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Rotenone

Human Health and Ecological Risk Assessment

FINAL REPORT

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADP	adenosine diphosphate
AEL	adverse-effect level
a.i.	active ingredient
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
calc	calculated value
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
DT_{50}	disappearance/degradation half-life
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IC_x	concentration causing X% inhibition of a process
IRED	Interim Reregistration Eligibility Decision
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill

LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MSMA	monosodium methanearsonate
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
PBPK	physiologically-based kinetic
PPE	personal protective equipment
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
TEP	typical end-use product
TGAI	Technical grade active ingredient
TIPA	Triisopropanolamine
TRED	Tolerance Reassessment Eligibility Decision
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8℃+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556℉-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter ($\mu g/cm^2$)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

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CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

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EXECUTIVE SUMMARY

Overview

Rotenone is a piscicide, a chemical used to kill fish. Rotenone, however, is also toxic to other groups of animals, including humans. At the molecular level, rotenone acts by inhibiting a specific metabolic pathway in animals, and this can lead to an increase in general oxidative damage. At the organ level, rotenone is a neurotoxin that causes degenerative changes in brain tissue that are characteristic of Parkinson's disease. Notwithstanding its toxicity to animals, rotenone is somewhat selective in the context of an aquatic application in that most species of fish are more sensitive to rotenone than are most species of aquatic invertebrates.

The U.S. EPA recently completed a review of rotenone uses and the potential risks associated with these uses. While rotenone had been registered as an insecticide for use on terrestrial crops, these uses are no longer permitted. In reviewing the piscicidal uses of rotenone, the U.S. EPA has recommended mitigation measures to reduce risk:

- Lowering the maximum application rate from 250 ppb to 200 ppb;
- The use of effective personal protective equipment by workers;
- Restricted access for members of the general public to treated areas;
- The use of potassium permanganate to detoxify rotenone.

At the time that this Forest Service risk assessment was prepared, the above recommendations had not been finalized and are not reflected on current labels for rotenone formulations. Assuming that these recommendations are implemented, the risks associated with the use of rotenone should be minimal. At the highest application rate (200 ppb), the upper bound hazard quotient for workers using PPE modestly exceeds the level of concern. At application rates that are more likely to be used in Forest Service programs – i.e., 50 to 150 ppb –hazard quotients for workers do not exceed the level of concern. If PPE is not used by workers, central and upper bound estimates of hazard quotients for workers exceed the level of concern. Members of the general public should not be exposed to significant levels of rotenone, so long as the above mitigation measures are implemented.

Aquatic applications of rotenone will entail exposures to both aquatic and terrestrial wildlife. There is no basis for asserting that rotenone is likely to have a direct toxic effect on terrestrial organisms. Fish mortality will most certainly occur in effective applications of rotenone to surface water. Mortality in some groups of aquatic invertebrates is also likely. The most sensitive groups of aquatic invertebrates appear to be zooplankton and some species of aquatic insects. Rotenone applications may have secondary effects on aquatic plants; however, direct toxicity to aquatic plants does not appear to be plausible. Depending on how secondary effects are measured, changes in the invertebrate community structure of surface waters may persist for a prolonged period of time. It is not clear, however, that these changes would necessarily be classified as adverse in terms of the ability of the ecosystem to support fish populations.

Program Description

Rotenone is used in Forest Service programs to eliminate unwanted or invasive species of fish in order to maintain suitable habitats for native and/or game fish species. Unlike many pesticides, rotenone is not synthesized commercially. Instead, rotenone and related compounds are extracted from the roots or other tissue of plants that naturally produce the compound. At the cellular level, rotenone acts by interfering with energy production. Both liquid and granular formulations of rotenone may be used in Forest Service programs. Some liquid formulations contain piperonyl butoxide, a compound that will inhibit the metabolism of rotenone and related compounds and hence will increase the toxicity of the formulation to fish. Granular formulations are essentially ground or pulverized preparations of the roots of the plants that produce rotenone. Rotenone is also available in bait pellet formulations but these are not used in Forest Service programs and are not considered in the current risk assessment.

Rotenone is also different from many other pesticides in that application rates are expressed as target concentrations in water rather than as lbs a.i./acre. For standing bodies of water such as ponds or lakes, application rates for rotenone range from 0.005 ppm (mg/L) to 0.2 ppm. For flowing water such as streams or rivers, the application rates range from 0.025 ppm to 0.1 ppm. While application rates are expressed as target concentrations, applications to standing water will involve calculations of the number of pounds of a formulation that must be applied to the water body depending on the depth of the water body. For streams, the applications are typically calculated as the amount of formulation that must be added to the stream per unit time depending on the flow rate of the stream. All of the product labels provide tables and equations for converting target concentrations to field application rates – i.e., pounds formulation per surface area of standing water or lbs formulation per unit time for flowing water.

Rotenone is not very persistent in water and field dissipation half-lives are reported in the range of less than one day to about 10 days. The rapid dissipation in water does not have a substantial impact on the effectiveness of rotenone because rotenone can kill fish very rapidly. The product labels recommend that rotenone concentrations should be kept in the lethal range for at least 2 hours. Recommended detoxification periods given on the product labels, however, range from 2 to 4 weeks. Alternatively, potassium permanganate can be used to break down (i.e., oxidize) rotenone very quickly and this method of rapid detoxification may be used in Forest Service programs. Because potassium permanganate can be toxic to fish, the risks associated with detoxification using potassium permanganate are considered quantitatively in the current risk assessment.

The amount of rotenone that might be used by the Forest Service in a given year cannot be estimated with precision and rotenone use is likely to vary with outbreaks of pest fish populations. The total use of rotenone in the United States has been estimated at about 20,000 pounds per year. Based on this total use estimate and admittedly sparse use statistics from the Forest Service, it seems likely that the use of rotenone as a piscicide in Forest Service programs will be minor compared the total use of rotenone as a piscicide by other organizations.

Human Health Risk Assessment

Hazard Identification

At the cellular level, rotenone is a metabolic toxin that interferes with the ability of mitochondria to chemically store energy within a cell – i.e., convert ADP to ATP. This effect results in both an energy deficit within the cell as well as an increase in general oxidative damage to the cell. While mitochondria could be affected by rotenone in any type of cell, the impact on nerve tissue is an endpoint of major concern. Numerous studies indicate that rotenone may cause specific damage to nerve cells, inducing gross signs of neurotoxicity in mammals similar to those associated with Parkinson's disease. Whether or not rotenone can be considered a cause of Parkinson's disease remains an open question that has little impact on the current risk assessment. It is clear that rotenone is neurotoxic, and this endpoint is of concern. Most studies demonstrating that rotenone can induce effects similar to those of Parkinson's disease were conducted using routes of exposure that are not directly germane to potential human exposures (e.g., intraperitoneal or intravenous injection as well as direct instillation into the brain); however, a recent study demonstrates that these effects can occur after oral dosing.

Rotenone is classified by the U.S. EPA as highly toxic after oral and inhalation exposures; yet, there appears to be no consistent pattern in its toxicity to various groups of mammals, except that females seem to be somewhat more sensitive than males. In rats, the LD_{50} is about 40 mg/kg body weight in females and 100 mg/kg body weight in males. With respect to human exposure, the estimated lethal dose is often cited between 300 and 500 mg/kg body weight; however, a relatively well-documented case report indicates a lethal dose of about 40 mg/kg body weight after the accidental poisoning of a young girl. With respect to mammals in general, very sketchy information indicates that rabbits may be somewhat less sensitive than other mammals to rotenone toxicity, whereas cats and dogs may be somewhat more sensitive than are other mammals.

The pharmacokinetics of rotenone in mammals are not well-characterized. While rotenone is often classified as a substance that is not well absorbed after oral exposure, it is able to cross the blood-brain barrier. Furthermore, its chemical properties suggest that rotenone should be well absorbed after oral exposure. The apparent slow rate of oral absorption sometimes attributed to rotenone may reflect rapid metabolism or at least a rapid breakdown in the gastrointestinal tract prior to absorption.

Of the available studies on rotenone, one study indicates that rotenone may be an endocrine disruptor in mammals, impacting testosterone production. Other studies assessing impacts on testosterone production are not available. There is no credible information suggesting that rotenone is a mutagen or carcinogen. Similarly, rotenone does not appear to have the potential to cause substantial dermal or ocular damage, although prudent handling practices dictate that dermal and ocular exposures should be avoided through the proper use of protective equipment.

Because rotenone is extracted from plant roots, commercial formulations of rotenone are complex mixtures of rotenone and other related plant material. It appears, however, that

the components of primary concern are rotenone and one other structurally similar compound, deguelin. Trichloroethylene is used in the extraction process for at least some formulations and small concentrations of trichloroethylene have been found in some rotenone formulations. The quantity of trichloroethylene in rotenone formulations does not appear to be toxicologically significant, based on both its toxicity and its concentration, relative to rotenone. Similarly, all liquid formulations of rotenone contain petroleum solvents, which are themselves complex mixtures. The composition of the petroleum solvents is well characterized in only three formulations. Among these three formulations, the composition of the petroleum solvents differ substantially; nevertheless, the petroleum solvents do not appear to be present in amounts that are toxicologically substantial relative to rotenone and other related compounds.

The U.S. EPA recommends the use of potassium permanganate to detoxify water treated with rotenone. If properly applied, potassium permanganate should not present any additional risk and should decrease risks associated with the use of rotenone as a piscicide. If improperly applied—i.e., applied in excess—the reduction in risk due to the destruction of rotenone should outweigh risks associated with the use of potassium permanganate.

Finally, all formulations of rotenone contain other related rotenoids and some formulations contain piperonyl butoxide, a compound that enhances the toxicity of rotenone. These materials are also listed as active ingredients on the product labels for rotenone formulations. Both other related rotenoids and piperonyl butoxide may contribute to the toxicity of rotenone formulations. Consequently, formulation-specific toxic equivalency factors ranging from 1.25 to 2.5 are developed and these factors are used in all exposure assessments to calculate joint exposures to rotenone, other related rotenoids, and piperonyl butoxide in units of rotenone equivalents.

Exposure Assessment for Human Health

All of the exposure assessments for workers as well as members of the general public are detailed in an EXCEL workbook that accompanies this risk assessment (Attachment 1). This workbook contains a set of worksheets on rotenone that details each exposure scenario discussed in this risk assessment as well as summary worksheets for both workers and members of the general public. Documentation for these worksheets is presented in SERA (2007b). The sections of the risk assessment on workers and the general public provide a plain language description of the worksheets. In addition, the sections discuss the rotenone specific data used in the worksheets.

As indicated in Table 2, there are several formulations of rotenone, including granular and liquid, and the formulations may be applied to ponds or streams. Exposure to rotenone for workers and members of the general public depends on the target concentration. For the current risk assessment, all exposure assessments are based on the application of a liquid formulation, CFT Legumine, at a target concentration of 0.2 ppm, which is the maximum application rate. The consequences of using lower application rates are discussed in the risk characterization (Section 3.4).

The different formulations of rotenone also contain differing amounts of other associated resins (i.e., rotenoids) and some formulations also contain piperonyl butoxide. As detailed in the hazard identification (Section 3.1.17), these compounds are considered using toxic equivalency factors (ranging from 1.25 to 2.5) to calculate rotenone equivalents which encompass the contribution of rotenone, other related resins, and piperonyl butoxide. Consequently, all doses derived in this exposure assessment are expressed in units of rotenone equivalents.

There are substantial uncertainties in the exposure assessments for workers. Since data are not available on worker exposure rates for aquatic applications of rotenone, the current risk assessment bases worker exposure rates on an aquatic application of 2,4-D. Uncertainties in the worker exposure rates are compounded by uncertainties concerning the use of personal protective equipment (PPE). While the U.S. EPA RED requires the use of personal protective equipment, waivers have been granted for applications of dilute solutions of some formulations. Thus, exposure estimates are made both with and without PPE. Worker exposures are estimated at about 0.003 (0.0013 to 0.0066) mg/kg body weight for workers not using PPE and 0.0003 (0.00012 to 0.00066) mg/kg body weight for workers who do use PPE. While the exposure methods used in this risk assessment differ from the approach taken by the U.S. EPA, which bases worker exposures on deposition data from ground application methods judged to be analogous to aquatic applications, the worker exposure rates used in the current risk assessment are similar to those used by the U.S. EPA in terms of the resulting hazard quotients. This detail is discussed further in the risk characterization for workers.

The major uncertainty in the exposure assessment for members of the general public involves the plausibility of any of the exposure scenarios. The U.S. EPA RED requires that access by members of the general public to treated sites be restricted. Along with the recommended use of potassium permanganate to detoxify rotenone, the restrictions on public access suggest that exposures to members of the general public will be minimal. Thus, all of the exposures developed for members of the general public should be regarded as extreme. As discussed further in the risk characterization, the non-accidental exposure of greatest concern involves the consumption of treated water by a small child for which the estimated dose is about 0.019 (0.011 to 0.028) mg/kg bw/day. This exposure and other exposures for the general public would occur only if the restrictions imposed by the U.S. EPA on the application of rotenone were not properly enforced.

Dose-Response Assessment for Human Health

Generally, the dose-response assessments used in Forest Service risk assessments adopt RfDs proposed by the U.S. EPA as indices of acceptable exposure. An RfD is basically defined as a level of daily exposure that will not result in any adverse effects in any individual over a specified period of time. The RfDs developed by the U.S. EPA are typically used directly in Forest Service risk assessments because the EPA RfDs generally provide a level of analysis, review, and resources that far exceed those that are or can be conducted in support of most Forest Service risk assessments. In addition, it is desirable for different agencies and organizations within the federal government to use concordant risk assessment values.

The current Forest Service risk assessment uses the most recent and the most conservative RfDs derived by the U.S. EPA. Specifically, this risk assessment adopts the acute RfD of 0.015 mg/kg bw/day and the chronic RfD of 0.0004 mg/kg bw/day derived in the recent Reregistration Eligibility Document prepared by the U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP 2007a). The acute RfD is based on a NOAEL of 15 mg/kg bw/day in mice from a developmental toxicity study. The chronic RfD is based on a lifetime dietary study with a dietary NOAEL of 7.5 ppm, equivalent to a daily dose of 0.0375 mg/kg bw/day. An uncertainty factor of 1000 is used with both of these NOAELs to derive the corresponding RfDs. The uncertainty factor of 1000 is generated by multiplying together separate factors of 10 for each of three factors considered as contributing to uncertainty: inter-species variability, intra-species variability, and uncertainties in the available data on rotenone. The factor for uncertainties in the available data reflects concern for the potential of rotenone to cause essentially permanent neurotoxic damage in pre-natal or early post-natal exposures, which might not induce observable adverse effects until late in life.

Dose-severity relationships for rotenone appear to be pronounced, particularly with respect to acute exposures. In the animal study on which the acute RfD is based, the ratio of the LOAEL to the NOAEL is only 1.6, which might suggest that a hazard quotient of 1.6 is associated with adverse effects, specifically fetal absorptions. Given the rather large uncertainty factor used to derive the RfD, however, this interpretation may be grossly conservative. Based on the acute lethal potency of rotenone confirmed in the available data on both experimental mammals and humans, acute hazard quotients of about 400 or less are not likely to be associated with potentially lethal effects. Information on acute lethal potency, however, is not useful in characterizing most of the non-accidental hazard quotients of concern, which only modestly exceed the RfD.

Risk Characterization for Human Health Effects

The risk characterization for rotenone is relatively simple and focuses on risks to workers. As with the exposure assessment, all hazard quotients are based on an application of CFT Legumine, at a target concentration of 0.2 ppm using a toxic equivalency factor of 1.25. Other formulations of rotenone – i.e., those formulations containing piperonyl butoxide – have toxic equivalency factor of up to 2.5 and this difference would lead to hazard quotients twice as high as those discussed below.

The recent RED prepared by the U.S. EPA's Office of Pesticide Programs requires that workers involved in the application of rotenone use personal protective equipment (PPE). If the specific PPE requirements outlined in the RED are implemented, only the upper bound hazard quotient at the maximum application rate exceeds the level of concern (HQ=1.7). If effective PPE is not used, hazard quotients exceed the level of concern; moreover, at the highest application rate, the upper bound of the hazard quotient is 17. While hazard quotient of 17 might not be associated with frank adverse effects, it would clearly amount to a highly imprudent exposure. The accidental exposure scenarios for workers result in HQ values that substantially exceed the level of concern, reaching an upper bound of 612. These accidental exposure scenarios are included in all Forest

Service risk assessments to evaluate the importance of proper handling of pesticides. For rotenone, it is apparent that aggressive steps are warranted in the event of accidental exposures or mishandling.

The risk quotients for members of the general public are similar to those for workers. At the maximum application rate of 0.2 ppm, the maximum acute hazard quotient for non-accidental scenarios is 1.9. The highest longer-term hazard quotient is 3. Both of these hazard quotients are associated with the consumption of contaminated water. In most Forest Service risk assessments, this exposure scenario is viewed as an *expected exposure*; however, this is not the case for rotenone. Owing to restrictions governing the access of the general public to treated sites during treatment and prior to detoxification with potassium permanganate, exposures for members of the general public are not expected to be significant.

Groups that may be at increased risk to rotenone exposures include women of child-bearing age and individuals with Parkinson's disease and perhaps other neurological disorders. While potassium permanganate is considered as a connected action, the use of potassium permanganate will mitigate several exposure scenarios that would otherwise be of concern, including exposures involving sensitive subgroups.

Ecological Risk Assessment

Hazard Identification

Since the use of rotenone covered in this risk assessment involves direct applications to surface waters, aquatic organisms are an obvious concern to the hazard identification for ecological effects. The hazard identification and even the risk characterization for fish is virtually a tautology: rotenone is a piscicide, and, if rotenone is applied at effective concentrations, fish will die. Not all fish, however, are equally sensitive to rotenone. The more sensitive species of fish, such as trout and bluegills, are likely to be killed by rotenone treatments at the lower bound of labeled application rates—i.e., from 5 to 7 ppb. Even the most tolerant species of fish are likely to be killed at the upper bound of the labeled application rate—i.e., 200 ppb. Because rotenone treatments typically last for only about 6 hours prior to detoxification with potassium permanganate, concentration-duration relationships are important. For fish, the temporal relationships indicate that 6-hour LC_{50} values are only a factor of 2-3 above the 96-hour hour LC_{50} values. As is true for mammalian exposure, concentration-response relationships for rotenone appear to be quite steep—i.e., the LC_{50} may not be much lower than the concentration that will cause 100% mortality in fish and may not be much higher than the concentration that will cause 0% mortality in fish.

Some aquatic invertebrates may also be adversely affected by rotenone applications at the labeled rates, and this is amply demonstrated in field studies. Aquatic invertebrates, however, have a much broader range of tolerances to rotenone than do fish. While the range of LC_{50} values among different fish species is about a factor of 40, the corresponding range in aquatic invertebrates spans a factor of about 10,000. The most sensitive group of invertebrates, small aquatic arthropods, are about as sensitive as the most sensitive fish species. Based on the available LC_{50} values, snails comprise the least

sensitive group of invertebrates and are more tolerant than fish to the toxicity of rotenone by factors of up to 1000. While the effects of rotenone on aquatic vegetation have not been studied extensively, aquatic plants appear to be insensitive to rotenone.

While the focus of the current risk assessment is on the toxicity of rotenone to aquatic organisms, potential risks to mammals and birds are considered quantitatively. In addition, information on terrestrial plants is useful in interpreting some of the data on aquatic plants. In the U.S. EPA ecological risk assessment (U.S. EPA/OPP 2006c), rotenone is classified as highly toxic to mammals, only slightly toxic to birds, and practically nontoxic to honeybees. The classification for mammals is clearly appropriate and consistent with the information detailed in the HHRA for the current Forest Service risk assessment.

The classification of rotenone as only slightly toxic to birds is consistent with the data considered in the EPA ecological risk assessment—i.e., LD_{50} values of 2200 and 1680 mg/kg body weight, respectively, for mallard ducks and pheasants. Additional information from the early study by Cutkomp (1943), however, suggests that other species of birds, particularly small birds, may be much more sensitive to rotenone exposure than are ducks, pheasants, and some other species. Based on relatively standard bioassays, the most sensitive species identified in the work by Cutkomp (1943) is the Eastern chipping sparrow for which the LD_{50} is 113 mg/kg body weight. Based on an atypical bioassay in which rotenone was administered to Eastern robins in prey items, doses of 25 mg/kg body weight and greater were lethal. The dose of 25 mg/kg body weight is somewhat lower than the dose of 30 mg/kg body weight used by the EPA to classify rotenone as highly toxic to mammals. Thus, there is some uncertainty in the hazard identification for birds; nonetheless, it seems plausible that some species of small birds may be sensitive to rotenone toxicity.

Similarly, the toxicity of rotenone to insects appears to be variable. Honeybees are relatively tolerant; however, other terrestrial insects (e.g., moths) may be more sensitive. Terrestrial plants are insensitive to rotenone, and the biochemical basis for this lack of sensitivity seems related to the presence of a NADH/NADPH dehydrogenase in plants that is insensitive to rotenone and that differs from the sensitive NADH/NADPH dehydrogenase found in animals.

Exposure Assessment for Ecological Risk Assessment

The exposure assessments for the ecological risk assessment generally parallel those used for the general public in the human health risk assessment. In other words, the exposure scenarios are similar in the basic assumptions concerning the application of rotenone. Differences in the estimated doses from those in the human health risk assessment are attributable to differences in body size and consumption rates for food or water. Also, as in the human health risk assessment, the exposure scenarios for terrestrial vertebrates are a subset of those used in most Forest Service risk assessments. Some exposure scenarios, such as the consumption of terrestrial vegetation, are not relevant to aquatic applications of rotenone. Lastly, all exposure assessments are based on the application of a liquid formulation, CFT Legumine, at a target concentration of 0.2 ppm (the maximum

application rate) and all exposures are based on rotenone equivalents that consider joint exposures to rotenone and other related rotenoids in CFT Legumine.

The exposure scenarios for terrestrial wildlife are summarized in Worksheet G01 of the EXCEL workbook that accompanies this risk assessment. The highest exposure scenarios involve the accidental spill of 200 gallons of a field solution into a small pond. The estimated doses for birds and mammals cover a relatively narrow range: about 1.25 to 13 mg/kg body weight. The expected non-accidental acute exposures are much lower, spanning a range from about 0.04 to 0.07 mg/kg body weight. Because rotenone will be detoxified with potassium permanganate, longer-term exposures are implausible. Nonetheless, longer-term exposures are estimated to assess the consequences of not using potassium permanganate. The range of the expected doses in the longer-term exposure scenarios for the consumption of contaminated water is very low: 0.0003 to about 0.01 mg/kg body weight/day. The longer-term consumption of contaminated fish by a fish-eating bird is much higher, ranging from 0.003 mg/kg bw/day to about 0.17 mg/kg bw/day.

Exposure of aquatic organisms to rotenone is taken as the nominal application rate or target concentration. In the EXCEL workbook that accompanies this risk assessment, the maximum application rate of 200 ppb is used. Using the toxic equivalency factor of 1.5 for CFT Legumine, maximum application rate of 200 ppb (rotenone) corresponds to 300 ppb rotenone equivalents. The consequences of using lower application rates are considered in the risk characterization.

Dose-Response for Ecological Risk Assessment

The specific toxicity values used in this risk assessment are summarized in Table 12, and the derivation of each of these values is discussed in the various subsections of the dose-response assessment (Section 4.3). The available toxicity data as well as the plausible exposure scenarios support separate dose-response assessments in five groups of organisms: terrestrial mammals, birds, fish, amphibians, and aquatic invertebrates. Different units of exposure are used for different groups of organisms, depending on how exposures are likely to occur and how the available toxicity data are expressed. Unlike the human health risk assessment, the toxicity values used in the ecological risk assessment involve different endpoints for different groups of organisms and different durations of exposure. These differences are necessitated by the nature of the available data on the different groups of organisms.

For terrestrial mammals, the toxicity endpoints correspond to the NOAEL values used in the human health risk assessment for the derivation of the acute and chronic RfDs—i.e., an acute NOAEL of 15 mg/kg body weight and a chronic NOAEL of 0.375 mg/kg body weight/day. Data on birds are highly variable, and a clear acute NOAEL cannot be defined. Consequently, a conservative but plausible LD_{50} of 113 mg/kg body weight is used to characterize acute risks in birds. Since chronic studies in birds are not available, the acute NOAEL in mammals is used to characterize chronic risks to birds.

The toxicity values used for aquatic species reflect the range of species sensitivity distributions detailed in the hazard identification for aquatic species. For fish as well as other aquatic organisms, the acute endpoints used for the dose-response assessment for aquatic organisms all involve LC_{50} values. While this approach is not preferred in most Forest Service risk assessments, it is used for rotenone because lethality best reflects the likely outcome of rotenone applications and because most of the available acute toxicity data on rotenone involve LC_{50} determinations. Risks associated with longer-term exposures are based on NOEC values for sensitive species, however, relative potency methods based on acute toxicity are used to estimate longer-term NOEC values for tolerant species.

Risk Characterization for Ecological Risk Assessment

Rotenone is an effective piscicide that is likely to kill fish when applied to surface waters at labeled application rates. There are differences in sensitivity among fish species, and these differences span a factor of about 40. Treatments with any formulations at the upper bound of the application rates for rotenone—i.e., 200 ppb—are likely to kill all but the most tolerant species of fish. Rotenone formulations containing piperonyl butoxide are likely to kill all species of fish, even the most tolerant. Rotenone can be viewed as a selective piscicide rather than a general aquatic biocide in that fish are more sensitive to rotenone than are most other aquatic organisms, with the exception of some species of zooplankton and small insects. Thus, while rotenone applications to surface water are expected to kill some invertebrates, extensive mortality due to the toxicity of rotenone among species of larger invertebrates is not expected. Despite the observation of secondary effects on aquatic plants, rotenone applications are not likely to directly affect aquatic plants. Depending on how secondary effects are measured, changes in the community structure of surface waters may persist for a prolonged period of time.

There is no basis for asserting that rotenone is likely to have a direct toxic effect on terrestrial organisms. Secondary effects are likely to occur in animals that consume fish as a substantial proportion of their diet. These changes, however, are likely to be transient.

1. INTRODUCTION

This document provides risk assessments for human health effects and ecological effects to support an assessment of the environmental consequences of using rotenone as a piscicide (an agent for killing unwanted species of fish) in Forest Service programs. Although rotenone had been used as an insecticide in some domestic agricultural applications, all non-piscicide uses of rotenone have been cancelled (U.S. EPA/OPP 2007a) and the Forest Service has and will use rotenone only as a piscicide.

Like other Forest Service risk assessments, this document has four chapters: the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with rotenone and its commercial formulations, an assessment of potential exposure, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2007a).

The series of human health and ecological risk assessments prepared for the USDA Forest Service are not, and are not intended to be, comprehensive summaries of all of the available information. Rotenone has been used as a commercial insecticide and piscicide for over 50 years and the open literature on rotenone is substantial.

In addition to standard literature searches of TOXLINE and AGRICOLA, this risk assessment considers the available reviews on rotenone. Much of the early literature on rotenone has been reviewed by Haley (1978) and additional reviews are available from the Extension Toxicology Network (EXTOXNET 1996), Hinson (2000), Mackenthun and Keup (1969), Ott (2008), and WHO (1990, 1992). Additional reviews on the use of rotenone to control unwanted species of fish have also been consulted (Cailteux et al. 2001; Entrix 2007; Finlayson et al. 2000; Ling 2003; Marking 1992; MSU 2006; Rotenone Stewardship Program 2008; Turner et al. 2007). These reviews have been used primarily to identify the primary literature. In addition to toxicity studies that are relatively standard for pesticides, there is a large body of literature available on the neurotoxicity of rotenone with particular emphasis on the use of rotenone as an animal model for Parkinson's disease. This literature has been extensively reviewed (e.g., Drechsel and Patel 2008; Gomez et al. 2007; Greenamyre et al. 2003; Hirsch et al. 2003; Hoglinger et al. 2006; Jenner 2001; Orr et al. 2002; Perier et al. 2003; Trojanowski 2003; Uversky 2004) and the relevance of this literature to the current risk assessment is addressed in Section 3.1.6 (Neurotoxicity).

The U.S. EPA's Office of Pesticide Programs has recently released the Registration Eligibility Decision (RED) for Rotenone (U.S. EPA/OPP 2007a). The RED is accompanied by a large number of supporting assessments prepared by the U.S. EPA as well as comments on these assessments submitted by rotenone suppliers, users of rotenone, and other interested parties. These documents (a total of 85) are available at the U.S. EPA's E-Docket for rotenone (http://www.regulations.gov, Docket Number EPA-HQ-OPP-2005-0494). In the preparation of this risk assessment, materials at the E-Docket have been reviewed and the relevant documents (listed in Section 5) from the E-Docket are considered.

The material in the EPA's E-Docket focus on the unpublished studies submitted to the U.S. EPA in support of the reregistration of rotenone. These studies are treated by the U.S. EPA as confidential business information (CBI), and full copies of these studies were not available for the current risk assessment. The key information from these studies, however, is summarized in the E-Docket.

In addition to information published in the open literature and available in the U.S. EPA E-Docket, a substantial amount of information on rotenone is available on the Internet – e.g., about 7-million hits at http://www.google.com/. For the most part, however, data derived from the Internet is not used unless the information is well documented. The most useful database for the risk assessment is the ECOTOX database compiled and reviewed by the U.S. EPA (U.S. EPA/ORD 2008). ECOTOX is also the main ecotoxicity database used by the Pesticide Action Network (PAN 2007). ECOTOX contains over 900 records on rotenone from over 100 citations. This information was screened and incorporated into the current risk assessment.

The Forest Service will update this and other similar risk assessments on a periodic basis and welcomes input from the general public on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

Almost no risk estimates presented in this document are given as single numbers. Usually, risk is expressed as a central estimate and a range, which is sometimes quite large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations, most of which are relatively simple. They are included in the body of the document.

Some of the calculations, however, are cumbersome. For those calculations, an EXCEL workbook, consisting of a set of worksheets, is included as an attachment to the risk assessment. The worksheets provide the detail for the estimates cited in the body of this document. SERA (2007b) provides documentation on the use of the EXCEL workbook.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

Rotenone is a pesticide that is used to kill fish – i.e., a piscicide. Specifically, rotenone is used in Forest Service programs to eliminate unwanted or invasive species of fish in order to maintain suitable habitats for native and/or game fish species. Unlike many pesticides, rotenone is not synthesized commercially. Instead, rotenone and related compounds are extracted from the roots or other tissue of plants that naturally produce the compound. At the cellular level, rotenone acts by interfering with energy production. Both liquid and granular formulations of rotenone may be used in Forest Service programs. Some liquid formulations contain piperonyl butoxide, a compound that will inhibit the metabolism of rotenone and related compounds and hence will increase the toxicity of the formulation to fish. Granular formulations are essentially ground or pulverized preparations of the roots of the plants that produce rotenone. Rotenone is also available in bait pellet formulations but these are not used in Forest Service programs and are not considered in the current risk assessment.

Rotenone is also different from many other pesticides in that application rates are expressed as target concentrations in water rather than as lbs a.i./acre. For standing bodies of water such as ponds or lakes, application rates for rotenone range from 0.005 ppm (mg/L) to 0.2 ppm. For flowing water such as streams or rivers, the application rates range from 0.025 ppm to 0.1 ppm. While application rates are expressed as target concentrations, applications to standing water will involve calculations of the number of pounds of a formulation that must be applied to the water body depending on the depth of the water body. For streams, the applications are typically calculated as the amount of formulation that must be added to the stream per unit time depending on the flow rate of the stream. All of the product labels provide tables and equations for converting target concentrations to field application rates – i.e., lbs formulation per surface area of water of lbs formulation per unit time.

Rotenone is not very persistent in water and field dissipation half-lives are reported in the range of less than one day to about 10 days. The rapid dissipation in water does not have a substantial impact on the effectiveness of rotenone because rotenone can kill fish very rapidly. The product labels recommend that rotenone concentrations should be kept in the lethal range for at least 2 hours. Recommendations for detoxification periods prior to restocking are given on the product labels and range from 2 to 4 weeks. Alternatively, potassium permanganate can be used to break down (i.e., oxidize) rotenone very quickly.

The amount of rotenone that might be used by the Forest Service in a given year cannot be estimated with precision and rotenone use is likely to vary with outbreaks of pest fish populations. The total use of rotenone in the United States has been estimated at about 20,000 pounds per year. Based on this total use estimate and admittedly sparse use statistics from the Forest Service, it seems likely that the use of rotenone as a piscicide in Forest Service programs will be minor compared the total use of rotenone as a piscicide by other organizations.

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

Rotenone is a naturally occurring chemical produced by various tropical plants such as the jewel vine (*Derris* spp.) and lacepod (*Lonchocarpus* spp). *Derris* is native to eastern Asia and the East Indies (Brooks and Price 1961) and the piscicidal and insecticidal properties of Derris root had been noted by the Chinese and Asian-Pacific cultures for centuries (Philippine Department of Agriculture 2006). While rotenone had been registered as an insecticide in the United States, all non-piscicidal uses of rotenone have been cancelled as of 2006 (U.S. EPA/OPP 2007a). Rotenone has been used as a piscicide in the United States and Canada since the mid-1930s (Lennon 1970) and has been registered as a piscicide in the United States since 1947 (U.S. EPA/OPP 2006c).

The biochemical mechanism of action of rotenone involves inference with the normal function of mitochondria, structures within cells that are involved in energy production. Specifically, rotenone inhibits electron transport of a mitochondrial component that effectively blocks the ability of the cell to store energy from the metabolism of nutrients – i.e., rotenone inhibits electron transport at NADH-ubiquinone oxidoreductase effectively uncoupling oxidative phosphorylation (Finlayson et al. 2000; Tomlin 2004; U.S. EPA/OPP 2006c).

The chemical structure of rotenone and related compounds is given in Figure 1 and a summary of the chemical and physical properties of rotenone is given in Table 1. Unlike most pesticides, rotenone is not synthesized in the manufacturing process. Instead, rotenone and related compounds are extracted from the roots or other tissue of plants that naturally produce the compound. The extraction process results in a material that contains both rotenone and other structurally related compounds, variously referred to as resins, extracts, and/or rotenoids. Thus, commercial formulations of rotenone express the content of the active ingredients as two separate percentages; that of rotenone as well as that of other resin extracts or rotenoids (Table 2).

The registered end-use formulations for Prentiss Incorporated and Foreign Domestic Chemicals are summarized in Table 2. Three suppliers of end-use formulations of rotenone piscicides are identified in the U.S. EPA RED (U.S. EPA/OPP 2007a): Prentiss Incorporated, Foreign Domestic Chemicals Corporation, and TIFA International LLC. Based on the labels database maintained by the U.S. EPA (http://oaspub.epa.gov/pestlabl/), Prentiss provides four liquid formulations, two powder formulations, and two pellet formulations and Foreign Domestic Chemicals Corporation provides one powder formulation. The EPA label web site also lists five formulations for TIFA International and three of which are end-use formulations: Chem Fish Regular, Chem Fish Synergized, and Cube Powder Fish Toxicant. While these formulations are listed at http://oaspub.epa.gov/pestlabl, this site does not contain copies of the product labels (as of February 15, 2008). In the conduct of this risk assessment, TIFA International was contacted and kindly provided copies of the relevant product labels and MSDSs (Cerciello 2008a).

One additional formulation of rotenone has been identified: CTF Legumine. This formulation is not discussed in the recent RED on rotenone (U.S. EPA/OP 2007a)

although the label for CTF Legumine is currently available at the EPA label web site. While the product is provided by CWE Properties, the distribution is done in collaboration with Prentiss and product labels and the MSDS for CTF Legumine are available at the Prentiss web site (http://www.prentiss.com/).

Two of the Prentiss formulations that are listed at the U.S. EPA label web site – i.e., Noxfish Fish Toxicant and Nusyn-Noxfish Fish Toxicant – are not included in the Prentiss web site. In terms of active ingredients, these two formulations are identical to CTF Legumine and Synpren-Fish Toxicant, respectively, both of which are listed at the Prentiss web site. It is not clear that Prentiss is still supplying Noxfish Fish Toxicant and Nusyn-Noxfish Fish Toxicant and these products may have been replaced with CTF Legumine and Synpren-Fish Toxicant, respectively.

In discussing the registered formulations of rotenone piscicides, the U.S. EPA identifies three active ingredients in rotenone formulations: rotenone itself, Derris resins other than rotenone, and cube resins other than rotenone (U.S. EPA/OP 2007a, p. 8). As summarized in Table 2, three liquid formulations – i.e., Nusyn-Noxfish Fish Toxicant, Synpren-Fish Toxicant, and Chem Fish Synergized – also list piperonyl butoxide as an active ingredient. As detailed further in Section 3.1.14 (Inerts and Adjuvants), piperonyl butoxide is an inhibitor of mixed-function oxidase, an enzyme system involved in the detoxification of rotenone. In rotenone formulations, piperonyl butoxide enhances the toxicity of rotenone by decreasing the rate of the metabolism/detoxification of rotenone (Section 3.1.3. Pharmacokinetics and Metabolism). In this respect, piperonyl butoxide may be regarded as an adjuvant.

Based on the information in the available MSDSs, the listed *Inerts* in rotenone formulations are summarized in Table 3. The term *Inerts* is used to concisely identify materials in the formulations that are not considered as active ingredients. As discussed below and detailed further in Section 3.1.14 (Inerts and Adjuvants), some of the listed inerts are potentially hazardous.

The granular and pellet formulations of rotenone contain no listed inerts. As discussed by Finlayson et al. (2000, p. 187), the powder formulations are made from ground plant-roots. While these formulations may contain fillers, no materials of concern appear to be added to the powder formulations.

Liquid formulations do contain inerts that must be listed on the MSDSs because the inerts are classified as toxic by one or more criterion. As summarized in Table 3, CTF Legumine, Synpren-Fish Toxicant, Prenfish Toxicant, Chem Fish Regular, and Chem Fish Synergized, all contain petroleum distillates.

Petroleum distillates are highly diverse mixtures of aromatic and aliphatic hydrocarbons and the specific blend of aromatic and aliphatic hydrocarbons will vary depending on the distillation and refining methods (e.g., Potter and Simmons 1998). The MSDSs for formulations supplied by TIFA (Chem Fish Regular and ChemFish Synergized) indicated only that the formulations contain variable mixtures of aromatic petroleum solvents. The

MSDS for Synpren-Fish Toxicant indicates that the formulation contains xylene class aromatics that have a somewhat lower molecular weight than the solvents contained in Prenfish Toxicant – i.e., naphthalenes and trimethylbenzene. CTF Legumine also contains petroleum distillates but no specific aromatics are identified on the MSDS for this formulation. This is consistent with promotional material on the Prentiss web site (http://www.prentiss.com/news.htm) indicating that CTF Legumine is a formulation with reduced concentrations of toluene, xylene, benzene and naphthalene. A reduction in aromatic hydrocarbons in CTF Legumine is also suggested in the product labels. A label for CTF Legumine approved for conditional use with an EPA approval date of April 23, 2003, indicates that the formulation contains aromatic hydrocarbons. An EPA approved label (without the conditional use qualifier) for August 9, 2007, however, indicates only that the formulation contains petroleum distillates. This does not offer assurance that all aromatics have been removed from CTF Legumine but it does suggest that the aromatics have been reduced to levels that are lower than those in the previous conditional use formulation.

In addition to the petroleum distillates that are intentionally added to the rotenone formulations, some liquid formulations of rotenone have been found to contain trichloroethylene (TCE). TCE is a commonly used extraction solvent (ATSDR 1997). While information on the solvent extraction processes used in preparing liquid formulations is not publically available – i.e., the processes are considered proprietary – the occurrence of TCE in some liquid formulations of rotenone suggests that TCE is used to extract rotenone from plant material. Nusyn-Noxfish has been reported contain TCE at concentrations of 10 to 1200 ppm or 0.001% to 0.12% (Finlayson et al. 2000, p. 112). TCE is a concern because this chemical is classified as a carcinogen, as discussed further in Section 3.1.14 (Inerts and Adjuvants).

2.3. APPLICATION METHODS

Rotenone may be applied directly to standing (lentic) bodies of water – e.g., ponds or lakes – as well as to flowing (lotic) bodies of water – e.g., rivers or streams. Either surface or subsurface applications may be made. The standard apparatus for making rotenone applications is not specified on the product labels but a very detailed discussion of application procedures and application equipment is provided in Chapter 3 (Technical Procedures) of Finlayson et al. (2000).

The product labels recommend that rotenone concentrations should be kept in the lethal range for at least 2 hours. Factors impacting the concentration/duration relationships for rotenone are discussed further in Section 4.3.3.1 (Toxicity to Fish).

After rotenone treatment, the product labels recommend a detoxification period of about 2 to 4 weeks. Alternatively, the water can be treated with chlorine or potassium permanganate (e.g. Cohen et al. 1960; Hockin et al. 1985; Mahon and Balon 1980). In the recent U.S. EPA reregistration eligibility document (RED), the Agency is requiring the use of potassium permanganate to detoxify residual concentrations of rotenone (U.S. EPA/OPP 2007a, p. 32). The use of potassium permanganate is addressed further in Section 3.1.16.2 of the current Forest Service risk assessment.

As detailed in the following section, the application rates for rotenone are specified as nominal concentrations of rotenone in water. Rotenone treated water will have a detectable taste and odor. The product labels suggest that treatment with activated charcoal can be used to remove the taste and odor. While not detailed on the product labels, the high K_{ow} for rotenone (about 14,000) suggests that rotenone will bind to activated carbon.

All product labels specify that surface water within ½ mile of a potable water intake or irrigation intake should not be treated with rotenone. The current product labels indicate that swimming is prohibited during treatment. As discussed further in Section 3.2.3 (Exposure Assessment for the General Public), the U.S. EPA/OPP (2007a, p. 32) has recommended additional post-application restrictions on swimming.

As noted in Table 2, two pellet formulations of rotenone are available, Grass Carp Management Bait and Common Carp Management Bait. Unlike the liquid and powder formulations, the bait formulations are designed for target/pest fish that can be trained to consume food at a specific location and specific period of time. The *application* method involves feeding a training bait (which does not contain rotenone) to carp until a large proportion of the population is habituated to feeding at the designated location and at the designated time. The treatment then involves feeding the fish the pellet formulation that contains rotenone with the expectation that the target population will be killed. This method of application appears to have the potential to be somewhat selective and bait feeding is used in some programs conducted in New Zealand (Gehrke 2003; Ling 2003; Row 2001). Bait feeding, however, does not appear to be used widely in the United States and this application method is not discussed in the otherwise detailed and comprehensive guidelines for the use of rotenone in fisheries management in the United States (Finlayson et al. 2000). Thus, bait feeding is not considered further in this risk assessment.

2.4. MIXING AND APPLICATION RATES

2.4.1. General Considerations

As summarized in Table 4, labeled application rates for rotenone are expressed as target concentrations in units of parts per million (ppm or mg/L) and the recommended application rates expressed as concentrations of rotenone range from 0.005 ppm (the lower bound of the range for selective treatments) to 0.25 ppm (the upper bound of the range for preimpoundment treatments above a dam). The application rates are identical on all rotenone labels for both liquid and powder formulations. In the recent RED, however, the U.S. EPA has lowered the maximum application rate from 0.25 ppm to 0.2 ppm (U.S. EPA/OPP 2007a, p. 19) and 0.2 ppm (200 ppb) is the maximum application rate considered in this current Forest Service risk assessment.

While all of the product labels list and give percentages for both rotenone and related resins as active ingredients, only the concentration of rotenone is used for calculating application rates. Similarly, for those formulations that contain piperonyl butoxide,

which is also listed as an active ingredient, only the concentration of rotenone is used to calculate application rates.

Liquid formulations of rotenone can be applied either diluted or undiluted. In slower moving streams or ponds, hand sprayers can be used with a 10% (w/w) aqueous dilution of the formulation. For more rapidly flowing streams, liquid formulations of rotenone can be applied as a drip for 4 to 8 hours.

Powder formulations can be applied in the same manner as liquid formulations after mixing the powder with water at a rate of one pound formulation per 3 to 10 gallons of water. No solvents or emulsifiers are recommended for use with powder formulations. Some powder formulations indicate that the formulations can be placed in a burlap sack and dragged behind a boat. This method would presumably apply only to standing bodies of water, although this is not specified on the product labels.

Computational details differ in the application of liquid and powder formulations to lentic bodies of water (e.g., ponds and lakes) and lotic bodies of water (e.g., streams and rivers) as discussed in the following four subsections. All of the product labels provide tables and equations for converting target concentrations to field application rates.

The specific methods used in generating the tables and equations on the product labels are not detailed in the product labels. In the preparation of this risk assessment, the tables and equations were reviewed and some inconsistencies as well as some apparent errors were noted. Some of the inconsistencies may be due to simple rounding errors and rounding errors are inherent in the types of calculations that are required. For example, the discussion below uses a conversion factor of 1,233,531.5 liters per acre-foot based on the conversion of acre-feet to gallons and gallons to acre-feet from Budavari (1989). Other methods of conversion will lead to slightly different results. The exact value for the metric conversion of 1 acre-foot is reported to be 1,233,481.8376 liters at http://online.unitconverterpro.com/. These very small differences, however, are insignificant.

The purpose of the following discussion is simply to provide a transparent explication of methods that can be used to calculate field application rates from target concentrations. Some discrepancies between the calculations presented below and the directions on the product labels are minor and may reflect simple rounding errors. Other discrepancies are more substantial and these appear to reflect errors in the product labels.

2.4.2. Liquid Formulations in Ponds and Lakes

For applications to standing bodies of water (i.e., ponds or lakes), all rotenone product labels for liquid formulations provide tables indicating the number of acre-feet covered by one gallon of formulation for a given target application rate. An acre-foot is a unit of volume equivalent to a one acre surface area that is one foot deep – i.e., 43,560 ft^3 which is equivalent to 325,900 gallons or 1,233,531.5 liters at 3.785 liters/gallon (Budavari 1989).

In the preparation of this risk assessment, the calculations given in these tables on the product labels were checked and discrepancies were noted. For example, the product label for CFT Legumine indicates that 1 gallon of CFT Legumine will cover 30 acre-feet at a target concentration expressed as rotenone of 0.005 ppm (i.e., 0.005 mg a.i./L).

The most direct way to check this calculation is to calculate the concentration of rotenone that would be reached in treating 1 acre-foot of water with one gallon of the formulation. This can be readily calculated from the density of the formulation given on the MSDS (8.506 lbs/gallon for CFT Legumine) and the proportion w/w of rotenone in the formulation (0.05 for CFT Legumine):

1 gallon x 8.506 lbs/gallon x 0.05 a.i. x 453,592.27 mg/pound /1,233,531.5 L ≈ 0.1564 mg a.i./L

Taking 0.1564 mg/L and dividing by the target concentration of 0.005 mg/L, this calculation indicates that one gallon of CFT Legumine would cover about 31.28 acre-feet [0.1564 mg a.i./L / 0.005 mg a.i./L], higher than the acre-feet indicated on the label by about 4% [31.28 acre-feet / 30 acre-feet = 1.0426].

The other values on the product label for the number of acre-feet covered at different target concentrations show identical discrepancies except for the value of 24 acre-feet at a target concentration of 0.007 ppm. Taking the concentration of 0.1564 mg a.i./L for one gallon added to one acre-foot of water, one gallon of the formulation would cover somewhat more than 22 acre-feet [0.1564 mg a.i. / 0.007 mg a.i./L = 22.34 acre-feet]. In this instance, the tabulated value on the label is lower than the calculated value by about 7% [22.34 acre-feet / 24 acre-feet = 0.9309]. While these discrepancies may be due partially to differences in rounding, variations of 4% to 7% are not trivial.

The Forest Service will follow label directions in making pesticide applications. Worksheet A01 of the EXCEL workbook that accompanies this risk assessment calculates the amount of formulation that would need to be applied to a body of water of a specified volume or flow rate using the information on the rotenone formulation – i.e., density of the formulation (lbs formulation/gallon) and the proportion (w/w) of rotenone in the formulation – rather than adopting the tables from the product labels.

The volume of the formulation in gallons is calculated as follows. By definition, the target concentration (***TC*** in mg a.i./L or ppm) is the amount of rotenone applied (in mg) divided by the volume of water in liters. Using common field units of measure and the appropriate conversion factors, the target concentration can be calculated as:

Equation 1

$$TC_{mga.i./L} = \frac{Gal_{Form} \times BD_{lb/gal} \times P_{a.i./Form} \times 453{,}592.27_{mg/lb}}{SA_{Acres} \times Dep_{ft} \times 1{,}233{,}531.5_{Liters/acrefoot}}$$

where

Gal_{Form} gallons of formulation required to reach the target concentration

BD bulk density of the formulation in pounds per gallon

P	the proportion (w/w) of rotenone in the formulation
SA	surface area of the water in acres
Dep	average depth of the water in feet
453,592.27	a constant for the number of milligrams in a pound
1,233,531.5	a constant for the number of liters in an acre-foot

By simple rearrangement of Equation 1, the number of gallons of formulation required to reach a given target concentration for a water body of a defined volume can be calculated as:

Equation 2

$$Gal_{Form} = \frac{TC_{mga.i./L} \times SA_{Acres} \times Dep_{ft} \times 1{,}233{,}531.5_{Liters/acrefoot}}{BD_{lb/gal} \times P_{a.i./Form} \times 453{,}592.27_{mg/lb}}$$

As discussed above, values generated by this equation, while mathematically correct, may differ from calculations based on adjustments to label directions by factors of up to 7%, depending on how the adjustments to the label directions are made.

As noted above, Equation 2 requires information on the bulk density of the liquid formulation – i.e., pounds formulation per gallon of formulation. Bulk density is typically indicated on the MSDS for a formulation. The bulk density is not included on the MSDS for Chem Fish Regular or Chem Fish Synergized (both formulations from TIFA). This information, however, has been provided by TIFA (Cerciello 2008b). MSDSs have not been located for two liquid formulations from Prentiss that appear to have active registrations – i.e., Noxfish Fish Toxicant and Nusyn-Noxfish Fish Toxicant (both formulations from Prentiss). As noted above, however, it is not clear that these formulations are still being marketed. While all of the liquid formulations listed in Table 2 are similar in that all formulations consist primarily of petroleum distillates (Table 3), the bulk densities that are reported range from 7.3 lbs/gallon to 8.506 lbs/gallon, differing by a factor of over 16% [8.506 / 7.3 = 1.1652]. Thus, it would not be appropriate to apply Equation 2 without information on the bulk density of the formulation that is being used.

