxic	MRID 143250	Supplemental
Avian Subacute Dietary Studies					
Ring-necked pheasant (*Phasianus colchicus*)	34.5	5-day LC_{50}: 1608 mg/kg diet	Slightly toxic	Acc. No. 248788	Supplemental
Honey bee Acute Toxicity					
Honey Bee (*Apis mellifera*) Contact	>95	LD_{50}: >60 µg a.i./bee	Practically non-toxic	Acc. No. 05001991	Acceptable
Honey Bee (*Apis mellifera*)/ 48 hour Oral	>95	LD_{50}: >30 µg a.i./bee	Practically non-toxic	Acc. No. 05001991	Acceptable

[1] Birds represent surrogates for terrestrial-phase amphibians and reptiles.
[2] Freshwater fish may be surrogates for aquatic-phase amphibians.
NA: Not Available

Ecological Incidents

Since the incident data system (IDS) was last searched in 2015 for the PF (USEPA 2015), no additional incidents have been reported for rotenone (accessed 07/09/19). In the IDS there are a total of six incident reports which were reported over the period from 1977 to 1999. Three of the incidents were classified as having occurred as a result of misuse of the compound, while for the remaining three the legality of use was undetermined. All but one of the reported incidents involved the loss of fish; however, one incident reported the loss of swans and geese. Since the incident occurred several days after the rotenone was applied, the report indicated that it was "unlikely" that the loss was associated with the rotenone application. Incidents may have occurred due to rotenone exposures but may not have been reported due to various factors, such as a lack of reporting, or a lack of observation of effects. Therefore, the lack of incident reports does not necessarily indicate the absence of incidents; however, the restricted use status of the compound coupled with its detailed SOPs may limit the extent of unintended exposure. No incidents were identified for rotenone in the aggregate database (accessed 06/28/18).

The incident data contained in the IDS indicate that the use of rotenone can result in unintended adverse effects on non-target fish and amphibians even with the regimented use prescribed in the SOPs associated with the labels.

Risk Characterization

Rotenone is a selective, non-specific botanical (isoflavone) used to kill fish. Potential environmental fate concerns are summarized in **Table 5**. The compound is not considered likely to volatilize and its primary route of abiotic degradation is through hydrolysis leading to the formation of rotenolone. The extent to which rotenolone dissipates is influenced by water pH and temperatures. Rotenone may also be subject to aqueous photolysis; however, there are insufficient data to characterize this route of degradation. The extent of degradation through biotic mechanisms is unknown since no acceptable guideline metabolism data are available to assess this potential route of degradation. Rotenone is expected to be moderately mobile to slightly mobile in the environment, indicating that some partitioning to benthic sediments may occur. While abiotic degradation may limit the amount of rotenone available for adsorption, it is unknown whether or to what extent the degradate rotenolone may sorb to sediment.

As stated in the preliminary Problem Formulation (USEPA 2015), the chemical's use at a treatment site at a maximum concentration of 200 µg a.i./L (previously 250 µg a.i./L) is intended to kill fish, and the RQs for freshwater fish and invertebrates and estuarine/marine invertebrates would be 103, 54, and 16.7, respectively, and exceed the acute risk LOC of 0.5 by factors of 206X, 108X, and 33X, respectively. The maximum treatment rate also exceeds the upper 95th percentile (79 µg a.i./L) of 96-hr median lethal concentrations for freshwater-fish species sensitivity distribution. The extent of acute mortality that will be inflicted on aquatic animals within a treatment area is uncertain; however, acute effects are expected. To the extent that lower treatment rates are used and less sensitive species are present than those used to evaluate risk in this assessment, the likelihood of acute mortality among non-target aquatic animals may be substantially lower.