2.4.3. Liquid Formulations in Streams and Rivers

As noted above, liquid formulations of rotenone are applied as a drip to streams or rivers for periods of 4 to 8 hours. The product labels for liquid formulations from Prentiss provide an equation for calculating the rate of drip for the formulation to the flowing body of water. On the product labels, this rate is designated as ***X***, the *application rate for the stream,* and the rate is expressed in units of cubic centimeters (cc) per minute. The general form of the algorithm is:

Equation 3

$$\boldsymbol{X = F\ C\ B}$$

Where the terms are defined as:

X	application rate for the stream in units of cubic centimeters of formulation per minute (equivalent to mL formulation/min),

F flow rate of the stream in units of cubic feet/second
C a constant
B target concentration in units of ppm formulation.

The constants given on the Prentiss product labels differ from formulation to formulation as indicated below:

Formulation	Constant, *C*, for Equation 3
CTF Legumine	1.699
Noxfish Fish Toxicant	1.699
Prenfish Toxicant Liquid	1.69
Nusyn-Noxfish Fish Toxicant	1.699
Synpren-Fish Toxicant	1.692

Given the structure of Equation 3 and the units for the defined values (i.e., ***X***, ***F***, and ***B***), the constant, C, must have units of $\mathbf{L_{Water}\ ml_{Form}\ sec\ /\ ft^3{}_{Water}\ mg_{Form}\ min}$. This can be demonstrated by rearrangement of Equation 3 solving for C:

Equation 4

$$\boldsymbol{C = X / F\ B}$$

and substituting the units for the defined values in Equation 4. This substitution yields:

Equation 5

$$C = \frac{\frac{mL_{Form}}{\min}}{\frac{Ft^3{}_{Wat}}{\sec} \times \frac{mg_{Form}}{Liter_{Water}}} = \frac{Liter_{Water}}{Ft^3{}_{Wat}} \times \frac{mL_{Form}}{mg_{Form}} \times \frac{\sec}{\min}$$

The number of liters per cubic feet of water (28.32 L/ft^3) and seconds per minute are fixed. The only formulation specific variable is the mL of formulation per mg of formulation. This can be calculated from the specific gravity of the formulation. Again using CFT Legumine as an example, the specific gravity of this formulation is given on the MSDS as 1.019 g/mL. Converting g to mg and taking reciprocal of the ratio yields [(1019 mg/mL)$^{-1}$ ≈ 0.0009814 mL/mg]. Using this value, the numeric value for the constant, C in Equation 3 through Equation 5, for CFT Legumine can be calculated as:

$$\boldsymbol{C} = 28.32\ \text{L/ft}^3 \times (1019\ \text{mg/mL})^{-1} \times 60\ \text{sec/min} = 1.6675.$$

This is less than the value given on the product label for CFT Legumine (i.e., 1.699) by about 2% [1.6675/1.699 ≈ 0.9815].

The rate at which a liquid formulation of rotenone should be applied to a stream based on the general equation for point source concentrations in a flowing body of water (e.g., SERA 2007c, Section 7.5) is:

Equation 6

$$\boldsymbol{TC}_{\text{mg a.i./L}} = \boldsymbol{a.i.}_{\text{mg}}/\text{min} \div \boldsymbol{Flow}_{\text{L}}/\text{min}$$

where ***TC*** is the target concentration of rotenone in units of mg/L, ***a.i.***/min is the rate at which rotenone must be added to the stream in units of mg a.i./minute, and ***Flow*** is the flow rate of the stream in units of L/minute. The ***a.i.*** term in Equation 6 can be expressed in terms of volume of formulation in milliliters (mL_{Form}) as:

Equation 7

$$\textbf{\textit{a.i.}}_{\text{ mg}} = \textbf{\textit{mL}}_{\text{Form}} \text{ x } \textbf{\textit{SG}}_{\text{g Form/mL Form}} \text{ x } 1000 \text{ mg/g x } \textbf{\textit{P}}_{\text{ a.i./Form}}$$

where:

P the proportion (w/w) of rotenone in the formulation

SG the specific gravity of the formulation in units of grams of formulation per mL of formulation.

Substituting a.i. in Equation 6 with the right hand side of Equation 7 yields:

Equation 8

$$\textbf{\textit{TC}}_{\text{mg a.i.}} = \textbf{\textit{mL}}_{\text{Form}}/\text{min x } \textbf{\textit{SG}}_{\text{g/mL}} \text{ x } 1000 \text{ x } \textbf{\textit{P}}_{\text{ a.i./Form}} \div \textbf{\textit{Flow}}_{\text{L}}/\text{min}$$

By definition, the application rate for the stream (***ApS***) in units of mL of formulation per minute is the term mL_{Form}/min in Equation 8. By rearrangement of Equation 8, this application rate can be expressed as:

Equation 9

$$\textbf{\textit{ApS}}_{\text{ mL Form/min}} = \textbf{\textit{TC}}_{\text{mg a.i.}} \text{ x } \textbf{\textit{Flow}}_{\text{L/min}} / (\textbf{\textit{SG}}_{\text{g/mL}} \text{ x } 1000_{\text{mg/g}} \text{ x } \textbf{\textit{P}}_{\text{a.i./Form}})$$

While Equation 9 could be used directly to calculate the application rate for the stream, the corresponding equation for lakes and ponds (Equation 2) uses bulk density (***BD*** in units of lb formulation/gallon formulation) rather than specific gravity (***SG*** in units of grams formulation per milliliter of formulation). Specific gravity can be derived from bulk density as:

Equation 10

$$SG_{g/mL} = BD_{lb/gal} \times \frac{453.5 g/lb}{3785 mL/gal} = BD_{lb/gal} \times 0.1198_{\frac{g \bullet gal}{lb \bullet mL}}$$

Substituting the right hand side of Equation 10 for SG in Equation 9 yields:

Equation 11

$$ApS_{mLForm/\min} = \frac{TC_{mga.i} \times Flow_{L/Min}}{BD_{lb/gal} \times 0.1198_{\frac{g \bullet gal}{lb \bullet mL}} \times 1000_{mg/g} \times P_{a.i./Form}}$$

Equation 11 is conceptually equivalent to Equation 3 but avoids the rounding errors in the implementation of Equation 3.

2.4.4. Powders Formulations in Ponds and Lakes

Powdered formulations differ from liquid formulations in that the labels for powdered formulations specify both the nominal concentration of rotenone in the formulation as well as the assayed or actual concentration of rotenone in the formulation. Because powdered formulations of rotenone consist primarily of ground plant root (Finlayson et

al. 2000, p. 113), the resulting concentration of rotenone in the powdered formulations will be variable and each batch of rotenone powder must be assayed for rotenone and the results of the assay are specified on the label that is released with the batch.

As with liquid formulations, the product labels for powdered formulations provide tables giving the number of acre-feet that are covered by one pound of formulation for a given application rate expressed as ppm rotenone (i.e., mg a.i./liter). The tables on the product labels also include target concentrations expressed in units of ppm of a 5% product. In general, the ppm units for a 5% formulation are simply 20 times those for rotenone – i.e., 1/0.05 = 20. The only exception is an apparent typographical error in the product label for Rotenone Fish Toxicant Powder (Prentiss, EPA Reg. No. 655-691). On this product label, the target ppm for selective treatment in terms of a 5% product is indicated as 1.3 ppm on the product label at the EPA web site as well as the product label at the Prentiss site. It appears that the intended value is 0.13 ppm, the value used on other labels for powdered formulations.

The tabulations on the product labels are correct within rounding differences of less than one percent, except for the target concentration of 0.007 ppm a.i. (mg rotenone/L). The product labels indicate that one pound of a 5% formulation will cover 2.8 acre-feet with a target concentration of 0.007 mg a.i./L. As detailed below, the correct value is 2.63 acre-feet, about 6% less than the value from the product labels [2.63 / 2.8 ≈ 0.9393].

The application rate tables on the product labels for powdered formulations are all based on a 5% formulation. All of the formulations, however, have nominal concentrations of 7.4% (w/w) rotenone. In addition, the powdered formulations are all assayed prior to release and the assayed concentration of rotenone is given on each label for a given batch of formulation that is released. Consequently, the tabulated application rates (except for the one that is in error) must be adjusted based on the assayed concentration of rotenone in each powdered formulation. These adjustments are relatively simple to make and the product labels provide reasonably clear directions.

The amount of a powdered formulation that must be applied to a lake or pond based on the dimensions of the body of water and the assayed proportion of rotenone in the powdered formulation is:

Equation 12

$$lb_{Form} = \frac{TC_{mga.i./L} \times SA_{Acres} \times Dep_{ft} \times 1{,}233{,}531.5_{Liters/acrefoot}}{P_{a.i./Form} \times 453{,}592.27_{mg/lb}}$$

where

lb_{Form}	pounds of formulation required to reach the target concentration,
TC	the target concentration in units of mg a.i./L,
P	the proportion (w/w) of rotenone in the powdered formulation, based on the results of the rotenone assay from the product label,
SA	surface area of the water in acres,
Dep	average depth of the water in feet,
453,592.27	a constant for the number of milligrams in a pound,
1,233,531.5	a constant for the number of liters in an acre-foot.

Equation 12 is identical to the corresponding equation for liquid formulations – i.e., Equation 2 – in that multiplying both sides of Equation 2 by the bulk density of the liquid formulation (***BD*** in units of lb_{Form}/Gal_{Form} in Equation 2) removes ***BD*** from the denominator of the right side of Equation 2 and converts gallons of formulation to pounds of formulation in the left side of Equation 2 – i.e., Gal_{Form} x lb_{Form}/Gal_{Form} = lb_{Form}.

As noted above, the product labels incorrectly indicate that 1 lb of a 5% formulation will cover 2.8 acre-feet at a target concentration of 0.007 mg a.i./L. The correct value is about 2.63 acre-feet. This can be demonstrated by rearranging Equation 10 to solve for acre-feet:

Equation 13

$$AcreFeet = SA_{Acres} \times Dep_{ft} = \frac{P_{a.i./Form} \times 453{,}592.27_{mg/lb}}{TC_{mga.i./L} \times lb_{Form} \times 1{,}233{,}531.5_{Liters/acrefoot}}$$

Setting P equal to 0.05, lb_{Form} equal to 1 lb, and the target concentration equal to 0.007 mg a.i./L, the calculated result is equal to about 2.6265 acre-feet. As also noted above, the other calculated values for acre-feet on the product labels are correct within very minor rounding differences of less than one percent.

2.4.4. Powders Formulations in Streams and Rivers

The product labels for powdered formulations provide the following algorithm for calculating the application rate (in units of pounds of formulation per second) for streams:

Equation 14

$$\boldsymbol{Rs}_{\,lb/sec.} = \boldsymbol{Rp}_{\,lb/acre\text{-}foot} \text{ x } \boldsymbol{C}_{\,acre\text{-}foot/cu\,.ft} \text{ x } \boldsymbol{F}_{\,cu\,ft/sec}$$

where

- ***R_s*** application rate for the stream in units of lb formulation/sec,
- ***R_p*** application rate for a pond in units of lb formulation/acre-feet ,
- ***C*** a constant, 1 acre-foot/43,560 ft^3, for converting acre-feet to cubic feet,
- ***F*** the stream flow rate in units of ft^3/second.

The label directions indicate that ***Rp***, the application rate for the pond, should be taken from the table on the product labels that give the number of acre-feet covered by one pound of the formulation for a given target concentration in unit of mg a.i./L or mg formulation/L.

As an example, the product label for Rotenone Fish Toxicant Powder applies Equation14 to calculate an application rate of 0.00031 lb formulation per second for a stream with a flow rate of 10 ft^3/second and a pond coverage value of 0.74 acres per pound which is associated with a target concentration of 0.025 mg a.i./L [1 lb formulation/0.74 acre-feet x 1 acre-foot/43,560 ft^3 x 10 ft^3/sec = 0.00031 lb formulation/second].

In a field application, however, the tables given on the product labels need to be adjusted for the assayed amount of rotenone in the powder formulation. In addition, as detailed in Section 2.4.3, some of the values in the tables on the product labels are not accurate.

A somewhat more direct approach can be based on the calculation of point source concentrations in a flowing body of water (Equation 6). The $\boldsymbol{a.i}_{mg}$ term in Equation 6 can be expressed as mg formulation based on the proportion (w/w) of rotenone in the formulation:

Equation 15

$$\boldsymbol{a.i.}_{mg} = \boldsymbol{mg}_{Form} \times \boldsymbol{P}_{a.i./Form\ (w/w)}$$

where $\boldsymbol{P}$ is the proportion (w/w) of rotenone in the formulation. For powder formulations, this value should be the assayed proportion of rotenone which is given on the label for the batch of formulation that is being used.

Substituting a.i. in Equation 6 with the right hand side of Equation 15 yields:

Equation 16

$$TC_{mg\ a.i./L} = \boldsymbol{mg}_{Form} \times \boldsymbol{P}_{a.i./Form\ (w/w)}/\text{min} \div \boldsymbol{Flow}_{L}/\text{min}$$

Rearrangement of Equation 16, solving for $\boldsymbol{mg}_{Form}$/min:

Equation 17

$$(\boldsymbol{mg}_{Form}/\text{min}) = \boldsymbol{TC}_{mg\ a.i./L} \times \boldsymbol{Flow}_{L}/\text{min} \div \boldsymbol{P}_{a.i./Form\ (w/w)}$$

Equation 17 can be converted to units of pounds formulation per minute by dividing both sides of Equation 17 by the number of milligrams in a pound:

Equation 18

$$Form_{lb/\text{min}} = \frac{TC_{mga.i./L} \times Flow_{L/\text{min}}}{P_{a.i./Form} \times 453{,}592.27_{mg/lb}}$$

This algorithm can be checked using the example discussed above from the product label for Rotenone Fish Toxicant Powder – i.e., a target concentration of 0.025 mg a.i./L, a proportion of rotenone in the formulation equal to 0.05, and a stream flow rate of 10 ft^3/second. A flow rate of 10 ft^3/second is equivalent to 600 ft^3/minute or 16,992 L/minute [28.32 L/ ft^3 x 600 ft^3 = 16,992 L]. Substituting 0.05 for $\boldsymbol{P}$, 16,992 for $\boldsymbol{Flow}$, and 0.025 for $\boldsymbol{TC}$ in Equation 18 yields 0.01873 pounds formulation per minute. This is equivalent to 0.000312175 lb formulation/second, equivalent within rounding errors to the value of 0.00031 lb formulation/second given in the example on the product label.

2.5. USE STATISTICS

Forest Service risk assessments attempt to characterize the use of a pesticides in Forest Service programs relative to the use of the pesticide by other organizations or in agricultural applications. The information on Forest Service use is taken from Forest Service pesticide use reports (http://www.fs.fed.us/foresthealth/pesticide/reports.shtml), and information on agricultural use is typically taken from use statistics compiled by the

U.S. Geologic Survey (http://water.usgs.gov/nawqa/pnsp/) and detailed pesticide use statistics compiled by the state of California (http://www.calepa.ca.gov/). No use statistics for rotenone are available at the USGS web site.

The USDA Forest Service tracks and reports its use of pesticides by management use objectives and by geographical areas referred to as "*Regions*". The Forest Service classification divides the United States into nine regions designated from Region 1 (Northern) to Region 10 (Alaska) (Figure 2). [Note: There is no *Region 7* in the Forest Service system.]

Over the period from 2000 to 2004, three rotenone applications are reported by the Forest Service, all of which occurred in 2004 in applications for fish eradication. As illustrated in Figure 2, one application occurred in Region 1 (Northern Region) and two applications occurred in Region 2 (Rocky Mountain Region). Two of the applications are reported in units of gallons and one application is reported in units of pounds. In all cases, the target concentrations cannot be calculated. As detailed in Section 2.4, the calculation of target applications required detailed information on the formulation used as well as the characteristics of the body of water. These are not provided in the summary statistics available in the Forest Service pesticide use reports. The California Department of Fish and Game has applied CTF Legumine on Forest Service facilities during February, 2007 (http://www.stpns.net/view_article.html?articleId=32443242155433325).

CDPR (2007) reports a total use of about 116 pounds of rotenone in California during 2006, the most recent year for which use statistics are available. All of the applications in California appear to involve crops. As noted in Section 2.2, all non-piscicidal uses of rotenone have been cancelled as of 2006 (U.S. EPA/OPP 2007a). Thus, these agricultural uses reported for California are no longer supported under the registration for rotenone.

As also noted in Section 2.2, rotenone has been used as a piscicide in the United States and Canada since the mid-1930s and some use statistics are available. During 1965, Lennon (1970) reports that nearly 700,000 pounds of rotenone were applied as a piscicide in 40 states. It is not clear if the 700,000 pound figure represents pounds of rotenone or pounds of rotenone formulations. McClay (2000) summarizes use statistics for rotenone in the U.S. and Canada in the decade from 1988 to 1997. A total use of 94,739 kg a.i. of rotenone is reported over the 10 year period is reported in McClay (2000). This use is equivalent to about 208,862 a.i. pounds over the 10 year period or about 21,000 pounds a.i. per year. McClay (2000) also notes a shift in use preference over the 10 year period from liquid to powdered formulations.

While the available statistics on the use of rotenone are somewhat sparse and the pesticide use data from the Forest Service are limited, the average use rate in the United States of about 21,000 pounds a.i./year reported by McClay (2000) suggests that the use of rotenone as a piscicide in Forest Service programs is likely to be minor compared the total use of rotenone as a piscicide by other organizations.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

At the cellular level, rotenone is a metabolic toxin that interferes with the ability of mitochondria to chemically store energy within a cell – i.e., convert ADP to ATP. This effect results in both an energy deficit within the cell as well as an increase in general oxidative damage to the cell. While mitochondria could be affected by rotenone in any type of cell, the impact on nerve tissue is an endpoint of major concern. Numerous studies indicate that rotenone may cause specific damage to nerve cells, inducing gross signs of neurotoxicity in mammals similar to those associated with Parkinson's disease. Whether or not rotenone can be considered a cause of Parkinson's disease remains an open question that has little impact on the current risk assessment. It is clear that rotenone is neurotoxic, and this endpoint is of concern. Most studies demonstrating that rotenone can induce effects similar to those of Parkinson's disease were conducted using routes of exposure that are not directly germane to potential human exposures (e.g., intraperitoneal or intravenous injection as well as direct instillation into the brain); however, a recent study demonstrates that these effects can occur after oral dosing.

Rotenone is classified by the U.S. EPA as highly toxic after oral and inhalation exposures; yet, there appears to be no consistent pattern in its toxicity to various groups of mammals, except that females seem to be somewhat more sensitive than males. In rats, the LD_{50} is about 40 mg/kg body weight in females and 100 mg/kg body weight in males. With respect to human exposure, the estimated lethal dose is often cited between 300 and 500 mg/kg body weight; however, a relatively well-documented case report indicates a lethal dose of about 40 mg/kg body weight after the accidental poisoning of a young girl. With respect to mammals in general, very sketchy information indicates that rabbits may be somewhat less sensitive than other mammals to rotenone toxicity, whereas cats and dogs may be somewhat more sensitive than are other mammals.

The pharmacokinetics of rotenone in mammals are not well-characterized. While rotenone is often classified as a substance that is not well absorbed after oral exposure, it is able to cross the blood-brain barrier. Furthermore, its chemical properties suggest that rotenone should be well absorbed after oral exposure. The apparent slow rate of oral absorption sometimes attributed to rotenone may reflect rapid metabolism or at least a rapid breakdown in the gastrointestinal tract prior to absorption.

Of the available studies on rotenone, one study indicates that rotenone may be an endocrine disruptor in mammals, impacting testosterone production. Other studies assessing impacts on testosterone production are not available. There is no credible information suggesting that rotenone is a mutagen or carcinogen. Similarly, rotenone does not appear to have the potential to cause substantial dermal or ocular damage, although prudent handling practices dictate that dermal and ocular exposures should be avoided through the proper use of protective equipment.

Because rotenone is extracted from plant roots, commercial formulations of rotenone are complex mixtures of rotenone and other related plant material. It appears, however, that the components of primary concern are rotenone and one other structurally similar compound, deguelin. Trichloroethylene is used in the extraction process for at least some formulations and small concentrations of trichloroethylene have been found in some rotenone formulations. The quantity of trichloroethylene in rotenone formulations does not appear to be toxicologically significant, based on both its toxicity and its concentration, relative to rotenone. Similarly, all liquid formulations of rotenone contain petroleum solvents, which are themselves complex mixtures. The composition of the petroleum solvents is well characterized in only three formulations. Among these three formulations, the composition of the petroleum solvents differ substantially; nevertheless, the petroleum solvents do not appear to be present in amounts that are toxicologically substantial relative to rotenone and other related compounds.

The U.S. EPA recommends the use of potassium permanganate to detoxify water treated with rotenone. If properly applied, potassium permanganate should not present any additional risk and should decrease risks associated with the use of rotenone as a piscicide. If improperly applied—i.e., applied in excess—the reduction in risk due to the destruction of rotenone should outweigh risks associated with the use of potassium permanganate.

Finally, all formulations of rotenone contain other related rotenoids and some formulations contain piperonyl butoxide, a compound that enhances the toxicity of rotenone. These materials are also listed as active ingredients on the product labels for rotenone formulations. Both other related rotenoids and piperonyl butoxide may contribute to the toxicity of rotenone formulations. Consequently, formulation-specific toxic equivalency factors ranging from 1.25 to 2.5 are developed and these factors are used in all exposure assessments to calculate joint exposures to rotenone, other related rotenoids, and piperonyl butoxide in units of rotenone equivalents.

3.1.2. Mechanism of Action

The mechanism of action of rotenone at the cellular/biochemical level is relatively well characterized. Rotenone interferes with oxidative phosphorylation, a fundamental process in living cells in which nutrients are oxidized and the energy of oxidation is stored by the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This process occurs in the mitochondria, discrete structures within a cell. The first step in this process involves the oxidation of NADH (reduced nicotinamide adenine dinucleotide) to NAD^+. This reduction is catalyzed within the mitochondria by NADH dehydrogenase (ubiquinone) which is also referred to as Complex I—i.e., the first step in oxidative phosphorylation (Michal 1999; Uversky 2004). While rotenone exposure will result in a decrease in ATP (i.e., an increase in ADP/ATP ratios), there is no indication that the toxicity of rotenone is based on bioenergetic deficits (Sherer et al. 2003; Uversky 2004).

The effect of the inhibition of NADH dehydrogenase resembles oxygen deprivation not because of a direct blockage of oxygen uptake but because the blockage of NADH

dehydrogenase prevents the use of oxygen in later stages of oxidative phosphorylation (Entrix 2007; Finlayson et al 2000; Fontenot et al. 1994; Oberg 1964). The net result of rotenone poisoning at the cellular level is similar to oxygen deprivation and leads to anaerobic metabolism with the formation of lactic acid leading to acidosis. As noted by Ling (2002), the effects of rotenone are similar to those of other agents that block or uncouple oxidative phosphorylation—e.g., antimycin, cyanide, and dinitrophenol.

While cell death may be attributed to oxygen deprivation (Fontenot et al. 1994), the inability of cells to use oxygen leads to increases in oxygen levels that in turn lead to increased oxidative stress and damage to the affected cells via reactive oxygen species such as superoxide (Chung et al. 2007; Crutchfield and Dluzen 2006; Lim et al. 2007; Keeney et al. 2006; Panov et al. 2005; Uversky 2004). The central role of oxidative stress to the toxicity of rotenone is also supported by studies indicating that antioxidants can reduce or prevent expressions of rotenone toxicity (Inden et al. 2007; Nehru et al. 2008).

3.1.3. Pharmacokinetics and Metabolism

3.1.3.1. General Considerations

Pharmacokinetics involves the quantitative study of the absorption, distribution, and excretion of a compound. Pharmacokinetics is important to this rotenone risk assessment for three reasons. First, many of the most plausible and quantitatively most significant exposure assessments (Section 3.2) involve dermal exposure, although most of the dose-response assessments (Section 3.3) used to interpret the consequences of dermal exposure involve oral exposure levels. Accordingly, it is necessary to understand the kinetics of both oral and dermal absorption so that dermal exposure assessments can be appropriately compared with oral dose-response assessments. Second, rotenone is a neurotoxic agent that can induce signs of toxicity similar to Parkinson's disease. As discussed further in Section 3.1.6, many of the studies used to characterize the neurotoxicity of rotenone involve parenteral administrations (i.e., subcutaneous infusion, intravenous administration, or direct installation into brain tissue). Thus, an understanding of the pharmacokinetics of rotenone is important in terms of assessing the qualitative and quantitative relevance of these studies to the hazard identification for potential human health effects. Finally, most of the plausible exposures to rotenone used for fish control (Section 3.2) will occur over a period of several hours, while most of the toxicity values available on rotenone (Section 3.3) are based on exposure periods of weeks to months. An understanding of the pharmacokinetics of rotenone can provide some insight to an interpretation of the applicability of existing toxicity values to the assessment of potential adverse effects from the use of rotenone as a piscicide.

The pharmacokinetics of rotenone is not well characterized, which is somewhat unusual for a pesticide like rotenone that has been in use for a prolonged period of time. The only detailed published study on the pharmacokinetics of rotenone is the report by Fukami et al. (1969) in which male mice were administered rotenone by gavage at 0.66 mg/kg body weight (12 µg of ^{14}C-rotenone in dimethyl sulfoxide). Total radioactivity was assayed in the expired air, urine, feces, and tissues at periods of 4 and 24 hours after dosing. Fukami et al. (1969) also report the metabolism of rotenone in rats but do not specify the dose

used. Signs of toxicity in rats and mice are not noted by Fukami et al. (1969). This study also examined the influence of inhibitors of cytochrome P450 mixed-function oxidases (e.g., piperonyl butoxide and SKF-525A) on the metabolism of rotenone by mice and rats. In these studies, rotenone appeared to be rapidly metabolized in the liver via cytochrome P450; whereas, metabolism in other organs appeared to be substantially slower than in the liver (Fukami et al. 1969, Table I, p. 1218). After 24 hours, approximately 20% of the radioactivity from the administered doses was recovered in the urine of both rats and mice (Fukami et al. 1969, Table V, p. 1223). Although Fukami et al. (1969, p. 1219) clearly indicate that the feces were assayed for radioactivity, the amount of residue in the feces of mice or rats is not reported. Most of the metabolites recovered by Fukami et al. (1969) are characterized as hydroxylated rotenoids or other water soluble metabolites.

The U.S. Fish and Wildlife Service submitted a pharmacokinetic study in rats to the U.S. EPA. While a full citation for this submission has not been identified, it appears that the study was submitted in 1984 and reviewed in detail by the U.S. EPA in 1985. A copy of the original study was not available for the current Forest Service risk assessment; however, the U.S. EPA kindly provided a copy of the 1985 review (Gardner 1985a). As noted in Gardener (1985a), this study involved both intravenous and gavage administrations of ^{14}C-rotenone to different groups of rats at a single dose 0.01 mg/kg body weight for the intravenous. study as well as single and multiple (14-day) doses of 0.01 and 5 mg/kg bw/day for the oral study. Unlike the published study by Fukami et al. (1969), the major route of excretion reported by Gardener (1985a) is fecal, with about 95% of the administered dose excreted in feces. Female rats excreted rotenone somewhat more slowly than males—i.e., 75% of the administered dose was excreted in the feces of male and female rats at 48 and 72 hours, respectively, after dosing. No substantial differences are reported among the doses or routes of exposure. While not detailed by Gardener (1985a), U.S. EPA/OPP (2005a) indicates that rotenone exhibited extensive enterohepatic circulation – i.e., re-absorption after transport from the liver to the gastrointestinal tract – and that urinary excretion was greater in females than in males, a factor that may account for the differences observed in male and female rats regarding the fecal excretion of rotenone.

The role of cytochrome P450 in the metabolism of rotenone has been clearly documented in the more recent study by Caboni et al. (2004), in which the human recombinant 3A4 and 2C19 isozymes were found to be more active than other isozymes. As discussed further in Section 3.1.15.1, the metabolites of rotenone are less toxic than rotenone itself (i.e., Fang and Casida 1999).

3.1.3.2. Absorption

The rate of rotenone absorption after oral exposures is not discussed quantitatively in the available literature. Nonetheless, rotenone is often characterized as poorly absorbed from the gastrointestinal tract (e.g., Entrix 2007; Ling 2003; Ott 2008; Turner 2007). This supposition may be based on the substantial differences in rotenone toxicity depending on the route of exposure demonstrated by Haag (1931) who observed that intravenous administration of rotenone was more toxic by a factor of about 1000, relative to oral

exposures. On the other hand, rotenone is highly lipophilic and is able to cross the blood-brain barrier and affect brain tissue (e.g., Uversky 2004), which suggests that rotenone should be readily absorbed. In the study by Fukami et al. (1969), only about 3.4% of the administered dose was recovered in the small intestine as unmetabolized rotenone (Fukami et al. 1969, Table V, 21.6% total dose x 16% rotenone). This finding is not consistent with the view that rotenone is poorly absorbed. Rotenone, however, may be subject to metabolism or at least reactivity within the gastrointestinal tract, as suggested by observations that rotenone damages the intestinal mucosa (Section 3.1.4). The inability to better characterize the gastrointestinal absorption of rotenone does not have a substantial impact on the current risk assessment under the assumption that gastrointestinal absorption by humans and experimental mammals will be similar.

No data are available on dermal absorption rates for rotenone, and this information gap is important to the current risk assessment because many of the exposure scenarios (Section 3.2) involve dermal exposure. Specifically, two types of dermal exposure scenarios are considered: immersion and accidental spills onto the skin surface. As detailed in SERA (2007a), the calculation of absorbed dose for dermal exposure scenarios involving immersion or prolonged contact with chemical solutions uses Fick's first law (zero-order absorption) and requires an estimate of the dermal permeability coefficient (K_p) expressed in cm/hour. For exposure scenarios like direct sprays or accidental spills, which involve deposition of the compound on the surface of the skin, first-order dermal absorption rates (k_a) expressed as a proportion of the deposited dose that is absorbed per unit time are used in the exposure assessment.

The U.S. EPA/OPP (2007a) uses a dermal absorption value of 9% for rotenone by analogy to fluazifop-butyl (U.S. EPA/OPP 2007a, p. 12) based on structural similarities as well as similar molecular weights. While not explicitly stated in the EPA assessment, the 9% absorption value represents an estimate of percent absorbed over a 1-day period and corresponds to a dermal absorption rate coefficient ($\boldsymbol{k_a}$) of about 0.094 day^{-1} [$\boldsymbol{k_a} = -\ln(1-\boldsymbol{P})/\boldsymbol{t}$, where $\boldsymbol{P}$ is the proportion of the absorbed dose over duration $\boldsymbol{t}$] or 0.0039 $hour^{-1}$. In the absence of experimental data, Forest Service risk assessments typically use quantitative structure-activity relationships to estimate both first-order dermal absorption rates and permeability coefficients (SERA 2007, Section 3.1.3.2). These algorithms are included in Worksheets B05 ($\boldsymbol{K_p}$) and B06 ($\boldsymbol{k_a}$) of the EXCEL workbook that accompanies this risk assessment (Attachment 1). As noted in Worksheet B06, the estimated k_a for rotenone is 0.0017 (0.0006 – 0.0051) $hour^{-1}$. This estimate is reasonably consistent with the approach taken by the U.S. EPA/OPP and the upper bound of 0.0051 $hour^{-1}$ (used to estimate upper bounds of risk) is somewhat more conservative. The K_p for rotenone is estimated at 0.0061 (0.0031 – 0.012) cm/hour. In the absence of any other data, this estimate of the K_p is used in all exposure scenarios involving zero-order absorption models, as discussed further in Section 3.2.

The available literature does not include data on the absorption of rotenone during inhalation exposures. As noted by U.S. EPA/OPP (2005a, p. 4), inhalation exposures are of particular concern to a rotenone risk assessment because they are analogous to intravenous exposures in that any inhaled compound goes directly into the bloodstream,

bypassing initial detoxification in the liver. The U.S. EPA (2007a) uses a default assumption that 100% of inhaled rotenone will be absorbed.

3.1.3.3. Excretion

While excretion rates are not used directly in either the dose-response assessment or risk characterization, excretion half-lives are often used in Forest Service risk assessments to infer the effect of longer-term exposures on body burden based on the *plateau principle* (e.g., Goldstein et al. 1974). The concentration of the chemical in the body after a series of doses (X_{Inf}) over an infinite period time can be estimated based on the body burden immediately after a single dose, X_0, by the relationship:

$$X_{Inf}/X_0 = 1 / (1- e^{-ke\ t^*)})$$

where t* is the interval between dosing.

As noted in Section 3.1.3.1, a pharmacokinetic study in rats indicates that about 75% of the administered dose is excreted the feces of male and female rats at 48 and 72 hours, respectively, after dosing. Using a first-order approximation, these excretion patterns correspond to elimination rates (k_e) of about 0.46 day^{-1} [k_e = -ln(1-*P*)/*t* = -ln(1-0.75)/3 days] to 0.7 day^{-1} [k_e = -ln(1-*P*)/*t* = -ln(1-0.75)/2 days]. Using these estimates of the k_e and a 1-day interval between doses (i.e., daily dosing), an increased body burden with infinite exposure, relative to the body burden after a single dose, would be a factor of about 2-2.7, suggesting that it is relatively unlikely that rotenone will accumulate in humans over periods of prolonged exposure. In addition, the estimates of relative body burden are likely to be overestimates because they are based only on fecal excretion.

For rotenone, however, the relative body burden probably does not provide a reasonable basis for inferring the consequences of prolonged exposure. As discussed in Section 3.1.6, neurotoxicity is an endpoint of major concern in the current risk assessment, and there is ample experimental data indicating that prolonged exposures to rotenone are likely to present a greater risk of neurotoxic effects, relative to comparable short-term exposures to rotenone. This pattern is not related to the accumulation of rotenone but instead to the cumulative damage to nervous system tissue, which has a remarkably low (and perhaps negligible) capacity for repair or regeneration of damaged or lost cells.

3.1.4. Acute Oral Toxicity

The general signs of rotenone poisoning are described in the early literature. As would be expected based on the cellular mechanism of action, the general signs of rotenone toxicity involve respiratory distress. Initially, a compensatory increase in respiratory rate is often noted. Because oxygen consumption is blocked at the cellular level, however, the increase in respiratory rate does not offset the blockage in oxygen consumption caused by rotenone, and the proximate cause of death may be characterized as respiratory failure (Haag 1931; Oliver and Roe 1957).

Secondary signs of toxicity include incoordination, emesis (in mammals that are capable of vomiting), and tremors (which may progress to convulsions or seizures at fatal doses).

Stomach enlargement and irritation to the gastric mucosa is also noted (Haag 1931; Harper et al. 2007; Lapointe et al. 2004) along with degenerative/fatty changes in the liver (Lapointe et al. 2004; Richter et al. 2007). Both the gastric irritation and liver damage may be associated with a general increase in cellular oxidative stress.

One type of acute toxicity information involves time-specific LD_{50} or LC_{50} values (i.e., doses or concentrations of a toxicant that result in or are estimated to result in 50% mortality of the test species during a specified exposure or observation period). These values can be viewed as an index of acute lethal potency. Information is also available on the acute neurological effects of rotenone from several routes of administration (Section 3.1.6) as well as acute dermal toxicity (Section 3.1.12) and acute inhalation toxicity (Section 3.1.13) of rotenone.

As summarized in Appendix 1, acute toxicity values by other routes of exposure (e.g., intravenous, intramuscular, and subcutaneous) are available from the early toxicity studies of Haag (1931). While intravenous studies are not generally used to quantitatively characterize risk, it is notable that the range of lethal intravenous doses in rabbits reported by Haag (1931)—i.e., 0.25-0.35 mg/kg body weight—is quite similar to the intravenous LD_{50} of 0.305 mg/kg body weight in rainbow trout (Erickson and Gingerich 1986).

For characterizing the acute risks associated with oral exposures to mammalian wildlife, the U.S. EPA/OPP (2006c) uses acute oral LD_{50} values of 102 mg/kg body weight in male rats and 39.5 mg/kg body weight in female rats. As noted in Section 3.1.3.1 (Pharmacokinetics), the lower LD_{50} value in female rats is associated with a lower excretion rate of rotenone (Gardner 1985a). As summarized in Appendix 1, the U.S. EPA/OPP (2006c) summarizes other toxicity studies of rotenone formulations that yield somewhat lower LD_{50} values in terms of rotenone exposure—e.g., 6.5 rotenone mg/kg body weight in female rats—and in terms of combined rotenone and other extracts—e.g., 13 mg/kg body weight in female rats. In all studies, female rats appear to be somewhat more sensitive than male rats.

The U.S. EPA ranks the potential of acute toxic risk, as well as risks of dermal toxicity, inhalation toxicity, eye irritation, and skin irritation, into four categories with Category I presenting the greatest risk and Category IV presenting the least risk (see SERA 2007a, Table 3-2). For oral toxicity, rotenone is classified as Category I based on the 39.5 mg/kg body weight LD_{50} in female rats.

Based on semi-quantitative patterns in the onset and duration of symptoms from *in vivo* studies, Haag (1931) suggests that dogs and cats may detoxify rotenone more slowly than do rodents and rabbits. Based on cell culture assays, Harper et al. (2007) suggests that larger mammals may be less sensitive than smaller mammals to rotenone, at least at the cellular level.

The approximate lethal dose of rotenone in humans is generally estimated to be between 300 and 500 mg/kg body weight (Lehman 1949; Lehman 1952; NRC 1986). De Wilde et

al. (1986) provide a relatively well-documented case report of fatal accidental poisoning of a 3-year-old girl in which the dose is estimated at 10 mL of an older liquid formulation, Galicide, that had been used on animals as an insecticide. Galicide contains 6% rotenone. Assuming a bulk density of 1 g/mL as an approximation, 10 mL of a 6% rotenone solution corresponds to 600 mg of rotenone. The body weight of the child is reported by De Wilde et al. (1986) as 15 kg. Thus, Wilde et al. (1986) calculate a lethal dose of 40 mg rotenone/kg body weight. This dose is virtually identical to the oral LD_{50} of 39.5 mg/kg body weight of rotenone in female rats (U.S. EPA/OPP 2006c).

The correspondence between the rotenone oral LD_{50} for female rats and the lethal dose in a young girl as well as the correspondence in intravenous LD_{50} values for mammals and fish may be coincidental. Nonetheless, the overall patterns in the acute lethal potency of rotenone do not suggest substantial species differences. This is discussed further in Section 3.3 (dose-response for human health) and Section 4.3.2.1 (dose-response for mammals in the ecological risk assessment).

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Systemic toxicity encompasses effects that a chemical has once the chemical is absorbed. Certain types of effects, however, are of particular concern to this risk assessment. Such special effects are considered in following subsections and include effects on the nervous system (Section 3.1.6), effects on the immune system (Section 3.1.7), developmental or reproductive effects (Section 3.1.8), and carcinogenicity or mutagenicity (Section 3.1.9). This section discusses the remaining studies on systemic toxic effects.

U.S. EPA/OPP (2006c, 2007a) summarizes a number of subchronic and chronic mammalian toxicity studies submitted by registrants in support of the registration and reregistration of rotenone. Other subchronic and chronic toxicity studies from the open literature are summarized in Appendix 1 to this Forest Service risk assessment. In terms of assessing the impact of exposure on potential human health effects, the most significant study is the chronic toxicity/oncogenicity study on which the U.S. EPA bases the chronic RfD (Section 3.3.2). In this study, rats were exposed to rotenone at dietary concentrations of 0, 7.5, 37.5, and 75 ppm for 2 years. The daily doses were estimated by the EPA at 0, 0.375, 1.88, and 3.75 mg/kg bw/day. The lowest dose, 0.375 mg/kg bw/day is classified as a NOAEL. Based on decreased body weight accompanied by decreased food consumption, the U.S. EPA classifies the dose of 1.88 mg/kg bw/day as the lowest observed adverse effect level (LOAEL) (U.S. EPA/OPP 2006c, Table 4.1b, p. 10). This study appears to be identical to the cancer bioassay summarized by Marking (1988).

At much higher dietary concentrations—i.e., 600 and 1200 ppm – Abdo et al. (1988) report decreased body weight gain in mice but not in rats. Decreased body weight is noted also in chronic studies with rotenone formulations and cubé resin (Brooks and Price 1961; Haag 1931; Hansen et al. 1965). As discussed further in Section 3.1.14.1 (Inerts), cubé resin is a non-end use form of rotenone extract which serves as the basis for preparing commercial formulations of rotenone.

3.1.6. Effects on Nervous System

There is a substantial body of literature concerning the use of rotenone to develop animal models for Parkinson's disease, and this literature is the subject of numerous published reviews (Drechsel and Patel 2008; Gomez et al. 2007; Greenamyre et al. 2003; Hirsch et al. 2003; Hoglinger et al. 2006; Jenner 2001; Orr et al. 2002; Perier et al. 2003; Trojanowski 2003; Uversky 2004). Interest in the ability of rotenone to cause Parkinson's disease is focused on two issues: the prevention of Parkinson's disease by limiting exposures to agents that may cause the disease and an understanding of the pathogenicity of Parkinson's disease with the goal of developing effective treatments for this condition. While both of these issues are important, the first issue is of primary concern to the current risk assessment. The following discussion of Parkinson's disease is based chiefly on the recent review by Drechsel and Patel (2008).

Parkinson's disease is a progressive degenerative neurological disorder characterized by resting tremor, rigidity, the inability to maintain posture, and generally slow movement. There are two general types of Parkinson's disease: familial and sporadic. Familial Parkinson's disease may occur early in life, and, as the name implies, has a clear genetic component—i.e., it runs in families. Sporadic Parkinson's disease tends to occur most frequently in the elderly with a prevalence of 1-2% in individuals who are 50 years old and about 5% in individuals who are 85 years old. The pathogenesis of Parkinson's disease involves the loss (progressive degeneration) of dopamine-secreting nerved cells in the middle section of the brain (substantia nigra). Dopamine is an important chemical in normal nervous system function (i.e., dopamine is a neurotransmitter), and the loss of dopamine in the brain is associated with overt signs of Parkinson's disease. The behavioral signs of Parkinson's disease are observed when about 60-70% of dopamine-secreting nerve cells are lost. Changes in the appearance of damaged nerve cells include the development of protein masses in the cytoplasm referred to as Lewy bodies, a characteristic feature of diseased nerve cells in Parkinson's disease (Le Couteur et al. 2002).

The cause or causes of Parkinson's disease are not well-understood. As noted above, the development of Parkinson's disease appears to involve both genetic predisposition (i.e., familial Parkinson's disease) and as well as environmental factors, including exposures to agricultural chemicals. Environmental factors may include relatively common agents such as cigarette smoking and the consumption of coffee (e.g., McCulloch et al. 2008) as well as general exposure to pesticides in populations of farmers (e.g., Brown et al. 2006). In terms of exposure to pesticides, the most consistent relationship noted in epidemiology studies is the positive correlation in the increased risk of the development of Parkinson's disease with the duration of pesticide exposure (Drechsel and Patel 2008). Nonetheless, no epidemiology studies specifically linking rotenone exposures to Parkinson's disease were encountered in the literature. Because pesticide exposures in farmers as well as other groups of individuals tend to involve exposures to many different pesticides as well as various other risk factors, the lack of an epidemiology study specifically linking rotenone to the development of Parkinson's disease should not be overly interpreted. In other words, no epidemiology studies are available indicating that populations exposed to

rotenone are at the same level of risk of Parkinson's disease as populations not exposed to rotenone.

Table 5 summarizes the experimental studies concerning the ability of rotenone to induce signs of toxicity consistent with the signs and symptoms of Parkinson's disease. This table summarizes the species tested, route of exposure, dose, duration of exposure, and a general indication of the endpoints observed: biochemical changes such as the inhibition of NADH oxidation or decreases in brain dopamine concentrations, morphological damage to brain tissue characteristic of Parkinson's disease, and gross signs of toxicity characteristic of Parkinson's disease. An early study by Ferrante et al. (1997) indicates damage to brain tissue; however, the specific nature of the damage was not characteristic of Parkinson's disease. Subsequently, Betarbet et al. (2000) noted specific damage to the midbrain of rats that appeared to be characteristic of Parkinson's disease. As noted in Table 5, both of these studies involved intravenous administration. While the study by Ferrante et al. (1997) involved higher doses of rotenone, the study by Betarbet et al. (2000) involved a longer period of exposure. While some additional studies indicate that single doses of rotenone caused midbrain damage (e.g., Crutchfield and Dluzen 2006), most of the studies reporting effects consistent with Parkinson's disease involve multiple doses, and note an association between the duration of exposure and the development of signs of toxicity consistent with Parkinson's disease (e.g., Antkiewicz-Michaluk et al. 2003; Bashkatova et al. 2004).

The strong duration-response relationship is consistent with the general association between the duration of pesticide exposure and the development Parkinson's disease in human populations. This consistency, however, may be trivial: most neurotoxic chemicals display a clear association between nerve damage and the duration of exposure, and this pattern is associated with the very slow rate of recovery in damaged nerve tissue.

All of the early studies and most of the subsequent studies on rotenone and Parkinson's disease involve routes of exposure that are not directly relevant to a human health risk—i.e., subcutaneous infusion, intravenous administration, or direct instillation into the brain. This detail was noted by Borzelleca (2001) in an early review of the Betarbet et al. (2000) study and is also noted by the U.S. EPA/OPP (2005a).

The recent study by Inden et al. (2007), however, reports Parkinson like effects in mice after oral administration of rotenone by gavage. As summarized in Appendix 1, Inden et al. (2007) treated mice with gavage doses of 0, 0.25, 1.0, 2.5, 5.0, 10 or 30 mg/kg rotenone for 28 days. At doses of 10 and 30 mg/kg bw/day, effects included degeneration of dopaminergic neurons as well as decreased endurance in a roto-rod test (a standard assay for motor function). Effects on dopamine neurons were sporadic at 10 mg/kg body weight but were seen in nearly all mice at 30 mg/kg body weight. Furthermore, Inden et al. (2007) discovered an accumulation of protein (synuclein) within viable neurons which may be consistent with Lewy body formation.

While the study by Inden et al. (2007) is clearly the most directly relevant publication to this risk assessment with respect to the experimental induction of signs of toxicity consistent with Parkinson's disease, it is also important to recognize that Inden et al. (2007) do not demonstrate that rotenone causes Parkinson's disease. Specifically, the Inden et al. (2007) publication states the following:

> *These results suggest that rotenone-treated mice may be useful for understanding the mechanism of DA*[dopamine] *neurodegeneration in PD* [Parkinson's disease] *and may be a model of the interaction of genetic and environmental factors involved in the pathogenesis of PD* (Inden et al., 2007, p. 1503).

Similarly, several of the researchers involved in the study of agents used in studying Parkinson's disease express reservations in the use of rotenone as an animal model for Parkinson's disease because of the broader spectrum of neurological effects induced by rotenone relative to the neurological effects seen in Parkinson's disease (Lapointe et al. 2004; Ravenstijn et al. 2008; Richter et al. 2007). Conversely, other researchers suggest that the available studies on rotenone provide a convincing or at least plausible basis for concluding that *"real life"* exposures to rotenone are likely to be associated with the development of Parkinson's disease (e.g., Alam and Schmidt 2002, p. 323).

Whether or not exposures to rotenone are likely to cause Parkinson's disease in humans cannot be unequivocally determined at this time. That rotenone can cause neurological damage is, nonetheless, evident, and neurotoxicity is an endpoint of concern in the current risk assessment. The study by Inden et al. (2007) impacts the current risk assessment in terms of the acute RfD. As discussed in U.S. EPA/OPP (2005a), the EPA did not require specific acute or developmental neurotoxicity studies on rotenone; however, it did recommend (but did not require) a subchronic inhalation neurotoxicity study. The rationale for this approach is discussed in U.S. EPA/OPP (2005a, p. 18) and is justified based on the lack of clinical signs of neurotoxicity in standard subchronic and chronic studies. The recommendation for an inhalation study is based on the likelihood that rotenone will be more rapidly absorbed after inhalation exposure, relative to oral exposure (see Section 3.1.3.2). The U.S. EPA (2005a; 2007a) derived an acute RfD based on a NOAEL of 15 mg/kg bw/day from a reproduction study. The Inden et al. (2007) study, however, suggests that adverse neurological effects, whether or not they are directly related to Parkinson's disease, may occur at oral doses as low as 10 mg/kg bw/day (LOAEL) with an apparent NOAEL of 5 mg/kg bw/day. This finding is considered further in Section 3.1.3 (Acute RfD).

3.1.7. Effects on Immune System

Various tests have been developed to assess the effects of chemical exposures on different types of immune responses, including assays of antibody-antigen reactions, changes in the activity of specific types of lymphoid cells, and assessments of the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells (SERA 2007a). Except for skin sensitization studies (Section 3.1.11.2),

specific studies concerning the effects of pesticides on immune function are not required for pesticide registration. In the U.S. EPA human health risk assessment of rotenone (U.S. EPA/OPP 2005a, 2006e, 2007a), potential effects on immune function are not addressed, except to note that rotenone does not appear to be skin sensitizer.

There is little information in the published literature on the potential of rotenone to cause effects on the immune system. *In vitro* assays conducted with cultured mouse spleen cells demonstrated a 65% inhibition of antibody formation (in response to sheet erythrocytes) with no loss of cell viability at a rotenone concentration of 10^{-7} M—i.e., 0.03944 mg/L— when the rotenone was applied at the initiation of cell culturing (Sabet and Hsia 1970). In a subsequent study (Sabet and Fridman 1972), rotenone inhibited *in vitro* antibody plaque formation in response to sheep erythrocytes in mouse spleen cells at 10^{-3} M (394 mg/L) [85% inhibition], 10^{-4} M (39.4 mg/L) [50% inhibition], and 10^{-5} M (3.94 mg/L) [12-15% inhibition] with rapid loss of cell viability. The reasons why the initial study by Sabet and Hsia (1970), reported only as an abstract, report a greater inhibition than the full publication by Sabet and Fridman (1972) are not apparent.

No studies or reports have been encountered in the literature on rotenone suggesting that rotenone may have an effect on pathogen resistance with *in vivo* exposures.

3.1.8. Effects on Endocrine System

Assessment of the direct effects of chemicals on endocrine function are most often based on mechanistic studies on estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding, or post-receptor processing). In addition, changes in structure of major endocrine glands—i.e., the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis—may also be indicative of effects on the endocrine system.