Benthic macroinvertebrate monitoring data indicate that repeated yearly rotenone treatments conducted in mountain streams in California did not substantially reduce aquatic invertebrate abundance and that aquatic communities tended to return to pretreatment conditions one to two years after treatment;

whether these aquatic communities achieved species diversity similar to pretreatment conditions is uncertain (Trumbo *et al*. 2000) The likelihood of chronic exposure following the treatment of streams is considered low since rotenone is typically applied only once per year and the chemical is continually diluted via displacement by inflowing water and by abiotic degradation; however, in lentic systems where rotenone is not intentionally deactivated and where only a portion of the water body has been treated, there is potential for chronic exposure. The extent to which rotenone's use as a piscicide will affect aquatic animals outside of the treatment area depends on the extent to which the chemical moves beyond the treatment area; however, SOPs have been modified to indicate that rotenone must be deactivated through the use of an oxidizing agent (specifically, potassium permanganate) in lotic treatment areas and in outflow from treated lakes, reservoirs, or ponds. Although not required by the label, relatively rigorous application and monitoring efforts may be conducted by resource managers using this compound and the SOPs require that rotenone be deactivated with potassium permanganate when there is a likelihood that the piscicide will move outside of the treatment area (*e.g*., see SOP 7 on Determining Need and Methods for Chemically Induced Deactivation; American Fisheries Society 2010). Additionally, rotenone is a Restricted Use Pesticide and the procedures and practices specified on the label and its associated SOPs are intended to reduce exposure and hence risk of non-target aquatic animal mortality by limiting the movement of rotenone outside of treatment areas to the extent possible. The use of potassium permanganate to deactivate rotenone can result in acute toxicity as well for aquatic organisms which are in close proximity to the deactivation station. A more detailed analysis of the risk associated with potassium permanganate alone is contained in the 2006 risk assessment (USEPA 2006) and the reader is referred to that assessment.

Given that rotenone is applied directly to water, the risk of terrestrial animal acute mortality is considered low because there are not likely to be any rotenone residues on terrestrial animal forage items, particularly since aerial application may no longer be used. The likelihood of acute adverse effects to birds is further reduced by the fact that rotenone is practically non-toxic to birds (which serve as surrogates for reptiles and terrestrial-phase amphibians) on an acute oral exposure basis and is slightly toxic to birds on a subacute dietary exposure basis. Depending on the treatment site, fishery management agencies may specify that dead fish be collected and buried; therefore, in these situations there is reduced opportunity for either birds or mammals to consume fish that may contain rotenone residues. However, even if birds or mammals were to consume fish killed by rotenone, there would be insufficient quantities of rotenone in the carcasses to represent a risk of acute mortality in terrestrial animals based measured residues in fish (USEPA 2006). Potential exposure through ingestion of rotenone-treated water was evaluated in the preliminary Problem Formulation using the Screening Imbibition Program (SIP; version 1.0). The model is intended to provide an upper bound of exposure to birds and mammals to pesticides through drinking water alone. Results from the Excel®-based tool indicated that drinking water exposure alone is not a potential concern for acute risk to birds or mammals (USEPA 2015). The available data also indicate that exposure through drinking water is not a potential concern for chronic risk to mammals; however, since no chronic toxicity data are available for birds, there are insufficient data to preclude chronic risk. However, the likelihood of chronic exposure through the diet and/or drinking water for terrestrial animals is also considered to be low because fish are either collected or shortly sink to the bottom of treated water and rapidly decompose.

In addition, effects on threatened and endangered species may be an important consideration for site-specific applications that are co-located with critical habitat. Rotenone is an important chemical used in

recovery efforts for endangered salmonid species by eliminating competing species. Based on the SOPs for rotenone, monitoring is to be conducted by resource managers prior to and after the use of rotenone as a piscicide and the SOPs indicate the steps for federal agencies and other users to take if endangered/threatened species are located in a projected treatment area. Previously, acute and chronic risk to estuarine/marine fish and invertebrates could not be determined because toxicity data were not available, and the possibility of risk from acute or chronic exposure to these receptors could not be precluded. However, the RED (USEPA 2007) stipulated that rotenone labels had to be updated to prohibit use in estuarine/marine environments, and the current SOP includes this restriction. Also, an evaluation of risk to aquatic plants could not be conducted since there were no pertinent toxicity data and the possibility of risk from exposure in treated waters could not be precluded. Subsequent to the RR PF, acute toxicity data have been submitted on an estuarine/marine invertebrate, *i.e*., the Eastern oyster (*Crassostrea virginica*). These data are discussed in the Ecological Effects Data section.