Disruption of the endocrine system during development may give rise to effects on the reproductive system, which may be expressed only after maturation. Consequently, multi-generation exposures are recommended for the toxicological assessment of suspected endocrine disruptors (SERA 2007a). A multi-generation reproduction study on rotenone is discussed in Section 3.1.9.2, and the effects of rotenone on gonadal tissue are discussed in Section 3.1.9.3.

As discussed in Section 3.1.5, several studies report weight loss in experimental mammals after exposure to rotenone (Brooks and Price 1961; Haag 1931; Hansen et al. 1965; Marking 1988). Moreover, body weight loss is the endpoint on which the chronic RfD is based (U.S. EPA/OPP 2007a). While changes (increases or decreases) in body weight might be associated with effects on endocrine function, body weight loss is a very common observation in toxicity studies and could be due to a variety of other factors secondary to general adverse effects. In addition, the loss of body weight is consistent with the biochemical mechanism of action, the inhibition of mitochondrial oxidative phosphorylation (Section 3.1.2). In the absence of any indication of effects on endocrine tissue, there is no basis for asserting that decreases in body weight are associated with changes in endocrine function.

Alam and Schmidt (2004b) report that intraperitoneal doses of 2 mg/kg bw/day to rats over a period of 30-60 days caused a decrease in plasma testosterone. The effect, which is also seen in Parkinson's disease, was attributed to diminished bioenergetics—i.e., a decrease in ATP in adrenal and testicular tissue—as well as general oxidative damage to adrenal and testicular tissue. The effect, however, did not appear to involve changes in thyroid or pituitary hormones. Nonetheless, an alteration in testosterone levels would clearly be regarded as a disruption in the endocrine system.

The U.S. EPA has yet to adopt standardized screen tests for endocrine disruptors. The Agency did conclude, however, that: *In the available toxicity studies on rotenone, there was no estrogen, androgen, and/or thyroid mediated toxicity shown* (U.S. EPA/OPP 2005a, p. 28). The Agency, however, did not address or cite the study by Alam and Schmidt (2004b).

3.1.9. Reproductive and Teratogenic Effects

3.1.9.1. Developmental (Teratology) Studies

Developmental studies are used to assess whether a compound has the potential to cause birth defects as well as other effects during prenatal development or immediately after birth. These studies typically entail gavage administration to pregnant rats, mice, or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are generally required for the registration of pesticides. Very specific protocols for developmental studies are established by U.S. EPA/OPPTS and are available at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized.

As summarized by U.S. EPA/OPP (2005a, 2007a), two teratology studies were submitted to the EPA in support of the registration of rotenone. One study was conducted in rats (referenced by the Agency as MRID 0144294) and the other study was conducted in mice (referenced by the Agency as MRID 00141707 for the main study and MRID 00145049 for the range-finding study). Both studies were classified by the U.S. EPA/OPP (2005a, Table 4.1b, p.7) as *acceptable/guideline*, indicating that the studies followed the above referenced EPA protocols and were conducted in an acceptable manner. In addition to the summaries of these studies provided in U.S. EPA/OPP (2005a, 2007a), the Agency kindly provided a detailed summary of these and other toxicity studies on rotenone (Gardener 1985b) for the preparation of the current Forest Service risk assessment.

The teratology study in rats involved dosing at 0, 0.75, 1.5, 3, and 6 mg/kg bw/day from Days 6-19 of gestation. Maternal effects—i.e., salivation and abnormal behavior—were noted in all dose groups. A 23% decrease in body weight gain as well as an increase in unossified sternabrae, relative to controls was noted at 6 mg/kg bw/day, and this dose was classified as a LOAEL. The rat NOAEL was identified by EPA as 3 mg/kg bw/day.

The teratology study in mice involved doses of 0, 3, 9, 15, 24 mg/kg/day on Days 6-17 of gestation. No adverse effects were noted in dams or offspring at 15 mg/kg bw/day. The developmental LOAEL was 24 mg/kg bw/day based on increased resorptions (3.8 versus

0.5 in controls) that were seen in the range-finding study. As discussed further in Section 3.3.3 (Acute RfD), the U.S. EPA/OPP (2007a) used the 15 mg/kg bw/day NOAEL as the basis for the acute RfD.

As summarized in Appendix 1, Spencer and Sing (1982) conducted a teratology study in rats using dietary rather than gavage exposure. The dietary concentrations ranged from 10 to 1000 ppm, corresponding to doses (based on measured food consumption and body weight) of 0.74-40 mg/kg bw/day from Days 6-15 of gestation. A decrease in fetal survival rate was noted at all but the lowest dose—i.e., the NOAEL was 0.77 mg/kg bw/day. This NOAEL is virtually identical to the NOAEL of 0.5-0.6 mg/kg bw/day from a reproduction study discussed in the following section.

3.1.9.2. Reproduction Studies

Reproduction studies involve exposing one or more generations of the test animal to the compound. The general experimental method involves dosing the parental (P or F0) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to mating, during mating, after mating, and through weaning of the offspring (F1). In a 2-generation reproduction study, this procedure is repeated with male and female offspring from the F1 generation to produce another set of offspring (F2). During these types of studies, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number, viability, and growth of offspring. As is the case with teratology studies, the U.S. EPA has very specific protocols for conducting multi-generation developmental studies (http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized).

U.S. EPA/OPP (2005c, 2007a) summarizes one acceptable/guideline reproduction study for rotenone in rats (referenced MRID 00141408). Although the EPA documentation does not identify a full citation to the study, this study appears to be identical to the reproduction study summarized by Marking (1988).

As with the teratology studies discussed in the previous subsection, a full copy of the one-generation reproduction study was not available for the current Forest Service risk assessment; however, a much more detailed summary of this study (Gardener 1985b) is available. The developmental study involved dietary exposures to 0, 7.5, 37.5, and 75 ppm rotenone in the diet. Based on measured body weight and food consumption, the dietary exposures corresponded to 0, 0.5-0.7 mg/kg bw/day (7.5 ppm), 2.4-3.7 mg/kg bw/day (37.5 ppm), and 4.8-8.1 mg/kg bw/day (75 ppm). The ranges in the daily doses reflect modest differences in food consumption and body weight between the sexes and generations.

The reproductive LOAEL was identified as 4.8-6.2 mg/kg bw/day (75 ppm, F0) based on decreases in live pups/litter in the F0 generation. This effect was also seen in the F1 generation. The corresponding reproductive NOAEL was identified as 2.4-3 mg/kg bw/day (37.5 ppm, F0). While 2.4-3 mg/kg bw/day is classified as a reproductive

NOAEL, a decrease in pup body weight was seen at this dose, and the NOAEL for offspring was established as 0.5-0.6 mg/kg bw/day.

Haag (1931) conducted a single generation reproduction study in guinea pigs. At a dietary concentration of 150 ppm, all young were either born dead or died within 5 days of birth. In a chick embryo screening assay, Roa and Chauhan (1971) noted a complete arrest of embryo development at 1 mg/L but no effect at 0.1 mg/L.

3.1.9.3. Target Organ Toxicity

As noted in Section 3.1.8 (Endocrine System), damage to gonadal tissue (ovaries or testes) can suggest an effect on endocrine function, and damage to these organs could be related to the adverse reproductive effects of rotenone, as discussed in the previous two subsections. While rotenone has been shown to decrease plasma testosterone levels (Alam and Schmidt 2004b), *in vivo* studies do show specific damage to gonadal tissue. In an *in vitro* mouse ovarian follicle culture system (Wycherley et al. 2005), rotenone arrested follicle growth at concentrations of 0.1, 0.5, and 1 µmol/L (i.e., 39, 197, and 394 µg/L).

3.1.10. Carcinogenicity and Mutagenicity

Mutagenicity assays are required by the U.S. EPA for the registration of pesticides. As summarized by U.S. EPA/OPP (2005a) and detailed further by Gardner (1985a), rotenone will arrest cell division; however, chromosomal damage has not been noted, and a full battery of mutagenicity assays submitted to the U.S. EPA did not provide an indication of mutagenic activity. Consistent with the studies submitted to the EPA, several mutagenicity screening assays in the published literature note arrested cell development (Barham and Brinkley 1976a,b; Meisner and Sorensen 1966) but no indication of mutagenicity (Amer and Aboul-ela 1985; Moriya et al. 1983; Waters et al. 1982). More recently, Johnson and Parry (2008) demonstrated that rotenone can induce aneuploidy (an abnormal number of chromosomes) through a disruption of the mitotic spindle. In addition, chromosome breaks and abnormal chromosome numbers were observed in cultured human lymphocytes (de Lima et al. 2005).

In terms of a quantitative significance to the human health risk assessment, carcinogenicity is an issue only if the *in vivo* data are adequate to support the derivation of a cancer potency factor. As reviewed by both U.S. EPA/OPP (2005a, 2007) and WHO (1990, 1992), chronic oral studies in rats and mice have failed to provide an indication that rotenone is carcinogenic. Thus, the U.S. EPA classifies the carcinogenic potential of rotenone in the lowest risk category: Group E (evidence of non-carcinogenicity for humans).

The only contrary report is provided by Gosalvez and Merchan (1973) in a brief *Letter to the Editor* in Cancer Research. These investigators report an increase in mammary tumors in female rats after intraperitoneal injections of rotenone at doses of 1.7 mg/kg body weight for 42 days. The tumors are characterized as: *adenomas with accentuated interstitial fibrosis and showed localized areas with adenocarcinomatous transformation* (Gosalvez and Merchan 1973). The Gosalvez and Merchan (1973) report is not

addressed in the EPA or WHO reviews, although WHO (1980) does cite the Gosalvez (1983) review suggesting that rotenone could be carcinogenic in vitamin-deficient animals.

While the Gosalvez and Merchan (1973) publication is acknowledged, the presence of negative mutagenicity studies, negative carcinogenicity studies by a more relevant route of exposure, the lack of any larger confirming studies over the 35 years since the publication of Gosalvez and Merchan (1973), as well as the judgments expressed by both the U.S. EPA and the World Health Organization, indicate that carcinogenicity is not an endpoint of concern for rotenone.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

3.1.11.1. Skin Irritation

The rotenone literature does not contain published studies or reports on skin irritation. The U.S. EPA evaluated skin irritation using relatively standard studies in which a pesticide is kept in contract with a shaved area of skin for 24 hours and dermal irritation is evaluated for a period of at least 72 hours. Rotenone evidenced a very low level of dermal irritation, and the EPA classifies the dermal irritation potential of rotenone as Category IV, the lowest hazard grouping (U.S. EPA/OPP 2005a; U.S. EPA/OPP 2006e). Relatively standard precautionary language on avoiding skin contact is included on all rotenone product labels and MSDSs.

3.1.11.2. Skin Sensitization

As with skin irritation, there are no published studies on the potential of rotenone to induce skin sensitization. U.S. EPA uses a standard assay for skin sensitization, just as it does for skin irritation. Rotenone is classified as having no indication of dermal sensitization (U.S. EPA/OPP 2005a; U.S. EPA/OPP 2006e). The EPA does not, however, use the Category I through IV classification system used for skin irritation studies to classify the degree of skin sensitization to rotenone.

3.1.11.3. Ocular Effects

Rotenone appears to have a low potential for eye irritation. On the basis of standard eye irritation studies in rabbits in which mild conjunctival irritation (reversible in 24 hours) was noted after direct instillation of rotenone, U.S. EPA/OPP (2005a, 2006e) classifies rotenone as Category IV, the lowest hazard grouping.

All liquid formulations of rotenone contain petroleum solvents, as discussed in Section 2 of this risk assessment, and it is plausible that the petroleum solvents would be more of an ocular irritant than rotenone itself. Accordingly, all product labels for rotenone formulations contain standard precautionary language on avoiding direct eye contact with the formulations.

3.1.12. Systemic Toxic Effects from Dermal Exposure

The potential for dermal toxicity is most often characterized by an LD_{50} value, and the EPA requires dermal LD_{50} studies for pesticide registration. The dermal toxicity studies cited in U.S. EPA/OPP (2005a, 2007a) include one which resulted in an acute dermal LD_{50} of >5000 mg/kg body weight in rabbits, which the EPA uses to classify the dermal toxicity of rotenone as Category IV, the least toxic classification.

The review by Gardener (1985b) does not summarize the dermal study which resulted in the LD_{50} of >5000 mg/kg body weight used by the EPA, but summarizes a dermal toxicity study involving a mixture of rotenone, pyrethrins, and an aromatic petroleum solvent in which the dermal LD_{50} in rabbits is 2000 mg/kg body weight. Hayes (1982, p. 83) cites an early dermal LD_{50} of 100 mg/kg body weight.

Discrepancies in LD_{50} values, particularly with values from older literature, are common. The reasons for the discrepancies in the available data on rotenone cannot be identified. Using the U.S. EPA/OPP (2005a) dermal absorption rate of 9%, a dermal LD_{50} of >5000 mg/kg body weight would result in an equivalent oral dose of >450 mg/kg body weight. The failure to observe substantial mortality after dermal exposure to an equivalent oral dose of 450 mg/kg body weight in rabbits is reasonably consistent with the early oral toxicity data reported by Haag (1931) in which rabbits survived single oral doses of up to 1250 mg/kg body weight.

3.1.13. Inhalation Exposure

As discussed in Section 3.1.3.2, rotenone is likely to be more toxic by inhalation than by oral exposure because inhalation exposures bypass initial metabolism and detoxification by the liver. Studies submitted to the U.S. EPA/OPP (2007a) in support of the registration of rotenone report 4-hour LC_{50} values of 0.0235 mg/L in male rats and 0.0193 mg/L in female rats. As with the acute oral studies, female rats appear to be somewhat more sensitive than male rats to inhalation exposure to rotenone. Based on these LC_{50} values, the U.S. EPA classifies the inhalation toxicity of rotenone as Category I, the most hazardous ranking.

The EPA expresses concern for inhalation exposures in workers applying rotenone as a piscicide, and, as noted in Section 2, the Agency now requires the use of a full-face respirator in workers involved in ground applications of rotenone (U.S. EPA/OPP 2007a, 2007d). Thus, while inhalation exposures to rotenone are a concern to the current Forest Service risk assessment, this hazard should be mitigated by the use of protective equipment. The impact of protective equipment is considered further in Section 3.2.2.1 (Workers, General Exposures).

3.1.14. Inerts and Adjuvants

3.1.14.1. Inerts

The U.S. EPA is responsible for regulating inerts and adjuvants in pesticide formulations. As implemented, these regulations affect only pesticide labeling and testing requirements. The term *inert* was used to designate compounds that do not have a direct toxic effect on

the target species. While the term *inert* is codified in FIFRA, some inerts can be toxic, and the U.S. EPA now uses the term *Other Ingredients* rather than *inerts* (http://www.epa.gov/opprd001/inerts/). For brevity, the following discussion uses the term *inert*, recognizing that *inerts* may be biologically active and potentially hazardous.

Several liquid formulations of rotenone list potentially hazardous compounds on the material safety data sheets (MSDS's) for the formulations and these compounds are summarized in Table 3. The MSDS's for the powdered formulations do not list any potentially hazardous inerts. As discussed in Section 2.2, the solid formulations of rotenone are essentially ground plant roots. These solid formulations contain other rotenoids, which are considered further in Section 3.1.15.2 (Impurities).

All of the liquid formulations of rotenone contain petroleum based products characterized as *petroleum distillates*, *xylene range aromatics*, or *aromatic petroleum products*. All of these solvents are complex and variable mixtures of aromatic and aliphatic compounds (e.g., ATSDR 1999). The MSDS's for the liquid formulations provide varying levels of detail in specifying the nature of the solvents used in the formulations. The MSDS's for Synpren-Fish Toxicant and Prenfish Toxicant identify many of the specific compounds in the petroleum products as well as the concentrations of the components in the solvent. Other formulations simply characterize the petroleum product as a *variable mixture*.

The differences in the reporting details in the MSDS's do not necessarily indicate that the petroleum products used in the different formulations do not contain the inerts identified in the other formulations. For example, and as discussed further below, 1,2,4-trimethylbenzene which is identified as an inert in both Prenfish Toxicant (at 32%) and Synpren-Fish Toxicant (at 1.7%). This compound is not identified as an inert in CTF Legumine. Fisher (2007), however, reports that 1,2,4-trimethylbenzene was detected in CTF Legumine at an average concentration of 30.7 mg/L (about 0.003%) with a range of 26-35 mg/L and naphthalene was detected at a concentration of 255.1 mg/L (0.02551%) with a range of 229-311 mg/L (Fisher 2007, Table 2, p. 10). While somewhat peripheral to the discussion of risk, it is noteworthy that the MSDS for CTF Legumine is not required to specify the concentration of 1,2,4-trimethylbenzene, because this compound is present at a very low concentration.

The assessment of whether or not the inerts are a concern is based both on considerations of relative potency—i.e., the potency of the inert relative to rotenone—and the amount of the inert present in the formulation relative to the amount of rotenone. Relative potency is defined quantitatively as the ratio of equitoxic doses (Finney 1971). Adopting the nomenclature of Finney (1971), potency ($\boldsymbol{\rho}$) is defined as the reciprocal of the RfD. In other words, the lower the RfD, the higher the potency. The relative potency of an inert with respect to rotenone is then defined as:

$$\boldsymbol{\rho} = 1/RfD_{Inert} \,/\, 1/RfD_{Rotenone} = RfD_{Rotenone} \,/\, RfD_{Inert}$$

A summary of the toxicities of the inerts relative to rotenone is presented in Table 6. While most of the exposure scenarios considered in this risk assessment involve very

brief periods of time, most of the calculations of relative toxicity are based on the chronic rather than the acute RfD because acute RfD values are not typically derived for compounds other than pesticides.

The only exception to the use of the chronic RfD is N-methylpyrrolidone. No RfD for this agent has been derived by the U.S. EPA; furthermore no comparable risk values (e.g., MRL's from ATSDR or ADI's from WHO) were found. N-methylpyrrolidone is identified as a compound of concern on MSDS's and has been cited as a concern by CalEPA (1999) and CalDHS (2006) based on developmental and reproductive toxicity data. Rather than excluding N-methylpyrrolidone from the quantitative comparison, a surrogate acute RfD of 1.25 mg/kg bw/day is derived based on the NOAEL of 125 mg/kg bw/day from the teratology study in mice by Saillenfait et al. (2001) and an uncertainty factor of 100. The toxicity relative to rotenone is then calculated using the acute RfD of 0.015 mg/kg bw/day from U.S. EPA/OPP (2007a) which is also based on a reproductive NOAEL and an uncertainty factor of 100.

As indicated in Table 6, the toxicity of the inerts in liquid formulations of rotenone is considerably lower than the toxicity of rotenone itself—i.e., ranging from factors of 0.00044 to 0.02—indicating that the inerts are less toxic than rotenone by factors of 50 to more than 2000. The most toxic inerts, relative to rotenone, are naphthalene (relative potency of 0.02), N-methylpyrrolidone (relative potency of 0.012), and 1,2,4-trimethylbenzene (0.008). While the toxicity of 1,2,4-trimethylbenzene is very low relative to rotenone, 1,2,4-trimethylbenzene is considered quantitatively in this discussion because it comprises 32% of the xylene range aromatics (90% of the formulation) in Synpren-Fish Toxicant—i.e., the formulation consists of 1,2,4-trimethylbenzene at a proportion of about 0.288 [0.9 x 0.32].

In considering the amount of a compound in a formulation, the potency-weighted amount of a compound (***ρAmt***) is taken as the proportion of the compound in the mixture (***π***) divided by the RfD:

$$\rho Amt = \pi / \text{RfD}.$$

Again, this is a standard method in the assessment of mixtures (e.g., Finney 1971; Mumtaz et al. 1994). The relative hazard (***RH***) of the inert with respect to rotenone is defined as the as potency-weighted amount for rotenone (***ρAmt*$_{\text{Rot}}$**) divided by the potency-weighted amount for the inert (***ρAmt*$_{\text{Inrt}}$**):

$$RH = \rho Amt_{\text{Rot}} / \rho Amt_{\text{Inrt}}.$$

The interpretation of relative hazard (***RH***) is straight forward. As ***RH*** increases, concern for the inert increases, and an ***RH*** of 1 indicates that the inert contributes as much toxicity to the mixture as rotenone. If more than one inert is considered, the relative hazards can be added. Thus, the total relative hazard (***RH*$_{Tot}$**) for a group of ***n*** inerts is calculated as:

$$RH_{Tot} = RH_1 + RH_2 + \ldots + RH_n$$

The application of this approach to rotenone formulations is modestly complicated by the occurrence of other *associated resins* in rotenone formulations as well as the addition of piperonyl butoxide in some formulations.

As noted in Table 2, *associated resins* are listed as an active ingredient on all product labels, and the percent of other associated resins ranges from 2.5 to 10% in the rotenone formulations. As discussed in Section 3.1.15.2 (Impurities), most of the constituents of the associated resins do not appear to be biologically active. A notable exception, however, is deguelin, which appears to be about half as toxic as rotenone (Cabizza et al. 2004) and is present in cubé resin at a concentration of about 22%, about half the concentration of rotenone (Fang and Casida 1999b). Other agents in cubé resin are less toxic than deguelin by at least a factor of 2 (Fang and Casida 1999b, Table 3 p. 2135). For a consideration of relative hazard, the amount of rotenone equivalents in a formulation is calculated as the proportion of rotenone plus the proportion of *associated resins* multiplied by 0.25. For example, Prenfish Toxicant contains 5% rotenone and 10% other resins (Table 2). For calculations of relative potency, the proportion of rotenone equivalents in Prenfish Toxicant is 0.075 (i.e., 0.05 + (0.10 x 0.25)).

Piperonyl butoxide must be handled somewhat differently. As discussed in Section 3.1.14.2 (Adjuvants), piperonyl butoxide is a synergist for rotenone in that piperonyl butoxide inhibits the metabolism and hence the detoxification of rotenone. Piperonyl butoxide will enhance the toxicity of rotenone, and this detail should be considered in the assessment of formulations that contain piperonyl butoxide. While it is difficult to quantify the enhancement, all formulations containing piperonyl butoxide contain only half as much rotenone as formulations that do not contain piperonyl butoxide. For the assessment of relative hazard, the proportion of piperonyl butoxide in the formulation is treated as an equivalent amount of the rotenone. Thus, all formulations that contain 2.5% rotenone with 2.5% piperonyl butoxide are treated as if they contained 5% rotenone. As detailed in Section 4.1.3.1 (Hazard Identification for Fish) in the discussion of the study by Marking and Bills (1976), this appears to be a reasonable assumption.

While the algorithms for implementing the consideration of relative hazard are not difficult, they are somewhat cumbersome. Consequently, the calculations are included in three custom worksheets (naphthalene, N-methylpyrrolidone, and 1,2,4-trimethylbenzene) which follow Worksheet A01 in the workbook that accompanies this risk assessment (Attachment 1). A summary of the analysis is given below:

	Relative Hazard		
	Synpren-Fish Toxicant	Prenfish Toxicant	CTR Legumine
Naphthalene	0	0.021	0.000082
Trimethylbenzene	0.037	0.0015	0.000004
N-methylpyrrolidone	0	0	0.019
TOTAL:	0.037	0.0225	0.019085

While this analysis could be extended to other inerts, the exercise would be trivial, because of the lower toxicity of the other inerts with respect to rotenone (Table 6) and the small amounts of the other known inerts in these formulations (Table 3). This analysis suggests that the inerts in the three rotenone liquid formulations listed above are not present in toxicologically significant amounts, relative to rotenone. In other words, for the three formulations on which the analysis can be conducted, the total hazard contribution of the inerts of greatest concern are below the potential hazard posed by rotenone by factors ranging from about 30 to greater than 50.

The significance of the petroleum solvents in other liquid formulations of rotenone—i.e., Chem Fish Synergized, Chem Fish Regular, Nusyn-Noxfish Fish Toxicant, and Noxfish Fish Toxicant—cannot be directly assessed because the compounds in the petroleum solvents are not clearly identified. In a review of rotenone formulations, Ott (2008) indicates that Nusyn-Noxfish will yield 145 ppb total trimethylbenzenes to achieve a rotenone concentration of 20 ppb—i.e., the concentration of total trimethylbenzenes in the formulation is a factor of about 7 higher than that of rotenone, which is similar to the concentration of 1,2,4-trimethylbenzene, relative to rotenone, in Synpren-Fish Toxicant— i.e., a factor of about 9.

The potential impact of inerts posed by the application of rotenone liquid formulations was also reviewed by Fisher (2007), Ott (2008), and Entrix (2007). None of these reviews suggests that the inerts in liquid formulations are likely to pose significant risks, relative to the risks posed by rotenone itself. While the U.S. EPA RED (U.S. EPA/OPP 2007a) does not assess the potential toxicity of the inerts in rotenone formulations, the risk assessment conducted by the Environmental Fate and Effects Division (U.S. EPA/OPP 2006c) does address inerts and concludes that:

> *... based on toxicity data collected on both technical grade rotenone (>95% active ingredient) and formulated end-product, the technical grade active ingredient is generally more toxic than formulated end-product [corrected for active ingredient] by at least a factor of two. These data suggest that for the formulated products tested and the toxicity endpoints measured, the inerts do not contribute substantially to the toxicity of the active ingredient.* (U.S. EPA/OPP 2006c, p. 11)

While the current risk assessment concurs with the other assessments, there are some differences between the current analysis and the analyses offered in these other reviews. For example, the review by Entrix (2007) uses the IRIS RfD for rotenone of 0.004 mg/kg/day (U.S. EPA/ORD 1988) rather than the more conservative RfD of 0.0004 mg/kg/day derived by the Office of Pesticide Programs (U.S. EPA/OPP 2007a). Similarly, the Entrix (2007) review uses an RfD of 0.5 mg/kg/day for 1,2,4-trimethylbenzene cited to an EPA provisional toxicity value. The analysis presented above uses a 10-fold more conservative risk value of 0.05 mg/kg bw/day from a Superfund assessment prepared by the U.S. EPA (U.S. EPA/Region 10 2002).

These differences in the analyses illustrate some of the problems associated with the assessment of inerts. The information on many inerts is incomplete, and a number of different toxicity values can be used in constructing comparisons between the toxicity of active and inert ingredients. The current risk assessment has evaluated the inerts following the same general principles applied in all Forest Service risk assessments – i.e., unless a compelling basis is apparent for doing otherwise, the most conservative risk values are used. Notwithstanding these differences among the analyses, there is no basis for asserting that inerts are a substantial concern relative to the toxicity of rotenone and related rotenoids.

At least some rotenone formulations contain low concentrations of trichloroethylene because trichloroethylene may be used to extract rotenone and related rotenoids from plant roots. Thus, while not classified as an inert, trichloroethylene could also contribute to the toxicity of rotenone formulations. As discussed in Section 3.1.15.3, however, the contribution of trichloroethylene to the toxicity of rotenone formulations appears to be very low and does not impact the above analysis of the inerts.

3.1.14.2. Adjuvants

As noted in Section 3.1.3. (Pharmacokinetics) and discussed further in Section 3.1.16 (Toxicological Interactions), piperonyl butoxide is a well-known inhibitor of mixed function oxidases, a group of enzymes that metabolize and hence detoxify rotenone (Section 3.1.15). Piperonyl butoxide may be considered an adjuvant in rotenone formulations—i.e., it enhances the toxicity of rotenone—rather than an inert. This appears to be the reason that piperonyl butoxide is listed as one of the active ingredients on product labels of formulations that contain piperonyl butoxide (Table 2).

At equivalent levels of rotenone and related rotenoids, exposures involving formulations that contain piperonyl butoxide are likely to be both more effective than other formulations and are also likely to pose a greater risk to both humans and nontarget species. It is difficult, however, to quantify the magnitude of this increased risk directly, particularly for humans, because the available toxicity studies on which the dose-response assessment can be based (Section 3.3) involve exposures only to rotenone. Toxicity studies involving co-exposure to rotenone and piperonyl butoxide in mammals that are comparable to the studies used in the dose-response assessment for mammals (Section 3.3) are not available.

In the assessment of the toxic contribution of inerts to rotenone formulations (Section 3.1.14.1), the assumption is made that the toxic contribution of piperonyl butoxide to rotenone formulations is equivalent to that of rotenone. In other words, a formulation that contains 2.5% rotenone with 2.5% piperonyl butoxide is treated as if it contained 5% rotenone. As illustrated in Figure 5 and discussed in Section 4.1.3.1.3, acute toxicity bioassays in fish by Marking and Bills (1976) support the assumption that piperonyl butoxide may be treated as an equivalent amount of rotenone in assessing the impact of piperonyl butoxide in rotenone formulations. As detailed in Section 3.1.17 (Impact of Impurities and Adjuvants), this assumption is incorporated into the current Forest Service risk assessment.

3.1.15. Impurities, Metabolites, and Contaminants

3.1.15.1. Metabolites

As discussed in SERA (2007, Sections 3.1.3.1), two types of metabolites may be considered in a risk assessment, *in vivo* metabolites and environmental metabolites. *In vivo* metabolites refer to compounds that may form within an animal after a chemical agent is absorbed. Environmental metabolites refer to compounds that may form in the environment as the result of biological and chemical processes.

While the metabolites of rotenone have not been studied as extensively as rotenone itself, metabolism is clearly a detoxification mechanism for rotenone (Fang and Casida 1998, 1999a,b). Rotenone is metabolized to more polar compounds by cytochrome P450 enzyme systems, a group of enzymes found in humans and most other animals. These more polar compounds are more readily excreted than rotenone. Fang and Casida (1999b) established that two specific isozymes of P450 (3A4 and 2C19) are the most active in the metabolism and detoxification of rotenone. As discussed in Section 3.1.14.2, the inclusion of piperonyl butoxide, a well-known inhibitor of P450 enzymes, is included in some formulations of rotenone to specifically block the metabolism and detoxification of rotenone by cytochrome P450.

In addition to the available experimental data, the environmental fate data on rotenone (Table 1) as well as many anecdotal reports and field studies (Appendix 7) clearly indicate that rotenone is rapidly degraded in the environment and that the degradation products are less biologically active than rotenone itself.

3.1.15.2. Impurities

As summarized in Section 2.2. of this risk assessment and detailed further in several reviews on rotenone (e.g., Orr et al. 2002; Ott 2008), rotenone itself is not commercially synthesized. Rotenone is obtained by processing the roots of plants such as *Derris* and *Lonchocarpus* species. Consequently, the materials from which rotenone formulations are made consist of complex mixtures of rotenone and other plant materials commonly referred to on the product labels as *other associated resins* or *other associated extracts*. The relative proportions of rotenone and related products in a commercial formulation will vary with the plant material from which the rotenone is obtained as well as the procedures used in processing the plant material. This variability is illustrated in Table 7, which lists *the non-end use* formulations of rotenone. The term *non-end use* designates formulations that are used as the basis for preparing the commercially available formulations but are not themselves applied directly in rotenone applications. As indicated in Table 7, these non-end use formulations may contain from 7.4 to 44.2% rotenone, and the ratios of rotenone to other associated materials range from 0.66 to 1.1.

Fang and Casida (1999b) assayed the potency of rotenone and 28 other compounds found in a cubé resin sample—i.e., a non-end use formulation—obtained from Peruvian *Lonchocarpus utilis* and *L. urucu*. The bioassays used include NADH:ubiquinone oxidoreductase (i.e., mitochondria Complex I as discussed in Section 3.1.2), the inhibition of phorbol ester-induced ornithine decarboxylase (a screening assay for cancer

inhibition), as well as cancer cell growth inhibition assays with two different cell types, mouse liver cancer cells and human epithelial breast cancer cells. These bioassays generally indicate that rotenone and deguelin (Figure 1) are substantially more toxic than the other compounds (Fang and Casida 1999b, Table 3, p. 2135). In all four assays, rotenone was found to be substantially more potent than any of the other compounds.

For the current risk assessment, the relative potencies from the NADH:ubiquinone oxidoreductase assay are most relevant because this endpoint is most directly related to the mechanism of action of rotenone (Section 3.1.2). In the NADH:ubiquinone oxidoreductase assays, the IC_{50} values for rotenone and deguelin were 4.4 and 6.9 nM, respectively, where nM indicates the concentration in nanomoles (moles x 10^{-9}). These two compounds were also present in cubé resin at the highest concentrations—i.e., 44% for rotenone and 22% for deguelin. The next three most potent compounds were a 12a-methoxy substituted rotenone (IC_{50}=16 nM), an 11-hydroxyl substituted deguelin (IC_{50}=18 nM), and a 12a,β-methoxyl substituted deguelin (IC_{50}=21 nM). Taking the standard definition of relative potency (Section 3.1.14.1), these compounds are less toxic than rotenone by factors of about 4-5.

The other compounds studied by Fang and Casida (199b) have IC_{50} values that range from 115 to >10,000 nM—i.e., they are less potent than rotenone by factors ranging from about 26 to greater than 2270. Fang and Casida (199b) do not specify the proportions of most of the rotenone and deguelin derivatives; they do, however, indicate that most of the compounds (and all of the compounds that are within a factor of 4-5 of rotenone's potency) were present at <0.5% each. Thus, in terms of mass-weighted relative potency, only rotenone and deguelin are present in toxicologically substantial amounts.

The toxicological significance of deguelin is also underscored by the Caboni et al. (2004) study in which rotenone and deguelin were assayed for the ability to induce Parkinson's disease-like symptoms in rats by subcutaneous injection. As indicated in Table 5, rotenone induced symptoms in rats at a dose of 3 mg/kg bw/day over a dosing period of up to 28 days. Deguelin had no effect at 3 mg/kg bw/day but did induce Parkinson's disease-like symptoms at a dose of 6 mg/kg bw/day for 16 days that were comparable to the symptoms observed with rotenone at 14 days (Caboni et al. 2004, Table 1, p. 1543). These *in vivo* results are consistent with the *in vitro* assay by Fang and Casida (1999b) indicating that deguelin is about half as potent as rotenone.

The toxicity of the compounds in rotenone formulations other than rotenone itself is of practical concern to the current risk assessment. Most risk assessments involving rotenone formulations (e.g., U.S. EPA/OPP 2007a) quantitatively consider only exposures to rotenone and do not quantitatively incorporate exposures to other related resin materials that may cause effects identical to those of rotenone. In addition and as summarized in Table 2, end-use formulations of rotenone contain other associated resins that vary from 2.5 to 11.1% of the formulation. If the other associated resins are toxicologically active, a case could be made that formulations with higher concentrations of other resin compounds should be regarded as more hazardous than formulations that contain lesser amounts of associated resin compounds. As detailed further in Section

3.1.17, the impact of associated resins in rotenone formulations is considered quantitatively in the current Forest Service risk assessment.

3.1.15.3. Contaminants

In at least some formulations of rotenone, trichloroethylene is used as a solvent in processing roots from *Derris* and *Lonchocarpus* species to obtain cubé resins which constitute the non-end use formulations of rotenone—i.e., those listed in Table 7 (e.g. Cabizza et al. 2004). Thus, trichloroethylene, when present in rotenone formulations, is considered as a contaminant or impurity rather than an inert or adjuvant because trichloroethylene is not intentionally added to rotenone end-use formulations but is present in these formulations as a consequence of the manufacturing process.

The concentrations of trichloroethylene in rotenone end-use formulations are very low. Fisher (2007) reports that trichloroethylene was found in samples of CFT Legumine at concentrations of 7.3 (0-29.1) mg/L—i.e. about 0.00073% (0% - 0.0029%)—and that the estimated concentration in a lake after the application of CFT Legumine is 0.0073 µg/L (about 7.3 parts per trillion). Finlayson et al. (2000) indicates that initial water concentrations of trichloroethylene could reach 1.4 ppb (1.4 µg/L) in water after an application of rotenone at a concentration of 2000 ppb—i.e., a factor of 10 greater than the maximum allowable application rate. With specific reference to Nusyn-Noxfish, Ott (2008) indicates that concentrations of trichloroethylene in water could reach 4 ppt (parts per trillion) at an application rate of 20 ppb (parts per billion) rotenone.

As reviewed by ATSDR (1997), trichloroethylene is a potential concern because it is both a toxic agent, primarily affecting the liver and nervous system, and because trichloroethylene is classified as a potential human carcinogen. The classification of trichloroethylene as a probable human carcinogen is based on an assessment from IARC (1997) which notes that there is limited evidence for the carcinogenicity of trichloroethylene in humans but sufficient evidence in mammals. Neither the U.S. EPA (U.S. EPA/ORD 1992a) nor any other government organization has derived a cancer potency factor for trichloroethylene.

U.S. EPA/ORD (1992a) also declined to derive an RfD for trichloroethylene because of limitations in the available toxicological data. For similar reasons, ATSDR (1997) declined to derive a chronic MRL (minimum risk level)—a chronic toxicity value comparable to a chronic RfD. ATSDR (1997), however, derived an acute MRL of 0.2 mg/kg/day based on a developmental toxicity study in mice. Analogous to the approach taken with N-methylpyrrolidone (Section 3.1.14.1), the potential toxicological significance of trichloroethylene with respect to rotenone can be assessed using the acute RfD for rotenone of 0.015 mg/kg bw/day from U.S. EPA/OPP (2007a), which is also based on a reproductive toxicity study. Based on these toxicity values, trichloroethylene is less toxic than rotenone by a factor of about 13 [0.2 mg/kg bw/day divided by 0.015 mg/kg bw/day].

Using the upper range of the proportion of trichloroethylene reported in CFT Legumine—i.e., 0.0000291 from Fisher (2007)—the mass-weighted relative potency of

trichloroethylene relative to rotenone is 0.000035. As with the calculations of the mass-weighted relative potency of the inerts, the details of this calculation are given in a custom worksheet following Worksheet A01 in Attachment 1. In other words, the contribution of trichloroethylene to the toxicity of CFT Legumine is a factor of over 28,000 below that of rotenone. While concentrations of trichloroethylene are likely be different in other formulations, the very small contribution of trichloroethylene to the toxicity of CFT Legumine suggests that trichloroethylene contamination in rotenone formulations is not toxicologically significant.

3.1.16. Toxicological Interactions

3.1.16.1. In Vivo Interactions

Toxicological interactions for rotenone are likely to be based on the oxidation of rotenone to less toxic compounds. The oxidation of rotenone may occur biologically, through metabolism or chemically through the intentional addition of potassium permanganate to water treated with rotenone. The biologically-based interactions are discussed in this subsection, and the detoxification of rotenone with potassium permanganate is discussed in the following subsection.

As discussed in Section 3.1.3.1, the primary metabolic pathways for rotenone involve detoxification by cytochrome P450 enzyme systems (Fukami et al. 1969). Piperonyl butoxide is a classic inhibitor of cytochrome P450 enzymes, which is the basis for the use of piperonyl butoxide in rotenone formulations (3.1.14.2. Adjuvants). Piperonyl butoxide and other compounds that are also metabolized by cytochrome P450 enzymes or compounds that bind tightly to cytochrome P450 enzymes may compete with rotenone, and this competition will enhance the toxicity of rotenone by inhibiting the detoxification of rotenone.

The quantitative significance of interactions with other compounds metabolized by cytochrome P450 depends on many factors including the binding affinity of the different compounds to cytochrome P450. In addition, many compounds that are metabolized by cytochrome P450 will also induce cytochrome P450 (e.g., Lewis et al. 1998). In other words, exposure to a compound that serves as a substrate for cytochrome P450 will often result in a series of processes that lead to increased amounts of cytochrome P450 in the organism. Thus, while concurrent exposures to rotenone and other substances that are metabolized by cytochrome P450 may enhance the toxicity of rotenone, sequential exposures may have the opposite effect. If cytochrome P450 is induced in an organism by a compound prior to exposure to rotenone, the higher levels of cytochrome P450 could result in the more rapid detoxification of rotenone. A final complication involves the specific isozymes of cytochrome P450. While cytochrome P450 is generally viewed as broad spectrum mixed-function oxidase, there are many varieties (isozymes) of P450, and the different isozymes have differing levels of affinity to various chemicals. As noted in Section 3.1.15.2 (Metabolites), two specific isozymes of P450 are most active in the metabolism of rotenone (Fang and Casida 1999b). Concurrent or sequential exposures to other agents that are metabolized most efficiently by isozymes different from those involved in the metabolism of rotenone might not result in a toxicologically significant interaction.

Other potential *in vivo* interactions between rotenone and other compounds are associated with rotenone's mechanism of action—i.e., the inhibition of mitochondrial complex I (Section 3.1.2). Many other chemicals inhibit mitochondrial complex I and thus could exacerbate the effects of concurrent exposure to rotenone. In terms of potential health effects in humans, ethanol is a complex I inhibitor, and co-exposure to rotenone and ethanol has been shown to influence the pattern of ethanol excretion in rats (Li et al. 2004). While differences in response may be noted with exposures to rotenone and other complex I inhibitors relative to rotenone alone, compounds with the same or similar modes of action will generally display additive toxicity as opposed to synergistic or antagonistic interactions (e.g., Finney 1972; Mumtaz et al. 1994). While additional experimental data on interactions between rotenone and other rotenoids or complex I inhibitors were not encountered in the literature, the joint action of rotenone deguelin and antimycin (another complex I inhibitor used as a piscicide) does appear to be additive in aquatic organisms (Schnick 1974).

Finally, as discussed in Section 3.1.2 (Mechanism of Action), many of the toxic effects of rotenone can be attributed to oxidative stress at the cellular level. Co-exposures to antioxidants (agents that inhibit oxidative stress) have been shown to antagonize the effects of rotenone (Inden et al. 2007; Nehru et al. 2008).

3.1.16.2. Detoxification with Potassium Permanganate

In addition to metabolic oxidation/detoxification, rotenone can be chemically oxidized, and hence detoxified, by a number of oxidizing agents, such as potassium permanganate ($KMnO_4$) and chlorine (Cl_2). The U.S. EPA (2007a, p. 32) is now requiring the use of potassium permanganate detoxification. Consequently, potassium permanganate is the only chemical detoxification agent considered in the current risk assessment.

The general approach in the use of potassium permanganate involves applying rotenone to a stream or lake, waiting for a specified period of time (typically a matter of hours) to allow rotenone to act on the target species, and then applying a sufficient amount of potassium permanganate to react with and detoxify the rotenone without resulting in a substantial residual concentration of permanganate anion (i.e., the oxidizer) in water. The kinetics of the reaction of potassium permanganate ($KMnO_4$) and rotenone in natural water are complex. In distilled water, a 1:1 ratio of $KMnO_4$ to rotenone is adequate for detoxification of rotenone (Finlayson et al. 2000). This result is to be expected in that the molecular weight of potassium permanganate (MW: 158 g/mole) is less than half that rotenone (MW: 394.4 g/mole). Thus a mass ratio of 1:1 would correspond to a molar ratio of about 2.5:1::$KMnO_4$:rotenone. Potassium permanganate, however, is a general oxidizing agent and will interact with and be consumed by other organics in natural water (e.g., tannins). Thus, $KMnO_4$:rotenone ratios of 2:1 to 4:1 are recommended in field applications (Finlayson et al. 2000; U.S. EPA/OPP 2007a). At the maximum target application rate of 200 ppb rotenone, potassium permanganate treatments at $KMnO_4$:rotenone mass ratios of 2:1 to 4:1 are equivalent to 400-800 ppb.

Workers are likely to be at the greatest potential hazard associated with the use of potassium permanganate. Because potassium permanganate is a strong oxidizing agent, it is irritating to the skin and respiratory tract and can cause severe eye damage on direct contact (ATSDR 2000). MSDS's for potassium permanganate (e.g., Fisher Scientific 2003) recommend the use of protective eye wear, gloves, and respirators.

If excess potassium permanganate is added to water, reducing agents such as sodium thiosulfate can be used to accelerate the neutralization of potassium permanganate in natural water (Engstrom-Heg 1972). As summarized by ATSDR (2000), excessive oral exposures to potassium permanganate can cause irritation to the gastrointestinal tract; furthermore, latent symptoms similar to Parkinson's disease were reported in a single case study. This incident, however, involved a dose (expressed as manganese equivalents) of 1.8 mg/kg/day over a 4-week period (ATSDR 2000, p. 119). The daily dose would be equal to a dose (expressed as equivalents of potassium permanganate) of about 5.2 mg/kg bw/day [1.8 mg manganese/kg/day x 158 g/mole divided by 54.9 g/mole]. Assuming a 70 kg body weight and a water consumption of 2 liters per day, the equivalent water concentration of potassium permanganate would be 182 mg/L [5.2 mg/kg bw/day x 70 kg / 2 L] or 182,000 ppb (µg/L). This is a factor of about 230 to 455 times the concentration of potassium permanganate that would be added to detoxify rotenone [182,000 ppb/(400 to 800 ppb) = 455 to 227.5].

Longer-term exposures to potassium permanganate will not occur because potassium permanganate will be consumed by rotenone and other organics, and there should be no substantial residual concentration of the permanganate ion – i.e., MnO_4^-. Nonetheless, the application of potassium permanganate will increase the concentrations of both potassium and manganese in water.

The application of potassium permanganate at concentrations ranging from 400 to 800 ppb could result in an increase in the concentrations of potassium (atomic weight of 39) by about 100 to 200 ppb (400 to 800 ppb x 39/158 = 98.7 to 197.4 ppb). This increase in potassium concentrations in water by 100 to 200 ppb is insubstantial relative to normal background concentrations of potassium in water of about 12,000 to 55,000 ppb (Molloy 2002).

The application of potassium permanganate at concentrations of 400 to 800 ppb also would increase the concentration of manganese (atomic weight of 54.9) by about 140 to 280 ppb (400 to 800 ppb x 54.9/158 = 138.99 to 277.97 ppb). As detailed by ATSDR (2000, p. 359), concentrations of manganese in surface water are highly variable, ranging from <0.3 ppb to 3230 ppb with average concentrations reportedly ranging from about 24 ppb to 59 ppb. Thus, unlike the case with potassium, the application of potassium permanganate to detoxify rotenone could result in a substantial increase in the concentration of manganese in surface water. The potential risks associated with this increase in the concentration of manganese in water is considered further in the following subsection.

3.1.16.3. Manganese Concentrations in Water

As summarized in ATSDR (2000), a large and complex literature is available on the toxicity of manganese and it is beyond the scope of the current risk Forest Service assessment on rotenone to independently reevaluate this literature. Nonetheless, a preliminary assessment can be based on the ATSDR (2000) review, the current chronic RfD for manganese (U.S. EPA/ORD 1995), a recent drinking water criteria developed by WHO (2004) and a consideration of manganese as an essential element (Institute of Medicine 2005).

While the Reregistration Eligibility Document (RED) prepared by the U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP 2007a) indicates that potassium permanganate detoxification is required, neither the RED nor supporting risk assessment documents (U.S. EPA/OPP 2005a, 2006b,d,e, 2007a,d) discuss the potential hazards associated with increased concentrations of manganese in water. Similarly, the U.S. EPA's Office of Drinking Water (U.S. EPA/ODW 2003) has also determined that manganese does not need to be regulated as a priority contaminant under the Safe Drinking Water Act.

One rationale given by U.S. EPA/ODW (2003) for not regulating manganese as a priority contaminant is that manganese is an essential element. U.S. EPA/ODW (2003) cites recommendations from the Institute of Medicine indicating that adequate intakes for manganese are 2.3 mg/day for men and 1.8 mg/day for women. The adequate intake values for men and women are identical to the adequate intakes of manganese given by the Institute of Medicine (2005). The Institute of Medicine (2005) also recommends somewhat higher adequate intakes for pregnant females (2 mg/day) and lactating females (2.6 mg/day). Much lower adequate intakes are recommended for infants (0.003 to 0.6 mg/day) and children (1.2 to 1.5 mg/day).

Notwithstanding the fact that manganese is an essential trace element, excessive exposures to manganese are a concern because manganese, like rotenone, can induce neurological effects that are similar to Parkinson's disease. These neurologic effects have been termed *manganism* or *manganese-induced Parkinsonism*. While the neurotoxicity of manganism is well-documented in humans after inhalation exposures, it is less clear that oral exposures to manganese will induced signs of neurotoxicity (ATSDR 2000, p. 49 and p. 114). As noted above, however, ATSDR (2000, p. 119) does summarize an incident in which the ingestion of potassium permanganate at doses equivalent to 1.8 mg manganese/kg bw/day or about 128 mg/day was associated with the development of neurotoxicity similar to Parkinson's disease.

Because of limitations in the available data on the toxicity of manganese after oral exposures, ATSDR (2000) declined to derive an oral minimal risk level (i.e., analogous to an oral RfD) for manganese. U.S. EPA/ORD (1995), however, has derived a chronic RfD for manganese of 0.14 mg/kg bw/day. Again assuming a 70 kg body weight, this RfD is equivalent to a daily dose of 9.8 mg/day [0.14 mg/kg bw/day x 70 kg]. Assuming a water consumption of 2 liters per day, the equivalent water concentration of manganese would be 4.9 mg/L [9.8/2 L] or 4900 ppb (µg/L). This concentration is above the

estimated increases in manganese associated with the use of potassium permanganate – i.e., 140 to 280 ppb – by factors of 17.5 to 35 [4,900 ppb divided by 140 to 280 ppb].

The above analysis, however, does not consider other sources of exposure to manganese. As noted in ATSDR (2000, p. 4), the normal daily intake of manganese is in the range of 1 to 10 mg/day. Taking the upper bound and using a body weight of 70 kg, the estimated daily dose of manganese is about 0.14 mg/kg bw/day [10 mg/day divided by 70 kg]. Thus, the upper bound of human exposures to manganese is equal to the RfD. Nonetheless, the occurrence of 280 ppb manganese in water – i.e., the upper bound that would be associated with the use of potassium permanganate to detoxify rotenone – would lead to an additional exposure of 0.008 mg/kg bw/day [0.280 mg/L x 2 L/day divided by 70 kg]. This additional exposure is a factor of 17.5 below the normal daily exposure [0.14 mg/kg bw/day divided by 0.008 mg/kg bw/day]. In terms of a hazard quotient, the upper range of normal exposures to manganese would be associated with an HQ of 1.0 [0.14 mg/kg bw/day divided by 0.14 mg/kg bw/day]. The addition of manganese from potassium permanganate would lead to an HQ of 1.06 [0.14 mg/kg bw/day + 0.008 mg/kg bw/day divided by 0.14 mg/kg bw/day].