Although the compound is likely to have an immediate effect on the aquatic community, monitoring data collected by the California Department of Fish and Game from 1990 to 1996 in Silver King Creek (Trumbo *et al.* 2000) indicate that the treated area community structure returns to pre-treatment conditions within approximately six months; however, the extent of effects is influenced by the concentration/duration of treatments, the area treated, non-target organism morphology/physiology and life history, whether refugia are available, and the extent to which there are sources of species immigration into the treatment area (Vinson *et al*. 2010). The long-term effects to more sensitive species and to aquatic communities downstream from the treatment sites are reduced given that the SOP stipulates that rotenone must be deactivated in water exiting treatment sites.

As noted in the preliminary Problem Formulation and previous RED for rotenone, the risk assessments concluded that although treatment rates are carefully monitored and adjusted to minimize impact to non-target aquatic organisms, treatment rates used in managing fish populations would impact non-target aquatic organisms (*e.g*., non-target fish, aquatic-phase amphibians and aquatic invertebrates). The extent of adverse effects from rotenone in the treatment area depends on the treatment rate and stream/river discharge rate and water temperature. Exposure to rotenone will also be attenuated outside of the treatment area by the new deactivation requirement in the SOP and by the cancellation of the aerial use. Although the compound is likely to have an immediate effect on the aquatic community, the data suggest that the treated area community structure returns to pre-treatment conditions within 1 to 2 years (USEPA 2007; Vinson *et al*. 2010).

Risk estimates for the piscicidal use of rotenone are based on the understanding that the revised standard operation procedures, which are linked to the label, no longer include aerial applications of rotenone and that rotenone is deactivated in lotic environments. The elimination of aerial applications and the deactivation of rotenone in lotic environments are both intended to prevent movement of the compound outside of the targeted treatment area. To the extent to which labels do not reflect these restrictions, the assessment of potential risks to non-target organisms may not be applicable.

Table 5. Potential Environmental Fate Concerns Identified for Rotenone.

Bioconcentration/ Bioaccumulation	Groundwater Contamination	Sediment	Persistence	Residues of Concern	Volatilization
No K_{ow}<1.0	No	Yes	No	Parent plus rotenolone	No

Data Gaps and Uncertainties

Although the problem formulation identifies both environmental fate and ecological effect data gaps for which data were required in a Generic Data Call-In (GDCI-071003-1584; USEPA 2017), the technical registrants submitted waiver requests for many of these studies (Finlayson and Skaar 2017). While some minor fate deficiencies were noted in the 2007 assessment, additional data were not expected to change the risk conclusions. EFED reviewed the waiver requests for three environmental fate data requirements and five ecological effect data requirements and recommended that all of the requests should be granted (USEPA 2017a). Environmental fate studies for which waivers were requested and granted include adsorption/desorption, hydrolysis, and anaerobic aquatic metabolism. Remaining environmental fate data gaps for which data were requested in the GDCI include photolysis in water, and aerobic aquatic metabolism. Ecological effects studies for which waivers were requested and granted include aquatic invertebrate acute toxicity (freshwater daphnids), fish acute toxicity (freshwater and marine), whole sediment acute toxicity invertebrates (freshwater), avian reproduction, and whole sediment chronic invertebrates (freshwater and marine). Ecological effects data gaps for which data were requested in the GDCI include oyster acute toxicity (shell deposition); this study has since been submitted. The study with the Eastern oyster is classified as supplemental due to low recoveries in mean-measured concentrations relative to nominal concentration; however, additional acute toxicity testing with the Eastern oyster is not being recommended.

Toxicity and risk from the rotenone degradate is an area of uncertainty. There is uncertainty regarding the potential mobility and formation rates of degradates relative to the parent compounds. However, when the compound is applied to moving water, it is deactivated using potassium permanganate (K_2MnO_4) as the water exits the treatment area.

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APPENDIX A. ECOLOGICAL EFFECTS CHARACTERIZATION

Toxicity testing reported in this section does not represent all species of bird, mammal, or aquatic organism. Only a few surrogate species for both freshwater fish and birds are used to represent all freshwater fish (2000+) and bird (680+) species in the United States. For mammals, acute studies are usually limited to Norway rat or the house mouse. Estuarine/marine testing is usually limited to a crustacean, a mollusk, and a fish. Also, neither reptiles nor amphibians are tested. The assessment of risk or hazard makes the assumption that avian toxicity is similar to terrestrial-phase amphibians and reptiles. The same assumption is made for fish and aquatic-phase amphibians.

Rotenone is practically non-toxic to honeybees on an acute contact exposure basis, is slightly toxic to birds on an acute oral and subacute dietary exposure basis and is highly toxic to mammals on an acute oral basis. A two-generation rat reproductive study with rotenone resulted in decreased body weight and body weight gain in both parental animals and pups. In addition, although several studies have linked sub-chronic rotenone exposure to Parkinson's disease-like symptoms in laboratory rats, the exposure methods used to obtain these results are not typically encountered through the current registered uses of rotenone. No chronic toxicity data were available to evaluate the effects of rotenone on birds and no data were available to evaluate the toxicity of rotenone to terrestrial plants.

For freshwater organisms, rotenone is highly toxic to fish and invertebrates on an acute exposure basis. In addition, chronic rotenone exposure to freshwater fish and invertebrates has been found to cause decreases in growth and reproduction, respectively. However, no registrant-submitted studies were available to evaluate the acute or chronic toxicity of rotenone to estuarine/marine fish or invertebrates. Also, no data are available to evaluate the toxicity of rotenone to aquatic plants.

Aquatic Effects Characterization of Rotenone Toxicity

Aquatic Animals

Freshwater Fish

Rotenone is very highly toxic to the cold water rainbow trout (96-h LC_{50} = 1.94 µg/L; MRID 439751-02) and warmwater bluegill sunfish (96-h LC_{50} = 4.9 µg/L; MRID 439751-01; **Table B1**). Acute toxicity testing using technical end-product (5% active ingredient) was also very highly toxic to rainbow trout (96-h LC_{50} = 11.5 µg/L; Acc. No: 121873) and bluegill (96-h LC_{50} = 56 µg/L; Acc. No. 121874). A rainbow trout early life-stage test produced a 32-day NOAEC of 1.01 µg/L (MRID 400633-02), based on reduced growth. Length of fish was reduced by roughly 20% at rotenone concentrations of 2.2 µg/L.

In a study where adult zebrafish (*Danio rerio*) were exposed to nominal rotenone concentrations of 2 µg/L for 4 weeks, no locomotor effects were observed (Bretaud *et al.* 2004). In the same study, larval zebrafish exposed to nominal rotenone concentrations of 5 and 10 µg/L (dissolved in dimethylsulfoxide) for 4 days (24 hr post-fertilization to 5 days post-fertilization) did not display any changes in their locomotor activity.

Larvae could be treated with up to 30 µg/L and performed similar to controls; however, at 50 µg/L, larvae showed what was described as a "degenerating phenotype" and died within 4 days of exposure. The authors attributed the lack of neurotoxic effects to the blood-brain barrier, i.e., limiting access of rotenone to central nervous system neurons, or rapid metabolism of rotenone in the periphery. These data suggest that in nonmammalian species and under slightly more realistic exposure conditions, rotenone did not result in neuron degeneration.