WHO (2004) has derived a drinking water criteria for manganese of 0.4 mg/L. This criteria is based on considerations of both the toxicity of manganese as well as other sources of exposure to manganese. Taking the upper range of the average concentrations of manganese in water – i.e., 59 ppb from ATSDR (2000) – the use of potassium permanganate to detoxify rotenone would result in an increase in manganese concentrations from 59 ppb to no higher than 339 ppb [280 ppb + 59 ppb] or 0.339 mg/L. This value approaches but does not exceed the WHO (2004) criteria of 0.4 mg/L. As noted above, however, manganese has been detected in water at concentrations of up to 3,230 ppb.

From the above preliminary analyses, it is apparent that hazards associated with the use of potassium permanganate to detoxify rotenone will generally not lead to increases in exposures to manganese that would exceed a level of concern. In areas with atypically high ambient concentrations of manganese in water, the use of potassium permanganate could result in an increase in exposures that would exceed the WHO (2004) guidelines. In areas with extremely high ambient concentrations of manganese in water – i.e., >3000 ppb – the use of potassium permanganate could exacerbate an already unacceptable exposure to manganese. While not explicitly addressed by the U.S. EPA, the impact of the use of potassium permanganate to detoxify rotenone entails a risk-benefit determination with the benefit being the detoxification of rotenone. Given the potential human health risks that are associated with the use rotenone as a piscicide (Section 3.4), detoxification of rotenone with potassium permanganate appears to be a generally prudent practice, consistent with the requirement in U.S. EPA/OPP (2007a).

3.1.17. Impact of Impurities and Adjuvants

As indicated in Table 2, rotenone formulations list active ingredients as not only rotenone itself but also as other associated resins (OAR). In addition, formulations that contain piperonyl butoxide also list piperonyl butoxide as an active ingredient. Nonetheless, the

application rates for rotenone are based only on the amount of rotenone in each formulation. Similarly, the U.S. EPA/OPP (2007a) risk assessment of rotenone is based on exposures to and the toxicity of rotenone and does not quantitatively consider the impact of other associated resins or piperonyl butoxide. In many respects, the decision by the U.S. EPA to base their risk assessment on rotenone alone is sensible. Rotenone is clearly the agent of greatest concern and the data supporting the risk assessment of rotenone is far more complete than the data supporting the risk assessment of other agents in rotenone formulations.

The current Forest Service risk assessment, however, will differ from the U.S. EPA risk assessment in that the contribution of other associated resins and piperonyl butoxide will be quantitatively considered. This approach is taken because the Forest Service has determined that the data on other associated resins and piperonyl butoxide is sufficient to support the quantitative assessment of these agents and that these agents should be considered under the requirements imposed on the Forest Service by NEPA.

The rationale for considering only associated resins and piperonyl butoxide rather than all agents contained in rotenone formulations is related to the apparent contribution of these agents to risk. In general, the use of pesticide formulations will involve exposures to other agents including inerts, adjuvants, metabolites, impurities, and contaminants. Metabolites are not a concern in the current Forest Service risk assessment on rotenone because metabolism is a detoxification process and there is no basis for asserting that *in vivo* or environmental metabolites of rotenone will increase risks associated with use of rotenone formulations (Section 3.1.15.1). Similarly, inerts (Section 3.1.14.1) and contaminants (Section 3.1.15.3) are not a quantitative concern in the current risk assessment because the available information indicates that these compounds are not present in amounts that would materially increase the quantitative assessment of risk – i.e., the hazard quotients. The impact of adjuvants and impurities, however, appears to be more substantial.

As detailed in Section 3.1.14.2 (Adjuvants), the impact of piperonyl butoxide on risks associated with exposures to rotenone formulations containing piperonyl butoxide may be addressed by assuming that piperonyl butoxide contributes to the formulation in a manner that is equal to that of rotenone. While no studies in mammals are available to directly assess the assumption, toxicity studies in fish (Section 4.1.3.1.3) do support the assertion that piperonyl butoxide in rotenone formulations acts as if it were an equivalent amount of rotenone.

The impact of impurities in rotenone formulations can also be addressed quantitatively. As detailed in Section 3.1.15.2 (Impurities), deguelin is the compound of greatest concern among the other associated resins in rotenone formulations (Fang and Casida (1999b); Caboni et al. 2004). In cubé resin assayed by Fang and Casida (1999b), deguelin was present at half of the concentration of rotenone. Based on the *in vitro* data from Fang and Casida (1999b) as well as the *in vivo* data from Caboni et al. (2004), deguelin appears to be half as potent as rotenone. Thus, using deguelin as a surrogate for the toxicity of the other associated resins, the contribution of the other associated resins may be taken as a

factor of 0.25 that of rotenone – i.e., 0.5 x 0.5 – because deguelin is present at half of the amount of rotenone and is only half as toxic as rotenone.

A quantitative consideration of the contribution of both other associated resins and piperonyl butoxide to the toxicity of rotenone formulations can be based on the assumption of dose-addition (Finney 1976) using an approach similar to that taken in the assessment of inerts (Section 3.1.14.1). Because all dose-response assessments considered in this risk assessment are based on rotenone, a toxic equivalency factor (***TEF***) for converting rotenone, other associated resins, and piperonyl butoxide to an equivalent amount of rotenone can be expressed as:

Equation 19

$$\boldsymbol{TEF} = 1 + (0.25 \times OAR_{\%}/Rt_{\%}) + PB_{\%}/Rt_{\%}$$

where:

0.25: a factor for converting other associated resins to equivalents of rotenone based on the data from Fang and Casida (1999b) as discussed above and detailed further below,

$OAR_{\%}$: the percentage of other associated resins in the formulation,

$Rt_{\%}$: the percentage of rotenone in the formulation,

PB%: the percentage of piperonyl butoxide in the formulation.

The toxic equivalency factors for each formulation covered in this risk assessment is given in the last column of Table 2. In addition, the above equation is implemented for all formulations covered in the current risk assessment in a custom worksheet, Worksheet TEF, in the workbook that accompanies this risk assessment. This custom worksheet follows the custom worksheets for the contaminants (Section 3.1.14.1) and immediately precedes the Worksheet A02.

Worksheet TEF is designed so that users can easily verify the TEFs given in the last column of Table 2 and modify the inputs if such modifications are needed in the future based on either additional data or the release of new formulations of rotenone. In each of the exposure worksheets given in Attachment 1, the dose or concentration of rotenone is multiplied by the formulation specific TEF given in Table 2. This approach quantitatively considers the potential contribution of other associated resins and piperonyl butoxide to the toxicity of the different rotenone formulations.

The derivation of Equation 19 for calculating TEFs is detailed below. While mathematically simple, the derivation of this equation may be viewed as somewhat tedious or trivial, depending on the readers background. The derivation is included below in the interest of transparency.

Using $\boldsymbol{Rot_{Eq}}$ to designate the rotenone equivalents in a formulation, $\boldsymbol{Rot_{Eq}}$ may be defined as:

Equation 20

$$\boldsymbol{Rot_{Eq}} = \boldsymbol{Rot_{\%}} + \boldsymbol{Rot_{OAR\%}} + \boldsymbol{Rot_{PB\%}}$$

where:

$Rot_{\%}$: the percentage of rotenone in the formulation,
$Rot_{OAR\%}$: the percentage of other associated resins (OAR) in the formulation expressed as rotenone equivalents,
$Rot_{PB\%}$: the percentage of other piperonyl butoxide (PB) in the formulation expressed as rotenone equivalents.

Under the assumption of dose addition (e.g., Finney 1976), relative potency ($\boldsymbol{\rho}$) is defined as the ratio of equitoxic toxic doses:

Equation 21

$$\rho = d_1 / d_2$$

where d_1 and d_2 are doses of two chemicals that cause an equivalent toxic effect. The term *equitoxic doses* refers to doses that will cause the same effect at the same incidence, magnitude, and/or severity. For example, LD_{50} values for two chemicals can be viewed as equitoxic. Under the assumption of dose-addition, relative potency can be used to convert any dose or amount of the chemical in the denominator (D_2) into an equivalent dose of the chemical in the numerator (D_1):

Equation 22

$$D_1 = \rho\ D_2.$$

Since piperonyl butoxide is treated as an equivalent amount of rotenone, the potency of piperonyl butoxide (ρ_{PB}) relative to rotenone is equal to 1. Thus, the calculation of $Rot_{PB\%}$ is very simple:

Equation 23

$$\boldsymbol{Rot_{PB\%}} = \rho_{PB} \text{ x } \boldsymbol{PO_{\%} = PO_{\%}.}$$

The derivation of $\boldsymbol{Rot_{OAR\%}}$ is somewhat more cumbersome. As noted in Section 3.1.15.2, deguelin induced Parkinson's disease-like symptoms at a dose of 6 mg/kg bw/day that were comparable to the symptoms induced by rotenone at a concentration of 3 mg/kg bw/day (Caboni et al. 2004). Thus, the potency of deguelin relative to rotenone is 0.5:

Equation 24

$$\rho_{Deg} = 3 \text{ mg/kg/day} / 6 \text{ mg/kg/day} = 0.5.$$

For any mixture with a given percentage of deguelin ($Deg_{\%}$), the equivalent percentage of rotenone ($Rot_{\%}$) can be calculated as:

Equation 25

$$Rot_{\%} = \rho_{Deg} \text{ x } Deg_{\%}$$

Based on the data provided by Fang and Casida (199b), the assumption is made that half of the other associated resins in rotenone formulations consist of deguelin. Based on the assumption that deguelin accounts for 50% of the other associated resins ($OAR_{\%}$),

Equation 26

$$Deg_{\%} = 0.5 \text{ x } OAR_{\%}.$$

By substituting Equation 26 into Equation 25, the rotenone equivalents for a given percentage of other associated resins ($\boldsymbol{Rot_{OAR\%}}$) can be calculated as:

Equation 27

$$\boldsymbol{Rot_{OAR\%}} = \rho_{Deg} \times Deg_{\%} = \rho_{Deg} \times 0.5 \times OAR_{\%} = 0.25\ OAR_{\%}.$$

Thus, Equation 20 may be rewritten as,

Equation 28

$$Rot_{Eq} = Rot_{\%} + 0.25\ OAR_{\%} + PB_{\%}.$$

The form of Equation 28, however, is not simple to apply in the current risk assessment. As detailed in Section 2, all application rates for rotenone formulations are expressed in units of rotenone alone. Thus, it is more convenient to define a toxic equivalency factor (TEF) as the rotenone equivalents in the formulation per unit of rotenone:

Equation 29

$$TEF = Rot_{Eq} / Rot_{\%}.$$

Substituting Equation 29 into Equation 28,

$$TEF = Rot_{Eq}/Rot_{\%} = (Rot_{\%} + 0.25\ OAR_{\%} + PB_{\%})/\ Rot_{\%}.$$

and then simplifying,

Equation 30

$$\boldsymbol{TEF} = 1 + 0.25\ \boldsymbol{OAR\%}/\boldsymbol{Rot\%} + \boldsymbol{PB\%}/\boldsymbol{Rot\%}.$$

This equation is identical to Equation 19, given at the start of this subsection. While the derivation of this equation is based on the percentages of rotenone, other associated resins, and piperonyl butoxide in each formulation, the TEF is unitless and the percentage calculations cancel out in Equation 30. Thus, as noted above, the TEF is applied to concentrations of rotenone in water in the calculation worksheets to derived concentrations of rotenone equivalents to considers the contribution of rotenone, other related resins, and piperonyl butoxide.

As also noted above, the data supporting the development of Equation 19 is not as complete as the data on rotenone. One limitation involves the handling of other associated resins. As detailed above, other associated resins are handled based on the toxicity of deguelin and the amount of deguelin noted in a sample of cubé resin assayed by Fang and Casida (1999b). As discussed in Section 3.1.15.2, Fang and Casida (1999b) noted other many other impurities which are not explicitly considered in the derivation of the TEF. This approach is taken because deguelin is the most toxic of the impurities and was present at far greater concentrations than other much less toxic components (i.e., 22% vs <0.5%). In addition, the relative potency for deguelin can be can be based on the *in vivo* data from Caboni et al. (2004) and this type of data is not available on the other impurities in rotenone formulations. Thus, while a case could be made for increasing the potency factor of 0.25 for other associated resins used in Equation 28, this would not have a substantial impact on the analysis.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

All of the exposure assessments for workers as well as members of the general public are detailed in an EXCEL workbook that accompanies this risk assessment (Attachment 1). This workbook contains a set of worksheets on rotenone that details each exposure scenario discussed in this risk assessment as well as summary worksheets for both workers and members of the general public. Documentation for these worksheets is presented in SERA (2007b). The sections of the risk assessment on workers and the general public provide a plain language description of the worksheets. In addition, the sections discuss the rotenone specific data used in the worksheets.

As indicated in Table 2, there are several formulations of rotenone, including granular and liquid, and the formulations may be applied to ponds or streams. Exposure to rotenone for workers and members of the general public depends on the target concentration. For the current risk assessment, all exposure assessments are based on the application of a liquid formulation, CFT Legumine, at a target concentration of 0.2 ppm, which is the maximum application rate. The consequences of using lower application rates are discussed in the risk characterization (Section 3.4).

The different formulations of rotenone also contain differing amounts of other associated resins (i.e., rotenoids) and some formulations also contain piperonyl butoxide. As detailed in the hazard identification (Section 3.1.17), these compounds are considered using toxic equivalency factors (ranging from 1.25 to 2.5) to calculate rotenone equivalents which encompass the contribution of rotenone, other related resins, and piperonyl butoxide. Consequently, all doses derived in this exposure assessment are expressed in units of rotenone equivalents.

There are substantial uncertainties in the exposure assessments for workers. Since data are not available on worker exposure rates for aquatic applications of rotenone, the current risk assessment bases worker exposure rates on an aquatic application of 2,4-D. Uncertainties in the worker exposure rates are compounded by uncertainties concerning the use of personal protective equipment (PPE). While the U.S. EPA RED requires the use of personal protective equipment, waivers have been granted for applications of dilute solutions of some formulations. Thus, exposure estimates are made both with and without PPE. Worker exposures are estimated at about 0.003 (0.0013 to 0.0066) mg/kg body weight for workers not using PPE and 0.0003 (0.00012 to 0.00066) mg/kg body weight for workers who do use PPE. While the exposure methods used in this risk assessment differ from the approach taken by the U.S. EPA, which bases worker exposures on deposition data from ground application methods judged to be analogous to aquatic applications, the worker exposure rates used in the current risk assessment are similar to those used by the U.S. EPA in terms of the resulting hazard quotients. This detail is discussed further in the risk characterization for workers.

The major uncertainty in the exposure assessment for members of the general public involves the plausibility of any of the exposure scenarios. The U.S. EPA RED requires

that access by members of the general public to treated sites be restricted. Along with the recommended use of potassium permanganate to detoxify rotenone, the restrictions on public access suggest that exposures to members of the general public will be minimal. Thus, all of the exposures developed for members of the general public should be regarded as extreme. As discussed further in the risk characterization, the non-accidental exposure of greatest concern involves the consumption of treated water by a small child for which the estimated dose is about 0.019 (0.011 to 0.028) mg/kg bw/day. This exposure and other exposures for the general public would occur only if the restrictions imposed by the U.S. EPA on the application of rotenone were not properly enforced.

3.2.2. Workers

3.2.2.1. General Exposures

The exposure assessments used for workers in most Forest Service risk assessments are based on a standard set of exposure scenarios used for herbicides and insecticides. Although these exposure assessments vary according to the available data for each chemical, the organization and assumptions used in the exposure assessments are standard and consistent. As detailed in SERA (2007a), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. Based on analyses of several different pesticides using various application methods, default exposure rates are typically estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial.

The application of rotenone to ponds or lakes as well as to streams or rivers involves application methods that are quite different from the application methods considered in most Forest Service risk assessments. The specific types of application methods are discussed in Section 2.4 of this Forest Service risk assessment and are detailed in several reviews and project summaries concerning rotenone applications to control pest fish (Cailteux et al. 2001; Entrix 2007; Finlayson et al. 2000; Ling 2003; Marking 1992; MSU 2006; Rotenone Stewardship Program 2008; Turner et al. 2007). Thus, the standard methods used in most Forest Service risk assessments are not applicable to aquatic applications of rotenone.

Again, the rotenone literature does not include worker exposure data involving aquatic applications of rotenone. There is, however, an available study on worker exposure rates associated with aquatic applications of 2,4-D (Nigg and Stamper 1983), as detailed in the recent 2,4-D risk assessment prepared for the Forest Service (SERA 2006). The study involved the application of a liquid formulation of 2,4-D by airboat handguns to control water hyacinths. The absorbed doses of 2,4-D were assayed in four workers as total urinary elimination over a 24-hour period. Occupational exposure rates for these workers were estimated at 0.0009 (0.0004 - 0.002) mg/kg body weight per lb handled.

While using 2,4-D data to estimate worker exposures to rotenone adds uncertainty to the risk assessment, there clearly are no other data to support the worker exposure assessment based on absorbed dose. As discussed in SERA (2007a), instead of an absorbed dose method for estimating worker exposure, the U.S. EPA typically uses a deposition-based

approach using data from the Pesticide Handlers Exposure Database (e.g., PHED Task Force 1995).

As noted by the U.S. EPA in their worker exposure assessment for aquatic applications of rotenone, PHED does not include deposition-based data on aquatic applications of rotenone. For that reason, the EPA uses surrogate data on other application methods—e.g., liquid low pressure handwand for applying liquid formulations from a backpack sprayer (U.S. EPA/OPP 2006e, p. 50 ff).

The EPA's judgment in selecting surrogate application methods appears to be reasonable based on the study by Nigg and Stamper (1983). The absorption-based worker exposure rates for aquatic applications derived from the Nigg and Stamper (1983) study—i.e., 0.0009 (0.0004 - 0.002) mg/kg body weight per lb a.i. handled—are between those generally used in Forest Service risk assessments for backpack workers [0.003 (0.0003-0.01) mg/kg body weight per lb handled/day) and workers involved in hydraulic ground broadcast applications [0.0002 (0.00001 - 0.0009) mg/kg body weight per lb handled/day] (SERA 2007a). Nonetheless, the use of surrogate deposition-based exposure estimates such as those used by the EPA does not appear to be any less tenuous than the direct use of the absorption-based estimates from Nigg and Stamper (1983). Thus, for the current Forest Service risk assessment, the worker exposure rates of 0.0009 (0.0004 - 0.002) mg/kg body weight per lb handled are used as the baseline (i.e., no PPE) worker exposure rates.

The current product labels for rotenone formulations do not specify a requirement for personal protective equipment (PPE). The U.S. EPA RED for rotenone, however, specifically adds the following requirements to product labels:

> *Registrants must update labels to require all handlers (except aerial applicators) and other individuals directly participating in the treatment to wear the following PPE in addition to baseline protection (long-sleeve shirt, long pants, socks and shoes): chemical resistant gloves, coveralls, and footwear; protective eyewear; and a full-face respirator that also provides eye protection. Aerial applicators must use an enclosed cockpit and wear long-sleeve shirt, long pants, shoes, and socks.* (U.S. EPA/OPP 2007a, p. 29)

This requirement implements the recommendations in the final human health effects Science Chapter for the EPA RED on rotenone. In this Science Chapter, the Health Effects Division of U.S. EPA/OPP expresses concern for workers involved in aquatic applications of rotenone (U.S. EPA/OPP 2006e). This concern is discussed further in the risk characterization for workers (Section 4.4.2) in the current Forest Service risk assessment. In assessing the impact of protective clothing, the U.S. EPA considered worker protection factors of 0.5 for double layers of clothing and 0.9 for respiratory protection (U.S. EPA/OPP 2006e, p. 50).

The efficiency of PPE—e.g., the extent to which the clothing retards deposition onto the skin of the worker—will vary with the nature of the application and the type of PPE used. A protection efficiency of about 90% is typical for many pesticides (Nigg 1998). Additional data on protection efficiencies are available in the U.S. EPA's Pesticide Handler's Exposure Database (PHED Task Force 1995) for various types of ground and aerial applications. High and low pressure hand wand applications as well as ground boom applications (i.e., application methods analogous to different types of aquatic applications) are associated with protections efficiencies from about 93% to greater than 99%, based on various configurations of PPE.

Notwithstanding the above quotation from EPA's RED, the status of the requirement to use PPE is unclear. For example, the suppliers of CFT Legumine appear to have petitioned the U.S. EPA to delete the requirement for PPE for individuals handling diluted solutions of CFT Legumine. In a letter from the Registration Division of OPP to the supplier of CFT Legumine, Peacock (2007) indicated that this request was approved by the Agency and that similar requests were granted for other rotenone formulations. This approval applies to dilutions of the formulation by 10-fold or greater. As discussed in Section 2.4.1, 10% dilutions are at the upper range of the recommended dilution rate for applications of most liquid formulations of rotenone.

Because it is unclear that PPE would be required and hence used in all applications of rotenone, two worker exposure scenarios are included in the EXCEL workbook that accompanies this risk assessment: Worksheet C01a which incorporates no factor for personal protective equipment and Worksheet C01b that includes a 90% efficiency factor for personal protective equipment.

Both the absorption-based (Forest Service) and deposition-based (EPA) worker exposure rates are based on the amount of material handled; furthermore, the exposure rates are not dependant on dilution. Since the application rate is expressed as a target concentration, the amount of rotenone that will be handled by a worker will depend only on the target concentration and the volume of water that is treated:

$$\text{Target Conc}_{\text{mg/L}} \times \text{Water Volume}_{\text{L}} = \text{Amount}_{\text{mg}}$$

In the EPA occupational assessment (U.S. EPA/OPP 2006d, Table 5, p. 13), the Agency uses the following assumptions:

> Pond: Up to 500 acre-ft/day are treated assuming a water depth of 5 ft. At one acre-ft = 43,560 ft^3 and with a 5 ft depth, the treatment is 217,800 ft^3. At 1 ft^3 = 28.32 L, the worker would treat 6,168,096 liters of water.
>
> Stream: 211,200 ft^3 (10560 feet long with a water body depth of 2 feet and a water body width of 10 feet). The water volume of 211,200 ft^3 corresponds to 5,981,184 liters of water.

To be consistent with the assumptions used by the EPA, Worksheet A1 in the EXCEL workbook that accompanies this risk assessment assumes that a worker will treat 6,000,000 liters of water per day with a target concentration of 200 ppb (0.2 mg/L).

As summarized in Worksheet C01a, the expected doses in workers without PPE are about 0.0030 (0.0013 to 0.0066) mg/kg body weight. The corresponding doses with PPE that is 90% efficient in reducing exposures (Worksheet C01b) are a factor of 10 lower: 0.00030 (0.00013 to 0.00066) mg/kg body weight. As indicated in Worksheets C01a and C01b, these doses are expressed in units of rotenone equivalents using a toxic equivalency factor (TEF) of 1.25 for CTF Legumine.

3.2.2.2. Accidental Exposures

Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route of exposure for pesticide applicators (Ecobichon 1998; van Hemmen 1992). Typical multi-route exposures are encompassed by the methods used in Section 3.2.2.1 on general exposures. Accidental exposures, on the other hand, are most likely to involve splashing a solution of the pesticide into the eyes or contaminating the surface of the skin.

There are various methods for estimating absorbed doses associated with accidental dermal exposure (SERA 2007a). Two general types of exposures are modeled in this risk assessment: those involving direct contact with a solution of the pesticide and those associated with accidental spills of the pesticide onto the surface of the skin. Any number of specific exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For this risk assessment, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarized in Worksheet E01, which references other worksheets in which the specific calculations are detailed.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute in a field solution of the pesticide or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of a chemical for any period of time. Nevertheless, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key assumption is that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a chemical solution. In both cases, the concentration of the chemical solution in contact with the skin and the resulting dermal absorption rate are basically constant.

For both scenarios (hand immersion and contaminated gloves), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA/ORD (1992), Fick's first law is used to estimate dermal exposure. As discussed in

Section 3.1.3.2, an experimental dermal permeability coefficient (k_p) for rotenone is not available. In the absence of experimental data, the K_p for a pesticide is estimated using the algorithm from U.S. EPA/ORD (1992b), which is detailed in Worksheet B05.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill onto the lower legs as well as a spill onto the hands. In these scenarios, it is assumed that a chemical solution is spilled on to a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the concentration of the chemical in the liquid), the first-order absorption rate, and the duration of exposure. For both scenarios, it is assumed that the contaminated skin is effectively cleaned after 1 hour.

3.2.3. General Public

3.2.3.1. General Considerations

3.2.3.1.1. Likelihood and Magnitude of Exposure

The likelihood that members of the general public will be exposed to rotenone in Forest Service applications appears to be low. Rotenone will not persist in the environment, treatment periods will occur only over a very short period of time, typically a few hours (Section 2), and residual rotenone will be eliminated through the use of potassium permanganate (Section 3.1.16.2). In addition, the U.S. EPA/OPP (2007a) is requiring the following risk mitigation measures:

> *...placard the treatment area to prohibit recreational access during treatment, swimming for at least 3 days following treatment, and consumption of dead fish taken from treatment area; and apply rotenone below the water's surface (except for aerial and backpack sprayer applications)*. U.S. EPA/OPP 2007a, p. 32.

Thus, many of the standard exposure scenarios discussed below are unlikely to occur. These exposure scenarios are included in the current risk assessment simply to illustrate which restrictions are most important.

Because of the conservative exposure assumptions used in the current risk assessment, the number of individuals who might be exposed to rotenone does not have a substantial impact on the characterization of risk presented in Section 3.4. As detailed in SERA (2007a, Section 1.2.2.2), the exposure assessments developed in this risk assessment are based on *Extreme Values* rather than a single value. Extreme value exposure assessments, as the name implies, bracket the most plausible estimate of exposure (referred to statistically as the central or maximum likelihood estimate) with extreme lower and upper bounds of plausible exposures.

This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed Individual* (MEI), sometime referred to as the *Maximum Exposed Individual* (MEI). As this name also implies, exposure assessments that use the MEI approach attempt to characterize the extreme but still plausible upper limit on exposure. This is a common approach to exposure assessment used by the U. S. EPA, other government agencies, as well as other organizations. In the current risk assessment, the upper bounds on exposure are all based on the MEI.

In addition to this upper bound MEI value, the Extreme Value approach used in this risk assessment also provides a central estimate of exposure and a lower bound on exposure. While not germane to the assessment of upper bound risk, it is worth noting that the use of the central estimate and especially the lower bound estimate is not intended to lessen concern. To the contrary, the central and lower estimates of exposure are used to assess the feasibility of mitigation—e.g., protective measures to limit exposure. Thus, the Extreme Value approach in the exposure assessment is part of an integrated approach designed to encompass plausible upper limits of risk for the most exposed and most sensitive individuals, regardless of the specific probabilities or number of exposures.

3.2.3.1.1. Summary of Assessments

The two types of exposure scenarios developed for the general public include acute exposure and longer-term or chronic exposure. As summarized in Worksheet E03, acute exposure scenarios are classified as either accidental or non-accidental. For many pesticides covered in Forest Service risk assessments, the non-accidental exposure scenarios may be classified as *Expected* exposure scenarios; however, this is not the case for rotenone owing to the extremely brief period between application and detoxification and the restrictions placed on public access to the treated area. Accordingly, all of the acute exposure scenarios can be considered as accidental in the sense that members of the general public should not be allowed into the treatment area.

Specific accidental scenarios are developed for the consumption of contaminated water or fish after an accidental spill. These scenarios should be regarded as extreme as well as implausible because of limitations placed on public access to sites that are treated with rotenone.

The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated water and fish. Again, however, these exposure scenarios should be accidental and highly implausible if rotenone is detoxified with potassium permanganate shortly after application.

Most Forest Service risk assessments also include scenarios for the consumption of contaminated vegetation or fruit as well as the direct spray of a small child and a woman. These scenarios are not included in the current risk assessment which only considers aquatic applications of rotenone. These exclusions are similar to the exposure assessment approach used by the U.S. EPA (2007a). Section designations for these excluded scenarios are given below as a matter of convenience for individuals who regularly use

many different Forest Service risk assessments—i.e., the section designations in all Forest Service risk assessments are consistent.

The exposure scenarios developed for the general public are summarized in Worksheet E03. As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany this risk assessment (Worksheets D01–D11). The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.2. Direct Spray

As noted Section in 3.2.3.1.1, direct spray scenarios are not relevant to aquatic applications of rotenone.

3.2.3.3. Dermal Exposure from Contaminated Vegetation

As noted Section in 3.2.3.1.1, scenarios involving dermal contact with contaminated vegetation are not relevant to aquatic applications of rotenone.

3.2.3.4. Contaminated Water

In terrestrial applications of pesticides, estimates of plausible concentrations in contaminated water can be elaborate and include modeling of runoff and leaching of the pesticide from contaminated soil, unintentional direct spray from aerial applications, or drift from either ground or aerial applications. For direct applications to water, most of these considerations are not relevant.

The estimated concentration in water is set to the target concentration. As noted above, the highest permitted target concentration, 0.2 ppm, is used in all exposure assessments. Applications of rotenone are likely to be inexact—i.e., there will be uncertainty and perhaps some error in estimating the volume of water to be treated, and the specific metering or application devices used may also be associated with a margin of error. While this degree of imprecision is more obvious for aquatic applications, uncertainties and errors in actual, as opposed to nominal, application rates are inherent in all pesticide applications. While detailed comparisons of actual versus nominal applications rates for rotenone are not commonly reported, the study by Chadderton et al. (2003, Table 2, p. 118) suggests that nominal concentrations of rotenone (i.e., the target application rate) will not be maintained for a prolonged period and will be reduced by a factor of about 2 within 3 hours of application. Thus, the use of the nominal target concentration for assessing risks to members of the general public, while consistent with the approach taken by the U.S. EPA/OPP (2007a), is likely to be conservative. This matter is discussed further in the risk characterization for members of the general public (Section 3.4.3).

As with all Forest Service risk assessments, accidental spill scenarios involve the spill of 200 gallons of a field solution into a small pond (0.25 acres in surface area and 1 meter deep). Estimated concentrations of rotenone in a field solution are given in Worksheet A01 for the range of dilution volumes specified on the product label. The doses

associated with the consumption of contaminated water after an accidental spill of rotenone are calculated in Worksheet D05.

As noted in Section 3.2.3.1.1 (Likelihood and Magnitude of Exposure), the accidental spill scenario is highly improbable with an application of rotenone. In addition, rotenone applications will typically involve contingency plans for handling accidental spills using potassium permanganate detoxification (e.g., Finlayson et al. 2000), as discussed in Section 3.1.16.2. Potassium permanganate detoxification is required by the U.S. EPA at least for most applications. Therefore, potassium permanganate should be readily available during aquatic applications of rotenone.

3.2.3.5. Oral Exposure from Contaminated Fish

Three sets of exposure scenarios are presented: one set for acute exposures following an accidental spill (Worksheets D08a and D08b), one set for acute exposures based on the target application rate (Worksheets D09c and D09d), and the other set for chronic exposures based on estimates of longer-term concentrations in water (Worksheets D09a and D09b). The two worksheets in each of the three sets are intended to account for consumption rates of caught fish among both the general population and subsistence populations. Details of these exposure scenarios are provided in Section 3.2.3.5 of SERA (2007).

In addition to estimated concentrations of the pesticide in water, scenarios involving the consumption of contaminated fish require information about the bioconcentration factor (BCF) in fish. As summarized in Table 1, structure-activity relationships suggest that the BCF for rotenone could be as high as 41.4 (Meylan and Howard 2007). This estimate, however, is based on the lipophilicity of rotenone and does not consider the toxicity of rotenone to fish. The study by Gilderhus et al. (1988) clearly indicates that higher concentrations of rotenone—i.e., concentrations lethal to fish—will result in BCF values of about 1.32, essentially no bioconcentration. A sublethal concentration, 5 ppb, resulted in much higher BCF values: 10.8 in fillet and 27.6 in whole fish. For exposures to contaminated fish, BCF values of 1.32 are used for acute exposures—i.e., exposures that occur during or shortly after treatment. A bioconcentration factor of 10.8 is used for longer-term exposures—i.e., fish exposed to residual sublethal concentrations of rotenone—under the assumption that an individual would only consume the fish fillet. The same acute BCF value is used in the ecological risk assessment; however, the higher BCF value for whole fish, 27.6, is used for the longer-term consumption of fish in the ecological risk assessment.

As discussed in Section 3.2.3.1.1 (Likelihood and Magnitude of Exposure), all of the exposure scenarios for the consumption of contaminated fish should be regarded as accidental, extreme, and implausible owing to exclusions placed on public access to treated areas and the recommendation that dead fish be removed from treated water. In addition to these restrictions, at least some individuals would be reluctant to consume dead or obviously poisoned fish.

3.2.3.6. Dermal Exposure from Swimming in Contaminated Water

To assess potential risks associated with swimming, an exposure assessment is developed for a young woman swimming for 1 hour in water treated at the target application rate (Worksheet D11). As discussed further below, this exposure scenario is implausible for applications of rotenone.

Conceptually and computationally, this exposure scenario is virtually identical to the contaminated gloves scenario used for workers (Section 3.2.2.2)—i.e., a portion of the body is immersed in an aqueous solution of the compound at a fixed concentration for a fixed period of time. The major differences in the two scenarios involve the concentration in water and the surface area of the body that is exposed. For the worker wearing contaminated gloves, the assumption is made that both hands are exposed to the field solution—i.e., the concentration of the compound in the solution being applied. For the swimmer, the assumption is made that the entire body surface area is exposed to the target application rate. Although the swimmer will not be immersed for 1 hour, the entire body surface is used both as a conservative approximation (i.e., the MEI) and to consider intermittent episodes during which the whole body might be immersed or at least wet.

As with the corresponding worker exposure scenario, the 1-hour period of exposure is somewhat arbitrary, and is intended as a unit of exposure estimate. In other words, the exposure and, consequently, the risk will increase or decrease linearly with the duration of exposure. Thus, a 2-hour exposure would lead to a hazard quotient that is twice as high as that associated with an exposure period of 1 hour.

As with all of the exposure scenarios for members of the general public, this exposure scenario is implausible. In addition to the general restrictions on access to the treated area, the U.S. EPA/OPP (2007a, p. 43) specifically notes that the treatment area must be posted with the following notices:

> *Recreational access (e.g., wading, swimming, boating, fishing) within the treatment area is prohibited while rotenone is being applied.*
> *Do not swim or wade in treated water for a minimum of 72 hours after the last application.*

In addition, the following requirements are imposed on the applicator (EPA/OPP 2007a, p. 28):

> *Through posting and access area closures, the Certified Applicator or designee under his/her direct supervision must prohibit swimming in treated areas during treatment and for 3 days thereafter (or until monitoring samples confirm rotenone concentrations in swimming areas are below 90 ppb for 3 consecutive samples taken no less than 4 hours apart).*

Finally, as with the consumption of contaminated fish, it is unrealistic to expect an individual to swim in water in which fish are obviously dead or dying.

3.2.3.7. Oral Exposure from Contaminated Vegetation

As noted in Section 3.2.3.1.1, scenarios involving the consumption of contaminated vegetation are not relevant to aquatic applications of rotenone.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Generally, the dose-response assessments used in Forest Service risk assessments adopt RfDs proposed by the U.S. EPA as indices of acceptable exposure. An RfD is basically defined as a level of daily exposure that will not result in any adverse effects in any individual over a specified period of time. The RfDs developed by the U.S. EPA are typically used directly in Forest Service risk assessments because the EPA RfDs generally provide a level of analysis, review, and resources that far exceed those that are or can be conducted in support of most Forest Service risk assessments. In addition, it is desirable for different agencies and organizations within the federal government to use concordant risk assessment values.

The current Forest Service risk assessment uses the most recent and the most conservative RfDs derived by the U.S. EPA. Specifically, this risk assessment adopts the acute RfD of 0.015 mg/kg bw/day and the chronic RfD of 0.0004 mg/kg bw/day derived in the recent Reregistration Eligibility Document prepared by the U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP 2007a). The acute RfD is based on a NOAEL of 15 mg/kg bw/day in mice from a developmental toxicity study. The chronic RfD is based on a lifetime dietary study with a dietary NOAEL of 7.5 ppm, equivalent to a daily dose of 0.0375 mg/kg bw/day. An uncertainty factor of 1000 is used with both of these NOAELs to derive the corresponding RfDs. The uncertainty factor of 1000 is generated by multiplying together separate factors of 10 for each of three factors considered as contributing to uncertainty: inter-species variability, intra-species variability, and uncertainties in the available data on rotenone. The factor for uncertainties in the available data reflects concern for the potential of rotenone to cause essentially permanent neurotoxic damage in pre-natal or early post-natal exposures, which might not induce observable adverse effects until late in life (Barlow et al. 2004).

Dose-severity relationships for rotenone appear to be pronounced, particularly with respect to acute exposures. In the animal study on which the acute RfD is based, the ratio of the LOAEL to the NOAEL is only 1.6, which might suggest that a hazard quotient of 1.6 is associated with adverse effects, specifically fetal absorptions. Given the rather large uncertainty factor used to derive the RfD, however, this interpretation may be grossly conservative. Based on the acute lethal potency of rotenone confirmed in the available data on both experimental mammals and humans, acute hazard quotients of about 400 or less are not likely to be associated with potentially lethal effects. Information on acute lethal potency, however, is not useful in characterizing most of the non-accidental hazard quotients of concern, which only modestly exceed the RfD.

3.3.2. Chronic RfD

U.S. EPA/OPP (2007a) derives a chronic RfD of 0.0004 mg/kg bw/day, based on a chronic/lifetime rat study involving dietary concentrations of 0, 7.5, 37.5, or 75 ppm rotenone, equivalent to oral doses of 0, 0.375, 1.88, or 3.75 mg/kg bw/day. No adverse effects and specifically no signs of neurotoxicity were noted at the dose of 0.375 mg/kg

bw/day. At a dose of 1.88 mg/kg bw/day, the effects included a decrease in body weight in male and female rats, accompanied by a decrease in food consumption in female rats only. The decrease in cumulative body weight gain was 10% in males and 31% in females, relative to controls. The decrease in food consumption was 9% in females (U.S. EPA/OPP 2006e, Table 4.1.3b, p. 21).

While decreased body weight gain may not always be considered an adverse systemic effect, particularly when weight loss is accompanied by a decrease in food consumption, the use of body weight to define the NOAEL of 0.375 mg/kg bw/day and the LOAEL of 1.88 mg/kg bw/day is clearly appropriate for rotenone. As noted in Section 3.1.2, rotenone will effectively uncouple oxidative phosphorylation at the cellular level; accordingly, the weight loss noted in rats is consistent with a decrease in food conversion efficiency at the level of the whole animal. The greater sensitivity in female rats, relative to males, is consistent with differences in acute oral toxicity (Section 3.1.4), acute inhalation toxicity (Section 3.1.13), and the slower elimination rate of rotenone by female rats, relative to male rats (Section 3.1.3.1).

In deriving the chronic RfD, the U.S. EPA/OPP (2007a) uses an uncertainty factor of 1000. This uncertainty factor is calculated as the product of three individual factors of 10 for inter-species variability, intra-species variability, and uncertainties in the available data on rotenone. As detailed in the HED Science Chapter on rotenone (U.S. EPA/OPP 2006e), the uncertainty in the database reflects the concern for the lack of a non-rodent (rabbit) developmental toxicity study (because rabbits are often the most sensitive species in developmental toxicity studies) as well as concerns for a fetal risk factor for conditions such as Parkinson's disease (Barlow et al. 2004). In other words, pre-natal or early post-natal exposures to agents causing essentially permanent neurotoxic damage might not induce overtly toxic effects until later life—i.e., increasing the prevalence of sporadic Parkinson's disease in aging populations, as discussed in Section 3.1.6.

While not specifically discussed in U.S. EPA/OPP (2006e), it is worth noting for clarity that *lifetime* feeding studies do not entail pre-natal or early post-natal exposures—i.e., the studies start with weanling animals. Similarly, multigeneration reproduction studies (Section 3.1.9.2), do involve pre-natal or early post-natal exposures but do not include observations of the test animals into old age.

No studies in the published literature report adverse effects at or below dietary concentrations of 7.5 ppm or daily doses of 0.375 mg/kg bw/day (Appendix 1). Thus, the chronic NOAEL selected by the U.S. EPA for the derivation of the chronic RfD appears to be appropriate.

Other chronic risk values (e.g., previous chronic RfDs and ADIs) have been derived for rotenone, and these values are discussed further in Section 3.3.4 (Dose-Severity Considerations). In the current Forest Service risk assessment, the U.S. EPA chronic RfD of 0.0004 mg/kg bw/day is used both to characterize risks in workers and longer-term exposures for members of the general public.

3.3.3. Acute RfD

In the recent RED on rotenone, the U.S. EPA/OPP (2007a) derives an acute RfD of 0.015 mg/kg bw/day. This acute RfD is intended to be protective of a sensitive subgroup (i.e, females between the ages of 13 and 49) exposed to a single acute (1-day) dietary concentration of a chemical. This subgroup, often used by the EPA, appears to reflect a particular concern for women of child-bearing age. Accordingly, these acute RfD values are often based on developmental studies (Section 3.1.9.1).

The RfD is based on the NOAEL of 15 mg/kg/day from the developmental toxicity study in mice, discussed in Section 3.1.9.1. As with the chronic RfD, the U.S. EPA/OPP uses an uncertainty factor of 1000, the rationale for which is identical to that for the chronic RfD discussed in Section 3.3.2.

The acute RfD uses information from both the range-finding phase of the developmental study in mice as well as the subsequent full study. Both phases of this study involved dosing pregnant females over a 12-day period—i.e., Days 6 to 17 of gestation. The NOAEL of 15 mg/kg bw/day is taken from the full-study. The corresponding LOAEL is taken from the range-finding study in which a dose of 24 mg/kg bw/day was associated with a 760% increase in resorptions, 3.8 in the dosed group versus 0.5 in the control group. The dose of 24 mg/kg bw/day was also associated with a 41% decrease in body weight gain (U.S. EPA/OPP 2006e, p. 19). The proximity of the NOAEL to the LOAEL is discussed further in Section 3.3.4 (Dose-Severity Relationships).

As noted in Section 3.1.9.1, a developmental toxicity study in rats was also submitted to the EPA, and, like the developmental study in mice, the rat study was classified as *Acceptable/Guideline*. In other words, the rat developmental study was conducted and documented in an acceptable manner that satisfied the Agency guidelines/protocols for developmental studies. In the rat study, dams were dosed at 0, 0.75, 1.5, 3, or 6 mg/kg/day. Based on the EPA review and classification of responses (U.S. EPA/OPP 2007e, p. 23 ff), adverse maternal effects (salivation and abnormal behavior) were noted at 0.75 mg/kg bw/day and adverse fetal effects (decreased body weight) were noted at 6 mg/kg bw/day. Thus, a maternal NOAEL was not established; the developmental NOAEL was 3 mg/kg bw/day.

While not discussed in detail by the U.S. EPA, the selection of the higher NOAEL of 15 mg/kg bw/day from the mouse reproduction study over the lower NOAELs or LOAELS from the rat reproduction study appears to reflect the standard practice of the Health Effects Division (HED) of OPP, which is to base acute/1-day RfDs only on NOAEL/LOAEL values that can be plausibly associated with a single/1-day dose. This standard practice is suggested in a comment in the HED Science Chapter indicating the reason that an acute RfD is not derived for groups other than women of child-bearing age: *An appropriate endpoint attributable to a single dose was not identified in the available studies, including the developmental toxicity studies* (U.S. EPA/OPP 2006e, p. 37).

In other words, the Agency intends the acute RfD to be protective of a single dose, 1-day exposure. In assessing developmental/teratology studies, a plausible but conservative assumption is generally made: adverse reproductive events, such as resorptions, could be associated with a sensitive stage in the development of the organism. Based on this conservative assumption, an adverse reproductive endpoint could be attributed to a single dose or a single day of exposure rather than to the entire course of treatment. In other words, the resorptions that occurred in the developmental study in mice could have all been related to adverse/lethal effects on the developing mice, which occurred solely because of a single dose given on 1 of the 12 days. The effects seen in the developmental study in rats, however, were more general in nature, which could lead to an assumption that they resulted from the multiple doses used and would not have been observed after a single dose.

The approach used by the EPA to derive the acute RfD may not seem to be the most conservative; nevertheless, it is based on a reasonable interpretation of the available developmental studies. While not specifically addressed in the EPA's acute RfD for rotenone, the distinction between single and multiple dose exposures is also appropriate in assessing the neurological effects of rotenone, given that the available data clearly indicate that multiple dose exposures are more likely to lead to adverse neurological effects than are equivalent single dose exposures (Section 3.1.6 and Table 5).

3.3.4. Dose-Severity Relationships

As summarized in the exposure assessment (Section 3.2), there is substantial uncertainty in the estimates of exposure and absorbed doses for workers and members of the general public. Particularly for members of the general public, there is also substantial uncertainty concerning the likelihood that the exposure scenarios will or could occur. Nonetheless, and as detailed further in Section 3.4 (Risk Characterization for human health effects), some of the standard exposure scenarios used in Forest Service risk assessments for both workers and members of the general public exceed the acute RfD of 0.015 mg/kg bw/day by substantial margins. In addition, some of the general exposure scenarios for workers, particularly workers not using PPE, exceed the chronic RfD by a substantial margin. Thus, some attempt must be made to characterize the health consequences of such exposures.

The dose-severity relationships considered in this discussion are summarized in Table 8, and the discussion itself is dominated by the atypically high uncertainty factor (1000 rather than 100) used by the U.S. EPA/OPP (2007a) as well as the apparently sharp increase in severity with dose in the animal studies on which the acute and chronic RfDs are based.

As discussed in Section 3.3.2, the recent chronic RfD from U.S. EPA/OPP (2007e) uses an animal NOAEL of 0.375 mg/kg bw/day and an uncertainty factor of 1000 to derive the chronic RfD of 0.0004 mg/kg bw/day. The current RfD for rotenone on IRIS—i.e., the Agency-wide RfD database maintained by U.S. EPA's Office of Research and Development—is based on the same study used by OPP and uses the same NOAEL (U.S. EPA/ORD 1988). The only difference between the two RfDs is the uncertainty factor:

1000 in the RfD from OPP and 100 in the RfD from ORD. Both of these RfDs are listed in Table 8 and both are compared with the OPP RfD that is also used in the current Forest Service risk assessment for characterizing risks associated with longer-term exposures.

The differences in the chronic RfDs from OPP and ORD are not related directly to dose-severity considerations but instead reflect the concern expressed by U.S. EPA/OPP (2006e, 2007a) for the potential neurological effects of rotenone. The difference in the RfDs also does not necessarily indicate a lack of agreement between OPP and ORD. The RfD on IRIS was developed in 1988, prior to the bulk of the literature on the neurotoxicity of rotenone (Table 6). As detailed in Section 3.1.6, the concern for the neurological effects of rotenone appear to be clearly justified, particularly with the recent report by Inden et al. (2007) that the Parkinson's disease-like effects of rotenone can be induced by oral exposure. Thus, while the higher RfD from U.S. EPA/ORD (1988) is acknowledged and included in Table 8, this does not suggest that hazard quotients of 10 based on the lower RfD from U.S. EPA/OPP, which is used in this Forest Service risk assessment, are *acceptable*. It does suggest, however, that hazard quotients of up to 10 might not be associated with frank adverse effects.

Of greater concern to this risk assessment is the apparently sharp dose-severity relationship for rotenone in both of the studies on which the RfDs are based. This is particularly evident with the acute RfD. The spacing between the NOAEL and the LOAEL—i.e., the LOAEL/NOAEL ratio—is often an artifact of the experimental design—i.e., the selection of doses used in the study. This is not the case with rotenone. The acute RfD is based on a combination of both a range-finding study (with doses of 0.75, 1.5, 3, 6, 12, or 24 mg/kg bw/day) and a full study (with doses of 0, 3, 9, or 15 mg/kg bw/day). While somewhat speculative, the expectation from the range-finding study appears to have been that the dose of 15 mg/kg bw/day would be an adverse effect level, given the effects seen in the range-finding study at 24 mg/kg bw/day—i.e., a substantial increase in resorptions. For a teratology study, which is most often focused on determining the ability of the chemical to induce developmental malformations, resorptions are a concern because they can mask teratogenic effects—i.e., a malformation may be so severe that the organism is not viable and is resorbed. For this reason, lowering the highest dose from 24 to 15 mg/kg bw/day for the full-study was sensible. That the dose of 15 mg/kg bw/day failed to induce any adverse effects was probably not expected, and the failure to note effects at 15 mg/kg bw/day suggests that the dose-severity relationship for rotenone may be pronounced. While somewhat peripheral to the discussion of mammalian risk, Chen and Farrell (2007) observed very steep dose-severity relationships in trout—i.e., no mortality at 5 ppb and complete mortality at 6.6 ppb.

The impact of the apparently steep dose-severity relationship on the current risk assessment for human health involves the interpretation of hazard quotients that are greater than 1. If the RfD is viewed as a reasonable estimate of a human threshold, the proximity of the animal NOAEL (15 mg/kg bw/day) to the animal LOAEL (24 mg/kg bw/day) could suggest that a hazard quotient of 1.6 constitutes a level of serious concern. On the other hand, if the RfD is regarded as a highly protective estimate—i.e., an

exposure that is likely to be far below a human threshold—then an HQ of 1.6 would not constitute a level of serious concern.

For many well-studied pesticides on the which the RfD is based on a non-reproductive endpoint, dose-severity relationships can be developed which suggest that hazard quotients of 10 or greater might not be associated with serious adverse effects. For rotenone, however, this type of assertion cannot be made.

As summarized in Table 8, mortality in rodents could be expected at acute hazard quotients of about 400—i.e., the lowest LD_{50} is about 6.5 mg/kg bw. Based on the lowest reported lethal dose in humans—i.e., 40 mg/kg bw—a hazard quotient greater than 2500 would suggest a potentially lethal exposure in sensitive human subgroups. While these very crude estimates have some impact on the assessment of extreme accidental exposures, they are of limited use in characterizing risks associated with many less severe exposure scenarios that result in risk quotients in the range of about 10 to 40.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterization for rotenone is relatively simple and focuses on risks to workers. As with the exposure assessment, all hazard quotients are based on an application of CFT Legumine, at a target concentration of 0.2 ppm using a toxic equivalency factor of 1.25. Other formulations of rotenone – i.e., those formulations containing piperonyl butoxide – have toxic equivalency factor of up to 2.5 and this difference would lead to hazard quotients twice as high as those discussed below.