Freshwater Invertebrates

Rotenone is very highly toxic to freshwater invertebrates (*Daphnia magna* 48-h EC_{50} = 3.7 µg/L; MRID 400633-03) on an acute exposure basis. In a 21-day full life-cycle study with *Daphnia magna*, the NOAEC was 1.25 µg/L (MRID 400633-03), based on roughly a 50% reduction in number of young produced.

Estuarine/Marine Fish

No registrant-submitted guideline studies were submitted to assess the toxicity of rotenone to estuarine/marine fish.

Estuarine/Marine Invertebrates

Since the last assessment, acute data have been provided on an estuarine/marine invertebrate, *i.e.*, the Eastern oyster (*Crassostrea virginica*) for the rotenone formulated end-use product Prentox™ Cube Resign (39.1% active ingredient). The study indicated that with a 96-hr IC_{50} of 12 µg ai/L the formulated product is very highly toxic to estuarine/marine invertebrates on an acute exposure basis. The study is classified as supplemental since it was conducted using formulated end-use product rather than technical grade active ingredient.

Aquatic Plants

No data are available to assess the potential toxicity of rotenone to aquatic plants.

Table B1. Rotenone toxicity values (µg a.i./L) for aquatic organisms.

Species	Toxicity Value (µg a.i./L)	Effects Endpoint	Exposure Duration	Toxicity Classification	Reference (classification)
Acute toxicity					
Freshwater Fish Rainbow Trout	LC_{50} = 1.94	Survival	96-hour	Very highly toxic	MRID 439751-02 (Acceptable)
Freshwater Invertebrates Daphnid	EC_{50} = 3.7	Survival	48-hour	Very highly toxic	MRID 400633-03 (Supplemental)
Estuarine/Marine Fish	NA	NA	NA	NA	NA
Estuarine/Marine Invertebrates	IC_{50}=12 39.1% a.i.	Shell Growth	96-hr	Very highly toxic	MRID 506711-01

Species	Toxicity Value (µg a.i./L)	Effects Endpoint	Exposure Duration	Toxicity Classification	Reference (classification)
Aquatic Plants	NA	NA	NA	NA	NA
Chronic Toxicity					
Freshwater Fish Rainbow Trout	NOAEC = 1.01	Growth	32-day	NR	MRID 400633-02 (Supplemental)
Freshwater Invertebrates Daphnid	NOAEC = 1.25	Reproduction	21-day	NR	MRID 400633-03 (Supplemental)
Estuarine/Marine Fish	NA	NA	NA	NA	NA
Estuarine/Marine Invertebrates	NA	NA	NA	NA	NA

NA = no guideline study was submitted by the registrant. No data were available in the literature.

NR = not relevant; EFED has not established toxicity classifications for chronic endpoints.

Terrestrial Effects Characterization of Rotenone Toxicity

The toxicity measures of effect used to characterize risks of rotenone exposure to mammals, birds, and non-target insects are summarized in **Table 3.16.**

Results of toxicity studies in mammals, birds, and honey bees indicate that rotenone is slightly toxic to birds on an acute oral and subacute dietary exposure basis, highly toxic to mammals on an acute exposure basis, and practically non-toxic to honeybees on an acute contact exposure basis. A two-generation rat reproductive study with rotenone resulted in decreased body weight and body weight gain in both parental animals and pups.