The recent RED prepared by the U.S. EPA's Office of Pesticide Programs requires that workers involved in the application of rotenone use personal protective equipment (PPE). If the specific PPE requirements outlined in the RED are implemented, only the upper bound hazard quotient at the maximum application rate exceeds the level of concern (HQ=1.7). If effective PPE is not used, hazard quotients exceed the level of concern; moreover, at the highest application rate, the upper bound of the hazard quotient is 17. While hazard quotient of 17 might not be associated with frank adverse effects, it would clearly amount to a highly imprudent exposure. The accidental exposure scenarios for workers result in HQ values that substantially exceed the level of concern, reaching an upper bound of 612. These accidental exposure scenarios are included in all Forest Service risk assessments to evaluate the importance of proper handling of pesticides. For rotenone, it is apparent that aggressive steps are warranted in the event of accidental exposures or mishandling.

The risk quotients for members of the general public are similar to those for workers. At the maximum application rate of 0.2 ppm, the maximum acute hazard quotient for non-accidental scenarios is 1.9. The highest longer-term hazard quotient is 3. Both of these hazard quotients are associated with the consumption of contaminated water. In most Forest Service risk assessments, this exposure scenario is viewed as an *expected exposure*; however, this is not the case for rotenone. Owing to restrictions governing the access of the general public to treated sites during treatment and prior to detoxification with potassium permanganate, exposures for members of the general public are not expected to be significant.

Groups that may be at increased risk to rotenone exposures include women of child-bearing age and individuals with Parkinson's disease and perhaps other neurological disorders. While potassium permanganate is considered as a connected action, the use of potassium permanganate will mitigate several exposure scenarios that would otherwise be of concern, including exposures involving sensitive subgroups.

3.4.2. Workers

The quantitative risk characterization for workers is presented in Worksheet E02 of the EXCEL workbook that accompanies this risk assessment (Attachment 1). As discussed in the exposure assessment for workers (Section 3.2.2), the hazard quotients are based on the maximum target application rate of 0.2 ppm.

3.4.2.1. General Exposures

For general exposures—i.e., exposures that might be anticipated in the aquatic application of rotenone—the risk characterization is dominated by the consideration of PPE. For workers using PPE, the central estimate of the hazard quotient (0.7) and lower bound of the hazard quotient (0.3) are below the level of concern (LOC=1). The upper bound of the hazard quotient is 1.7, modestly exceeding the level of concern. Based on the dose-severity relationships for rotenone (Section 3.3.4), this hazard quotient is below the hazard quotient of 5, the HQ associated with the animal LOAEL on which the chronic RfD is based (Table 8). Because the hazard quotient is linearly related to the application rate, the upper bound of the hazard quotient would reach but not exceed the level of concern at an application rate of about 0.12 ppm (120 ppb). As summarized in Table 4, an application rate of 0.12 ppm would encompass most of the types of applications for which rotenone is labeled. The only exceptions are the upper bound target application rates for bullheads and carp (0.2 ppm) and the upper bound of the target application rates for pre-impoundment treatment above a dam. Thus, for most of the types of applications that would be made in Forest Service programs, the hazard quotients for workers using PPE would not exceed the level of concern.

As discussed in the worker exposure assessment, the use of PPE is required in the RED prepared by the U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP 2007a), but waivers of this requirement have been granted since the RED was released (e.g., Peacock 2007). Thus, worker exposure assessments were also conducted using baseline values — i.e., regular clothing with no PPE. In this instance, the central estimate of the hazard quotient is 7 with a range from 3 to 17. In order for the upper bound of these hazard quotients to reach but not exceed the level of concern, the application rate would need to be about 0.012 ppm [0.2 ppm / 17]. As summarized in Table 4, this application rate would encompass only the lowest labeled rates—i.e., from 0.005 to 0.007 ppm for selective treatment (presumably of sensitive species of pest fish).

The U.S. EPA/OPP (2007a) uses a different method to estimate worker exposure from that used in the current Forest Service risk assessment, and the risk characterizations from EPA are more severe than those given in the current Forest Service risk assessment. The EPA uses a Margin of Exposure (MOE) method in which the acceptable margin of exposure is 1000—i.e., equivalent to the uncertainty factor used in deriving the RfD. Thus, an MOE of 100—i.e., a factor of 10 below the target MOE—would correspond to a hazard quotient of 10 in this Forest Service risk assessment. As summarized in U.S. EPA/OPP (2007a, pp. 19-20), the baseline MOEs derived by the U.S. EPA range from about 0.51 to 440. These MOEs would correspond to hazard quotients from about 2 to 2000. The very low MOEs (high HQs) in the EPA risk assessment are associated with larger areas than those used in the current risk assessment as well as the selection of different surrogate application methods. With PPE (gloves, double layer clothing, and a respirator with 90% efficiency), the MOEs derived by the U.S. EPA are greater than 1000—i.e., corresponding to HQ values of less than 1 in this Forest Service risk assessment.

Thus, the current Forest Service risk assessment is consistent with U.S. EPA/OPP (2007a) in suggesting that the effective use of PPE is prudent over the range of application rates that would typically be used for rotenone.

3.4.2.2. Accidental Worker Exposures

The risk quotients associated with wearing contaminated gloves lead to hazard quotients that are much higher than those associated with the general levels of exposure anticipated for routine applications of rotenone. In these scenarios, the variables that determine risk are the concentration of rotenone in the field solution, the surface area of the skin in contact with the field solution, and the duration of exposure. All of these factors are linearly related to risk. Thus, the actual exposures of a worker wearing contaminated gloves for 1 hour are 60 times greater than those for a worker wearing contaminated gloves for 1 minute (Worksheet E01). The hazard quotients are not precisely different by a factor of 60, because hazard quotients in the range of 0.1 and higher are rounded to one significant place.

The upper bound of the risk quotients associated with accidental spills on to the surface of the hands or legs lead to hazard quotients that exceed the level of concern (LOC=1.0) and are higher than those associated with general exposures for workers wearing effective PPE. The central and lower bound estimates of the hazard quotients are below the level of concern.

Any number of more or less severe accidental exposure scenarios could be constructed. The 1-minute and 1-hour scenarios for rotenone are consistent with exposure scenarios used in all other Forest Service risk assessments and are intended to serve only as an indication of the potential consequences of imprudent handling of pesticides.

For rotenone, it is apparent that aggressive steps are warranted in the event of accidental exposures.

3.4.3. General Public

The risk characterizations for members of the general public are summarized in Worksheet E04 and are based on the estimates of exposure from Worksheet E03. As emphasized in the exposure assessment for members of the general public (Section 3.2.3), U.S. EPA/OPP (2007a) requires that effective measures be taken to preclude access of members of the general public to the treatment area. In addition, the EPA generally requires detoxification of rotenone with potassium permanganate. Consequently, aquatic applications of rotenone should be conducted in a way that ensures that exposure and its consequential risk to members of the general public is minimal. All of the risk quotients given in Worksheet E04 and discussed below would involve instances in which the requirements imposed by the U.S. EPA on public access to treated sites are not properly implemented.

The non-accidental acute exposure scenarios modestly exceed a level of concern (with a central estimate HQ of 1.3 an upper bound HQ of 1.9) for a child drinking contaminated water from a lake or stream. As discussed in the exposure assessment for this scenario

(Section 3.2.3.5), using the target application rate probably overestimates plausible acute exposures, based on the differences between nominal and measured concentrations noted by Chadderton et al. (2003). These HQ values are based on a concentration of rotenone in water of 200 ppb, the highest application rate considered in this risk assessment. The HQ is linearly related to the application rate. Thus, the application rate associated with an HQ of 1 (i.e., at but not above the level of concern) is 105 ppb [200 ppb/1.9]. As summarized in Table 4, the application rate of 105 ppb is above most the application rates that would be used for rotenone.

The chronic risks associated with longer-term concentrations of rotenone in surface water are 0.6 (0.1 to 3). The upper bound HQ of 3 is based on a concentration in water of about 39 ppb rotenone equivalents (Worksheet B04b). This exposure scenario is implausible because of limitations imposed by the U.S. EPA on public access to treated waters as well as the requirement to detoxify treated waters with potassium permanganate (Section 3.1.16.2).

None of the non-accidental risk quotients for the consumption of contaminated fish exceed a level of concern by a substantial margin – i.e., the highest HQ is 1.2. The lack of risk associated with scenarios for the consumption of contaminated fish is consistent with human experience in the centuries old use of rotenone as a piscicide used for harvesting fish from surface water (Section 2.2).

The accidental exposure scenarios all involve the spill of 200 gallons of a field solution into a small pond. The highest upper bound of the hazard quotients—i.e., HQ of 363—approaches the magnitude of the hazard quotients for accidental worker exposures. Again, these accidental exposure scenarios will not occur in a properly managed rotenone application, and they are included in this risk assessment both for consistency with other Forest Service risk assessments and to assess the potential impact of inadvertent errors or accidents in handling rotenone. Should a serious accident occur, the restrictions involved in public access to treated sites as well as the availability of potassium permanganate to detoxify rotenone would reduce the potential for adverse effects to members of the general public.

3.4.4. Sensitive Subgroups

Women of child-bearing age, particularly women who are pregnant, as well as individuals that have a predisposition to develop Parkinson's disease are groups that appear to be at increased risk from exposure to rotenone. As detailed in Section 3.3.3, rotenone exposures are associated with fetal resorptions in mice, and the acute RfD for rotenone is specifically intended to protect women of child-bearing age. As discussed in U.S. EPA/OPP (2006e), the fetus may be at special risk as well, not only because of potentially lethal effects (i.e., resorption) but because of the potential for longer-term neurological effects that might not be displayed until later in life.

Individuals with Parkinson's disease are a group identified as being at special risk because of the ability of rotenone to cause neurological damage resembling the effects of Parkinson's disease (Section 3.1.6). Whether or not rotenone causes Parkinson's disease

is not clear; nonetheless, it is evident that rotenone causes neurological damage. Because Parkinson's disease is more prevalent among the elderly, they may also be a sensitive subgroup.

3.4.5. Connected Actions

Because the U.S. EPA/OPP (2007e) recommends the use of potassium permanganate to detoxify rotenone, the use of potassium permanganate is a connected action under the National Environmental Policy Act (NEPA). The potential risks associated with the use of potassium permanganate are discussed in further detail in Section 3.1.16.2. While no chemical is without risk, the U.S. EPA/OPP (2007a) recommends the use of potassium permanganate to reduce the greater potential risks of rotenone exposure to the general public and nontarget species. As discussed above in this risk characterization for human health (Section 3.4) and as reiterated in the risk characterization for ecological effects (Section 4.4), the use of potassium permanganate will mitigate several exposure scenarios that would otherwise be of concern.

3.4.6. Cumulative Effects

Cumulative effects may involve either repeated exposures to an individual agent or simultaneous exposures to the agent of concern (in this case rotenone) and other agents that may cause the same effect or effects by the same or a similar mode of action. The U.S. EPA/OPP (2007a) does not specifically address cumulative risks for rotenone. As discussed in Section 3.1.16.1 (*In Vivo* Interactions), exposures to several different compounds could either enhance or diminish the toxicity of rotenone, depending on the nature of the agent and the sequence of exposure. Other agents having the same mode of action as rotenone would probably have an additive effect on the toxicity of rotenone.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

Since the use of rotenone covered in this risk assessment involves direct applications to surface waters, aquatic organisms are an obvious concern to the hazard identification for ecological effects. The hazard identification and even the risk characterization for fish is virtually a tautology: rotenone is a piscicide, and, if rotenone is applied at effective concentrations, fish will die. Not all fish, however, are equally sensitive to rotenone. The more sensitive species of fish, such as trout and bluegills, are likely to be killed by rotenone treatments at the lower bound of labeled application rates—i.e., from 5 to 7 ppb. Even the most tolerant species of fish are likely to be killed at the upper bound of the labeled application rate—i.e., 200 ppb. Because rotenone treatments typically last for only about 6 hours prior to detoxification with potassium permanganate, concentration-duration relationships are important. For fish, the temporal relationships indicate that 6-hour LC_{50} values are only a factor of 2-3 above the 96-hour hour LC_{50} values. As is true for mammalian exposure, concentration-response relationships for rotenone appear to be quite steep—i.e., the LC_{50} may not be much lower than the concentration that will cause 100% mortality in fish and may not be much higher than the concentration that will cause 0% mortality in fish.

Some aquatic invertebrates may also be adversely affected by rotenone applications at the labeled rates, and this is amply demonstrated in field studies. Aquatic invertebrates, however, have a much broader range of tolerances to rotenone than do fish. While the range of LC_{50} values among different fish species is about a factor of 40, the corresponding range in aquatic invertebrates spans a factor of about 10,000. The most sensitive group of invertebrates, small aquatic arthropods, are about as sensitive as the most sensitive fish species. Based on the available LC_{50} values, snails comprise the least sensitive group of invertebrates and are more tolerant than fish to the toxicity of rotenone by factors of up to 1000. While the effects of rotenone on aquatic vegetation have not been studied extensively, aquatic plants appear to be insensitive to rotenone.

While the focus of the current risk assessment is on the toxicity of rotenone to aquatic organisms, potential risks to mammals and birds are considered quantitatively. In addition, information on terrestrial plants is useful in interpreting some of the data on aquatic plants. In the U.S. EPA ecological risk assessment (U.S. EPA/OPP 2006c), rotenone is classified as highly toxic to mammals, only slightly toxic to birds, and practically nontoxic to honeybees. The classification for mammals is clearly appropriate and consistent with the information detailed in the HHRA for the current Forest Service risk assessment.

The classification of rotenone as only slightly toxic to birds is consistent with the data considered in the EPA ecological risk assessment—i.e., LD_{50} values of 2200 and 1680 mg/kg body weight, respectively, for mallard ducks and pheasants. Additional information from the early study by Cutkomp (1943), however, suggests that other

species of birds, particularly small birds, may be much more sensitive to rotenone exposure than are ducks, pheasants, and some other species. Based on relatively standard bioassays, the most sensitive species identified in the work by Cutkomp (1943) is the Eastern chipping sparrow for which the LD_{50} is 113 mg/kg body weight. Based on an atypical bioassay in which rotenone was administered to Eastern robins in prey items, doses of 25 mg/kg body weight and greater were lethal. The dose of 25 mg/kg body weight is somewhat lower than the dose of 30 mg/kg body weight used by the EPA to classify rotenone as highly toxic to mammals. Thus, there is some uncertainty in the hazard identification for birds; nonetheless, it seems plausible that some species of small birds may be sensitive to rotenone toxicity.

Similarly, the toxicity of rotenone to insects appears to be variable. Honeybees are relatively tolerant; however, other terrestrial insects (e.g., moths) may be more sensitive. Terrestrial plants are insensitive to rotenone, and the biochemical basis for this lack of sensitivity seems related to the presence of a NADH/NADPH dehydrogenase in plants that is insensitive to rotenone and that differs from the sensitive NADH/NADPH dehydrogenase found in animals.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals

As summarized in the human health risk assessment (see Section 3.1), a substantial amount of information is available on the toxicity of rotenone to mammals. For many chemicals, systematic or allometric relationships are apparent between body weight and toxicity (e.g., Boxenbaum and D'Souza 1990). For some chemicals, larger mammals are more sensitive than smaller mammals, and the opposite relationship is true for other chemicals. As discussed in Section 3.3.1, the data on rotenone do suggest modest differences among species; however, these differences do not appear to be clearly related to body weight. For example, the early studies of Haag (1931) indicate that rabbits are more tolerant than small rodents to rotenone exposure and that larger mammals, like dogs and cats, may be somewhat more sensitive than rodents because they appear to eliminate rotenone more slowly. This assessment, however, is based on intravenous studies in small numbers of animals (Appendix 1), and the differences do not seem substantial. In addition, the longer-term toxicity studies in rats and dogs are remarkably similar. In a 6-month feeding study in dogs summarized in Gardener (1985b), the NOAEL was 0.4 mg/kg bw/day, and the endpoint for the LOAEL of 2 mg/kg bw/day was decreased body weight. The dog NOAEL is virtually identical to both the rat NOAEL of 0.375 mg/kg bw/day on which the chronic RfD is based and the rat LOAEL of 1.88 mg/kg bw/day which, like the dog study, is based on decreased body weight (Section 3.3.2).

A lack of systematic differences among species is also reflected in similar estimates of lethal doses for rats and humans. For example, the LD_{50} value used by U.S. EPA/OPP (2006c) to characterize the toxicity of rotenone in female rats is 39.5 mg/kg body weight. This value is virtually identical to the estimated lethal dose of rotenone for a young girl after accidental ingestion of a rotenone formulation—i.e., 40 mg rotenone/kg body weight (De Wilde et al. 1986).

Thus, among mammalian species, the differences in sensitivity to rotenone toxicity appear to be modest. Accordingly, as discussed in Section 4.3.2, only one dose-response assessment is made for mammals. This approach is similar to the one taken by Environmental Fate and Effects Division (U.S. EPA/OPP, EFED) in the recent ecological risk assessment for rotenone (U.S. EPA/OPP 2006c). Based on the LD_{50} of 39.5 mg/kg body weight in female rats (see Section 3.1.3), EFED classifies rotenone as highly toxic to mammals (U.S. EPA/OPP 2006c, Table 3.18, p. 56).

Field studies in the published literature do not provide a clear association between rotenone applications and effects on mammalian wildlife. Similarly, U.S. EPA/OPP (2006c) does not report any incident data for rotenone involving species of mammalian wildlife.

4.1.2.2. Birds

Most of the available studies from the primary literature as well as some data extracted from the EPA ecological risk assessment (U.S. EPA/OPP 2006c) are summarized in Appendix 2. The many available reviews on rotenone toxicity focus mostly on mammals or aquatic species. Very little toxicity data are available on birds. Based on subacute dietary studies by Hill et al. (1975), summarized in Appendix 2, U.S. EPA/OPP (2006c) classifies rotenone as slightly toxic to birds.

Haag (1931) suggests that with respect to rotenone exposure, pigeons are much more tolerant than mammals. This conclusion is based on an intravenous lethal dose of 1 mg in pigeons and further studies involving dosing pigeons with rotenone in capsules. In the capsule studies, doses ranging from 200 to 500 mg caused only vomiting, and lower doses (not specified) caused no apparent adverse effects. Haag (1931) does not specify the species or body weight of the pigeons used in these studies. Generally, the body weights of conventionally studied pigeons (i.e., *Columba livia*, the Rock Dove or feral pigeon) is about 270 g (Sibley 2000, p. 260). Using the body weight of 270 g, the intravenous dose of 1 mg/bird corresponds to about 3.7 mg/kg body weight, which is much higher than the lethal intravenous doses reported for mammals—i.e., from 0.2 to 0.65 mg/kg body weight (Appendix 1).

Again, if 270 g is assumed to be the body weight of a pigeon, the rotenone doses of 200 to 500 mg reported by Haag (1931) in the capsule studies correspond to a doses of about 740 to 1850 mg/kg body weight. These doses, which caused only vomiting in pigeons, are only somewhat lower than the gavage LD_{50} values in birds —i.e., 1680 to 2200 mg/kg body weight from an unpublished study by Tucker (1968, MRID 143250)—submitted to the U.S. EPA/OPP (2006c). The LD_{50} values cited by U.S. EPA are similar to the LD_{50} values for rotenone cited in Tucker and Crabtree (1970)—i.e., >2000 mg/kg body weight for mallards and >1414 mg/kg body weight for pheasants. In Tucker and Crabtree (1970), however, the toxic material is reported as 32.38% cubé resins, and it is unclear whether the doses refer to the resin or to rotenone.

Cutkomp (1943) conducted somewhat unusual studies in which Eastern robins were fed derris dust (0.75% rotenone) incorporated into various prey items. Some birds survived

doses of 3-15 mg/kg body weight, while others died after doses of 8-34 mg/kg body weight. No birds survived doses of 25 mg/kg body weight, which would correspond to a rotenone dose of 0.1875 mg/kg body weight, substantially below any oral lethal doses reported in mammals.

Cutkomp (1943) also conducted studies in which several other species of birds were exposed to rotenone in capsule form. As summarized in Appendix 2, these studies consist of both bioassays with relatively few animals (i.e., analogous to range-finding studies) as well as bioassays with larger numbers of animals (i.e., analogous to full studies). In the full studies, the LD_{50} values range from 113 mg/kg body weight for the Eastern chipping sparrow to 3077 mg/kg body weight for 28-day-old chickens.

As is true for mammalian exposure to rotenone, the LD_{50} values from Cutkomp (1943) do not suggest a clear pattern in sensitivity among species based on differences in body weight. What is more, Cutkomp (1943) does not report the body weights of the test species. Using data from Dunning (1993), the typical body weight of the most sensitive species—i.e., the chipping sparrow, *Spizella passerina*—is about 12.3 g (Dunning 1993, p. 287). This species weighs much less than some of the more tolerant species, like older chickens and pheasants. Nonetheless, one of the more tolerant species (LD_{50} = 853 mg/kg body weight) is the English sparrow (*Passer domesticus*), which has a typical body weight of about 28 g (Dunning 1993, p. 287). A more consistent pattern in the data from Cutkomp (1943) is that younger birds of the same species are more sensitive than older birds to rotenone toxicity. [See the data on chickens and pheasants in Appendix 2.] Whether or not the difference in sensitivity is attributable to differences in size or other factors is unclear.

4.1.2.3. Terrestrial Invertebrates

Some important nontarget terrestrial insects do not appear to be sensitive to rotenone while other species (primarily pest species) do appear to be more sensitive to rotenone. Until recently, rotenone was registered as an insecticide to control several species of crop insects (U.S. EPA/OPP 2006c). Presumably, this detail indicates that rotenone is an effective insecticide at sufficiently high application rates to terrestrial vegetation. Delaney and Wilkins (1995) report a 72-hour LC_{50} of 2 $\mu g/cm^2$ rotenone in the diamond-moth on treated leaf surfaces. The residue rate of 2 $\mu g/cm^2$ corresponds to a terrestrial application rate of only about 0.18 lb a.i./acre, which is similar to the application rate of 0.22 lb a.i./acre rotenone that had been used on some vegetable crops prior to the cancellation of rotenone as an insecticide for use on crops (U.S. EPA/OPP 2006c, Table 3.4, p. 32).

Cell culture assays also indicate that rotenone can be an effective and perhaps selective insecticide. Based on cell growth inhibition assays using cells from the Egyptian cotton leaf worm and mouse fibroblast cells, rotenone was more potent in insect cells than in mammalian cells by a factor of 5 (EC_{50} values for growth inhibition of 10^{-8} M vs. 2 $x10^{-7}$ M). In cell bioassays using mosquito cell cultures, rotenone was the most potent of 20 common insecticides (including malathion, lindane, and DDT) in inhibiting cell growth (Mitsuhashi et al. 1970).

Based on a standard contact bioassay, however, the LD_{50} of rotenone in honeybees is >60 µg/bee. The EPA uses this dose to classify rotenone as *Practically Non-toxic* to bees (U.S. EPA/OPP 2006c, p. 57). Using a body weight of 0.093 g (0.000093 kg) for the honey bee (USDA/APHIS 1993), the LD_{50} of >60 µg/bee corresponds to a dose of >645 mg/kg body weight.

Haag (1931) indicates that maggots (species not specified) were not adversely affected by rotenone sprinkled on food; however, neither the food material nor the rotenone concentration is specified in the study. Haag (1931) also suggests that rotenone may be an effective treatment for parasitic worms in hogs, which is similar to the assessment made more recently by Kotze et al. (2006).

4.1.2.4. Other Terrestrial Organisms

Although not directly relevant to issues regarding the potential impact of aquatic applications of rotenone, information about the effects of rotenone on terrestrial plants and bacteria is useful for interpreting the toxicity data on aquatic plants (Section 4.1.3.4). Assays of mitochondrial activity in red beetroots, potatoes, and soybeans indicate that plant mitochondria are relatively insensitive to rotenone. Furthermore, the relative insensitivity is attributed to the presence of an NADH/NADPH dehydrogenase in plants which is insensitive to rotenone (Menz and Day 1996). In addition, rotenone is not an effective inhibitor of respiration in yeast (*Saccharomyces cerevisiae*) (Walker 1990) and does not appear to be cytotoxic in broad beans, except at saturated solutions (Amer and Mikhael 1986). The observation that terrestrial plants and microorganisms are relatively insensitive to the effects of rotenone is consistent with field observations that aquatic applications of rotenone do not adversely affect aquatic plants (Section 4.1.3.4).

One study is available on the toxicity of rotenone to the brown tree snake, *Boiga irregularis*, an invasive pest reptile in Guam. Gavage administration of rotenone caused mortality in the tree snake at a doses of 1.25 mg/kg bw (1/5 animals) and doses of 2.5 to 40 mg/kg bw (5/5 animals). When incorporation into the diet, however, at doses equivalent to 100 to 200 mg/kg bw, no mortality was noted in treated snakes (Johnston et al. 2001).

4.1.3. Aquatic Organisms

4.1.3.1. Fish

4.1.3.1.1. General Considerations

As would be expected for a commercial piscicide that has been used for many years, the toxicity of rotenone to fish has been studied in great detail. Standard published toxicity studies are summarized Appendix 4. The U.S. EPA considers numerous toxicity studies submitted in support of the registration of rotenone, and these unpublished studies are summarized in risk assessment documents prepared by U.S. EPA/OPP (2006c, 2007a). In addition, the literature on rotenone includes several reviews on the toxicity of rotenone to fish (Haley 1978; Hinson 2000; Ling 2003; Ott 2008) as well as on specific

applications of rotenone to control unwanted fish species (Entrix 2007; Finlayson et al. 2000; Marking 1992; Rotenone Stewardship Program 2008; Turner 2007).

Composing a hazard identification for fish on a compound intended to kill fill may seem to be a somewhat simple, self-evident, and perhaps pointless exercise. If rotenone is applied at effective concentrations, fish (and perhaps all fish) will die. Nonetheless, there are relevant issues to be addressed in an ecological risk assessment concerning the risks of rotenone exposure to fish, and they include: the range of sensitivities among species, the relationship between treatment time and toxicity, the residual toxicity of rotenone—i.e., how long treated water will remain toxic—and the use of potassium permanganate to detoxify rotenone.

Most Forest Service risk assessments do not specifically address efficacy. In general, Forest Service risk assessments attempt to assess the range of sensitivities in groups of aquatic organisms, including fish. Subsequently, separate toxicity values are derived for sensitive and tolerant species in the dose-response assessment (Section 4.3.3.1). While this general approach is maintained in the current risk assessment, the efficacy of rotenone is relevant to the hazard identification for fish in terms of the sensitivities of nontarget species relative to target species. As noted in Table 4, typical application rates for rotenone range from 25 to 100 ppb, the maximum application rate is 200 ppb, and application rates for *selective treatment* range from 5 to 7 ppb.

The full range of applications rates for rotenone—i.e., from 5 to 200 ppb—appears to encompass the range of sensitivities for most species of fish. As illustrated in U.S. EPA/OPP (2006c, Figures 4.1 and 4.2, p. 84), the range of 96-hour LC_{50} values for both technical grade rotenone and rotenone formulations spans a range of concentrations (expressed as rotenone) of about 2-100 ppb.

4.1.3.1.2. Species Sensitivity

Figure 3 in this Forest Service risk assessment illustrates the species sensitivity distribution for fish based on studies using technical grade rotenone (expressed as TGAI or technical grade active ingredient). Figure 3 includes all of the data in EPA Figure 4.1 as well as additional data from studies in Appendix 4. All of the specific data points used in Figure 3 are summarized in Table 9. For rotenone, the 96-hour LC_{50} value may not be the most appropriate duration for comparisons. As summarized in Section 2, rotenone concentrations are typically maintained in treated water for much shorter periods of time. The 96-hour LC_{50} value is used for initial estimates of interspecies variability simply because this duration is the most commonly reported toxicity value in the literature. The toxicity of rotenone over shorter periods of exposure is discussed further below.

In Figure 3 as well as in subsequent plots of species sensitivity distributions discussed in this risk assessment, the x-axis plots the toxicity value (in this case the 96-hour LC_{50}) and the y-axis plots the corresponding cumulative frequency associated with the toxicity value. For example, the first point in Figure 3 is the 96-hour LC_{50} of 1.94 ppb in trout reported in the EPA risk assessment (U.S. EPA/OPP 2006c, MRID 439751-02). There are a total of 19 points in Figure 3. Thus, the LC_{50} of 1.94 ppb on the x-axis has a

corresponding cumulative frequency of about 0.0525 (1/19). The second point, also in trout, is an LC_{50} of 2.9 ppb, and this point is plotted with a cumulative frequency of about 0.105 (2/19). Each of the subsequent ordered sets of LC_{50} values and cumulative frequency are plotted in a similar manner. While species sensitivity distributions can be used quantitatively (e.g., Posthuma et al. 2002), this type of use entails assumptions concerning the random selection of species. In all of the species sensitivity distributions given in this risk assessment, the species selected for study are dominated by standard test species used for pesticides (e.g., rainbow trout, fathead minnows, and bluegill sunfish). Thus, species sensitivity distribution plots in the current risk assessment are used only to illustrate patterns in the data.

As illustrated in Figure 3, rainbow trout are the most sensitive species of fish. Four bioassays in rainbow trout were conducted with rotenone, and the 96-hours LC_{50} values range from 1.94 to 5.8 ppb (Chen and Farrell 2007). The magnitude of this variability is relatively modest—i.e., about a factor of 3—and is commonly seen in comparisons of bioassays conducted by different investigators, at different times and with different populations of animals (e.g., Buhl 2002, p.24 ff; Schimmel 1981). As illustrated in Figure 3, the sensitivities of trout to rotenone overlap with the sensitivity of other common test species such as the fathead minnow and bluegill sunfish. Carp and some other cyprinids such as goldfish are among the most tolerant species of fish. The overall range of sensitivities among species in terms of the 96-hours LC_{50} values spans a factor of about 40—i.e., a lower bound of 1.94 ppb and an upper bound of 80 ppb.

The range of application rates or target concentrations for rotenone—i.e., from 5 to 200 ppb—encompasses the reported 96-hours LC_{50} values for most species of fish. While the groupings of species on which acute toxicity data are available do not necessarily reflect the variability of all fish, the available data suggest that the application rate range for *selective treatment* (5-7 ppb) would be effective for fish species not commonly classified as target species—i.e., trout.

While most of the LC_{50} studies summarized in Table 9 and illustrated in Figure 3 do not report the slope of the concentration-response curves, most of the LC_{50} values given in Appendix 4 have a rather narrow range. Confidence intervals for LC_{50} values depend on the slope of the concentration-response curve as well as random scatter (e.g., Finney 1971). The slope is inversely related to inter-individual variability in a population, with steeper slopes indicating less variability which in turn leads to narrower confidence intervals given similar patterns of random scatter. As noted by Chen and Farrell (2007) the concentration-response relationship for rotenone is very steep: the study indicates that a concentration of 5 ppb resulted in no mortality, while a concentration of 6.6 ppb resulted in 100% mortality. Although this example may be extreme, the steep concentration-response relationship is consistent with the apparently steep dose-severity relationship in mammals (Section 3.3.4) as well as the apparently steep dose-severity relationship in aquatic invertebrates (Section 4.3.3.3).

4.1.3.1.3. Inerts and Adjuvants

As noted in Section 3.1.14 (Inerts and Adjuvants), there is little basis for asserting that inerts contributed substantially to the toxicity of rotenone formulations. Moreover, the EPA ecological risk assessment (U.S. EPA/OPP 2006c) specifically notes that rotenone formulations are generally less toxic than rotenone itself. The relationship of formulation toxicity to the toxicity of technical grade rotenone (TGAI) to rainbow trout is illustrated in Figure 4. Two sets of points are plotted in Figure 4—triangles represent bioassays of rotenone TGAI, and diamonds represent various formulations. The data used in Figure 4 are summarized in Table 10 for the formulations and in Table 9 for the TGAI.

As illustrated in Figure 4, the data points for the formulations are shifted substantially to the left of the corresponding TGAI, indicating that the toxicity of the formulations is generally less than the toxicity of rotenone itself. This pattern is consistent with the generalization suggested by U.S. EPA/OPP (2006c) that inerts in rotenone formulations do not contribute substantially to toxicity. As also illustrated in Figure 4, however, most of the least toxic formulations reported in the literature do not appear to be formulations that are currently used, which is clearly the case with the formulation data reported by Tooby et al. (1975)—i.e., formulations such as Dectinol, Murphy's Liquid Derris, and Bugge's Liquid Derris. Some of the other data points used in Figure 4 are taken from U.S. EPA/OPP (2006c), and it is not clear if these formulations are currently in use. In addition, some of the currently used formulations—i.e., Noxfish and Chemfish Regular—appear to have toxicity values similar to those of the TGAI.

Marking and Bills (1976) specifically assayed differences in toxicity to rainbow trout for three rotenone formulations, two of which appear to correspond with formulations still in use. The three formulations tested by Marking and Bills (1976) are specified as Noxfish (5% rotenone), Noxfish-Pro (2.5% rotenone and 2.5% piperonyl butoxide), and rotenone powder (33% rotenone). The Noxfish formulation used by Marking and Bills (1976) has the same percentage of rotenone as Noxfish Fish Toxicant (Table 2). The Noxfish-Pro formulation used by Marking and Bills (1976) has the same amount of rotenone and piperonyl butoxide as Nusyn-Noxfish Fish Toxicant as well as other synergized formulations (Table 2). The 33% rotenone powder used by Marking and Bills (1976) does not correspond to any end-use formulation (Table 2) but has a rotenone concentration similar to some non-end use formulations (Table 7). The advantage in using the Marking and Bills (1976) data in assaying differences in rotenone formulations is that the bioassays were all conducted in the same laboratory (U.S. Fish and Wildlife Service, Le Crosse, Wisconsin) using the same experimental methods. In addition, Marking and Bills (1976) provide time-course data—i.e., LC_{50} values for 1, 3, 6, 24, and 96 hours.

An analysis of the data from Marking and Bills (1976, Table 9) is provided in Figure 5 of the current Forest Service risk assessment. In this analysis, the assumption tested is that there is no significant difference in toxicity, expressed as TGAI, among the three formulations. Thus, the LC_{50} data are pooled and fit to a standard log-log function:

$$\text{Log}_{10}(LC_{50}) = \boldsymbol{a}\ \text{Log}_{10}(\text{Hours}) + \boldsymbol{b}$$

where ***a*** and ***b*** are model parameters. A complication in this analysis is that the Noxfish-Pro formulation contained both rotenone (2.5%) and piperonyl butoxide (2.5%). As discussed in Section 3.1.14.1 (Inerts), an additional assumption is made that a piperonyl butoxide/rotenone mixture is equivalent to an equal mass of rotenone. Thus, in the statistical analysis, the LC_{50} values reported by Marking and Bills (1976) for Noxfish-Pro are doubled, as illustrated in Figure 5 with large open hexagons for the unadjusted values and small triangles for the adjusted values. Finally, since Marking and Bills (1976) did not test TGAI rotenone, the LC_{50} of 1.94 ppb for TGAI rotenone is included in Figure 5 only to illustrate that the regression of the formulation is consistent with the toxic potency of rotenone.

As summarized in Figure 5, the combined data fit the following model:

$$Log_{10}(LC_{50}) = -0.45\ Log_{10}(Hours) + 1.22$$

with an r^2 of 0.90 and a p-value of 0.0000002—i.e., the model accounted for about 90% of the variability in the data and the fit was highly significant. Thus, this analysis supports the suppositions that the toxicity of the formulations can be accounted for by rotenone and that piperonyl butoxide, at least in 1:1 mixtures with rotenone, behaves as an equivalent amount of rotenone itself.

In addition to supporting two suppositions about the toxicity of rotenone formulations—i.e., the utility of the TGAI transformation and the equivalence of piperonyl butoxide to the TGAI—the study by Marking and Bills (1976) is also useful for examining the relationship of duration of exposure to toxicity. Removing the log-transformation from the model fit in Figure 5, the relationship of the LC_{50} to duration is:

$$LC_{50} = 16.5\ Hours^{-0.45}$$

where 16.5 is equivalent to $10^{1.22}$. Since most rotenone treatments will be followed by detoxification after about 6 hours, the relationship of the 6-hour LC_{50} to the 96-hour LC_{50} is of interest. For trout—i.e., the species used in generating the above equation—this ratio can be calculated by substitution:

$$\text{6-h } LC_{50}/\text{96-h } LC_{50} = 7.36 / 2.12 = 3.47$$

More generally, the relationship can be simplified as:

$$t_1\ LC_{50}/t_2\ LC_{50} = 16.5 \times t_1^{-0.45} / 16.5 \times t_2^{-0.45} = t_1^{-0.45} / t_2^{-0.45} = (t_1/t_2)^{-0.45}$$

The 3.47 estimate based on the regression does somewhat overestimate the actual ratios based on the LC_{50} values reported in Marking and Bills (1976)—i.e., an average of 2.26 with a standard deviation of 0.56. This overestimate is due to a slight curvilinearity in the data, as illustrated in Figure 5.

4.1.3.1.4. General Concentration-Time Relationships

In trout, the 6-hour LC_{50} values appear to be about a factor of 2-3 higher than the 96-hour LC_{50} values. In terms of assessing the efficacy of 6-hour treatment periods over the range of application rates for rotenone—i.e., from 5 to 200 ppb—the 6-hour trout LC_{50} of about 7 ppb is only marginally relevant because trout are a highly sensitive species. Based on the overall species sensitivity distribution for rotenone (Figure 3 and Table 9), the highest 96-hour LC_{50} value is 80 ppb. Assuming that the relationship for trout holds for more tolerant species, a 6-hour application rate at the highest labeled rate for rotenone would be lethal to relatively tolerant species – i.e., 200 ppb / 3 = 66.6 ppb. The upper bound of the typical application rate for streams of 100 ppb (Table 4) would also be lethal to the great majority (about 95%) of the species. Based on the labeled rates for ponds, the upper bound of the typical application rate is only 50 ppb (Table 4). Assuming that the 6-hour LC_{50} for target species is a factor of about 2-3 times the 96-hour LC_{50}, a target application rate of 50 ppb might be ineffective over a 6-hour treatment period.

Data for assessing the effects of the duration of exposure and concentration of rotenone in controlling target species, particularly estimates of 6-hour LC_{50} values is very limited. Marking and Bills (1976) provide LC_{50} values for Noxfish in 21 species of fish; furthermore, for 16 of the 21 species, toxicity values are given for durations of 3, 6, 24, and 96 hours. These data are summarized in full in Appendix 4 as Supplemental Table 1. As with the trout data from Marking and Bills (1976), the bioassays on these 21 species are ideal for assessing temporal relationships, because many of the variables involved in assessing interspecies relationships—e.g., different formulations, holding conditions, experimental methods, etc.— are identical in the data presented by Marking and Bills (1976).

A major disadvantage of the Marking and Bills (1976) report, however, is that the units of the LC_{50} values are not specified. While the investigators state that the LC_{50} values for the formulation comparison in trout are expressed in mg a.i./L, the other toxicity values in this study are not explicitly identified mg a.i./L or mg formulation/L. A review of the values suggests that they are reported in units of mg formulation/L; however, this is not certain. Nevertheless, as illustrated above, the uncertainty regarding the units of measure is not crucial for estimating the slope of the concentration-time relationship—i.e., the units of the LC_{50} cancel out in taking the ratio of one duration to that of another.

The data for the 16 species with full time-course toxicity values are illustrated in Figure 6. The dashed lines in Figure 6 are plotted using the slope from the trout data discussed above (i.e., -0.45) and are included only for comparison. Overall, the time-course for the 16 species of fish are similar to that for trout, and the average of the 6-hour LC_{50} to the 96-hour LC_{50} is 2.64 (SD 1.08). This value is intermediate between the average value of 2.26 for the three formulations in trout, discussed above, and the value based on the slope of -0.45—i.e., 3.47. Thus, the generalization that the 6-hour LC_{50} is likely to be from 2 to 3 times higher than the 96-hour LC_{50} seems to hold for a large number of species. The specific ratios of the 6-hour LC_{50} to the 96-hour LC_{50} values based on the data provided by Marking and Bills (1976) range from about 1 to 5. As detailed in Section 4.1.3.3, a

substantially different and more marked concentration-time relationship is apparent in invertebrates exposed to rotenone.

4.1.3.1.5. Detoxification with Potassium Permanganate

As discussed in Section 3.1.16.2, the U.S. EPA requires the detoxification of rotenone with potassium permanganate at least under some circumstances (U.S. EPA/OPP 2007a). The use and efficacy for potassium permanganate detoxification of rotenone is amply documented in the literature (e.g., Engstrom-Heg 1972; Marking and Bills 1976; Mahon and Balon 1980).

In both the human health and ecological risk assessments, the required use of potassium permanganate substantially limits any concern associated with longer-term exposures. While longer-term exposures are not a substantial concern for members of the general public (Section 3.4.3), longer-term exposures would be a concern for sensitive species of fish, invertebrates, and perhaps amphibians, if effective detoxification with potassium permanganate were not used (Sections 4.4.3).

Nevertheless, there are potential risks associated with the use of potassium permanganate to neutralize rotenone. The detoxification of rotenone by potassium permanganate is effective because potassium permanganate is a strong oxidizing agent. As a strong oxidizing agent, potassium permanganate can cause substantial damage to aquatic organisms exposed to the permanganate anion at high concentrations. The toxicity of potassium permanganate to fish is not well studied, relative to the toxicity of rotenone. The reported 96-hour LC_{50} values for fish exposure to potassium permanganate range from 750 to 4920 ppb (U.S EPA/OPP 2006c, p. 58 ff). While concentrations as low as 750 ppb are reported as LC_{50} values, potassium permanganate is also used to prevent or treat diseases in fish in recreational or commercial ponds, and the recommended therapeutic application rate for a long-term treatment is 2000 ppb.

Based on the recommended $KMnO_4$:rotenone ratios, ranging from 2:1 to 4:1 (Finlayson et al. 2000; U.S. EPA/OPP 2007a), potassium permanganate might be applied at target concentrations of up to 800 ppb to detoxify rotenone at the maximum application rate of 200 ppb. While this rate is below the recommended therapeutic rate of 2000 ppb, the data from U.S. EPA/OPP (2006c) suggest that 800 ppb might be toxic to some fish.

That potassium permanganate constitutes a serious or substantial hazard, however, is not clear. If potassium permanganate is properly applied, the permanganate anion will be rapidly consumed by rotenone and other organic material in the water; accordingly, risks to fish and other aquatic organisms would be minimal. As noted by Finlayson et al. (2000, p. 119), an algorithm for estimating the target concentration of potassium permanganate as a multiplier (***M***) of the target concentration of rotenone can be developed using the data from Engstrom-Heg (1972):

$$M = 1 + 0.002\ (TA - 20) + 0.5\ OD$$

where TA is total alkalinity (as ppm $CaCO_3$) and OD is the organic demand (as ppm).

The likelihood of adverse effects to fish and other aquatic organisms associated with the misapplication/over use of potassium permanganate is difficult to assess quantitatively, but the risks seem to be remote. Incident reports of adverse effects in nontarget aquatic organisms associated with applications of rotenone frequently involve applications in which insufficient rather than excess amounts of potassium permanganate were applied (U.S. EPA/OPP 2006c, pp. 83, 94, 184-185; Finlayson et al. 2000). Incidents of adverse effects associated with applications of excess potassium permanganate have not been encountered.

4.1.3.2. Amphibians

Few studies, relative to those in fish, are available on the toxicity of rotenone and rotenone formulations to aquatic phase amphibians. The available studies are summarized in Appendix 5. As noted in Appendix 5, one of the major limitations in interpreting the available studies involves the distinction between concentrations reported as rotenone (TGAI) and those reported as formulation. The only three exceptions are the studies by Haag (1931), Hashimoto and Nishiuchi (1981), and Holcombe et al. (1987), all of which report toxicity values as concentrations of rotenone.

Assessments of potential risks to amphibians are thus based on relatively sparse data, and the assessments tend to vary. McCoid and Bettoli (1996) suggest that larval amphibians may be very susceptible to rotenone. On the other hand, the study by Ling (2003) suggests that larval amphibians, in general, appear to have sensitivities similar to those of the most tolerant species of fish, and a similar assessment is offered by Haque (1971).

While the data for making quantitative comparisons between fish and amphibian sensitivities are limited, the assessment by Ling (2003) appears to be correct. The early work of Haag (1931) indicates that exposures for *several hours* to 2 ppm rotenone, which is equivalent to 2000 ppb, caused mortality in frogs (*Rana pipiens*). The most directly comparable data in fish are the 3-hour LC_{50} values of 4.53-8.7 ppb rotenone in rainbow trout (Marking and Bills1976, Table 9). Based on 96-hour LC_{50} values in tolerant species of fish—i.e., 80 ppb—and the general slope of the concentration-duration relationship for rotenone (i.e., -0.45), a 96-hour LC_{50} would correspond to a 3-hour LC_{50} of about 380 ppb [80 ppm x $(3/96)^{-0.45}$]. Thus, the lethal concentration of 2000 ppb reported by Haag (1931) is consistent with the assessment that amphibians, relative to tolerant fish species, may be as, and perhaps more, tolerant to rotenone exposure. The apparent relative tolerance of amphibians relative to fish is also suggested by 48-hour LC_{50} of 330 ppb in the Japanese common toad, with is a factor of about 4 greater than the LC_{50} of 80 ppb for the most tolerant species of fish (Section 4.1.3.1.2).

Notwithstanding the above, the assessment by McCoid and Bettoli (1996) that amphibians may be very susceptible to rotenone is supported by a comparative toxicity study in *Rana sphenocephala* and several species of invertebrates conducted by Chandler and Marking (1982). As noted in Section 4.1.3.1.4, the study by Marking and Bills (1976) is among the most extensive in fish but has limited use in quantitative estimates of risk because the study does not clearly state whether the reported LC_{50} values are given

as the mass of rotenone or the mass of the formulation. A similar situation exists in the study by Chandler and Marking (1982) on the toxicity of rotenone to the larvae of *Rana sphenocephala* as well as a large number of invertebrates. Nonetheless, in terms of relative toxicity, the study by Chandler and Marking (1982) can be used to assess differences in sensitivity between *R. sphenocephala* and several species of invertebrates. In other words, in terms of making comparisons among species, it does not matter if the LC_{50} values are reported as units of rotenone or as units of formulation. As summarized in Appendix 5, Chandler and Marking (1982) report 1-hour to 96-hour LC_{50} values for *R. sphenocephala* from 0.830 to 0.500 mg/L. As discussed in detail in Section 4.1.3.3, these toxicity values are comparable to the most sensitive species of invertebrates in the Chandler and Marking (1982) study—i.e., ostracods (with a 96-hour LC_{50} of 0.34 mg/L) and caddisfly larvae (with a 96-hour LC_{50} of 0.604 mg/L).

While the units in the Chandler and Marking (1982) study are unclear, a review of numerous studies conducted by the Fish and Wildlife Service (i.e., Bills and Marking 1988; Bills et al. 1981; Chandler and Marking 1979,1982; Marking 1988, 1982; Marking and Bills 1976, 1981; Marking et al. 1984) suggests that the common practice was to report toxicity data on formulations in units of formulations. Although it is impossible to determine whether this practice was undertaken by Chandler and Marking (1982), it is, nonetheless, the conservative/protective assumption. Under that assumption, the 96-hour LC_{50} of 0.500 mg/L (500 ppb) for *R. sphenocephala* using a 5% formulation of rotenone corresponds to an LC_{50} of 25 ppb as rotenone. By comparison to 96-hour LC_{50} values in fish (Table 9), the sensitivity of *R. sphenocephala* to rotenone would be classified as intermediate between sensitive and tolerant species of fish.

4.1.3.3. Aquatic Invertebrates

4.1.3.3.1. General Considerations

While the number of relatively standard toxicity studies (i.e., LC_{50} determinations) in aquatic invertebrates (Appendix 6) is substantially less than the number of similar studies in fish (Appendix 4), the number of LC_{50} estimates for various groups of aquatic invertebrates is sufficient to characterize the toxicity of rotenone. The overall pattern in toxicity indicates that small zooplankton are as sensitive as sensitive species of fish to rotenone, and that other groups, like larger arthropods and mollusks are much less sensitive.

The standard toxicity studies on aquatic invertebrates are supported by numerous field studies (Appendix 7) on the effects of rotenone applications in streams and ponds. In addition, this literature tends to focus on effects in aquatic invertebrates. Accordingly, the hazard identification for aquatic invertebrates is similar to that for fish in that the hazards are more or less self-evident. If rotenone is applied at application rates sufficient to kill fish, adverse effects on some groups of aquatic invertebrates will occur, although most field studies suggest that the affected populations of aquatic invertebrates will recover.

4.1.3.3.2. Species Sensitivity

As with fish (Section 4.1.3.1), the interpretation of the variability in the acute toxicity of rotenone to aquatic invertebrates is complicated by variability in the toxicity of rotenone TGAI as well as differences in the toxicity of various rotenone formulations. Also, as with fish, some very detailed studies on the toxicity of rotenone to aquatic invertebrates do not clearly indicate whether the reported toxicity values are in units of TGAI or in units of formulation (e.g., Chandler and Marking 1982); thus, the quantitative use of these studies to assess risks for invertebrates exposed to rotenone is limited.

The most common measure of the acute toxicity in aquatic invertebrates is the 48-hour $LC_{50,}$ rather than the 96-hour LC_{50} most commonly reported in fish. The 48-hour LC50 values for rotenone TGAI in aquatic invertebrates are summarized in Table 11. Phylogenetically, aquatic invertebrates are a more diverse group of organisms than are fish, and this diversity is reflected in the available toxicity data on technical grade rotenone. As noted for fish in Section 4.1.3.1, the range of 96-hour LC_{50} values for fish spans a factor of about 40—i.e., from 1.94 to 80 ppb (Table 9). As indicated in Table 11, the range of 48-hour LC_{50} values in aquatic invertebrates spans a factor of about 10,000—i.e., from 3.7 to 40,000 ppb.

The wide range of toxicity values for aquatic invertebrates is clearly associated with different subgroups of aquatic invertebrates. The specific pattern is illustrated in Figure 7 using the data from Table 11. Figure 7 illustrates the species sensitivity distributions for three subgroups: Cladocera (small arthropods), other larger arthropods, and snails. *Daphnia magna*, a small cladoceran arthropod commonly used in aquatic toxicity studies, appears to be about as sensitive to rotenone as are sensitive species of fish. Other small cladocerans (i.e., *Daphnia pulex* and *Simocephalus serrulatus*) appear to be somewhat more tolerant to rotenone than even tolerant species of fish. Larger arthropods such as dragonfly, stonefly, and amphipods are much more tolerant than fish to rotenone by about 2 orders of magnitude. The most tolerant group of invertebrates appears to be the snails, which are more tolerant than fish by about 3 orders of magnitude. This overall pattern of sensitivity is similar to findings in the early toxicity studies of Hamilton (1941). While the studies by Hamilton (1941) are not reported in great detail, the overall ranking of sensitivity in the studies is: *Daphnia* ≈ *Leptodora* (another cladoceran) ≈ *Diaptomus* (a copepod) > *Estheria* (a dipteran) > leaches > amphipods > *Planria* (a flatworm).

In addition to differences in sensitivity to rotenone, aquatic invertebrates differ from fish in terms of concentration-duration relationships. As discussed in Section 4.1.3.1.4 and illustrated in Figure 6, the 6-hour to 96-hour LC_{50} ratios for different fish species span a relatively narrow range: a factor from about 2 to 3. As illustrated in Figure 8, the corresponding ratios in aquatic invertebrates tend to be much greater—i.e., an average of about 10 with a range from about 3.7 to 34. In other words, relative to the 96-hour LC_{50}, exposures of aquatic invertebrates to rotenone must be substantially greater in a 6-hour exposure period to induce the same level of mortality. This detail has practical significance to the current risk assessment because only relatively short treatment periods will be used in aquatic applications of rotenone. This approach will tend to diminish effects in aquatic invertebrates to a greater extent than in fish. Thus, rotenone can be

considered to be at least somewhat selective as a piscicide relative to its ability to adversely affect aquatic invertebrates both in terms of LC_{50} values and concentration-duration relationships.

4.1.3.3.3. Field Studies

When applied to streams, terms such as *catastrophic drift* (Lintermans and Raadik 2001) and *explosive* drift (Cook and Moore 1969) have been used to describe the effects of rotenone on aquatic invertebrates. In other words, after rotenone is applied to streams, large numbers of invertebrates will be displaced, and large increases in invertebrate numbers will be noted in drift nets—i.e., nets that are placed across sections of streams to monitor invertebrate populations (e.g., Cook and Moore 1969; Dudgeon 1990; Lintermans and Raadik 2001; Magnum and Madrigal 1999; Morrison 1977).

Similarly, when rotenone is applied to ponds, very large decreases in zooplankton—i.e., invertebrates such as daphnids that tend to reside in the water column—are noted (Anderson 1970; Burress 1982; Linn 2002; Neves 1975; Shapiro and Wright 1984). While impacts on benthic organisms (i.e., organisms that reside in the subsurface) are typically less severe than impacts on zooplankton (Dudgeon 1990; Houf and Campbell 1977), adverse effects on some groups such as midges, clams, and worms have been noted (Burress 1982; Oglesby 1964; Serns 1979). In some cases, eventual increases in populations or size distributions of planktonic invertebrates may be noted; however, these increases appear to be secondary to a reduction in fish populations (Sanni and Waervagen 1990; Stenson 1973).

Recovery of invertebrate populations is reported in most field studies that monitor the populations over a prolonged period of time. The reported recovery periods may range from weeks (Neves 1975), to months (Cook and Moore 1969; Linn 2002; Lintermans and Raadik 2001) or even years (Anderson 1970; Morrison 1977). Some studies involving relatively short post-application observations periods, report a lack of full recovery (e.g., Burress 1982; Oglesby 1964). On the other hand, Magnum and Madrigal (1999) report that some macroinvertebrate populations did not fully recover (in the sense that some groups of macroinvertebrate populations were missing) over a 5-year observation period after and application of rotenone (150 ppb) to a river.

The widely varying durations for reported recovery periods may be attributable as much to differences in the definitions of recovery as to differences in the actual patterns of recovery. In some cases, the nature of the recovery may be incomplete in that long-term shifts in invertebrate populations may occur (Blakely et al. 2005; Prejs et al. 1997; Sanni and Waervagen 1990; Stenson 1973). The extent to which these differences in recovery patterns are attributable to differences in the nature and extent of the treatments and/or differences in the initial structure of the invertebrate communities is unclear.

4.1.3.4. Aquatic Plants

As discussed in Section 4.1.2.4, toxicity studies in terrestrial plants indicate that plants are insensitive to rotenone because of the presence of a mitochondrial NADH/NADPH

dehydrogenase which is not inhibited by rotenone. While this mechanism has not been demonstrated in aquatic plants and the toxicity of rotenone to aquatic plants has not been carefully studied, relative to rotenone toxicity in other aquatic organisms, there is no basis for asserting that rotenone is likely to have any direct toxic effect on aquatic plants, except at very high concentrations. At a concentration of 500 µM rotenone (about 197,000 ppb), decreased mitochondrial energy production was observed in a marine alga, *Nanochlorpsis gaditana* (Huerta et al. 2002). The EC_{50} for growth inhibition in *Tetraselmis suecica*, another marine alga, is 723,000 ppb (Gilbert et al. 1992). Sawant et al. (1995) assayed methanol extracts of *Derris scandens* for effects on bacterial and algal toxicity, and while growth inhibition was noted at high concentrations of the methanol extract (i.e., 300 µg/mL or 300,000 ppb), the extracts were not analyzed for rotenone concentrations.

As summarized in Appendix 7, most aquatic field studies generally report no direct effects on aquatic plants. Secondary effects, primarily algal blooms, are associated with adverse effects on zooplankton grazers (Anderson 1950; Sanni and Waervagen 1990). An exception to this pattern of algal blooms is the report by Shapiro and Weight (1984), which reported a decrease in phytoplankton abundance. This effect, however, appears to have been associated with a decrease in fish populations (consumers of zooplankton) rather than a direct effect of rotenone on the algae. The decrease in fish numbers led to an increase in the zooplankton population, with consequent increased grazing and a decrease in algal populations.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

The exposure assessments for the ecological risk assessment generally parallel those used for the general public in the human health risk assessment. In other words, the exposure scenarios are similar in the basic assumptions concerning the application of rotenone. Differences in the estimated doses from those in the human health risk assessment are attributable to differences in body size and consumption rates for food or water. Also, as in the human health risk assessment, the exposure scenarios for terrestrial vertebrates are a subset of those used in most Forest Service risk assessments. Some exposure scenarios, such as the consumption of terrestrial vegetation, are not relevant to aquatic applications of rotenone. Lastly, all exposure assessments are based on the application of a liquid formulation, CFT Legumine, at a target concentration of 0.2 ppm (the maximum application rate) and all exposures are based on rotenone equivalents that consider joint exposures to rotenone and other related rotenoids in CFT Legumine.

The exposure scenarios for terrestrial wildlife are summarized in Worksheet G01 of the EXCEL workbook that accompanies this risk assessment. The highest exposure scenarios involve the accidental spill of 200 gallons of a field solution into a small pond. The estimated doses for birds and mammals cover a relatively narrow range: about 1.25 to 13 mg/kg body weight. The expected non-accidental acute exposures are much lower, spanning a range from about 0.04 to 0.07 mg/kg body weight. Because rotenone will be detoxified with potassium permanganate, longer-term exposures are implausible. Nonetheless, longer-term exposures are estimated to assess the consequences of not using potassium permanganate. The range of the expected doses in the longer-term exposure scenarios for the consumption of contaminated water is very low: 0.0003 to about 0.01 mg/kg body weight/day. The longer-term consumption of contaminated fish by a fish-eating bird is much higher, ranging from 0.003 mg/kg bw/day to about 0.17 mg/kg bw/day.

Exposure of aquatic organisms to rotenone is taken as the nominal application rate or target concentration. In the EXCEL workbook that accompanies this risk assessment, the maximum application rate of 200 ppb is used. Using the toxic equivalency factor of 1.5 for CFT Legumine, maximum application rate of 200 ppb (rotenone) corresponds to 300 ppb rotenone equivalents. The consequences of using lower application rates are considered in the risk characterization.

4.2.2. Terrestrial Animals

All exposure scenarios for terrestrial animals are summarized in Worksheet G01 in the EXCEL workbook that accompanies this risk assessment (Attachment 1). As with the exposure assessments for members of the general public (Section 3.2.3), the exposure assessments for terrestrial animals are a subset of those typically included in Forest Service risk assessments. Rotenone will be applied directly to surface water; consequently exposure scenarios concerning the consumption of contaminated vegetation

or fruit, the direct spray of a small mammal, and the consumption of a sprayed small mammal by a predator are not included in the ecological risk assessment.

An important difference between the ecological and human health exposure assessments involves the plausibility of exposure. While specific measures must be taken to limit access of the general public to treated waters, it is impossible to impose such restrictions on terrestrial wildlife. Nonetheless, the use of potassium permanganate detoxification subsequent to rotenone treatment will have an impact on wildlife exposure similar to that for the general public—i.e., longer-term exposures to rotenone will not occur. The longer-term exposure scenarios developed in this section should be regarded as accidental in the sense that longer-term exposures will not occur in properly conducted rotenone applications involving prompt detoxification with potassium permanganate.

While not all standard exposure scenarios are relevant to rotenone applications, the section designations for the excluded scenarios are given below as a matter of convenience for individuals who regularly use many different Forest Service risk assessments—i.e., the section designations in all Forest Service risk assessments are consistent.

4.2.2.1. Direct Spray

This scenario is not relevant to aquatic applications.

4.2.2.2. Contact with Contaminated Vegetation

This scenario is not relevant to aquatic applications.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey

This scenario is not relevant to aquatic applications.

4.2.2.4. Ingestion of Contaminated Water

Since ingestion of contaminated water by terrestrial wildlife is likely to occur, three sets of exposure scenarios, each involving water consumption by a small mammal and a small bird, are included for an accidental spill (Worksheets F05a and F05b), the peak expected concentration in water (Worksheets F06a and F06b), and the longer-term consumption of contaminated water (Worksheets F07a and F07b). The accidental spill scenario is identical to that considered in the exposure assessment for members of the general pubic (Section 3.2.3.4). Also like the exposure assessment for members of the general public, the peak concentration in surface water is taken as the target application rate. Although longer-term exposures are unlikely, they are considered based on a 90-day average using the target application rate and the estimated field dissipation half-lives in surface water of 2 (0.5-10) days. Note that although Worksheets F07a and F07b calculate the longer-term doses based on water consumption estimates for a small mammal and a small bird, respectively, both of these worksheets use the longer-term concentrations in water calculated in Worksheet B04b.

All of these exposure scenarios are conservative—i.e., will overestimate risk—because the estimated water intake is based on metabolic water requirements, and the assumption

is made that the mammal or bird gets all of its water from the contaminated water body. In most instances, both mammals and birds may obtain a significant fraction of their metabolic water requirements from natural food sources—e.g., vegetation or prey. As discussed further in Section 4.4 (Risk Characterization), these conservative assumptions have no impact on the interpretation of risk because the resulting hazard quotients are far below the level of concern.

4.2.2.5. Oral Exposure from Contaminated Fish

The consumption of contaminated fish by a fish-eating bird is handled similarly to the corresponding exposure scenarios for human health (Section 3.2.3.5). As with the exposure scenarios in the human health risk assessment, three specific exposure scenarios are provided based on an accidental spill (Worksheet F08), expected peak concentrations (Worksheet F09a), and expected longer-term concentrations (F09b).

The only exception involves the bioconcentration factor (BCF) used for the longer-term exposure scenario. In the human health risk assessment, the longer-term BCF is taken as 10.8 based on bioconcentration in fish muscle—i.e., fish fillet—under the assumption that most members of the general public will not consume the entire fish. For wildlife, the assumption is made that the entire fish is consumed. Thus, a higher BCF of 27.6 is used based on bioconcentration factors in whole fish (Gilderhus et al. 1988).

4.2.3. Terrestrial Plants

Exposure scenarios for terrestrial plants are not relevant to aquatic applications.

4.2.4. Soil Organisms

Exposure scenarios for soil organisms are not relevant to aquatic applications. Exposures to benthic aquatic species are considered in the assessment for aquatic species (Section 4.2.5).

4.2.5. Aquatic Organisms

For the direct application of rotenone to water, expected peak exposures to aquatic organisms are based on the target concentration; the water concentrations for accidental spills and longer-term concentrations of rotenone in water are based on the same values used in the exposure assessment for mammals (Section 4.2.2.4). As in the human health risk assessment, the EXCEL workbook that accompanies this risk assessment is based on the highest allowable application rate, 200 ppb. Using the toxic equivalency factor of 1.5 for CFT Legumine, maximum application rate of 200 ppb (rotenone) corresponds to 300 ppb rotenone equivalents. The consequences of using lower application rates are discussed in the risk characterization (Section 4.4).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The specific toxicity values used in this risk assessment are summarized in Table 12, and the derivation of each of these values is discussed in the various subsections of the dose-response assessment. The available toxicity data as well as the plausible exposure scenarios support separate dose-response assessments in five groups of organisms: terrestrial mammals, birds, fish, amphibians, and aquatic invertebrates. Different units of exposure are used for different groups of organisms, depending on how exposures are likely to occur and how the available toxicity data are expressed. Unlike the human health risk assessment, the toxicity values used in the ecological risk assessment involve different endpoints for different groups of organisms and different durations of exposure. These differences are necessitated by the nature of the available data on the different groups of organisms.

For terrestrial mammals, the toxicity endpoints correspond to the NOAEL values used in the human health risk assessment for the derivation of the acute and chronic RfDs—i.e., an acute NOAEL of 15 mg/kg body weight and a chronic NOAEL of 0.375 mg/kg body weight/day. Data on birds are highly variable, and a clear acute NOAEL cannot be defined. Consequently, a conservative but plausible LD_{50} of 113 mg/kg body weight is used to characterize acute risks in birds. Since chronic studies in birds are not available, the acute NOAEL in mammals is used to characterize chronic risks to birds.

The toxicity values used for aquatic species reflect the range of species sensitivity distributions detailed in the hazard identification for aquatic species. For fish as well as other aquatic organisms, the acute endpoints used for the dose-response assessment for aquatic organisms all involve LC_{50} values. While this approach is not preferred in most Forest Service risk assessments, it is used for rotenone because lethality best reflects the likely outcome of rotenone applications and because most of the available acute toxicity data on rotenone involve LC_{50} determinations. Risks associated with longer-term exposures are based on NOEC values for sensitive species, however, relative potency methods based on acute toxicity are used to estimate longer-term NOEC values for tolerant species.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals

Most Forest Service risk assessments use the same toxicity values for mammals that are used in the human health risk assessment. In other words, the NOAEL values that are derived for the acute and chronic RfDs are used to characterize risks to mammalian wildlife. This approach is typically more conservative than the approach taken by the U.S. EPA, which generally uses acute LD_{50} values to characterize acute risks to mammals and reproductive NOAEL values to characterize chronic risks to mammals. For rotenone, the standard Forest Service approach is taken. Acute risks are based on the NOAEL of 15 mg/kg/day from the developmental toxicity study in mice (Section 3.3.3), and chronic

risks are based on the NOAEL of 0.375 mg/kg body weight/day from a lifetime feeding study in rats (Section 3.3.2).

Exposures to rotenone will occur only over a very short period of time—i.e., a matter of a few hours—because of the use of potassium permanganate to detoxify rotenone as well as dilution and degradation. Thus, a case can be made that the standard Forest Service approach is grossly conservative. The acute RfD is based on a study involving multiple exposures during the gestation period, and the chronic RfD is based on a lifetime feeding study. While this argument has merit, the conservative values used in this Forest Service risk assessment do not impact the risk characterization. As noted in Section 4.4, risks to mammals are far below the level of concern even at the highest application rate of 200 ppb.

4.3.2.2. Birds

As discussed in Section 4.1.2.2, U.S. EPA/OPP (2006c) classifies rotenone as *slightly toxic* to birds, based on an oral LD_{50} of 1680 mg/kg body weight and a dietary LC_{50} of 1608 ppm in pheasants. A somewhat more conservative approach is taken in the current Forest Service risk assessment. Based on the study by Cutkomp (1943), the current risk assessment uses the LD_{50} of 113 mg/kg body weight for the Eastern chipping sparrow. While the studies by Cutkomp (1943) are not as fully detailed as the more standardized and better-documented studies used by EPA, Cutkomp (1943) tested a large number of relevant species of avian wildlife.

The decision to take this somewhat more conservative approach is based on the commonalities noted in the toxicity of rotenone to a wide range of species. As discussed in Section 4.1.2.1, intravenous toxicity data in mammals and fish suggest virtually identical susceptibilities to rotenone. While some species of birds, such as pheasants, do appear to be more tolerant to rotenone than mammals, most of the toxicity data reported by Cutkomp (1943) are presented in sufficient detail to be credible and suggest that some species of birds may be as sensitive as some mammalian species to rotenone.

The approach taken in selecting the oral LD_{50} of 113 mg/kg body weight is not the most conservative approach that could be taken. As also noted in 4.1.2.2, Cutkomp (1943) briefly summarizes a study in robins in which rotenone was administered in prey items, and reports that the lethal oral doses to robins was about 0.1875 mg/kg body weight. This dose is much lower than any reported lethal doses by oral exposure in mammals or other species of birds.

No data are available on the chronic toxicity of rotenone in birds. This lack of information has only a minor impact on the current risk assessment owing to the implausibility of longer-term exposures. As a protective approximation, the chronic NOAEL of 0.375 mg/kg body weight/day for mammals (Section 4.3.2.1) is used to characterize longer-term risks for birds. As discussed in Section 4.4, this highly protective approach has no impact on the risk characterization because the resulting hazard quotients are far below the level of concern.

4.3.2.3. Terrestrial Invertebrates

No dose-response assessment is developed for terrestrial invertebrates because rotenone will be applied only to surface water. While incidental exposures are possible, substantial impacts on terrestrial invertebrates are not likely.

4.3.2.4. Terrestrial Plants (Macrophytes)

As with terrestrial invertebrates, no dose-response assessment is made for terrestrial vegetation because the likelihood of exposures to rotenone during aquatic applications is remote. In addition, the hazard identification for terrestrial plants is essentially negative—i.e., there is no basis for asserting that rotenone will adversely affect terrestrial plants.

4.3.3. Aquatic Organisms

4.3.3.1. Fish

Forest Service risk assessments generally prefer to base dose-response assessments for fish as well as other aquatic organisms on NOAEL values rather than LC_{50} values. This approach is not taken for acute exposures to rotenone for two reasons. First, the focus of the toxicity studies in fish (Appendix 4) is on acute lethal potency. This focus is sensible in terms of assessing both the efficacy of rotenone as well as the selectivity of rotenone. Second, there is little point in focusing on relatively subtle endpoints for deriving a NOAEL because these endpoints are not relevant to the use of rotenone—i.e., to kill fish.

As discussed in Section 4.1.3.1.2 and summarized in Table 9, the range of species sensitivity to rotenone in fish is well defined. The acute LC_{50} of 1.94 µg/L in rainbow trout is used to assess effects in sensitive species of fish. This is the same toxicity value used in U.S. EPA/OPP (2006c, MRID 439751-02). For tolerant species of the fish, the acute LC_{50} of 40 µg/L in goldfish from the study by Gersdorff and Smith (1940) is used to characterize risks. This is not the highest reported LC_{50}. As indicated in Table 9, the U.S. EPA reports an LC_{50} of 80 µg/L in fish identified only as *Mozambique* (U.S. EPA/OPP 2003c, Figure 4.1). The EPA, however, does not reference the source of this LC_{50} value and the species of fish referenced is unclear. In addition, the toxicity value of 40 µg/L is more representative of tolerant species of fish, such as mosquito fish, carp, and the pond loach for which well-documented toxicity values are available.

For longer-term exposures, the trout NOEC of 0.00101 mg/L is used. This value is identical to the value used in U.S. EPA/OPP (2006c) and is based on the early life-stage study in trout submitted to the EPA in support of the reregistration of rotenone. This toxicity value is taken as the NOEC for sensitive species of fish. No longer-term toxicity studies are available on presumably tolerant species. As noted in the discussion of the species sensitivity distribution for fish, the range of sensitivities spans a factor of about 40 (Section 4.1.3.1.2, Table 9). Based on this relative potency, the longer-term NOEC of 0.00101 mg/L is adjusted upward by a factor of 40 to 0.04 mg/L, and this toxicity value is used as a surrogate for tolerant species of fish.

4.3.3.2. Amphibians

As discussed in Section 4.1.3.2, there are relatively few studies on the toxicity of rotenone to amphibians in the rotenone literature. Furthermore, many of these studies are not reported in detail, and the data are subject to different interpretations: some interpretations suggesting that amphibians may be relatively insensitive to rotenone and other interpretations suggesting that amphibians may be as sensitive as some species of fish to rotenone toxicity.

In the ecological risk assessment conducted by the U.S. EPA (U.S. EPA/OPP 2006c, p. 109), the Agency notes a lack of data on amphibians and elects to use data on sensitive species of fish as a surrogate for aquatic phase amphibians. While the data discussed in Section 4.1.3.2 are not considered in U.S. EPA/OPP (2006c), using fish as surrogates for amphibians is not unreasonable given the uncertainties in the available amphibian data.

Given concern for the impact of pesticides on amphibians, Forest Service risk assessments generally attempt to characterize risks to amphibians whenever possible. While the data on amphibians are relatively sparse, relative to data on fish and invertebrates, separate dose-response assessments for amphibians are proposed for acute exposures. The most sensitive amphibian endpoint reported is the 24-hour LC50 of 5 ppb (0.005 mg/L) in salamanders (Hamilton 1941), and this value is used to characterize risks in potentially sensitive species of amphibians. The highest approximate lethal dose is 2000 ppb (2 mg/L) reported by Haag (1931).

No data are available for characterizing the risks to amphibians of longer-term exposures to rotenone.

4.3.3.3. Aquatic Invertebrates

The variability in the sensitivity of aquatic invertebrates to rotenone is much more substantial than that seen in fish. As illustrated in Figure 7, separate dose-response assessments could be made for very sensitive small zooplankton, larger crustaceans, and snails. Additionally, semi-quantitative or qualitative assessments could be made for other groups of invertebrates (4.1.3.3.2). As noted in Section 4.1.3.3.3 (Field Studies), field observations may be more useful for presenting a realistic assessment of risks to aquatic invertebrates because the available field studies incorporate considerations of habitat (planktonic vs benthic organisms) as well as recovery.

Thus, hazard quotients are presented only for tolerant and sensitive species, and the risk characterization is elaborated with the consideration of field studies in Section 4.4.3.3. As illustrated in Figure 7 and detailed in Table 11, the most sensitive species of aquatic invertebrates is *Daphnia magna*, and the lowest reported LC_{50} of 3.7 ppb (Rach et al. 1988) is used to characterize acute risks to sensitive species of aquatic invertebrates.

Snails are the most tolerant group of invertebrates based on the available data. The highest LC_{50} for this group is 40 mg/L—i.e., *Aplexa hypnorum* from the study by Holcombe et al. (1987). For the dose-response assessment, however, the LC_{50} of 6.8 mg/L in *Physa acuta* (Nishiuchi and Yoshida 1972) is used to characterize risk. This

approach is taken to be consistent with the conservative methods used in all Forest Service risk assessments—i.e., the approach recognizes the relative insensitivity of snails but uses the most sensitive species in this tolerant subgroup for characterizing risk.

The only chronic toxicity data available on aquatic invertebrates is the NOEC of 0.00123 mg/L (1.23 ppb) in *Daphnia magna*. It should be noted that this chronic NOEC is very close to the acute LC_{50} of 3.7 ppb in *Daphnia magna*. This proximity is consistent with the relatively steep dose-severity relationship in mammals (Section 3.3.4) as well as the apparently steep dose-response relationship in fish (Section 4.1.3.1.2).

Data are not available on chronic effects in tolerant species of aquatic invertebrates exposed to rotenone. A surrogate chronic NOEC of 2000 ppb is based on the ratio of acute toxicity values for aquatic invertebrates [1.23 ppb x 6800 ppb / 3.7 ppb = 2261 ppb] rounded to one significant place.

4.3.3.4. Aquatic Plants

No dose-response relationship is proposed for aquatic plants. As discussed in Section 4.1.3.4, there is no basis for asserting that aquatic plants are sensitive to rotenone; furthermore, the field studies provide sufficient evidence that effects on aquatic plants are not plausible.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

Rotenone is an effective piscicide that is likely to kill fish when applied to surface waters at labeled application rates. There are differences in sensitivity among fish species, and these differences span a factor of about 40. Treatments with any formulations at the upper bound of the application rates for rotenone—i.e., 200 ppb—are likely to kill all but the most tolerant species of fish. Rotenone formulations containing piperonyl butoxide are likely to kill all species of fish, even the most tolerant. Rotenone can be viewed as a selective piscicide rather than a general aquatic biocide in that fish are more sensitive to rotenone than are most other aquatic organisms, with the exception of some species of zooplankton and small insects. Thus, while rotenone applications to surface water are expected to kill some invertebrates, extensive mortality due to the toxicity of rotenone among species of larger invertebrates is not expected. Despite the observation of secondary effects on aquatic plants, rotenone applications are not likely to directly affect aquatic plants. Depending on how secondary effects are measured, changes in the community structure of surface waters may persist for a prolonged period of time.

There is no basis for asserting that rotenone is likely to have a direct toxic effect on terrestrial organisms. Secondary effects are likely to occur in animals that consume fish as a substantial proportion of their diet. These changes, however, are likely to be transient.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals

The risk characterization for mammals is simple and unambiguous: there is no basis for asserting that adverse effects are plausible in large or small mammals when rotenone is applied at the highest application rate considered in this risk assessment, 200 ppb.

For acute exposure scenarios, the hazard quotients for mammals range from 0.002 (the acute consumption of contaminated water at the expected peak concentration) to 0.5 (the upper bound of the hazard quotient associated with the consumption of contaminated water after an accidental spill of rotenone into a small pond). This range is below the level of concern (1.0) by factors of 2 to 500.

As discussed in the exposure assessments for both the human health risk assessment as well as the ecological risk assessment, longer-term exposures to rotenone are implausible because treated waters will be detoxified with potassium permanganate within hours after rotenone is applied. Thus, the chronic hazard quotients for mammals as well as other groups considered in this ecological risk assessment would be associated with a misapplication of rotenone.

For chronic exposures, the only exposure assessment considered for mammals is the consumption of contaminated water. These hazard quotients range from 0.0008 to 0.02

with a central estimate of 0.003. These hazard quotients are below the level of concern by factors ranging from 100 to 1250.

This risk characterization for mammals is consistent with the risk characterization presented in U.S. EPA/OPP (2007a), which found no basis for asserting that adverse effects in mammals are plausible. The exposure assessments used by U.S. EPA, however, differ somewhat from those used in the current Forest Service risk assessment. The U.S. EPA does not provide a drinking water scenario. Instead, the EPA provides a risk characterization based on the consumption of fish by a piscivorous mammal. For this exposure scenario (U.S. EPA/OPP 2007a, p. 24), the Agency uses an estimated dose of 37 µg/kg body weight and an LD_{50} of 30.4 mg/kg body weight to characterize risk, which corresponds to a hazard quotient of 0.0012 [0.037 mg/kg body weight / 30.4 mg/kg body weight], somewhat below the range of acute hazard quotients derived in the current Forest Service risk assessment—i.e., 0.002 to 0.5. Adjusting the toxicity value from the LD_{50} to the acute NOEC of 15 mg/kg body weight, the resulting risk quotient would be 0.002 [0.037 mg/kg body weight / 15 mg/kg body weight], identical to the lower range of the risk quotients derived in this risk assessment.

The application of any effective piscicide, including rotenone, is likely to decrease prey availability for mammals that consume fish as a substantial part of their diet. This alteration is likely to lead to either shifts in the populations of some mammals and/or changes in feeding behavior. The impact and significance of these changes are likely to vary over time and vary among different species of piscivorous mammals.

4.4.2.2. Birds

The risk characterization for birds is similar to that of mammals in that no hazard quotients exceed unity. The interpretation of the acute hazard quotients for birds, however, differs from that in mammals in that the hazard quotients are calculated using an estimated LC_{50} for sensitive species of birds—i.e., 113 mg/kg body weight as summarized in Table 12—rather than an NOEC. This consideration, however, has very little impact on the qualitative risk characterization for two reasons. First, as detailed below, all of the risk quotients are very low. Second, as noted in the dose-response assessment for mammals, fish, and invertebrates (which is based on more extensive data than are available on birds), rotenone appears to have very steep dose-response and dose-severity relationships. Taking mammals as an example, the NOAEL in mammals (15 mg/kg body weight) is only a factor of about 2 below the LD_{50} in mammals (30.4 mg/kg body weight) used for risk characterization by the U.S. EPA.

The acute hazard quotients for birds range from 0.0006 (the consumption of contaminated water after the application of rotenone at the target application rate of 200 ppb) to 0.1 (the upper bound associated with the consumption of contaminated water after an accidental spill). These acute hazard quotients are below the level of concern by factors ranging from about 10 to about 1667. Because these hazard quotients are based on the highest application rate considered in this risk assessment—i.e., 200 ppb—the use of lower application rates would lead to lower hazard quotients; consequently, the use of lower application rates is not considered further in the risk characterization for birds.

The hazard quotients associated with longer-term exposures are also very low, ranging from 0.001 (the lower bound for the consumption of water by a small bird) to 0.4 (the consumption of contaminated fish by a predatory bird). These hazard quotients are below the level of concern by factors of about 2.5 to 1000.

This risk characterization for birds is consistent with the risk characterization presented in the EPA RED (U.S. EPA/OPP 2007a) as well as the more detailed ecological risk assessment prepared by EPA OPP (U.S. EPA/OPP 2006c).

4.4.2.3. Terrestrial Invertebrates

As detailed in the exposure assessment and dose-response assessment, significant exposures to terrestrial invertebrates during aquatic applications of rotenone are not plausible. Consequently, no quantitative risk characterization for terrestrial insects is made. Nonetheless, there is no basis for asserting that substantial or significant effects on terrestrial invertebrates are likely. This rationale also applies to terrestrial plants and soil microorganisms.

4.4.2.4. Terrestrial Plants

See Section 4.4.2.3.

4.4.2.5. Soil Microorganisms

See Section 4.4.2.3.

4.4.3. Aquatic Organisms

4.4.3.1. Fish

As with terrestrial species, the quantitative risk characterization for fish and other aquatic organisms is expressed as the hazard quotient, and the hazard quotients for aquatic organisms are given in Worksheet G03 of the EXCEL workbook that accompanies this risk assessment (Attachment 1). As with other risk characterization worksheets, Worksheet G03 is based on the maximum application rate considered in this risk assessment, 200 ppb (rotenone) or 250 ppb as rotenone equivalents for CTF Legumine (i.e., TEF = 1.25).

While extensive and very detailed information is available on the toxicity of rotenone to fish, and some of the analyses of these data are modestly complex (Section 4.1.3.1), the risk characterization for fish is extraordinarily simple. If rotenone is applied at effective application rates, fish will die. As noted in Worksheet G03, the hazard quotient for sensitive species of fish for treatments of rotenone is about 130. Given the apparently steep concentration-response relationships for rotenone (Section 4.1.3.1.2) as well as the very high hazard quotient for sensitive species of fish, it is likely that mortality will be 100% for all sensitive fish in waters treated at the target application rate of 200 ppb rotenone. Generally, this is the intended result of rotenone applications.

For tolerant species of the fish, however, the hazard quotient associated with an application rate of 200 ppb rotenone is only 0.6. In a risk assessment for a non-piscicide, low HQ values for fish would be regarded as desirable. For a piscicide, however, HQ values of less than 1 might suggest limited efficacy for some species of tolerant fish. While efficacy is a somewhat peripheral consideration to this risk assessment, potential differences in the efficacy of different formulations for rotenone may be important for some applications. As summarized in Table 2 and detailed in Section 3.1.17, formulations of rotenone that contain piperonyl butoxide (TEF values of 2.25 to 2.5) are likely to be about twice as potent as formulations that do not contain piperonyl butoxide (TEF values of 1.25 to 1.5). As discussed in Section 2.4 and summarized in Table 4, all formulations of rotenone have the same labeled application rates, and the upper bound rate of 200 ppb rotenone set by U.S. EPA/OPP (2007a) applies to all rotenone formulations. Thus, an application rate of 200 ppb rotenone for a formulation containing piperonyl butoxide could be equivalent in efficacy to using an application rate of about 400 ppb for a formulation that does not contain piperonyl butoxide. The hazard quotients presented in this risk assessment are all based on applications of CTF Legumine, a formulation that does not contain piperonyl butoxide (Table 2). Thus, if rotenone is to be applied for the eradication of fish that may be at the upper bound of the species sensitivity distribution for rotenone (Figure 3), consideration could be given to using formulations of rotenone that contain piperonyl butoxide.

For the accidental spill of rotenone into a small pond, a standard accidental scenario used in all Forest Service risk assessments, the hazard quotients range from 12 to about 120 for tolerant species of fish and about 2500 to 25,000 for sensitive species of fish. Since these hazard quotients are based on LC_{50} values, considerations of the different formulations are of little consequence.

Based on the hazard quotients for longer-term exposures, tolerant species would not likely be at risk, with HQ values ranging from 0.005 to 0.1, but sensitive species would be at risk, with HQ values ranging from 1 to 21. Chronic exposures to rotenone, however, should not be relevant for two reasons: first, potassium permanganate detoxification will prevent longer-term exposures, and, second, most fish would not survive acute exposures. Accordingly, the quantitative risk characterization for longer-term exposures has little relevance.

Because rotenone will not remain in the treated water for a prolonged period of time, natural recovery of fish populations is plausible. Fish recovery is noted in some field studies, however, as discussed further in Section 4.4.3.3, most recovery studies focus on invertebrate populations. The likely reason for this focus is that recovery of most fish populations will occur by planned restocking of fish as part of the rotenone treatment program. The effective recovery of insectivorous fish populations will probably be limited not by residual rotenone but the recovery period needed for invertebrate populations.

4.4.3.2. Amphibians

As discussed in Section 4.3.3.2, the available toxicity data on amphibians are much less complete and more difficult to interpret than the toxicity data on fish. The U.S. EPA/OPP (2005c, 2007a) suggests that risks to aquatic phase amphibians should be assessed based on the risk characterization for fish, which is a reasonable approach.

As summarized in Worksheet G03, the HQ values for amphibians are virtually identical to those for fish. Because the toxicity values used for amphibians are only slightly higher than those used for fish, the hazard quotients are quite similar across the range of considered exposure scenarios. If rotenone is applied at concentrations that will kill fish, amphibians are likely to die as well.

Unlike fish, attempts to restock amphibian populations are not likely to be made routinely, if at all. While natural recovery of amphibian populations after rotenone treatment will probably occur, the rates of recovery in amphibian populations cannot be quantified.

4.4.3.3. Aquatic Invertebrates

While the risk characterizations for fish and amphibians are virtually identical, the risk characterization for aquatic invertebrates is substantially different. The toxicity of rotenone to a relatively wide variety of aquatic invertebrates has been determined, and the sensitivity of aquatic invertebrates to rotenone varies to a much greater extent than the variability in fish. The most sensitive groups of aquatic invertebrates are small zooplankton, such as the cladocerans and perhaps other small arthropods.

For sensitive species of aquatic invertebrates, the quantitative characterization of risk is very similar to that of sensitive species of fish. At the application rate of 200 ppb, the hazard quotient for sensitive invertebrates is 68, about half of the corresponding HQ for fish (129). Thus, when rotenone is applied at effective concentrations, it is virtually certain that substantial mortality will occur in small zooplankton. Based on field studies, particularly those in streams, it is also likely that substantial mortality/drift will occur in several groups of small aquatic insects.

Populations of tolerant species of invertebrates are not likely to be adversely affected by rotenone. The risk quotients for tolerant species of invertebrates are based on snails because this is the group on which the best toxicity data are available. Based on early and much less well-reported studies, it is likely that other groups of invertebrates that would not be substantially affected by rotenone include flatworms, leaches, and some larger species of arthropods, including aquatic beetles.

A reduction in the population of small zooplankton may lead to a transient increase in algae due to decreased grazing pressure. Field studies indicate that the duration of the impact of decreased grazing—i.e., the recovery period for small zooplankton—is highly variable. Some field studies suggest that small zooplankton populations can recover quickly. Small zooplanktons have very short life spans and correspondingly short reproductive cycles. In addition, small zooplankton will often evidence a sharp rise in

reproductive rates following a period of stress. Furthermore, the removal of fish, a major predator group of zooplankton, may facilitate the rebound of zooplankton populations.

Other field studies, however, indicate that *full recovery* may not be observed over a period of several years (Appendix 7). The practical significance of these reports is not simple to assess. Changes can occur over a period of several years in any ecosystem, and it is difficult to demonstrate that an apparent failure to recover after a stress event, such as rotenone application, is associated only with the stress event as opposed to other changes in the environment. In addition, rotenone treatment has been noted to cause shifts in species composition within various groups of aquatic invertebrates.

While changes in species composition in a pond or stream may be attributable to rotenone treatment, shifts in species composition may not necessarily lead to gross changes in the community structure that would be considered adverse. In other words, the purpose of rotenone applications is to cause changes in the fish community, replacing less desirable fish (e.g., invasive species) with more desirable fish. Changes in fish populations are likely to lead to changes in invertebrate species composition as well as changes in other groups within the aquatic community. Whether or not these changes are *acceptable* or *desirable* is an issue that must be addressed in formulating wildlife management programs.

4.4.3.4. Aquatic Plants

While exposures of aquatic plants to rotenone will occur, the hazard identification for aquatic plants indicates that rotenone will not have any direct adverse effect on plant species. Thus, no quantitative risk characterization is developed for this group of organisms. As noted above, effects on fish or zooplankton may lead to increases in aquatic vegetation, but these changes are likely to be transient.

5. REFERENCES

NOTE: The initial entry for each reference in braces {} simply specifies how the reference is cited in the text. The final entry for each reference in brackets [] indicates the internal tracking category identifying the source of the reference. These categories are listed below.

SET00	**Preliminary publications identified prior to formal literature search. [n=9]**
SET01	**Initial TOXLINE Screen – general and recent neurotoxicity/Parkinson's Disease [n=45]**
SET02	**Initial TOXLINE Screen – fish [n=20]**
SET03	**Initial TOXLINE Screen – invertebrates and algae [n=17]**
SET04	**Studies on rotenone listed in the U.S. EPA ECOTOX database available at: http://cfpub.epa.gov/ecotox. [n=74]**
SET05	**Supplemental studies based on a screen of bibliographies in Sets 1 to 4 [n=17].**
SET06	**Pre-peer review update literature search [n=8].**
SET07	**Additional tree-searching [n=9].**
SET08	**Additional tree-searching [n=9].**
SET09	**Papers added in post-peer review revisions.**
EPA	**Copies of EPA memoranda prepared after the RED courtesy of Dirk Helder, U.S. EPA/OPP, Special Review and Reregistration Division. Received July 22, 2008.**
FOIA01	**FOIA to the U.S. EPA for two Cleared Reviews on Rotenone, June 19, 2008.**
Internet	**Various reports on rotenone from specified web sites.**
Rev	**Review comments on previous versions of this risk assessment.**
Sec	**Summaries from secondary source.**
Std	**Standard references used in most Forest Service risk assessments.**
E-Docket	**These are from the following E-Dockets developed by U.S. EPA. To get the complete listing of items available, go to http://www.regulations.gov/search/ index.jsp and enter the docket number in the Search box. Docket Number for Rotenone is: EPA-HQ-OPP-2005-0494. At total of 85 documents are available. Documents currently under review at listed below.**

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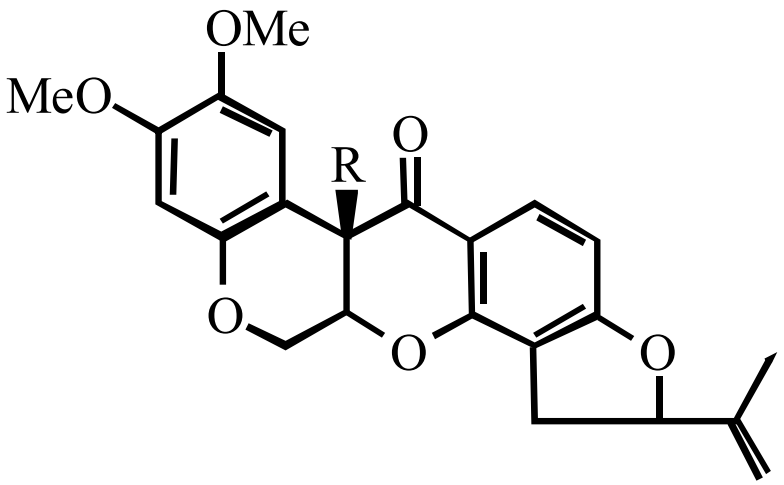
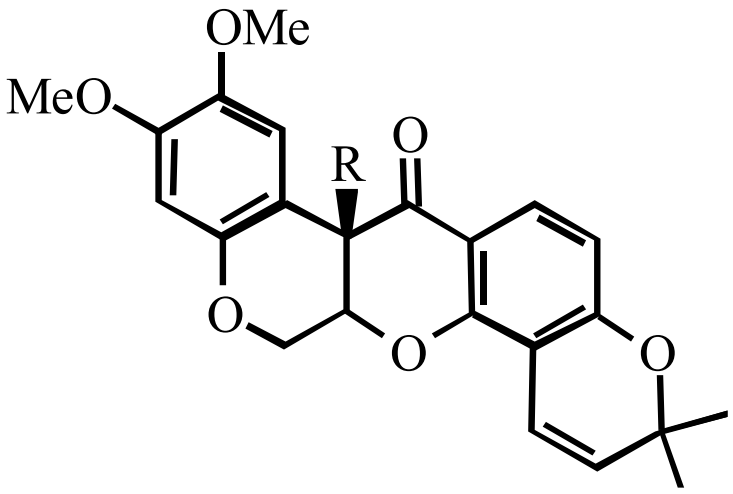


Rotenone: R = H
Rotenolone: R = OH



Deguelin: R = H
Tephrosin: R = OH

Figure 1: Chemical Structure of Rotenone and Related Plant Extracts
Modified from Figure 1 in Fang and Casida 1999b

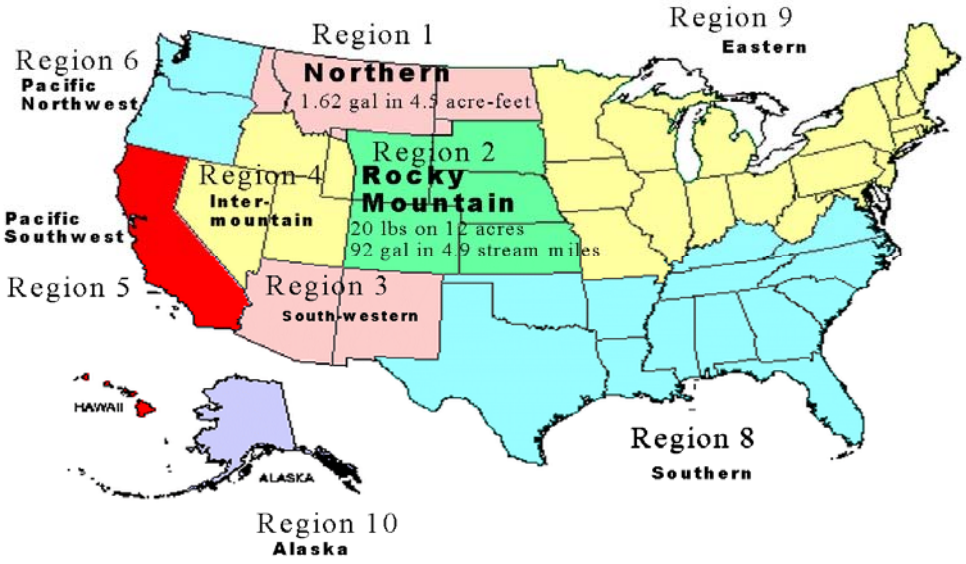


Figure 2: Use of Rotenone in Forest Service Programs in 2004

Source: http://www.fs.fed.us/ foresthealth/pesticide/reports.shtml

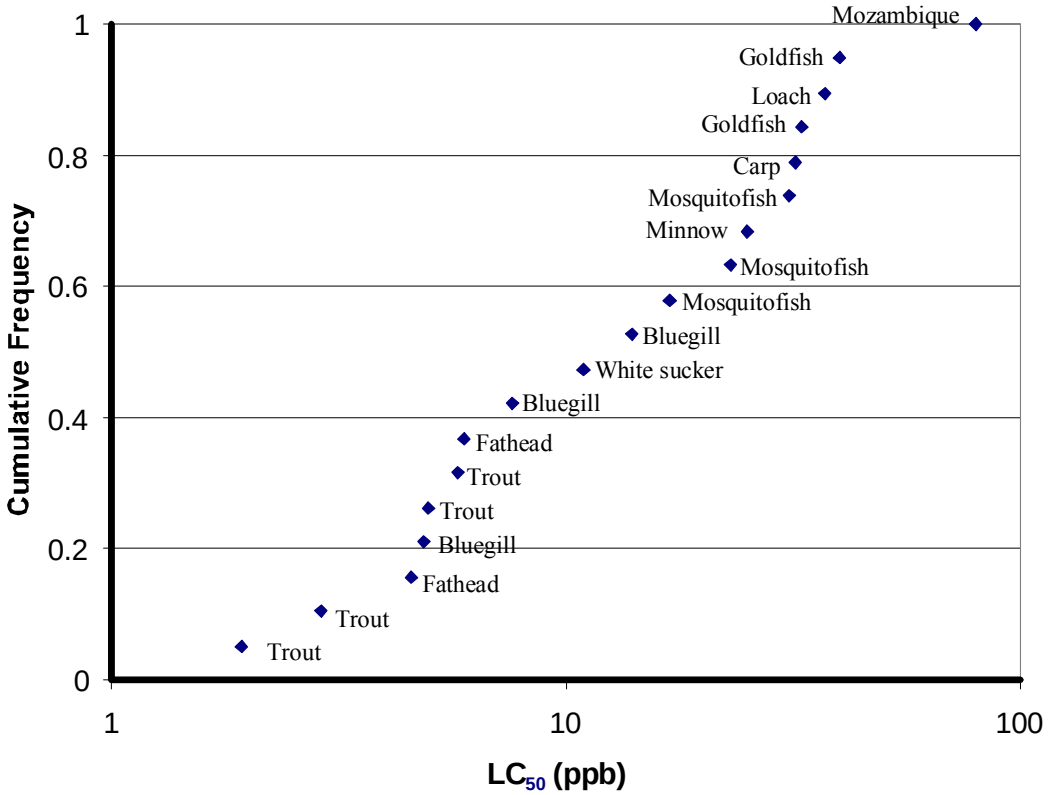


Figure 3: Species Sensitivity Distribution of Rotenone (TGAI) in Fish
See Table 9 for data and Section 4.1.3.1 for discussion.

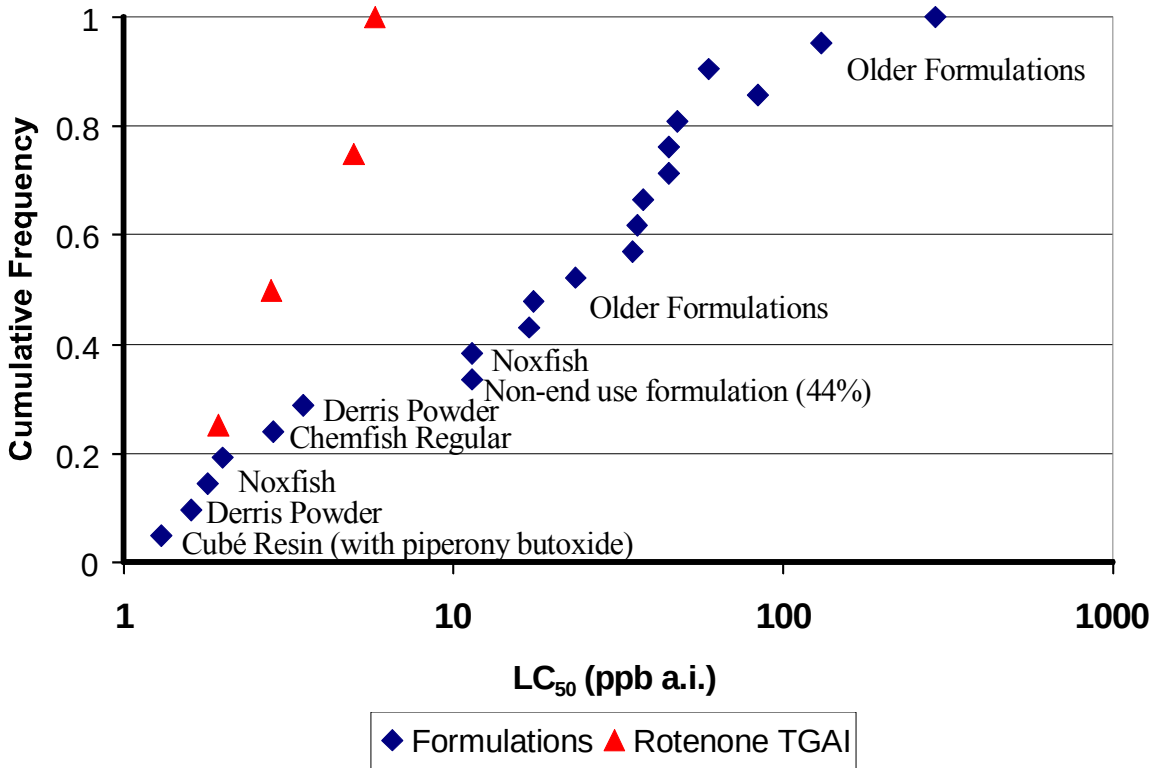


Figure 4: Comparative Toxicity of Rotenone TGAI and Formulations to Rainbow Trout
See Table 9 for TGAI data, Table 10 for formulation data, and Section 4.1.3.1 for discussion.