Terrestrial Animals

Mammals

Rotenone was more toxic to female rats (LD_{50} = 39.5 mg/kg) compared to male rats (LD_{50} = 102 mg/kg), based on an acute oral exposure (MRID 00145496; **Table B2**). In a two-generation rat reproductive study, adult and offspring toxicity was indicated by decreased body weight (MRID 00141408). An NOAEC of 7.5 mg/kg (0.5 and 0.6 mg/kg/day for male and female, respectively) was determined based on decreased F1 and F2 pup body weight and body weight gain. The offspring toxicity LOAEL for rotenone in male and female rats was 35.7 ppm (2.4 and 3.0 mg/kg/day for male and females, respectively), based on decreased body weight (10 - 50%) and body weight gain (20 - 60%) in both generations (MRID 00141408).

In a study by Betarbet *et al.* (2000; MRID 452795-01), Lewis rats were continuously infused with rotenone dissolved in dimethylsulfoxide (DMSO) and polyethylene glycol (PEG) via jugular vein cannula for one week to more than five weeks and produced behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease. A rotenone concentration of 2 to 3 mg/kg/day induced a neurological pathology similar to that of Parkinson's disease. Intravenous rotenone exposure induced specific degenerative brain lesions in nigrostriatal dopinergic neurons and resulted in clinical signs that included hypoactivity, unsteady gait, and hunched posture. The purpose of this study was to develop a model for Parkinson's disease rather than study the toxicity of rotenone. Although effects of rotenone on Lewis rats were observed, the utility of this study is complicated by the exposure method and duration, as well as the carrier solvent used. DMSO is a solvent that is known to facilitate the movement of compounds across the cell membrane. Also, the route of exposure, *i.e.*, continuous infusion into the jugular vein and duration of exposure, *i.e*., 55 days, would not likely be encountered from the labeled uses of rotenone. Direct infusion into the jugular vein would initially by-pass the liver where rotenone may undergo biotransformation. An analogous route of exposure where the liver is initially by-passed would be possibly following inhalation of rotenone. However, to inhale the quantity of rotenone in the co-solvent (DMSO) used to illicit brain lesions would be highly unlikely. As a result, EFED is uncertain regarding the relevancy of this study to the ecological risk assessment of rotenone.

Formulated product toxicity testing data were available on three products used as applications to water for fishery resource management purposes. All of the formulations tested (**Table B3**) were less toxic than technical grade active ingredient on an acute oral exposure basis. One of the formulations tested, *i.e.*, Chem Fish Regular contains 5% cube root extractables plus 5% rotenone; however, the formulation (LD_{50}=294.8 mg/kg bw) was roughly 3 times less toxic than technical grade (LD_{50}=102 mg/kg bw) in male rats. For females, formulated end-product (LD_{50}=130 mg/kg bw) was also roughly 3 times less toxic than technical grade (LD_{50}=39.5 mg/kg bw). These data suggest that cube root extractables do not contribute appreciably to the toxicity of rotenone.

It is noteworthy that similar to the technical grade rotenone, all of the formulated products tested were more toxic to female rats than to male rats by factors ranging from 1.6 to 5.0X.

Table B2. Rotenone toxicity values for terrestrial organisms.

Species	% a.i.	Toxicity Value	Toxicity Category	Reference	Study Classification
Mammalian Acute Oral Toxicity					
Rat (*Rattus norvegicus*)	99.2%	LD_{50}: 102 mg a.i./kg (Males) 39.5 mg a.i./kg (Females)	Highly toxic	MRID 00145496	Acceptable
Mammalian Chronic Toxicity					