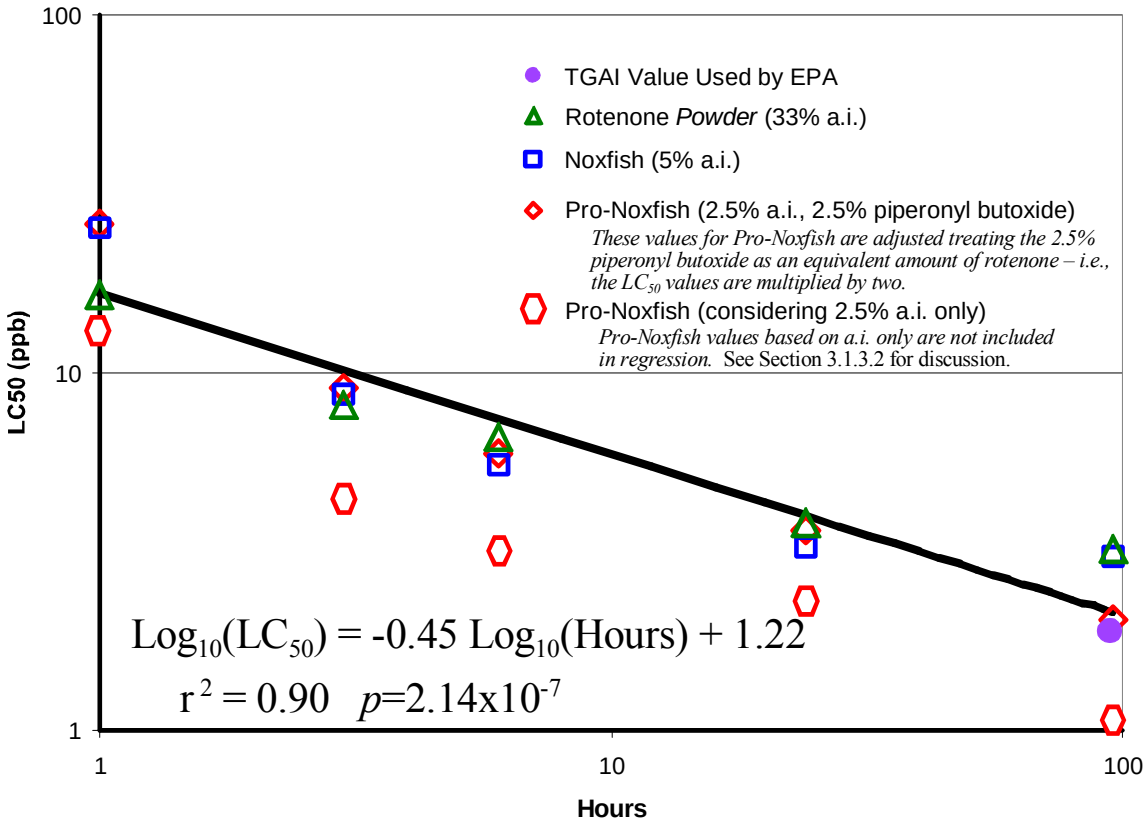


Figure 5: Comparative toxicity of rotenone TGAI to three rotenone formulations

The 1, 3, 6, 24, and 96-hour LC_{50} values for the formulations are taken from Marking and Bills (1976, Table 9). The 96-hour LC_{50} value for rotenone a.i. is taken from U.S. EPA/OPP 2006c, MRID 439751-02. See Section 3.1.3.2 for discussion.

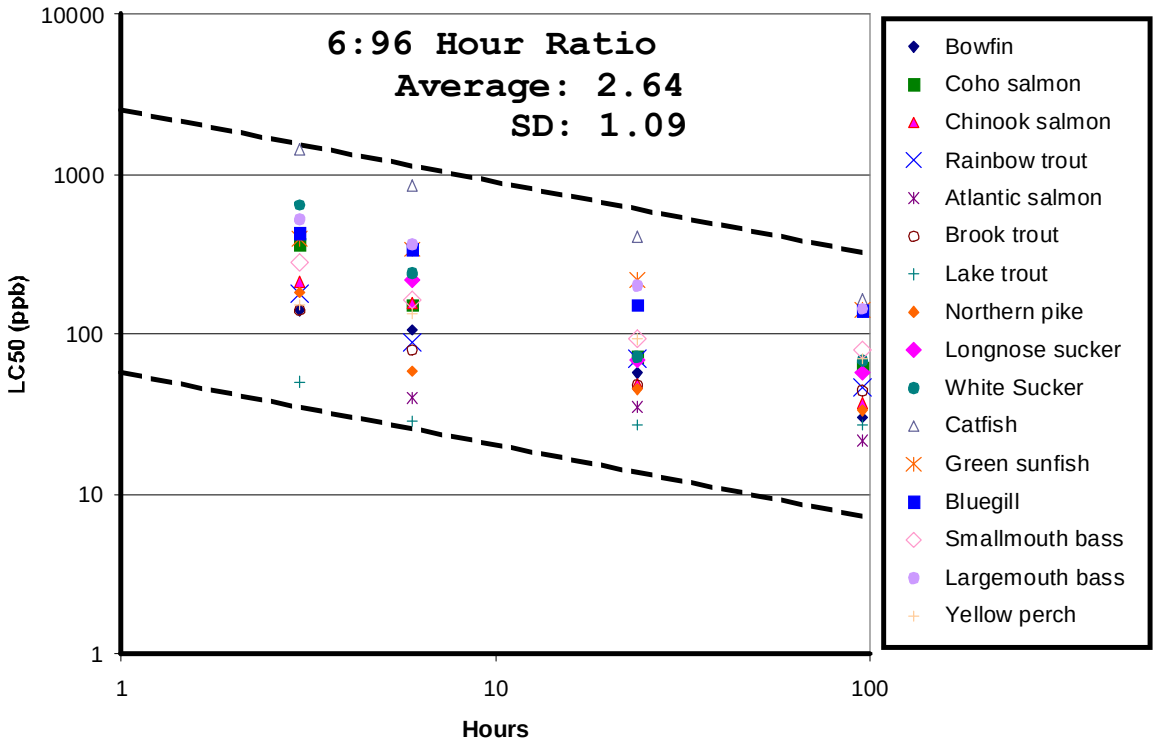


Figure 6: Concentration-Duration Relationships in Fish

The 3, 6, 24, and 96-hour LC_{50} values for the formulations are taken from Marking and Bills (1976, Table 1). This table is presented in Supplemental Table 1 of Appendix 4. See Section 3.1.3.2 for discussion.

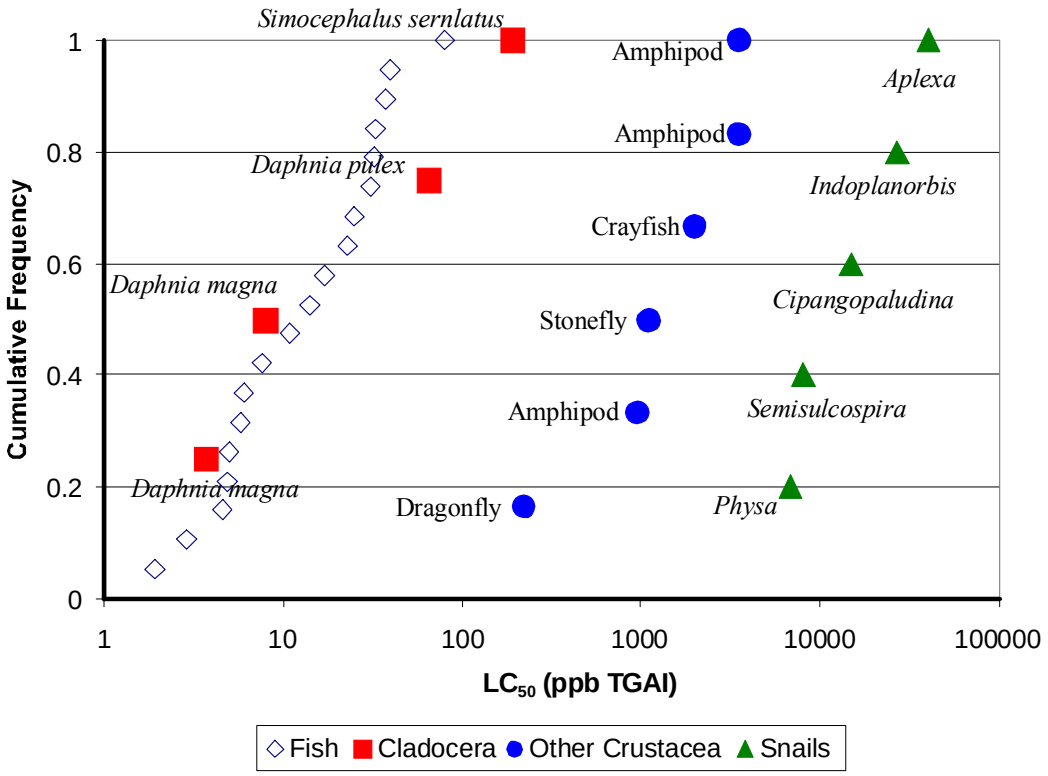


Figure 7: Species Sensitivity Distributions for Rotenone (TGAI) in Aquatic Invertebrates and Fish

Note that labels for fish are omitted. Data for fish are given in Table 9 and illustrated in detail in Figure 3 with labels for the different types of fish. Data for invertebrates are given in Table 11.

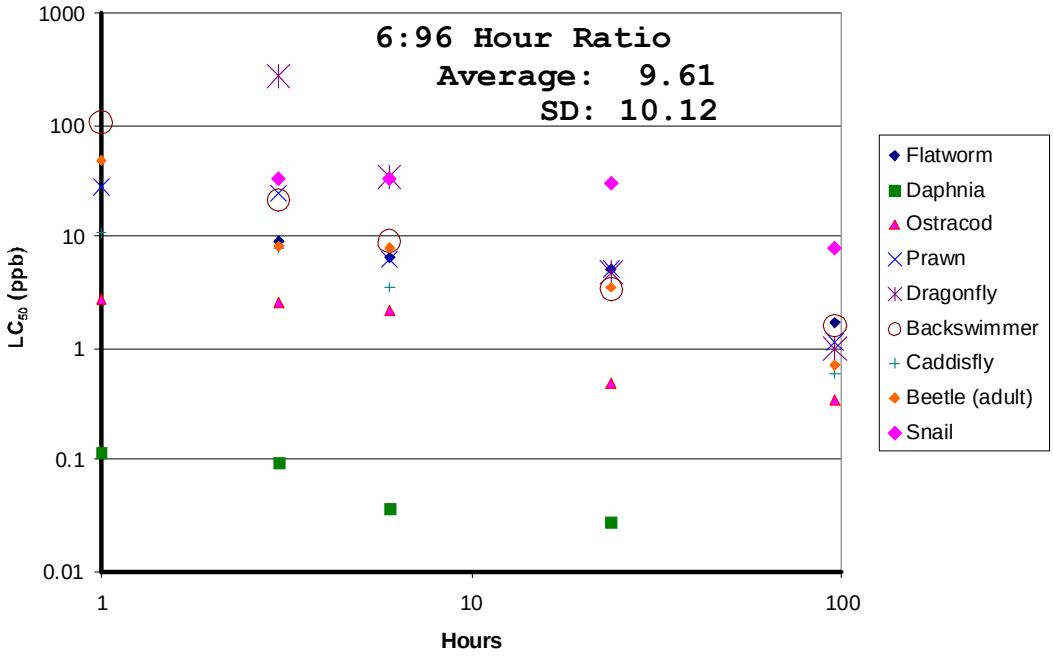


Figure 8: Concentration-Duration Relationships in Aquatic Invertebrates

The 1, 3, 6, 24, and 96-hour LC_{50} values are taken from Chandler and Marking (1982, Table 1). This table is presented as Supplemental Table 1 of Appendix 6. See Section 4.1.3.3 for discussion.

Table 1: Physical and chemical properties of Rotenone

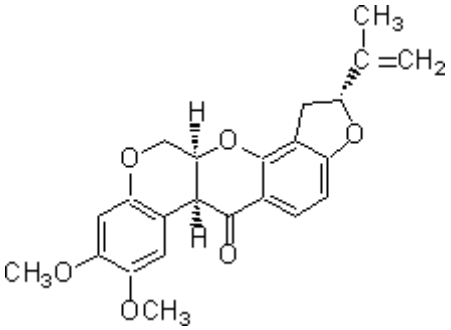
Property	Value [1]	Reference
Nomenclature		
Common Name	Rotenone	Tomlin 2004
IUPAC Name	(2R,6aS,12aS)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromeno[3,4-b]furo [2,3-h]chromen-6-one	Tomlin 2004
CAS Name	[2R-(2α,6aα,12aα)]-1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl) [1]benzopyrano[3,4-b]furo[2,3-h][1]benzopyran-6(6aH)-one	Tomlin 2004
Synonyms	Plant extract: derris root; tuba-root; aker-tuba Plant: barbasco; cubé; haiari; nekoe; timbo	
CAS number	83-79-4	Tomlin 2004
Structure		Tomlin 2004
Bioconcentration	Bluegills: 25.4 (head); 11 (viscera); 26 (carcass).	Gingerich and Rach 1985
	Bluegills: 27.9 (head); 10.8 (viscera); 27.6 (carcass).	U.S. EPA/OPP 2006c, MRID 455801073 [pre-publication of Gingerich and Rach 1985]
	Fish: 0.68 to 1.32 (cold water), 0.8 (warm water) Crayfish: 1.58 (cold water), 0.35 (warm water) Mussels: 2.88 (cold water), 4.24 (warm water)	Gilderhus et al. 1988
	Oysters: 177 (4-day exposure to 26 ppb)	Samuelsen et al. 1988
	41.4 [QSAR estimate. Not application to rotenone. See Section 3.2.3.5 for discussion.]	Meylan and Howard 2007
Density	0.67 (fluffed), 0.78 (packed)	Tomlin 2004
Foliar halftimes	1.4 hours (with volatilization) 2.9 hours (excluding volatilization) Natural sunlight	U.S. EPA/OPP 2006c
	2 days	Knisel and Davis 2000
Foliar washoff fraction	0.05	Knisel and Davis 2000
Henry's law constant	1.12 x10^{-13} atm-m^3/mole (QSAR)	Howard and Meylan 2007
K_{oc}	100,000	Augustijin-Beckers et al. 1994; Knisel and Davis 2000

Table 1: Physical and chemical properties of Rotenone

Property	Value [1]			Reference
K_d and K_{oc}	Texture	Kd	Koc	U.S. EPA/OPP 2006c, MRID 470152009, Table 3.11, p. 45-46.
	Fine sand (2.32% OC)	71.6	3086	
	Sand (1.16% OC)	37.6	3241	
	Silt loam (2.18% OC)	80.92	3712	
	Recommended value: 1263 L/kg OC			
log K_{ow}	4.16 [K_{ow}= 14,454]			Tomlin 2004
	4.1 [K_{ow}= 12,589]			Hansch et al. 1995
Melting point	163 °C; 181 °C (dimorphic)			Tomlin 2004
Molecular formula	$C_{23}H_{22}O_6$			Tomlin 2004
Molecular weight (g/mole)	394.4			Tomlin 2004
Photolysis	Decomposes rapidly			Tomlin 2004
Sediment-Water halftimes				
SMILES Notation	`COc1cc2OC[C@H]3Oc4c5C[C@@H](Oc5ccc4C(=O)[C@H]3c2cc1OC)C(=C)C`			Tomlin 2004
Soil halftimes (NOS)	1 to 3 days			EXTOXNET 1996
	3 days			Augustijin-Beckers et al. 1994; Knisel and Davis 2000
Soil halftimes (aerobic)	12 days [estimated as 2x aquatic metabolism]			U.S. EPA/OPP 2006c
Soil halftimes (anaerobic)				
Soil photolysis	2.9 hours [surrogate value based on foliar half-life]			U.S. EPA/OPP 2006c, MRID 41125402
	Loam DT_{50} = 7 hours Silt clay loam DT_{50} = 5 hours Silt clay loam DT_{50} = 6 hours			Cavoski et al. 2007
U.S. EPA Docket Number				
Vapor pressure	<1 mPa (20 °C)			Tomlin 2004
	6.9 x 10^{-10} torr			U.S. EPA/OPP 2006c
Vegetation/plant halftime	4 days (olives via photolysis)			Cabras et al. 2002
Water halftime (field dissipation)	Initial/target conc: 0.25 ppm 23 hours (cold water pond) 10.6 hours (warm water pond)			U.S. EPA/OPP 2006c, MRID 4558020-73 (pre-publication of Gilderhus et al. 1988)
	Cold water pond at 0.25 ppm initial conc. Water column: DT_{50} 10.3 days Warm water pond at 0.25 ppm initial conc. Water column: DT_{50} 0.94 days			Gilderhus et al. 1988
Water halftime (NOS)	1 to 3 days			EXTOXNET 1996
	10.3 days (cold water) 0.94 days (warm water)			Gilderhus 1982
Water hydrolysis halftime	12.6 days (pH 5) 3.2 days (pH 7) 2.0 days (pH 9)			U.S. EPA/OPP 2006c, MRID 000141409
Water, aquatic metabolism	6 days			U.S. EPA/OPP 2006c

Table 1: Physical and chemical properties of Rotenone

Property	Value [1]	Reference
Water photolysis halftime	191 days (2 meter depth, well mixed)	U.S. EPA/OPP 2006c
	21 hours (top 1 cm surface)	
	Nearly all of the toxicity of the compound is lost in 5 to 6 days of spring sunlight or 2 to 3 days of summer sunlight.	EXTOXNET 1996
	Calculated direct photolysis half-lives of 1.1 year (summer conditions) to 3.1 years (winter conditions) at a water depth of 0.5 meters and a concentration of 50 µg/L.	Draper 2002
Water solubility (mg/L)	0.142 mg/L (20 °C) [given as 0.142 µg/ml]	Tomlin 2004
	0.2 mg/L [value used by EPA]	Augustijn-Beckers, 1994; Knisel and Davis 2000
	15 mg/L	USDA/ARS, EXTOXNET 1996

Table 2: Commercial End-Use Formulations of Rotenone Piscicides

Trade Name, Manufacturer	EPA Reg. No., Label Approval Date [a]	Rotenone (% w/w)	Other Associated Resins/ Extracts (% w/w)	Piperonyl Butoxide (% w/w)	Density of Formulation (from MSDS)	Exposure Adjustment Factor
Liquid Formulations						
CTF Legumine, CWE Properties	75338-2, 8/9/2007	5%	5%		8.506 lbs/gal.	1.25
Noxfish Fish Toxicant, Prentiss.	655-805, 6/28/2001	5%	5%		Not available	1.25
Chem Fish Regular, TIFA	82397-1, no date	5%	5%		7.37 lb/gal	1.25
Prenfish Toxicant Liquid, Prentiss	655-422, 9/30/2002	5%	10%		7.78 lbs/gal.	1.5
Nusyn-Noxfish Fish Toxicant, Prentiss	655-804, 4/20/2001	2.5%	2.5%	2.5% [b]	Not available	2.25
Chem Fish Synergized, TIFA	82397-2, no date	2.5%	2.5%	2.5% [d]	7.3 lb/gal	2.25
Synpren-Fish Toxicant, Prentiss	655-421, 5/17/2001	2.5%	5%	2.5% [b]	7.48 lbs/gal.	2.5
Powder Formulations						
Rotenone Fish Toxicant Powder, Prentiss.	655-691, 1/28/2003	7.4%	11.1%		14 lbs/ft^3	1.375
Prentox Cube Powder Fish Toxicant, Prentiss	655-806, 4/20/2001	7.4%	11.1%		0.24 gm/cm^3 to 0.45 gm/cm^3	1.375
Cube Powder Fish Toxicant, Foreign Domestic Chem.	6458-6, 11/7/1997	7.4%	11.1%		Not specified	1.375
Chem-Sect Brand Cube Powder Fish Toxicant, TIFA	82397-5, no date	7.4%	11.1%		Not specified	1.375
Pellet Formulations						
Grass Carp Management Bait [e], Prentiss Inc.	Pellets, 655-795, 6/18/2001	2.64%	3.36%	0.5% [c]		
Common Carp Management Bait [e], Prentiss Inc.	Pellets, 655-803, 8/1/2001 [e]	2.64%	3.36%	0.5% [c]		

[a] Unless otherwise specified, the date of the most recent approved label on the U.S. EPA/OPP label site, http://oaspub.epa.gov/pestlabl/, current as of February 6, 2008. Labels and MSDS for CWE and Prentiss products available at: http://www.prentiss.com/. Labels and MSDSs as well as formulation densities for TIFA products provided by TIFA (Cerciello 2008a,b).

[b] Equivalent to 2.0% [Butylcarbityl] [6-propylpiperonyl] ether and 0.5% related compounds.

[c] Equivalent to 0.4% [Butylcarbityl] [6-propylpiperonyl] ether and 0.1% related compounds.

[d] Equivalent to 2.35% [Butylcarbityl] [6-propylpiperonyl] ether and 0.15% related compounds.

[e] Atypical application rates. Amount of bait to apply is dependent on the population of target organisms and their response to trainer baits.

[f] See Section 3.1.17 for a discussion of the derivation and use of Exposure Adjustment Factors.

Table 3: *Inerts* Contained in End-use Liquid Formulations of Rotenone

Formulation (% of formulation classified as inerts) [a]	Inerts: Name, CAS No.	Inert % by Weight
CTF Legumine (90%) [a]	N-Methylpyrrolidone, 872-50-4 [b]	9.8%[c]
	Petroleum distillates, NOS	NOS
	1,2,4-Trimethyl Benzene, 95-63-6	0.003% [c]
	Naphthalene, 91-20-3	0.02551% [c]
Synpren-Fish Toxicant (90%)[a]	Xylene range aromatics, 64742-95-6	<= 90%
	1,2,4-Trimethyl Benzene, 95-63-6	32%
	Mixed xylenes, 1330-20-7	3%
	Cumene, 98-82-8	1.5%
	Ethyl benzene, 100-41-4	0.5%
Prenfish Toxicant (85%) [b]	Aromatic petroleum solvent, 64742-94-5	<= 80%
	Naphthalene, 91-20-3	9.9%
	1,2,4-trimethylbenzene, 95-63-6	1.7%
	Acetone, 67-64-1	<= 7.5%
	Emulsifier #1 (NOS)	1.5%
	Emulsifier #2 (NOS)	4.5%
	MSDS Comments: Petroleum solvent: The supplier reports that inhalation of high vapor concentrations (over 1,000 ppm) may cause nervous system effects such as headaches, dizziness, anesthesia and respiratory tract irritation. Surfactant: Causes severe eye irritation, which could lead to permanent eye damage. Prolonged or repeated skin contact may cause discomfort and local redness. Mist can irritate the respiratory tract, experienced as nasal discomfort and discharge with chest pain and coughing.	
Chem Fish Synergized (92.5%)	Aromatic petroleum solvent (variable mixture)	≈85 100 ppm
Chem Fish Regular	Aromatic petroleum solvent (variable mixture)	≈85 100 ppm / 90%

[a] Information taken from MSDS's unless otherwise specified. No hazardous inert ingredients are listed on the MSDSs for powder and pellet formulations.

[b] California Proposition 65: WARNING: This product contains chemicals known to the State of California to cause cancer or birth defects or other reproductive harm.

[c] Information on inerts in CTF Legumine from Fisher (2007).

Table 4: Labeled Application Rates for Rotenone to Surface Water

Use	Application Rate (ppm or mg/L)
Ponds and Lakes	
Selective treatment	0.005 – 0.007
Normal Use	0.025 – 0.05
Bullheads and Carp	0.05 – 0.1
Bullheads and Carp (rich organic ponds) [a]	0.1 – 0.2
Pre-impoundment treatment above dam	0.15 – 0.2 [b]
Streams	
Normal Use [c]	0.025 – 0.1

[a] Several product labels do not give a range and indicate a target concentration of 0.1 ppm. The range of 0.1 to 0.2 ppm is taken from the product label for Prenfish Toxicant. See Table 2 for a listing of formulations covered by this risk assessment.

[b] All current labels for rotenone formulations indicate a maximum application rate of 0.25 ppm. In the U.S. EPA RED, however, the maximum application rate has been lowered to 0.2 ppm (U.S. EPA/OPP 2007a, p. 19).

[c] Application rates for streams were evaluated at a maximum of 50 ppb in the RED (U.S. EPA/OPP 2007a, p. 10) and this is discussed further in U.S. EPA/OPP (2007c). Several product labels prepared after the publication of the RED specify application rates of up to 0.1 ppm or 100 ppb.

Table 5: Summary of studies on rotenone as a model for Parkinson's Disease

Species	Route [a]	Dose mg/kg bw [b]	Duration [c]	Response [d]			Reference
				Bio-chem	Morph	Signs	
Rats	i.p.	1.5, 2.5	2 m	+	+	+	Alam and Schmidt 2002
Rats	i.p.	2.5	48 d	+		+	Alam Schmidt 2004a
Rats	brain	6µg	20 min	+		+	Alam et al. 2004
Rats	s.c.	12	1 d	-		-	Antkiewicz-Michaluk et al. 2003
Rats	s.c.	12	7 d	+		+	
Rats	brain	2 µg	N.S.	+			Antkiewicz-Michaluk et al. 2004
Rats	s.c.	10	1 d	-			
Rats	s.c.	10	7 d	+			
Rats	s.c.	1.5	1 to 10 d	-			Bashkatova et al. 2004
Rats	s.c.	1.5	20 to 30 d	+		+	
Rats	i.v.	2.5-2.75	1-5 w	+	+	+	Betarbet et al. 2000
Rats	s.c.	3	5 w	+	+		Betarbet et al. 2006
Rats	s.c.	3	5-6 d	+	+		Caboni et al. 2004
Mice	i.p.	0.65	single	-			Crutchfield and Dluzen 2006
Mice	i.p.	1.3, 2.6	single	+			
Rats	i.v.	10-18	7-9 d	-	-		Ferrante et al. 1997
Rats	i.v.	2-3.5	21 d	+		+	Fleming et al. 2004
Rats	s.c	2-3.5	21 d	+		+	
Rats	i.v.	2.5	28 d		+	+	Garcia-Garcia et al. 2005
Mice	oral	0.25-5	28 d		-		Inden et al. 2007
		10, 30	28 d		+		
Rats	s.c	2.5	8 d		-	-	Lapointe et al. 2004
Rats	s.c.	3	5 d		+	+	Luo et al. 2007
Rats	s.c.	2	35 d	+		+	Nehru et al. 2008
Rats	s.c.	3	6 d	+			Panov et al. 2005
Rats	s.c.	3	28 d		-/+	+	Ravenstijn et al. 2008
Rats	brain	0.5–5µg	28 d		+		
Mice	s.c.	2.5-5	30-45 d		-	+	Richter et al. 2007
Mice	nasal	2.5	30 d		-	-	Rojo et al. 2007
Rats	i.p.	1.5, 2.5	20-60 d	+		+	Schmidt and Alam 2006

[a] brain (intracerebral), i.p. (intraperitoneal), s.c. (subcutaneous), oral (gavage), nasal (nasal instillation to mimic inhalation exposure).
[b] doses as mg/kg bw except for injections/instillation into the brain. For the later, the dose units per animal/brain are specified.
[c] m (months), min (minutes), d (days), w (weeks), N.S. (duration intracerebral injection not specified).
[d] Biochem (biochemical changes characteristic of Parkinson's Disease); Morph (morphologic changes to the brain characteristic of Parkinson's Disease); Signs (frank signs of toxicity characteristic of Parkinson's Disease). A plus sign (+) indicates an effect and a minus sign (-) indicates no effect. A blank indicates that no observations were made for the particular endpoint.

Table 6: Toxicity of Identified Inerts in Rotenone Formulations Relative to Rotenone

Inert, CAS No.	Toxicity Value	Citation	Toxicity Relative to Rotenone [a]
Acetone, 67-64-1	RfD: 0.9 mg/kg/day	U.S. EPA/ORD 2003a	0.00044
Cumene, 98-82-8	RfD: 1 mg/kg/day	U.S. EPA/ORD 1997	0.0004
Ethylbenzene, 100-41-4	RfD: 0.1 mg/kg/day	U.S. EPA/ORD 1998a	0.004
N-methylpyrrolidone, 872-50-4	Surrogate acute RfD of 1.25 mg/kg bw/day[b].	Footnote b.	0.012 [b]
Naphthalene, 91-20-3	RfD: 0.02 mg/kg/day	U.S. EPA/ORD 1998b	0.02
1,2,4-Trimethyl benzene, 95-63-6	MRL: 0.05 mg/kg/day	U.S. EPA/ Region 10, 2002	0.008
Xylenes (mixed), 1330-20-7	RfD: 0.2 mg/kg/day	U.S. EPA/ORD 2003b	0.002

[a] Unless otherwise specified, the relative toxicity is based on the chronic RfD for rotenone – i.e., 0.0004 mg/kg/day from U.S. EPA/OPP 2007a – divided by the RfD for the *inert*.

[b] No chronic RfD for N-methylpyrrolidone. A surrogate RfD of 1.25 mg/kg bw/day based on a reproductive NOAEL of 125 mg/kg bw/day from Saillenfait et al. (2001) using an uncertainty factor of 100. The toxicity relative to rotenone is based on the acute RfD for rotenone of 0.015 mg/kg bw/day which is based on a reproductive NOAEL of 15 mg/kg bw/day and an uncertainty factor of 100.

Table 7: Non-End Use Formulations of Rotenone Powder

Trade Name, Manufacturer	EPA Reg. No., Label Approval Date [a]	Rotenone (% w/w)	Other Associated Resins/ Extracts (% w/w)	Ratio of Rotenone to Other Material
Powdered Cube Root	655-807, 4/20/2001	7.4%	11.1%	0.66
Brittle Extract of Cube Root	655-808, 4/20/2001	44.2%	44.2%	1
PRENTOX Cube Powder,	655-3, 6/28/2005	8.74%	13.11%	0.66
PRENTOX Cube Resins	655-69, 6/28/2005	44.2%	44.2%	1
Cube Root Powder, Foreign Domestic Chem. Corp. [b]	6458-1, 4/18/1999	7.4%	11.1%	0.66
Rotenone Extract, Foreign Domestic Chem. Corp.	6458-5, 2/26/1999	44.2%	44.2%	1
Chem Sect Brand Cube Powder, TIFA [c]	82397-3, No date	8%	8%	1
Chem Sect Brand Rotenone Resins, TIFA [c]	82397-4, No date	44%	40%	1.1

[a] The date of the most recent approved label on the U.S. EPA/OPP label site, http://oaspub.epa.gov/pestlabl/, current as of February 6, 2008.

[b] Parts of label at EPA site not legible. Some details taken from June 6, 2001 label.

[c] Labels and MSDSs provided by TIFA (Cerciello 2008).

Table 8: Dose-Severity Relationships for Rotenone

NOTE: The dose-severity relationships detailed in this table and discussed in Section 3.3.4 should not be interpreted as suggesting that exposures above the acute RfD of 0.015 mg/kg bw or the chronic RfD of 0.0004 mg/kg bw/day are acceptable.

Dose (mg/kg bw)[a]	Correspond-ing Hazard Quotient	Organism (number of individuals): Effect	Reference
ACUTE			
0.015	1	Acute RfD for sensitive population, women of child bearing age from animal NOAEL of 15 mg/kg day	Section 3.3.3
0.024	1.6	Based on animal LOAEL of 24 mg/kg/day with uncertainty factor of 1000.	Section 3.3.4
0.24	16	Based on animal LOAEL of 24 mg/kg/day with uncertainty factor of 100.	Section 3.3.4
6.5	433	Lowest lethal oral dose in mammals (female rats).	Appendix 1
40	2666	Lowest lethal oral dose in humans.	De Wilde et al. 1986
300	20,000	Lower range of typical estimated lethal doses for human.	Section 3.1.4
CHRONIC			
0.0004	1	Chronic RfD based on an animal NOAEL of 0.375 mg/kg bw/day divided by 1000.	Section 3.3.2
0.002	5	Animal LOAEL of 1.88 mg/kg bw/day (decreased body weight) divided by 1000.	Section 3.3.2
0.004	10	Chronic RfD for rotenone on IRIS: NOAEL/100.	Section 3.3.4
0.02	50	Animal LOAEL, decreased body weight, divided by 100 from study on which the chronic RfD is based.	Section 3.3.4

Table 9: Toxicity of Rotenone (TGAI) to Various Species of Fish

Species	96-hour LC_{50} (ppb)	Reference/Note
Rainbow trout (*Oncorhynchus mykiss*)	1.94	U.S. EPA/OPP 2006c, MRID 439751-02
Rainbow trout (*Oncorhynchus mykiss*)	2.9	U.S. EPA/OPP 2003c, Figure 4.1.
Fathead minnows (*Pimephales promelas*)	4.6	Broderius et al. 1995
Bluegill (*Lepomis macrochirus*)	4.9	U.S. EPA/OPP 2006c, MRID 439751-01
Rainbow trout (*Oncorhynchus mykiss*)	5	Holcombe et al. 1987
Rainbow trout (*Oncorhynchus mykiss*)	5.8	Chen and Farrell 2007
Fathead minnows (*Pimephales promelas*)	6	Holcombe et al. 1987
Lepomis	7.6	U.S. EPA/OPP 2003c, Figure 4.1.
White sucker (*Catostomus commersonii*)	11	Holcombe et al. 1987
Bluegill (*Lepomis macrochirus*)	14	Gingerich and Rach 1985
Mosquitofish (*Gambusia affinis*)	17	Fabacher and Chambers 1972
Gambusia	23	U.S. EPA/OPP 2003c, Figure 4.1.
Freshwater minnow (NOS)	25	Schaut 1939
Mosquitofish (*Gambusia affinis*)	31	Fabacher and Chambers 1972
Carp (*Cyprinus carpio*)	32	Hashimoto and Nishiuchi 1981 [48-h]
Goldfish (*Tanakia tanago*)	33	Hashimoto and Nishiuchi 1981 [48-h]
Pond loach (*Misgurnus anguilicaudatus*)	37	Hashimoto and Nishiuchi 1981 [48-h]
Goldfish (*Carassius auratus*)	40	Gersdorff and Smith 1940
Mozambique (NOS)	80	U.S. EPA/OPP 2003c, Figure 4.1.

Table 10: Toxicity of rotenone formulations in rainbow trout (*Oncorhynchus mykiss*)

Formulation (% a.i.)	LC_{50} (ppb) [a]		Note	Reference
	Form	a.i.		
Cubé resin (4.85%)	27	1.3	Contained PB (20%)	Bridges and Cope 1965
Derris powder (6.5%)	N.S.	1.6	24-hour LC_{50}	Rowe-Rowe 1971
N.S. (5%)	N.S.	1.8	Acc. No: 121875	U.S. EPA/OPP 2006c
Noxfish (5%)	N.S.	2		Waller et al. 1993
ChemFish Regular (5)	57	2.85		Howland 1969
Derris powder (1%)	350	3.5		Skadsen et al. 1980
N.S. (44%)	26	11.44		Mayer and Ellersieck 1986
Noxfish (5%)	N.S.	11.5	Acc. No: 121873	U.S. EPA/OPP 2006c
Liquid Derris #1 (5%)	340	17	hard water[b]	Tooby et al. 1975
Liquid Derris #2 (5%)	350	17.5	hard water[b]	Tooby et al. 1975
Dactinol (5%)	470	23.5	soft water[b]	Tooby et al. 1975
N.S. (5%)	N.S.	35	Accession No. 121886	U.S. EPA/OPP 2006c
N.S. (2.55%)	N.S.	36.2	MRID 400633-01	U.S. EPA/OPP 2006c
N.S. (5%)	N.S.	38	Accession No. 89907	U.S. EPA/OPP 2006c
N.S. (5%)	N.S.	45	MRID 400633-01	U.S. EPA/OPP 2006c
N.S. (6.8%)	N.S.	45	Accession No.89904	U.S. EPA/OPP 2006c
N.S. (5%)	N.S.	48	Accession No.121822	U.S. EPA/OPP 2006c
N.S. (5%)	N.S.	84	Accession No.121882	U.S. EPA/OPP 2006c
Liquid Derris #2 (5%)	1200	60	hard water[b]	Tooby et al. 1975
Liquid Derris #1 (5%)	2600	130	hard water[b]	Tooby et al. 1975
Dactinol (5%)	5,800	290	hard water[b]	Tooby et al. 1975

PB: Piperonyl butoxide

DOC: Dissolved organic carbon.

[a] LC_{50} values are for 96-hours except for those from Tooby et al. (1975) which are for 48-hours and Rowe-Rowe (1971) which is a 24-hour LC_{50}. All studies from the U.S. EPA/OPP taken from Table D.5.

[b] Soft water = 20 mg/L as calcium carbonate. Hard water = 270 mg/L as calcium carbonate. Liquid Derris #1 = Murphy's Liquid Derris, Liquid Derris #2 = Bugge's Liquid Derris. All toxicity values from Tobby et al. (1975) are 48-hour LC_{50}s.

Table 11: Toxicity of Rotenone (TGAI) to Various Species of Aquatic Invertebrates

Species	48-hour LC_{50} (ppb)	Reference/Note
Cladoceran (*Daphnia magna*)	3.7	Rach et al. 1988
Cladoceran (*Daphnia magna*)	8	Holcombe et al. 1987
Cladoceran (*Daphnia pulex*)	65	Claffey and Costa 1974
Cladoceran (*Simocephalus serrulatus*)	190	Sanders and Cope 1966
Dragonflies *(Basiaeschna janata)*	220	Watkins and Tartar 1975
Amphipod (*Gammarus fasciatus*)	950	Claffey and Costa 1974
Stoneflies (*Pteronarcys californica*)	1100	Sanders and Cope 1968
Crayfish (*Cambarus bartoni*)	2000	Claffey and Costa 1974
Amphipod (*Gammarus lacustris*)	3520	Nebeker and Gaufin 1964
Amphipod (*Gammarus lacustris*)	3500	Sanders 1969
Snail (*Physa acuta*)	6800	Nishiuchi and Yoshida 1972
Snail (*Semisulcospira libertine*)	8000	Nishiuchi and Yoshida 1972
Snail (*Cipangopaludina malleata*)	15000	Nishiuchi and Yoshida 1972
Snail (*Indoplanorbis exustus*)	27000	Nishiuchi and Yoshida 1972
Snail (*Aplexa hypnorum*)	40000	Holcombe et al. 1987

Table 12: Summary of Toxicity Values used in Ecological Risk Assessment

(all amounts expressed as a.i.).

Organism Group/Duration		Endpoint	Toxicity Value	Reference
Terrestrial Organisms				
Acute				
	Mammals	NOAEL	15 mg/kg bw	Section 4.3.2.1
	Birds (Sparrow)	LD_{50}	113 mg/kg bw	Cutkomp 1943
Longer-term				
	Mammals	0.375	mg/kg bw/day	Section 4.3.2.1
	Birds	0.375	mg/kg bw/day	Same as mammals
Aquatic Organisms				
Acute				
Amphibians				
	Sensitive (Salamander)	24-hour LC_{50}	0.005 mg/L	Hamilton 1941
	Tolerant (*Rana pipiens*)	Lethal exposure	2.0 mg/L	Haag 1931
Fish	Sensitive (Trout)	96-hour LC_{50}	0.00194 mg/L	Section 4.3.3.1 (RED Table 3.15)
	Tolerant (Goldfish)	96-hour LC_{50}	0.4 mg/L	Section 4.3.3.1
Invertebrates	Sensitive (*Daphnia*)	48-hour LC_{50}	0.0037 mg/L	Section 4.3.3.3 (RED Table 3.15)
	Tolerant (Snail)	24-hour LC50	6.8 mg/L	Nishiuchi and Yoshida 1972
Algae	Sensitive	N/A	N/A	Section 4.3.3.4
	Tolerant	N/A	N/A	Section 4.3.3.4
Macrophytes		N/A	N/A	Section 4.3.3.4
Longer-term				
Amphibians	Sensitive	N/A	N/A	Section 4.3.3.2.
	Tolerant	N/A	N/A	Section 4.3.3.2.
Fish	Sensitive (Trout)	NOEC growth	0.00101 mg/L	Section 4.3.3.1
	Tolerant	NOEC growth	0.04 mg/L	Section 4.3.3.1
Invertebrates				
	Sensitive (*Daphnia*)	NOEC reproduction	0.00123 mg/L	Section 4.3.3.3
	Tolerant	NOEC reproduction	2 mg/L	Relative potency

List of Appendices

Species	Exposure	Response	Reference
ACUTE ORAL			
Rats, Gavage			
Rat (*Rattus norvegicus*)	99.2% a.i.	LD_{50}: 102 mg/kg (M) LD_{50}: 39.5 mg/kg (F)	U.S. EPA/OPP 2006c , p. 56, MRID 00145496, acceptable. Used by EPA in acute assessment of mammals.
White rats (NOS)	Rotenone (NOS)	LD_{50} = 132-1500 mg/kg	Tomlin 2004
Rat (NOS)	Rotenone (NOS)	LD_{50} = 60-132 mg/kg	Hayes 1982
Rat (NOS)	Prentox Grass Carp Bait, 2.6% rotenone and 0.5% piperonyl butoxide.	LD_{50} values as formulation 1550 mg/kg (M) 970 mg/kg (F) LD_{50} values as rotenone 40.3 mg/kg (M) 25.2 mg/kg (F)	U.S. EPA/OPP 2006c , p. 56, MRID 42981701
Rat (NOS)	Chem Sect Chem Fish Regular, 5% rotenone 5% cube root extractables	LD_{50} values as formulation 294.8 mg/kg (M) 130.3 mg/kg (F) LD_{50} values as rotenone 14.7 mg/kg (M) 6.5 mg/kg (F) LD_{50} values as rotenone & extract 29.5 mg/kg (M) 13.0 mg/kg (F)	U.S. EPA/OPP 2006c , p. 56, MRID 43127001
Rat (NOS)	Chem Sect Cube Root Powder Toxicant, 8.08% rotenone	LD_{50} values as formulation >1049 mg/kg (M) > 209 mg/kg (F) LD_{50} values as rotenone >84.8 mg/kg (M) >16.9 mg/kg (F)	U.S. EPA/OPP 2006c , p. 56, MRID 44849201
Other species			
White mice (NOS)	Rotenone (NOS)	LD_{50} = 350 mg/kg	Tomlin 2004
Rabbit (NOS)	Rotenone in ethylene glycol	400, 800, and 1250 mg/kg: 1 animal per dose, all survived. 1600 and 2000 mg/kg bw: 1 animal per dose, both died.	Haag 1931
Rabbit (NOS)	Rotenone (NOS)	3000 mg/kg	Cutkomp 1943
Guinea pigs (NOS)	Rotenone in ethylene glycol	50 mg/kg bw: no mortality 75 or 100 mg/kg bw: mortality	Haag 1931
Guinea pigs (NOS)	Rotenone (NOS)	LD_{50} = 50 to 200 mg/kg	Cutkomp 1943
ACUTE INTRAVENOUS			
Rat (NOS)	Rotenone (NOS)	LD_{50} = 0.2-0.3 mg/kg	Hayes 1982
Rabbit (NOS)	Rotenone (NOS)	LD_{50} = 0.35-0.65 mg/kg	Hayes 1982

Species	Exposure	Response	Reference
Rabbit (NOS)	Rotenone in ethylene glycol	0.25 mg/kg bw: 1/3 died 0.30 mg/kg bw: 1 animal exposed and survived. 0.35 mg/kg bw: 3/3 died	Haag 1931
Cat (NOS)	Rotenone, oil solution (NOS)	0.65 mg/kg: Fatal	Haag 1931
Dogs (NOS)	Rotenone in ethylene glycol	0.5 mg/kg bw: 1/3 died 0.6 mg/kg bw: 0/1 died 0.65 mg/kg bw: 3/3 died	Haag 1931
ACUTE DERMAL			
Rabbits (NOS)	Rotenone (NOS)	LD_{50}>5.0 g/kg	Tomlin 2004
Rabbits (NOS)	Rotenone (NOS)	LD_{50} = 100-200 mg/kg	Hayes 1982
SUBCUTANEOUS			
Guinea pigs (NOS)	Rotenone in ethylene glycol	Minimum lethal dose: 16 mg/kg bw	Haag 1931
Rabbits (NOS)	Rotenone in ethylene glycol	Minimum lethal dose: 20 mg/kg bw	Haag 1931
INTRAMUSCULAR			
Guinea pigs (NOS)	Rotenone in ethylene glycol	Minimum lethal dose: 7 mg/kg bw	Haag 1931
Rabbits (NOS)	Rotenone in ethylene glycol	Minimum lethal dose: 5 mg/kg bw	Haag 1931
ACUTE INTRAPERITONEAL			
Mouse (NOS)	Rotenone (NOS)	LD_{50} = 5.4 mg/kg	Hayes 1982
Guinea pigs (NOS)	Rotenone in ethylene glycol	Minimum lethal dose: 2 mg/kg bw	Haag 1931
Guinea pigs (NOS)	Rotenone (NOS)	LD_{50} = 13 mg/kg	Hayes 1982
Guinea pigs (NOS)	Rotenone, oil solution (NOS)	Minimal lethal dose: 2 mg/kg	Hayes 1982
ACUTE INHALATION			
Rats, males and females (NOS)	Rotenone (NOS) via inhalation	LD_{50} = 0.0235 mg/L (males) LD_{50} = 0.0194 mg/L (females)	U.S. EPA/OPP 2007a, MRID 42153701

Species	Exposure	Response	Reference
Short Term Multiple Gavage (other than developmental studies)			
F344 rats, males, 5 weeks old	0, 40, 200, or 400 mg/kg bw rotenone in 0.5 mL of 5% gum arabic via gavage for 5 consecutive days.	Significant elevation of phase II enzymes, glutathione S-transferase (GST) and quinone reductase (QR) in liver and colon.	Yoshitani et al. 2001

Species	Exposure	Response	Reference
C57BL/6 mice, 20-25 g	Gavage doses of 0, 0.25, 1.0, 2.5, 5.0, 10 or 30 mg/kg for 28 days. 0.5% carboxymethyl cellulose vehicle, 5 mL/kg bw.	At doses of 10 and 30 mg/kg/bw/day, degeneration of dopaminergic neurons. Signs of motor impairment consistent with Parkinson's disease. Toxicity reduced by 4-phenylbutyrate and dopamine. No effects at doses of 5 mg/kg bw/day for 28 days. No changes in body weight. Decrease endurance on roto-rod test but data not detailed.	Inden et al. 2007

Subchronic Dietary (15 days to 90 days)

Species	Dose/Exposure	Response	Reference
F344 rats, males, 5 weeks old	500 ppm rotenone in the diet for 4 weeks	Significant inhibition of ACF formation induced by azomethane (20 mg/kg/ bw 1/week for 2 weeks).	Yoshitani et al. 2001
Rabbits (NOS)	Rotenone	150 mg/kg bw for 6 weeks. No apparent adverse effects	Haag 1931
Beagles, 4-5 months old, 30/sex/dose group	Rotenone (a.i. not specified) administered in gelatin capsules at daily doses of 0, 0.4, 2.0, or 10 mg/kg for 26 weeks. **Note**: The study does not specify the a.i. of the rotenone and indicates that *Details of the studies and records of all the original data are bound in volumes on file at the National Fisheries Research Center* in Wisconsin.	Major signs of toxicity at 10 mg/kg included diarrhea or soft stools that persisted throughout the course of the study, decreased food consumption, weight loss during the first 2 months of exposure, mild anemia, and decreases in blood glucose, total lipids, and cholesterol. At the 2.0 mg/kg dose the signs of toxicity were the same as described above, but relatively mild. No treat-related effects were observed on urinalyses or histopathological evaluations at any dose level. NOEL = 0.4 mg/kg	Marking 1988
Beagles (NOS), groups of four	Fixed dietary concentrations of ≥ 0.52 mg/kg rotenone (NOS) for 28 months	*No unusual symptoms*	Hansen et al. 1965 (summarized in Marking 1988)

Reproduction Studies

Species	Dose/Exposure	Response	Reference
Charles River CD(SD)BR rats, 4 weeks old, 15 males and 25 males/dose group	Rotenone (97-98% pure) incorporated into the diet at concentrations of 0, 7.5, 37.5, or 75.0 mg/kg and fed continuously to two successive generations.	No effects observed on reproduction. NOEL for toxicity = 7.5 mg rotenone/kg feed. Treatment-related decreases in average body weights of parental males and females was observed at week 13 and continued throughout the study; bodyweights of F_0 and F_{1a} generation male rats exposed to 37.5 and 75.0 mg/kg were significantly lower than those of control animals; mean litter size at birth of the $F1_a$ and $F2_a$ litter was smaller in the high-dose group, relative to controls. No significant effects observed on litter data; offspring did not show signs of physical or behavioral abnormalities.	Marking 1988 Also summarized by U.S. EPA/OPP 2006c , p. 56, MRID 00141408, acceptable. Used for chronic assessment of mammals by EPA.
Rats, Sprague-Dawley, decidualized, pseudopregnant, 10/dose group	0, 10, 100, 200, 250, 500, 750, or 1000 ppm rotenone (purity not specified) in diet from days 6 to 10 of gestation Table 1 provides data on mg/kg bw doses: 0.74, 7.08, 14.1, 15.9, 26, 32.8, and 40.9 mg/kg bw/day as rotenone	Reduced body and uterine weights, relative to controls. Clinical signs of toxicity included lethargy, ataxia, and rough unkempt fur at doses of 750 and 1000 ppm. Apparent NOEL = 500 ppm (clinical signs of toxicity and body weight loss) Maternal NOEL = 200 ppm (body weight loss and clinical signs of toxicity) Developmental NOEL = 10 ppm (decreased fetal survival)	Spencer and Sing 1982
Rats, Sprague-Dawley, pregnant, 7/dose group	0, 10, 100, 200, 400, 600, or 800 ppm rotenone (purity not specified) in diet from days 6 to 15 of gestation See Table 2 for doses of rotenone as mg/kg bw: 0, 0.77, 8.1, 12.8, 16.5, 19.2, and 22.8 mg/kg bw	Effects similar to those described above observed at doses of 600 and 800 ppm; no resorptions occurred but the fetal survival rate was reduced at all doses and was significant at doses of 8.1 mg/kg bw or greater. Apparent NOEC: 0.77 mg/kg bw/day.	Spencer and Sing 1982

Chronic Studies

Species	Dose/Exposure	Response	Reference
Fischer 344/N rats, 50/sex/dose group, 13 weeks old	Rotenone (>98% pure) Exposure Period: 103 weeks Dietary concentrations: 0, 38, or 75 ppm	No treatment-related effects on survival, mean body weights or food consumption. Treatment-related effects included an increased incidence of parathyroid gland adenomas in males at 75 ppm (4/44), relative to controls (1/44). A statistically significant increase in subcutaneous tissue neoplasms (fibromas, fibrosarcomas, sarcomas, myxosarcomas, and neurofibrosarcomas) only in females at 38 ppm was not attributed to rotenone administration because there was no apparent dose/response trend and because the statistical significance was based on the combination of tumors of different morphology. No treatment-related nonneoplastic lesions were observed in rats. Equivocal evidence of carcinogenicity in male rats; no evidence of carcinogenicity in females rats.	Abdo et al. 1988
$B6C3F_1$ mice, 50/sex/dose group, 5 weeks old	Rotenone (>98% pure) Exposure Period: 103 weeks Dietary concentrations: 0, 600, or 1200 ppm	Survival rate significantly higher (47/50) among high dose (1200 ppm) males, relative to controls and other treated rats; mean body weights significantly decreased in all treated mice (5-30% lower), relative to controls; no observed effects of treatment on food consumption. Significant decreases ($p<0.01$) in the incidence of neoplasms of the liver (1/50), relative to controls (12/47) and subcutaneous tissue observed in high-dose (1200 ppm) male mice; no significant differences observed in tumor incidence at any site in female mice.	Abdo et al. 1988

Abdo et al. 1988 (*continued*)
Unusually low rate of liver tumors in male $B6C3F_1$ mice considered an effect of rotenone administration.