Species	% a.i.	Toxicity Value	Toxicity Category	Reference	Study Classification
Rat (*Rattus norvegicus*) Two-generation reproductive study	97.9%	NOAEL = 7.5 mg/kg (0.5 and 0.6 mg/kg/day for males and females, respectively) based on decreased body weight and body weight gain	--	MRID 00141408	Acceptable
Avian Acute Oral Studies					
Mallard duck (*Anas platyrhynchos*)	32.4	LD_{50} = 2200 mg/kg	Practically nontoxic	MRID 143250	Supplemental
Ring-necked pheasant (*Phasianus colchicus*)	32.4	LD_{50} = 1680 mg/kg	Slightly toxic	MRID 143250	Supplemental
Avian Subacute Dietary Studies					
Mallard duck *(Anas platyrhynchos*)	34.5	5-day LC_{50}: 2600 ppm	Slightly toxic	Acc. No. 248788 (Hill *et al.*, 1975)	Supplemental
Ring-necked pheasant (*Phasianus colchicus*)	34.5	5-day LC_{50}: 1608 ppm	Slightly toxic	Acc. No. 248788 (Hill *et al.*, 1975)	Supplemental
Japanese quail (*Coturnix japonica*)	34.5	5-day LC_{50}: 1882 ppm	Slightly toxic	Acc. No. 248788 (Hill *et al.*, 1975)	Supplemental
Honey bee Acute Contact Toxicity					
Honey bee (Apis mellifera)	> 95	LD_{50}: > 60 µg a.i./bee	Practically non-toxic	MRID 05001991 (Stevenson JH, 1978)	Acceptable
Honey bee (Apis mellifera)/ 48 hour	Technical	2.4 µg a.i./bee elicited 12% mortality	–	MRID 00036935 (Atkins EL *et al.*, 1975)	Supplemental - Check this study

Table B3. Acute oral toxicity of formulated end-products of rotenone to rats.

Formulation	% rotenone	Toxicity	MRID
Prentox Grass Carp Management Bait	2.6% rotenone 0.5% piperonyl butoxide 95% inerts	males: 1550 mg/kg bw females: 970 mg/kg bw	429817-01
Chem Sect Chem Fish Regular	5% rotenone 5% cube root extractables 90% inerts	males: 294.8 mg/kg bw females: 130.3 mg/kg bw	431270-01
Chem Sect Cube Root Powder Toxicant	8.08% rotenone 91.92% inerts	males: >1049 mg/kg bw females: >209 mg/kg bw	448492-01

Birds

Avian acute oral toxicity data are available for the mallard duck and ring-necked pheasant. In these studies, only female birds were tested. The LD_{50}s for the mallard duck and ring-necked pheasant, based on formulated product (32.4% a.i. rotenone), were 2200 mg/kg and 1680 mg/kg, respectively (**Table 3.18**; MRID 143250). Regurgitation occurred at concentrations above 1500 mg/kg. Based on these data, rotenone is classified as slightly toxic to birds and the taxa for which they serve as surrogates (reptiles and terrestrial phase amphibians) on an acute oral exposure basis.

Subacute dietary toxicity studies on formulated product (34.5% rotenone) have been conducted using ring-necked pheasants (*Phasianus colchicus*), Japanese quail (*Coturnix japonica*), and mallard ducks (*Anas platyrhynchos*). Toxicity (LD_{50}) values for 5-day subacute dietary toxicity studies in the three species are 1608, 1882, and 2600 ppm, respectively (**Table 3.18**; ACC No. 248788). Based on the most sensitive species tested, i.e., ring-necked pheasants, rotenone is classified as slightly toxic to birds on a subacute dietary exposure basis.

No chronic toxicity data were available to assess the chronic effects of rotenone on birds.

Terrestrial Invertebrates

Acute contact and oral toxicity studies in honey bees (Apis mellifera) using technical grade rotenone (95% a.i) yielded LD_{50} values of greater than 60 µg a.i./bee (MRID 05001991) and greater than 30 µg a.i./bee (MRID 05001991), respectively. Based on these results, rotenone is classified as practically non-toxic to honey bees on an acute contact and oral exposure basis.

Terrestrial Plants

No toxicity studies were submitted by the registrant to evaluate the toxicity of rotenone to terrestrial plants.

APPENDIX B. LISTED SPECIES

The Planning and Standard Operating Procedures (SOPs) for the Use of Rotenone in Fish Managment Rotenone SOP Manual, 2nd Edition (American Fisheries Society) specifies the process used by applicators to address potential impacts to federally listed threatened or endangered species. According to the SOP, Federal agencies and others should contact their local National Oceanic and Atmospheric Administration (NOAA) office and U.S. Fish and Wildlife Service (USFSW) Ecological Services office for assistance approximately 6 months in advance of a planned rotenone application.

The SOP specifies that Section 7(a)(2) of the Endangered Species Act (ESA) requires all federal agencies to ensure their actions do not jeopardize existence of listed species or adversely modify or destroy their critical habitat. Actions include all activities and programs of any kind authorized, funded, or carried out, in whole or in part, by a federal agency. To ensure this Section 7 mandate is fulfilled, federal agencies must follow procedures prescribed in regulation. In brief, if no listed species are present or will not be affected in any manner, no further consultation is needed. If federally listed species are present and "may be affected," the action agency must assess the impacts upon such species. If their biological assessment (BA) indicates listed species "may be affected but are not likely to be adversely affected" consultation may be concluded informally with written concurrence from the USFWS or NOAA, the administrators of the ESA. If the action is "likely to adversely affect" listed species, formal consultation is required. The culmination of formal consultation is a written biological opinion (BiOp) that puts forth "jeopardy" or "no jeopardy" determination. In the former, the biological opinion identifies reasonable and prudent alternatives, which must be taken to avoid jeopardy. In all cases where incidental take is likely to occur, the biological opinion includes an "Incidental Take Statement," which provides exemption for the incidental take of listed species.

Section 9 of the ESA prohibits take of listed species. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct without a permit from the USFWS or NOAA, as appropriate.

Federal agencies conducting rotenone projects in waters with threatened and endangered species need to determine whether such projects will affect listed species. The agencies may contact their local NOAA office and USFWS Ecological Services at www.fws.gov/offices/ offices for assistance in fulfilling their Section 7 requirements. Non-federal entities conducting rotenone projects in waters with federally listed species, should contact their local Ecological Services office to determine whether a take permit is required prior to commencement of their work.

As the SOP is linked to the rotenone labels and conditions/requirements for addressing potential risks to listed species is stipulated in the SOP, users are required to follow the necessary procedures where Federally listed species or their designated critical habitat may be affected. Therefore, the labels for rotenone ingredients are compliant with the Endangered Species Act.

APPENDIX C. ENDOCRINE DISRUPTOR SCREEENING PROGRAM

As required by FIFRA and the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA reviews numerous studies to assess potential adverse outcomes from exposure to chemicals. Collectively, these studies include acute, subchronic and chronic toxicity, including assessments of carcinogenicity, neurotoxicity, developmental, reproductive, and general or systemic toxicity. These studies include endpoints which may be susceptible to endocrine influence, including effects on endocrine target organ histopathology, organ weights, estrus cyclicity, sexual maturation, fertility, pregnancy rates, reproductive loss, and sex ratios in offspring. For ecological hazard assessments, EPA evaluates acute tests and chronic studies that assess growth, developmental and reproductive effects in different taxonomic groups. As part of the Draft Ecological Risk Assessment for Registration Review, EPA reviewed these data and selected the most sensitive endpoints for relevant risk assessment scenarios from the existing hazard database. However, as required by FFDCA section 408(p), rotenone is subject to the endocrine screening part of the Endocrine Disruptor Screening Program (EDSP).

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

Under FFDCA section 408(p), the Agency must screen all pesticide chemicals. Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. A second list of chemicals identified for EDSP screening was published on June 14, 2013[1] and includes some pesticides scheduled for registration review and chemicals found in water. Neither of these lists should be construed as a list of known or likely endocrine disruptors. Rotenone is not on List 1. For further information on the status of the EDSP, the policies and procedures, the lists of chemicals, future lists, the test guidelines and Tier 1 screening battery, please visit our website at http://www.epa.gov/endo/.

[1] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0477-0074 for the final second list of chemicals.