Chronic Studies

Species	Dose/Exposure	Response	Reference
Mice, two F_1 hybrid strains, 7 days old, 18/strain/sex/dose group	Rotenone (purity not specified) by gavage from 7 days old until weaning; in feed at 0 or 1.0 mg/kg for 18 months post weaning.	No adverse effects observed. **Note:** This study is summarized in CalEPA 1997. I added the citation to list of references to get from Paul.	Innes et al. 1969 (Cited in CalEPA 1997)
Fischer 344 rats, 6 weeks old, 40/sex/dose group	Dietary exposure to 0, 7.5, 37.5, or 75.0 mg rotenone/kg of feed for 24 months. **Note**: The study does not specify the a.i. of the rotenone and indicates that *Details of the studies and records of all the original data are bound in volumes on file at the National Fisheries Research Center* in Wisconsin.	NOEL = 7.5 mg/kg in diet. No significant clinical signs of toxicity observed at any dose; significantly lower body weights observed in males and females treated with 37.5 or 75.0 mg/kg (however, decreased food consumption by females in 37.5 and 75.0 mg/kg dose groups may have accounted for the effects on body weight gain); treatment-related effects on total protein and albumin observed in the high-dose females and higher serum urea nitrogen levels in females in the mid- and high-dose groups were observed in the absence of corresponding histopathological findings; no effects were observed on hematology, blood chemistry, urinalysis, or histology of treated rats.	Marking 1988
F344 rats, males, 5 weeks old	500 ppm rotenone in diet for 4 weeks (initiation phase) and 34 weeks (post initiation phase)	During post-initiation phase, rotenone reduced the frequency of colonic adenocarcinoma (60% vs 19%).	Yoshitani et al. 2001
Carworth rats, males and females, 20 rats/dose group	0 or 100 ppm Pro-Noxfish (2.5% rotenone (100%) in drinking water for 70 weeks. Concentration of rotenone given as 0.0125 ppm.	Decreased body weight gain (about 11% in males and 12% in females). Decreased water consumption (58% of controls in males and females combined). No remarkable organ pathology.	Brooks and Price 1961
Carworth rats, males and females, 20 rats/dose group	Detoxified Pro-Noxfish as above with an exposure period of 50 weeks. Detoxification specified as: *exposing a 100-ppm aqueous suspension of Pro-Noxfish in 12-gallon glass bottles to sunlight and aeration until no colorimetric test for rotenone remained* (study p. 51).	Decreased body weight gain in males (about 8%). Body weight in females was somewhat higher than controls (about 7%). No substantial decrease in water consumption (about 97% of controls). No remarkable organ pathology.	Brooks and Price 1961

Chronic Studies

Species	Dose/Exposure	Response	Reference
Osborne-Mendel rats, males and females, 50 rats/group	0, 50, 100, 250, 500, or 1000 ppm cube powder (5.80% rotenone) in diet for 2 years.	No treatment-related effects on hematology, gross pathology or histopathology at any dose level. NOEL = 50 ppm based on decreased weight gain.	Hansen et al. 1965
Rats (NOS)	2, 5, 25, or 50 ppm for 104 weeks	*Tissue damage* at 5 ppm; gross effects at 25 ppm. **Note**: This is summarized from CalEPA 1997 and the citation was added to the list of refs to get from Paul.	Lehman 1952
Rats, albino, females, 35 ± 2 days old, 100 ± 1g, 4 series of 10	1.7 µg/g bw rotenone (K&K Labs, Plainveiw, NY) dissolved in 0l mL sunflower oil via daily i.p. injections for 42 days. (Total administered dose = 9.1 ± 1.6 mg rotenone/rat.	Mortality = 80 in 1st series and 90% in three other series. Tumor incidence: 100% mammary tumors appearing 6-11 months post treatment in 1st series; 60% mammary tumors at 10 months post-treatment of continuing observation period. Controls in all series had 0% mammary tumors. Mammary tumors were encapsulated and did not show signs of metastasis; tumor-bearing rats did not show signs of liver damage or alterations in endocrine organs; and 4-5/30 tumors were transplanted successfully, but were slow growing taking 7-12 months to fully develop. **Note**: This is summarized in CalEPA 1997, and I was able to download the study from the *Cancer Research* website. We must add the citation to the Bib.	Gosalvez and Merchan 1973
Beagle dogs, 2/sex/group	0, 50, 150, or 400 ppm Cube powder (rotenone 5.8%) for 28 months.	No adverse effects; no NOEL established. **Note**: This is summarized from CalEPA 1997 and the citation was added to the list of refs to get from Paul.	Hansen et al. 1965
Dogs (NOS), n=5	Rotenone	10 mg/kg bw/day: 3 dogs died in 7-31 days. Others survived to 102 days but one had severe weight loss. 5 mg/kg/day for 30 days: slight increase in body weight with no signs of toxicity.	Haag 1931

Species	Exposure	Effects	Reference
Acute Dietary (5-day)			
Japanese quail, 14 days old, 10/test concentration	Rotenone (purity 34.5%) in 5-day diets	5-day LC_{50} = 1882 ppm (95% CI = 1418-2497 ppm)	Hill et al. 1975
Ring-necked pheasant, 10 days old, 10/test concentration	Rotenone (purity 34.5%) in 5-day diets	5-day LC_{50} = 1608 (95% CI = 1365-1875 ppm)	Hill et al. 1975
Mallard, 10 days old, 10/test concentration	Rotenone (purity 34.5%) in 5-day diets	5-day LC_{50} ≈2600 ppm	Hill et al. 1975
Eastern Robin (*Turdus migratorius*), 3 to 10 days old	Derris dust (0.75% rotenone) on four prey items: caterpillar, cankerworm, cabbage worm, and silkworm	Doses Causing No mortality: 3 mg/kg bw, 5 mg/kg bw, 12 mg/kg bw, 15 mg/kg bw. Doses Causing Mortality: 8 mg/kg bw, 8 mg/kg bw, 25 mg/kg bw, 30 mg/kg bw, 34 mg/kg bw.	Cutkomp 1943 See Table 4, p. 243 of paper.
Intravenous Injection			
Pigeons (NOS)	1 mg rotenone (NOS) per bird. Note: The body weight of the pigeons are not specified. See Section 4.1.2.2 for discussion.	Minimum lethal dose Symptoms similar to dogs and cats. Vomiting a common response. Pigeons recovered from sublethal doses more rapidly than mammals.	Haag 1931
Capsules			
Pigeons (NOS)	200 to 500 mg per bird. Note: The body weight of the pigeons are not specified. See Section 4.1.2.2 for discussion.	Vomiting but no other adverse effects. Lower doses (not specified) caused no adverse effects.	Haag 1931
Acute Gavage			
Mallard (NOS)	Rotenone (NOS)	Oral (NOS) LC_{50} = 2200 mg/kg	U.S. EPA/OPP 2006c, MRID 143250
Pheasant (NOS)	Rotenone (NOS)	Oral (NOS) LC_{50} = 1680 mg/kg	U.S. EPA/OPP 2006c, MRID 143250

Species	Exposure	Effects	Reference
Gelatin Capsules: All below are nestling birds of about 3 to 10 days old. See Table 1 in Cutkomp 1943)			
Eastern Yellow Warbler (*Dendroica aestiva aestiva*)	Rotenone (*chemically pure*, NOS)	Mortality: 1,470 to 10,000 mg/kg bw. No Mortality: 110 to 361 mg/kg bw.	Cutkomp 1943
Eastern Meadowlark (*Sturnella magna magna*)	Rotenone (*chemically pure*, NOS)	Mortality: 129 to 355 mg/kg bw	Cutkomp 1943
Cedar Waxwing (*Bornbycilla cedrorum*)	Rotenone (*chemically pure*, NOS)	Mortality: 200 mg/kg bw	Cutkomp 1943
Prairie Horned Lark, (*Otocoris alpestris praticola*)	Rotenone (*chemically pure*, NOS)	Mortality: 230 mg/kg bw	Cutkomp 1943
Least Flycatcher, (*Empidonax minimus*)	Rotenone (*chemically pure*, NOS)	Mortality: 322 to 333 mg/kg bw	Cutkomp 1943
Eastern Cowbird, (*Molothrus ater*)	Rotenone (*chemically pure*, NOS)	Mortality: 380 mg/kg bw	Cutkomp 1943
Eastern Mourning Dove, (*Zenaidura macroura*)	Rotenone (*chemically pure*, NOS)	Mortality: 414 mg/kg bw Missing (possible mortality): 97 mg/kg bw	Cutkomp 1943
Pigeon, (*Columba livia*)	Rotenone (*chemically pure*, NOS)	Mortality: 145 to 526 mg/kg bw Survived: 98 mg/kg bw	Cutkomp 1943
Gelatin Capsules: All below are were nestling birds of about 3 to30 days old except for English Sparrows, which were adults. See Table 2 in Cutkomp 1943)			
Eastern Chipping Sparrow, (*Spizella passerina*)	Rotenone (*chemically pure*, NOS)	Median lethal dose: 113 mg/kg bw	Cutkomp 1943
Eastern Song Sparrow, (*Melospiza melodia*)	Rotenone (*chemically pure*, NOS)	Median lethal dose: 130 mg/kg bw	Cutkomp 1943
Eastern Robin (*Turdus migratorius*)	Rotenone (*chemically pure*, NOS)	Median lethal dose: 195 (94-407) mg/kg bw	Cutkomp 1943
English Sparrow (*Passer domesticus*)	Rotenone (*chemically pure*, NOS)	Median lethal dose: 199 (185-214) mg/kg bw	Cutkomp 1943
Chickens (NOS), 5 days old	***Derris extract, 25% rotenone***	Median lethal dose: 247 (166-366) mg/kg bw (dose expressed as rotenone)	Cutkomp 1943
Chickens (NOS), 5 days old	Rotenone (*chemically pure*, NOS)	Median lethal dose: 996 (563 – 1,747) mg/kg bw	Cutkomp 1943
Chickens (NOS), 28 days old	Rotenone (*chemically pure*, NOS)	Median lethal dose: 3,077 mg/kg bw	Cutkomp 1943

Species	Exposure	Effects	Reference
English Sparrow (*Passer domesticus*)	Rotenone (*chemically pure*, NOS)	Median lethal dose: 853 mg/kg bw	Cutkomp 1943
Pheasant (*Phasianus colchicus*), 5 days old	Rotenone (*chemically pure*, NOS)	Median lethal dose: 850 mg/kg bw	Cutkomp 1943
Pheasant (*Phasianus colchicus*), 30 days old	Rotenone (*chemically pure*, NOS)	Median lethal dose: 1,190 mg/kg bw	Cutkomp 1943
Longer/Reproduction Term			
No longer term studies available in open literature or in EFED Science Chapter (U.S. EPA/OPP 2006c)			
Teratology Studies			
Chick embryos, at 5 developmental stages, 10-16/stage	0.5, 0.8, or 1.0 µg/mL rotenone (purity not specified) for 15 minutes.	Treatment arrested development at some stages, especially stages 4 and 5 (NOS). ATP was effective in reversing the effects *anticipated by the mechanism of action of rotenone on the mitochondrial respiratory chain.*	Rao and Chauhan 1971 (Cited in CalEPA 1997)

Appendix 3: Toxicity to Terrestrial Invertebrates

Grouped by bees, earthworms, and other and then alphabetically by author within each group.

Species	Exposure	Effect	**Reference**
Bees			
Honey bee (Apis mellifera)	Contact bioassay, >95% a.i.	Contact LD_{50}: >60µg/bee Used by EPA to classify rotenone as *Practically non-toxic* to honeybees.	U.S. EPA/OPP 2006c, MRID 05001991
Honey bee (Apis mellifera)	2.4 µg a.i./bee	12% mortality	Atkins et al. 1975; U.S. EPA/OPP 2006c, MRID 00036935
Earthworms			
Predominantly *Poa annua* L./*Lolium perenne* L. Turf based on a loam soil (pH 5.4).	17.5% Derris dust in soil: 175 kg rotenone (dust-able powder 100% ; Murphy Ltd) in 1000 kg sand/ha applied at Shipley Golf Club, Shipley, West Yorkshire	Principal earthworm species identified: *Allolobophora* spp. Assuming 7.4% rotenone and total rotenoids of 18.5% (Table 1 of the current risk assessment), the exposure involved 1.295% rotenone (12,950 ppm) or 32,375 ppm total rotenoids. Inhibition of cast production of up to 48.9% of control values after about 2 months with recovery (107.5% of control value by 1 year after treatment.	Baldwin and Bennett 1990

Note on organization: Three tables are included for freshwater acute, freshwater chronic, and saltwater acute. Following the initial entry for Marking and Bills (1976) in the freshwater acute table, all entries are sorted by species and then reference.

Note on units: To facilitate quality control checks of the values summarized below with the corresponding publications, the units given for the various entries reflect the units reported in the corresponding publication. 1 ppm = 1,000 ppb = 1 mg/L = 1000 µg/L.

Note on Formulations: Several studies – e.g., Bridges and Cope 1965 and Tooby et al. 1975 – express results in units of formulation rather than a.i. Again, the entries below are expressed as in the corresponding publication. Toxicity values are compared to a.i. equivalents of rotenone as needed in the body of the risk assessment.

Freshwater Fish – Acute

Species	Exposure	Effects	Reference
21 Species of freshwater fish (***see supplemental Table 1 below***)	Noxfish (emulsifiable concentrate containing 5%). Toxicity values reported in units of formulation.	3-hour LC_{50} values: 50.0-1410 µg/L **least sensitive**: goldfish/carp/fathead minnow/black bullhead **most sensitive**: lake trout 6-hour LC_{50} values: 28.3-1190 µg/L **least sensitive**: goldfish/black bullhead **most sensitive**: lake trout 24-hour LC_{50} values: 16.5-400 µg/L **least sensitive**: goldfish **most sensitive**: walleye 96-hour LC_{50} values: 21.2 – 497 µg/L **least sensitive**: goldfish **most sensitive**: Atlantic salmon	Marking and Bills 1976
American eel (*Anguilla rostrata*), black eel stage, total length = 97.2 mm	Noxfish (5% rotenone), recommended application rate not specified (according to Table 1 of study).	96-hour LC_{50} = 50.49 µg/L (95% CI = 35.49-65.57 µg/L) Noxfish was extremely toxic to the black eel: ≥75 µg/L caused 100% mortality.	Hinton and Eversole 1979
Bluegill (*Lepomis macrochirus*), 4.5-5.5 cm (total length), 2.00 ± 0.34g, 20/test concentration	Rotenone (purity >98%) exposure via continuous flow proportional diluter	24-hour LC_{50} = 14.0 µg/L (95% CI = 10.5-18.6) 96-hour LC_{50} = 10.9 µg/L (95% CI = 8.6-13.8)	Gingerich and Rach 1985

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Bluegill (*Lepomis macrochirus*), average length: 38 mm; average weight: 0.6 g	Rotenone formulation: Cubic resin extract (33.7% rotenone): 14.39% Piperonyl butoxide: 19.71% Tergitol: 24.90% Aerosol OT: 4.74% Oil Yellow G Extra: 4.74% Xylene: 31.52% Formulation contained 4.85% rotenone	24-hour LC_{50} = 26 (23-29) µg/L 48-hour LC_{50} = 23 (20-25 µg/L 96-hour LC_{50} = 23 (20-25) µg/L **The test results are expressed as the weight of the formulation in µg/L of test water.**	Bridges and Cope 1965
Bluegill sunfish, 1.9 g, 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 96 hours	96-hour LC_{50} = 0.014 mg/L (95% CI = 0.013-0.015 mg/L)	Holcombe et al. 1987
Bluegill, 0.60 g	Rotenone (44.00% technical material) Static exposure	24-hour LC_{50} = 26 µg/L (95% CI = 23-29 µg/L) 96-hour LC_{50} = 22.5 µg/L (95% CI = 20-25 µg/L)	Mayer and Ellersieck 1986
Bluegills (*Lepomis macrochirus*)	70-160 ppb ChemFish Regular (5% liquid formulation of rotenone) for 96 hours	48-hour LC_{50} = 122.0 ppb (95% CI = 114.0-130.0 ppb) 96-hour LC_{50} = 114.0 ppb (95% CI = 105-124 ppb)	Howland 1969
Bluegills (*Lepomis macrochirus*)	Technical grade	96-hour LC_{50} = 4.9 µg/L	U.S. EPA/OPP 2006c, MRID 439751-01
Bluegills (*Lepomis macrochirus*)	End use product (NOS), 96 hours	96-hour LC_{50} = 56 (51.9 – 60.5) µg/L	U.S. EPA/OPP 2006c, Accession No. 121874
Bluegills (*Lepomis macrochirus*)	24-hour exposure to concentrations of 3 µg/L to 17 µg/L in specialized tanks designed to record gill movement.	Minimum lethal concentration: 10 µg/L. No increase in ventilatory frequency except with fish *in extremis*.	Carlson 1990

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Brook trout (*Salvelinus fontinalis*), green eggs, 24-hours post fertilization, 25/test chamber	Noxfish (5% rotenone) in soft water under static test conditions	24-hour LC_{50} = 4.24 mg/L (95% CI = 3.27-5.49) 96-hour LC_{50} = 3.40 mg/L (95% CI = 2.74-4.22)	Olson and Marking 1975
Carp (*Cyprinus carpio*)	Technical grade rotenone	48-hour LD_{50} =0.032 mg/fish	Hashimoto and Nishiuchi 1981
Carp (*Cyprinus carpio*), average total length 6.0 cm, 2.5 g, 10 fish/test	Technical grade rotenone Oral exposure: pesticide added to powdered diet and consumed within 15 minutes; mortality observed 48 hours after feeding. Topical application: pesticide dissolved in distilled water, acetone, or tetrahydrofuran to achieve dose of 5 µL of solvent. Pesticide solution was applied to anesthetized fish by micrometer syringe onto the gill lamella; mortality observed 48 hours after treatment. Contact toxicity test: fish exposed to water concentration of 10 L of test solution.	48-hour oral LD_{50} = 6.5 mg/fish 48-hour dermal LD_{50} =0.014 mg/fish 48-hour LD_{50} =0.032 mg/fish	Hashimoto and Fukami 1969
Carp, Bighead (*Aristichthyes noblis*)	Noxfish (containing 5% rotenone)	96-hour LC_{50} = 0.0437 ppm (95% CI =0.0349-0.0547)	Marking and Bills 1981
Grass carp (*Ctenpharyngodon idella*)	Noxfish (containing 5% rotenone)	96-hour LC_{50} = 0.0852 ppm (95% CI = 0.0759-0.957)	Marking and Bills 1981
Channel catfish (*Ictalurus punctatus*), 0.8-1.2 g	Rotenone formulation: Noxfish (5% a.i.) in static tests	48-hour LC_{50} = 0.0073 mg/L (95% CI = 0.0030-0.0080 mg/L)	Waller et al. 1993

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Channel catfish (*Ictalurus punctatus*), average length: 43 mm; average weight 0.5 g	Rotenone formulation: Cubic resin extract (33.7% rotenone): 14.39% Piperonyl butoxide: 19.71% Tergitol: 24.90% Aerosol OT: 4.74% Oil Yellow G Extra: 4.74% Xylene: 31.52% Formulation contained 4.85% rotenone	24-hour LC_{50} = 33 (30-37) µg/L 48-hour LC_{50} = 29 (25-33) µg/L 96-hour LC_{50} = 28 (24-32) µg/L The test results are expressed as the weight of the formulation in µg/L of test water.	Bridges and Cope 1965
Channel catfish, 0.50 g	Rotenone (44.00% technical material) Static exposure	24-hour LC_{50} = 3.3 µg/L (95% CI = 2.8-3.9 µg/L) 96-hour LC_{50} = 2.6 µg/L (95% CI = 2.1-3.2 µg/L)	Mayer and Ellersieck 1986
Channel catfish, 0.70 g	Rotenone (44.00% technical material) Static exposure	24-hour LC_{50} = 5.8 µg/L (95% CI = 4.2-7.9 µg/L) 96-hour LC_{50} = 2.8 µg/L (95% CI = 1.9-4.1 µg/L)	Mayer and Ellersieck 1986
Chinook salmon (*Oncorhynchus tshawytscha*), green eggs, 24-hours post fertilization, 25/test chamber	Noxfish (5% rotenone) in soft water under static test conditions	24-hour LC_{50} >3.00 mg/L 96-hour LC_{50} >3.00 mg/L 192-hour LC_{50} >3.00 mg/L	Olson and Marking 1975
Common carp (*Cyprinus carpio*)	Noxfish (containing 5% rotenone)	96-hour LC_{50} = 0.0500 ppm (95% CI = 0.0411-0.0608)	Marking and Bills 1981
Common carp (*Cyprinus carpio*), 1-year-old, 121-168 g, 18-22 cm, 12/tank, 2 tanks/dose level	Test chemical: 97% pure rotenone in 100 g gelatin Dose levels: 0.0, 7.0, 7.6, 8.3, 9.1, and 10 mg/kg of fish Administration: single bolus dose; gavage Surfactant: 10 g polysorbate 80 (Tween 80; Sigma) to enhance absorption via the intestine.	48-hour LD_{50} = 8.1 mg/kg of fish (95% CI = 7.7-8.5 mg/kg) All mortality occurred within the first 16 hours of exposure.	Fajt and Grizzle 1993

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Dwarf tilapia (*T. sparrmanii*), 41 mm (32-50 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0073 ppm	Rowe-Rowe 1971
Dwarf tilapia (*T. sparrmanii*), 77 mm (51-84 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0098 ppm	Rowe-Rowe 1971
Fathead minnows (*Pimephales promealas*)	Rotenone Formulation: Noxfish (5% a.i.)	Control toxicity value (0 g *Elodea*): 24-hour LC_{50} = 10.7 µg/L 96-hour LC_{50} = 3.4 µg/L Control toxicity value (0 g suspended bentonite): 24-hour LC_{50} = 12.1 µg/L 96-hour LC_{50} = 8.0 µg/L **Note**: These toxicity values are the control values for the study involving the effects of Canadian waterweed or suspended clay on the toxicity of rotenone to fathead minnows.	Gilderhus 1982
Fathead minnows (*Pimephales promelas*), juveniles (26- to 34-days old)	Rotenone (NOS) in continuous flow-through systems	96-hour LC_{50} = 0.0046 mg/L	Broderius et al. 1995
Fathead minnows, 0.2 g, 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 96 hours	96-hour LC_{50} = 0.006 mg/L (95% CI = 0.004-0.009 mg/L)	Holcombe et al. 1987
Fresh-water minnow (NOS)	Rotenone (chemically pure)	24-hour LC_{50} = 0.025 ppm	Schaut 1939
Goldfish (*Carassius auratus*)	Rotenone stock solution	8-hour LC_{50} = 0.0400 mg/L	Gersdorff and Smith 1940
Goldfish (*Carassius auratus*), mean length of 43 mm, mean weight of 2.5 g, 10 fish total	Rotenone prepared in the laboratory from *Derris elliptica* Test concentration = 0.075 mg/L	Survival time for goldfish exposed to 0.075 mg/L rotenone ranged from 93 to 133 minutes, with a mean survival time of 115 minutes.	Gersdorff 1930
Goldfish, tanago	Rotenone, technical grade	48-hour LC_{50} = 0.033 ppm	Hashimoto and Nishiuchi 1981

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Guppies, adult males, 18-25 mm long, 10/test	Rotenone (NOS)	0.5 mg/L for 1 day had no immediate effect, but caused some distress after 2 hours; 3/10 fish died after 8 hours 1.0 mg/L for 6 hours had no immediate effect, but caused listlessness after 1 hour; 10/10 fish died within 2 ½ hours.	Jones 1975
Harlequin fish (*Rasbora heteromorpha*), 1-3 cm	Dactinol (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 9.5 mg/L	Tooby et al. 1975
Harlequin fish (*Rasbora heteromorpha*), 1-3 cm	Murphy's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 3.2 mg/L	Tooby et al. 1975
Harlequin fish (*Rasbora heteromorpha*), 1-3 cm	Bugge's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone	24-hour LC_{50} = 1.8 mg/L	Tooby et al. 1975
Lake trout (*Salvelinus namaycush*), 24-hours post fertilization, 25/test chamber	Noxfish (5% rotenone) in soft water under static test conditions	24-hour LC_{50} >1.00 mg/L 96-hour LC_{50} >1.00 mg/L 192-hour LC_{50} >0.250 mg/L	Olson and Marking 1975
Largemouth bass (*Micropterus salmoides*), 60 mm (55-65 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0036 ppm	Rowe-Rowe 1971
Medaka (*Oryzias latipes*), Japanese killifish	Rotenone, technical grade	48-hour LC_{50} = 0.030 ppm	Hashimoto and Nishiuchi 1981
Minnow (*Barbus anoplus*) 40 mm (30-50 mm)	Derris powder containing 6.5% rotenone	LC_{50} = 0.0023 ppm	Rowe-Rowe 1971
Minnow (*Barbus gurneyi*) 51 mm (35-60 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0516 ppm	Rowe-Rowe 1971

Freshwater Fish – Acute

Species	Exposure	Effects	Reference
Mosquitofish (*Gambusia affinis*), wild caught in different parts of Mississippi	Rotenone (NOS), 24 hour exposure period	Resistant populations: LC_{50} of 31 µg/L Sensitive populations: LC_{50} of 17 µg/L Resistance associated with greater mixed function oxidase activity.	Fabacher and Chambers 1972
Mouthbreeder (*Pseudocrenilabrus philander*), 65 mm (40-105 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0088 ppm Some of the females were carrying eggs, alevins, or fry in their mouths:	Rowe-Rowe 1971
Rowe-Rowe 1971: Additional Notes **Eggs**: at a test concentration of 0.15 ppm derris solution, female expectorated 63 eggs, 33 of which were not removed from the test solution. Within 35 hours of exposure the eggs started to change color, and after 48 hours of exposure, development in all the eggs ceased and some eggs began to decompose. All eggs removed from the test solution to fresh water hatched. **Alevins**: at a test concentration of 0.15 ppm derris solution, female expectorated 42 alevins, of which 14 were not removed from the test solution. All alevins survived 48 hours of exposure; however, 10 died after day 5, two died on day 6, and the remaining two died after 10 days of exposure. During exposure, the alevins were unable to swim in an upright position and remained on the bottom of the aquarium swimming only on their sides until they died. Most alevins removed from the test solution survived and developed normally. **Fry**: at a test concentration of 0.18 ppm, groups of 19, 31, and 29 fry were expectorated. All fry died by 24 hours. 24-hour adult mortality was 100% in the first and second groups and 60% in the third group.			
Mozambique tilapia (*Tilapia mossambica*), 67 mm (50-90 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0103 ppm	Rowe-Rowe 1971
Natal yellowfish or scaly (*Barbus natalensis*), 47 mm (35-55 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0036 ppm	Rowe-Rowe 1971
Pond loach (*Misgurnus anguilicaudatus*)	Rotenone, technical grade	48-hour LC_{50} = 0.037 ppm	Hashimoto and Nishiuchi 1981
Rainbow trout	Rotenone (>98% pure) by **intravenous injection** (into the caudal vein of un-anesthetized fish) *NOTE: IV LD50 of 0.305 mg/kg is virtually identical to that in mammals (i.e., 0.2 to 0.65 mg/kg as summarized in Hayes 1982).*	Estimated 6- hour LD_{50} =305 µg/kg (95% CI = 254-364 µg/kg) No mortality observed at 225 µg/kg; 2/8 fish died 2 hours after treatment with 275 µg/kg. Signs of toxicity (periods of increased ventilation and pronounced coughing) were observed in most treated fish within the first 15 minutes after treatment.	Erickson and Gingerich 1986

Freshwater Fish – Acute

Species	Exposure	Effects	Reference
Rainbow trout (*Onchorhynchus mykiss*), juveniles, wet body mass of 3.2 ± 0.7 g, fork length of 6.4 ± 0.4 cm. 10/concentration	Acute Lethal Toxicity Tests: Five concentrations of rotenone (95-98%) ranging from 5.00 to 7.75 µg/L; Vehicle: DMF Four 96-hour static renewal tests: Range finder and definitive static renewal 96-hour LC_{50} test; Static renewal 96-hour LC_{50} test in the presence of 0.2% (w/v) Fraser River sediments; Static renewal 96-hour LC_{50} test in the presence of 0.2% (w/v) Fraser River sediments plus 5 mg/L humic acids (dissolved organic carbon); Static renewal 96-hour LC_{50} test in the presence of 0.2% (w/v) Fraser River sediments plus 10 mg/L humic acids (dissolved organic carbon)	Extremely small margin between 0% lethality (5.0 µg/L) and 100% mortality (6.6 µg/L); 96-hour LC_{50} = 5.80 µg/L Signs of toxicity were rapid and included pronounced burst of ventilation and locomotion, loss of equilibrium, and erratic swimming, followed by sinking to the aquarium bottom and continued opercula movements at a slower pace. The 96-hour LC_{50} was unchanged with the addition of Fraser River sediments. Dissolved organic carbon (DOC) from humic acids significantly increased the rotenone 96-hour LC_{50}: LC_{50} = 6.55 µg/L (DOC = 3.0 mg/L) LC50 = 7.75 µg/L (DOC = 4.0 mg/L) Investigators suggest that rotenone adsorption onto the DOC decreased its bioavailability.	Chen and Farrell 2007
Rainbow trout (*Onchorhynchus mykiss*), juveniles, wet body mass of 3.2 ± 0.7 g, fork length of 6.4 ± 0.4 cm. 10/concentration	Swimming Performance Test: Test concentrations: 0, 3.0, 4.0, or 5.0 µg/L rotenone (95-98%) Exposure period: 2, 4, 6, 12, 16, 24, or 48 hours	Threshold for impairment of critical swimming performance = 3.0 µg/L (p=0.029); exposure to higher concentrations did not cause further impairment, and the adverse effect was not time-dependent.	Chen and Farrell 2007
Rainbow trout (*Onchorhynchus mykiss*), juveniles, wet body mass of 3.2 ± 0.7 g, fork length of 6.4 ± 0.4 cm. 10/concentration	Effects on Routine Oxygen Uptake: Test concentrations: 0, 1.5, 2.5, 3.0, or 3.5 µg/L Exposure period: 1 hour	Exposure caused a significant decrease in peak active oxygen uptake at all exposure concentrations without affecting routine oxygen uptake.	Chen and Farrell 2007

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Rainbow trout (*Onchorhynchus mykiss*), juveniles, wet body mass of 3.2 ± 0.7 g, fork length of 6.4 ± 0.4 cm. 10/concentration	Effects on Excess Post Exercise Oxygen Consumption (EPOC): Test concentrations: 0, 1.0, 3.0, 4.0, 5.0, or 6.0 µg/L Procedure: Trout were individually chased and then exposed to the rotenone test concentrations to monitor initial post exercise oxygen consumption during a 40-minute recovery period.	Exposure to 4.0 or 5.0 µg/L, but not 6.0 µg/L, significantly (p=0.002) decreased post exercise oxygen consumption (Mo_{2MAX}) without affecting EPOC.	Chen and Farrell 2007
Rainbow trout (*Salmo gairdneri*)	10-70 **ppb** ChemFish Regular (5% liquid formulation of rotenone) for 96 hours. Results appear to be reported as formulation.	48-hour LC_{50} = 57.0 ppb (95% CI = 51.3-63.4 ppb) 96-hour LC_{50} = 57.0 ppb (95% CI = 51.3-63.4 ppb)	Howland 1969
Rainbow trout (*Salmo gairdneri*), 0.8-1.2 g	Rotenone formulation: Noxfish (5% a.i.) in static tests	48-hour LC_{50} = 0.0020 mg a.i./L (95% CI = 0.0018-0.0023 mg a.i./L)	Waller et al. 1993
Rainbow trout (*Salmo gairdneri*), 81 mm (69-102 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.0016 ppm	Rowe-Rowe 1971
Rainbow trout (*Salmo gairdneri*), average length: 33mm; average weight: 0.3 g	Rotenone formulation: Cubé resin extract (33.7% rotenone): 14.39% Piperonyl butoxide: 19.71% Tergitol: 24.90% Aerosol OT: 4.74% Oil Yellow G Extra: 4.74% Xylene: 31.52% Formulation contained 4.85% rotenone	24-hour LC_{50} = 31 (28-35) µg/L 48-hour LC_{50} = 28 (24-34) µg/L 96-hour LC_{50} = 27 (23-31) µg/L The test results are expressed as the weight of the formulation in µg/L of test water.	Bridges and Cope 1965
Rainbow trout (*Salmo gairdneri*), yearlings	Commercially available rotenone (1% in derris powder	96-hour LC_{50} = 0.350 ppm	Skadsen et al. 1980

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Dactinol (5% rotenone) under standard constant flow-through conditions in water with a hardness of 270 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 7.3 mg/L 48-hour LC_{50} = 5.8 mg/L Concentrations as formulation.	Tooby et al. 1975
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Dactinol (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 0.58 mg/L 48-hour LC_{50} = 0.47 mg/L	Tooby et al. 1975
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Murphy's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 270 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 3.1 mg/L 48-hour LC_{50} = 2.6 mg/L	Tooby et al. 1975
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Murphy's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone.	24-hour LC_{50} = 0.39 mg/L 48-hour LC_{50} = 0.34 mg/L	Tooby et al. 1975
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Bugge's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 270 mg/L, expressed as calcium carbonate. Solvent probably acetone	24-hour LC_{50} = 1.6 mg/L 48-hour LC_{50} = 1.2 mg/L	Tooby et al. 1975
Rainbow trout, (*Salmo gairdneri*), yearlings, 3.0 cm	Bugge's Liquid Derris (5% rotenone) under standard constant flow-through conditions in water with a hardness of 20 mg/L, expressed as calcium carbonate. Solvent probably acetone	24-hour LC_{50} = 0.39 mg/L 48-hour LC_{50} = 0.35 mg/L	Tooby et al. 1975
Rainbow trout, 0.30 g	Rotenone (44.00% technical material) Static exposure	24-hour LC_{50} = 31 µg/L (95% CI = 27-36 µg/L) 96-hour LC_{50} = 26 µg/L (95% CI = 20-32 µg/L)	Mayer and Ellersieck 1986

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
Rainbow trout, 4.5 g, 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 96 hours	96-hour LC_{50} = 0.005 mg/L (95% CI = 0.004-0.006 mg/L)	Holcombe et al. 1987
Rainbow trout	Technical grade, 96 hour exposure	96-hour LC_{50} = 1.94 (1.7-2.2) µg/L	U.S. EPA/OPP 2006c, MRID 439751-02
Rainbow trout	Noxfish	96-hour LC_{50} = 11.5 (10.14 – 13.05) µg/L	U.S. EPA/OPP 2006c, Accession No. 121873
Red-chested tilapia (*T. melanopleura*), 68 mm (55-100 mm)	Derris powder containing 6.5% rotenone	24-hour LC_{50} = 0.012 ppm	Rowe-Rowe 1971
Silver carp (*Hypopthalmichthyes molitrix*	Noxfish (containing 5% rotenone)	96-hour LC_{50} = 0.0558 ppm (95% CI =0.03388-0.0803)	Marking and Bills 1981
Simocephalus serrulatus, 1st instar	Rotenone (44.00% technical material) Static exposure	48-hour EC_{50} = 310 µg/L (95% CI = 239-402 µg/L)	Mayer and Ellersieck 1986
Spotted snakeheads (*Channa punctata*), three large or four small fish/tank	Rotenone (NOS) in well water in unaerated tanks	At 2.0 ppm, average mortality was 75% at 24 hours and 100% at 48 hours At 2.5 ppm, mortality was 100% at 24 hours. Detoxification, determined by the survival of carp fry, required 6 days at 2.5 ppm.	Perschbacher and Sarkar 1989
Striped bass (*Morone saxatilis*), fingerlings, 35-51 mm long, 2/test container	Cube root (5% rotenone)	No mortality at 0.001 ppm; 100% mortality at 0.01 ppm	Hughes 1973
Striped bass (*Morone saxatilis*), larvae, 10/test container	Cube root (5% rotenone)	No mortality at 0.001 ppm; 100% mortality at 0.01 ppm	Hughes 1973

Freshwater Fish – Acute			
Species	**Exposure**	**Effects**	**Reference**
White sucker, 4.1 g, 10/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 96 hours	96-hour LC_{50} = 0.011 mg/L (95% CI = 0.009-0.014 mg/L)	Holcombe et al. 1987
Zebrafish (*Danio rerio)* fry	Rotenone technical (purity not specified) 5 or 10 µg/L for 4 days	No effects on locomotor activity.	Bretaud et al. 2004
Zebrafish (*Danio rerio)* fry	Rotenone technical (purity not specified) 30 or 50 µg/L for 4 days	No effects on morphology at 30 µg/L. All fish died at 50 µg/L.	Bretaud et al. 2004

Appendix 4, Supplemental Table 1:
Toxicity of Noxfish to fish in standardized laboratory tests at 12° C
(taken from Marking and Bills 1976). *See Section 4.1.3.1 for discussion.*

	LC_{50} and 95% confidence interval (µg/ L) at			
Organism	**3 hours**	**6 hours**	**24 hours**	**96 hours**
Rowfish (*Amia calva)*	141 114-174	106 82.5-136	57.5 50.4-65.5	30.0 23.7-38.0
Coho salmon (*Oncorhynchus kisutch*)	358 --	152 105-219	71.6 63.1-81.3	62.0 54.8-70.2
Chinook Salmon (*O. tshawytscha*)	212 171-262	156 137-177	49.0 44.3-54.2	36.9 33.9-40.2
Rainbow trout (*Salmo gairdneri*)	175 160-191	86.9 --	68.9 56.2-84.4	46.0 32.6-64.9
Atlantic salmon (*S. salar*)	61.5 53.4-70.8	40.0 33.6-70.8	35.0 29.7-41.2	21.5 15.5-29.8
Brook trout (*Salvelinus fontinalis*)	141 124-160	79.7 69.2-91.8	47.0 42.2-52.3	44.3 41.1-47.7
Lake trout (*S. namaycush*)	50.0 38.6-64.7	28.3 21.0-38.0	26.9 19.8-36.5	26.9 19.8-36.5
Northern pike (*Esox lucius*)	181 160-204	58.2 52.5-64.5	44.9 31.4-64.3	33.0 26.6-41.0
Goldfish (*Carassius auratus*)	-- --	-- --	-- --	497 412-600
Carp (*Cyprinus carpio*)	-- --	270 254-287	84.0 74.7-94.4	50.0 41.1-60.8
Fathead minnow (*Pimephales promelas*)	-- --	1190 917-1453	400 291-549	142 115-176
Longnose sucker (*Catostomus catostomus*)	388 332-454	218 141-337	67.2 59.3-76.1	57.0 51.9-62.6
White sucker (*C. commersoni*)	630 452-878	238 186-304	71.9 64.0-80.8	68.0 54.0-85.6
Black bullhead (*Ictalurus melas*)	-- --	-- --	665 516-856	389 298-507
Channel catfish (*I. punctatus*)	1410 1139-1745	840 717-984	400 234-684	134 138-196
Green sunfish (*Lepomis cyanellus*)	389 332-456	332 249-443	218 197-241	141 114-174
Bluegill (*L. macrochirus*)	424 335-537	336 245-461	149 124-178	141 133-149
Smallmouth bass (*Micropterus dolomieui*)	277 219-350	165 --	93.2 85.1-102	79.0 70.7-88.2
Largemouth bass *(M. slamoides*)	514 449-588	360 305-425	200 131-305	142 115-176
Yellow perch (*Perca flavescens*)	150 126-179	134 120-149	92.0 80.1-106	70.0 59.8-82.0
Walleye *(Stizostedion vitreum vitreum*)	136 103-176	52.4 46.8-58.7	16.5 15.2-17.9	-- --

Freshwater Fish – Longer term exposures			
Species	**Exposure**	**Effects**	**Reference**
Rainbow trout (*Salmo gairdneri*), eyed eggs and early larval stages	Crystalline technical grade rotenone (96.47% pure) Duration of continuous flow-through exposure: 32* days (egg stage to fry stage) Nominal test concentrations: 1.0-10.0 µg/L Mean measured (± SD) concentrations: 0, 1.01±0.09, 2.21 ±0.266. 2.75±0.424, 4.37±0.092, 5.32±0.197, 7.52±0.577, or 10.0±0.436 µg/L *Materials and Methods section of study indicates that exposure was 28 days; everywhere else in the study, the exposure duration is defined as 32 days.	No adverse effect on eyed eggs or hatching; all eggs hatched on the 5th or 6th day of exposure. 90% mortality within 15 days among larvae exposed to 2.75 µg/L; 100% mortality within 5 days among all larvae exposed to concentrations ≥4.37 µg/L. Growth was significantly less ($p<0.05$) in fry that survived exposure to 2.21 or 2.75 µg/L, relative to controls; all surviving larvae reached swim-up-stage and appeared to be searching for food. 32-day LC_{50} = 2.08 µg/L (95% CI = 1.98-2.18) 32-day LC_{01} = 1.00 µg/L (95% CI = 0.894-1.12)	Bills et al. 1988 Summarized in U.S. EPA/OPP 2006c as MRID 400633-02 from a 1986 report by Bills et al. **OPP used a NOAEC of 1.01 µg a.i./L for longer-term effects in fish.**
Zebrafish (*Danio rerio)*	Rotenone technical (purity not specified) 2 µg/L for 4 weeks	No effect.	Bretaud et al. 2004

Saltwater Fish - Acute			
Species	**Exposure**	**Effects**	**Reference**
Two-spotted goby (*Gobiusculus flavescens*), larvae	Exposure to 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, or 10.0 ppm rotenone (mixture manufactured by Gullvik in Sweden and *almost identical with Pro-Noxfish*) **NOTE**: Gullvik is an emulsifiable concentrate containing 2.65% pure rotenone and an equal amount of a sulfoxide synergist.	36-hour LC_{50} = 0.1 ppm 16-hour LC_{50} = 0.25 ppm 5-hour LC_{50} = 0.5 ppm	Naess et al. 1991

Saltwater Fish - Acute			
Species	**Exposure**	**Effects**	**Reference**
Four species of marine reef fish: Bermuda Porgy (D*iplodus bermudensis*), Long-spine Squirrelfish (*Holocentrus rufus*), French Grunt (*Haemulon flavoleneatum*), and Blue stripped Grunt (*Haemulon sciurus*)	Test concentrations of 5, 25, 50, 75, or 250 µg/L rotenone (extracted from Cube root, 46.6%) for 45 minutes.	*Haemulon flavoleneatum* and *Holocentrus* have low tolerance to rotenone (see tables of ventilation rates in study); whereas, D*iplodus bermudensis* and *Haemulon sciurus* have a greater tolerance to rotenone. Exposure to 5 or 25 µg/L test concentration caused depression of the ventilator rate, which may indicate an avoidance response common to teleosts exposed to environmental toxins; exposure to >25 µg/L test concentration caused an oscillating ventilatory response, which also may be an avoidance response; at the highest test concentrations (NOS), exposure resulted in increased variability of ventilator patterns.	Wingard and Swanson 1992
Saltwater Fish - Chronic			
Four species of Atlantic reef fish (*Haemulon scirurus*, *H. fiavolineatum*, *Holocentrus rufus*, and *Mugil curema*)	5, 25, 50, or 75 µg/L rotenone (extracted from Cube root, 46.6%) until *ultimate lethality*.	At dose ≥50 µg/L, all four species showed a dose-dependent decreased *rate and amplitude*, monitored by opercular impedance electrodes routed to an A/D recording system. Investigators conclude that the sensitivity of these marine test species to rotenone toxicity is similar to that of freshwater species. **Note**: This is an abstract of an efficacy study and does not provide a lot of detail.	Swanon et al. 1989

General note: Except for the studies by Holcombe et al. 1987 and Hashimoto and Nishiuchi 1981, it is unclear if the concentrations reported in this appendix refer to rotenone or to the formulation.

Species	Exposure	Effects	Reference
Aquatic Exposures			
Southern leopard frog larva (*Rana sphenocephala*)	Static tests involving 1- to 96- hour exposure to Noxfish (emulsifiable concentrate containing 5% rotenone) Controls: acetone or untreated water	1-hour LC_{50} = 0.830 mg/L (CI = 0.795-0.867 mg/L) 3- hour LC_{50} = 0.775 (CI = 0.740-0.812) 6-hour LC_{50} = 0.635 (CI = 0.596-0.677) 24-hour LC_{50} = 0.580 (CI = 0.494-0.680) 96-hour LC_{50} = 0.500 (CI = 0.423-0.591) Above values are concentrations of formulation as reported by Chandler and Marking (1982) and not a.i.	Chandler and Marking 1982
Southern leopard frog, tadpole (*Rana pipiens*)	Powdered derris in water (5% rotenone)	Lethal concentration = 100 µg/L Corresponds to 5 µg/L rotenone	Hamilton 1941
Southern leopard frog, adult (*Rana pipiens*)	Noxfish (5% w/w)	24-hour LC_{50} = 240 µg/L 96-hour LC_{50} = 240 µg/L	Farringer 1972
Southern leopard frog, adult (*Rana pipiens*)	Noxfish (NOS)	24-hour LC_{50} = 1200 µg/L 96-hour LC_{50} = 290 µg/L	Farringer 1972
Southern leopard frog, adult (*Rana pipiens*)	Dri-Noxfish (20% powder)	24-hour LC_{50} = 1460 µg/L 96-hour LC_{50} = 920 µg/L	Farringer 1972
Southern leopard frog, adult (*Rana pipiens*)	Dri-Noxfish (NOS)	24-hour LC_{50} = 1580 µg/L 96-hour LC_{50} = 640 µg/L	Farringer 1972
Tiger salamander (*Ambystoma tigrinum*)	Powdered derris in water (5% rotenone)	Lethal concentration = 100 µg/L after metamorphosis. 16.6 µg/L were *"toxic but not fatal"*	Hamilton 1941
Japanese Common Toad, tadpole (*Bufo bufo japonicas*)	Rotenone, technical grade	48-hour LC_{50} = 0.33 ppm (a.i.)	Hashimoto and Nishiuchi 1981
Tadpoles (*Xenopus*), 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 96 hours	96-hour LC_{50} >0.040 mg a.i./L This study involves the simultaneous exposure of multiple species in separate compartments of an individual exposure tank. Table 5 of the study ranks the tested species, including fish and invertebrates, in order of their sensitivity.	Holcombe et al. 1987
Frog (NOS)	Rotenone , oil solution (NOS)	LD_{50} = 4 mg/kg This appears to be a study of adult frogs (i.e., terrestrial phase exposure).	Hayes 1982

Note: Freshwater species followed by saltwater species in separate tables. Tables sorted by author.

Freshwater Acute

Species	Exposure	Effects	Reference
Amphipod (*Gammarus fasciatus*), 10/test concentration	Rotenone (95.0%), 0.009-3.3 ppm (at approximately 10% intervals), 48 hour exposure.	48-hour $LC_{50} \approx 0.95$ ppm Microscopic examination revealed no change in structure or deterioration of gills.	Claffey and Costa 1974
Amphipod *(Gammarus fasciatus)*, immature	Rotenone (44.00% technical material)	24-hour $LC_{50} = 6000$ µg/L (95% CI =5000-7200 µg/L) 96-hour $LC_{50} = 2600$ µg/L (95% CI = 2100-3200 µg/L)	Mayer and Ellersieck 1986
Amphipod (*Gammarus lacustris*)	Technical grade rotenone	96-hour $LC_{50} = 3.52$ ppm	Nebeker and Gaufin 1964
Amphipod (*Gammarus lacustris*)	Rotenone, technical grade	Estimated acute toxicity values: 24-hour $LC_{50} = 6000$ µg/L (95% CL = 5000-7200 µg/L) 48-hour $LC_{50} = 3500$ µg/L (95% CL = 2900-4300 µg/L) 96-hour $LC_{50} = 2600$ µg/L (95% CL = 2100-3200 µg/L)	Sanders 1969
Bivalve, Pearl mussels (*Margaritifera margaritifera*), 9 medium- to large-sized mussels/test aquarium	Rotenone formulation: Gullviks' rotenone (manufactured in Sweden) which is almost identical to Pro-Noxfish (2.5% rotenone and 2.5% sulfoxide) Test concentrations: 0, 5, 10, 15, 20, 30, 40, or 50 ppm Exposure duration: 12 hours	No mortality at 30 ppm; at ≥40 ppm, mussels survived treatment, but died less than 1 week post exposure.	Dolmen et al. 1995 [Field study portion summarized in Appendix 7]
Bivalve, Unionid mussel (threehorn wartyback, *Obliquaria reflexa*), 30-50 mm	Rotenone formulation: Noxfish (5% a.i.) in static tests	48-hour $LC_{50} > 1.0$ mg/L* 48- hour ***post-exposure**** $LC_{50} = 0.518$ mg/L (95% CI = 0.421-0.636 mg/L) *<50% mortality in highest test concentration. **mussels held in untreated (reference) water for an additional 48 hours.	Waller et al. 1993

<table>
<tr><th>Species</th><th>Exposure</th><th>Effects</th><th>Reference</th></tr>
<tr><td>Bivalve, Zebra mussel (Dreissena polymorpha), 20-25 mm</td><td><u>Rotenone formulation</u>: Noxfish (5% a.i.) in static tests</td><td>48-hour LC_{50} = 0.219 mg/L
(95% CI = 0.131-0.365 mg/L)

48- hour post-exposure* LC_{50} = 0.228 mg/L
(95% CI = 0.157-0.329 mg/L)

*mussels held in untreated (reference) water for an additional 48 hours.</td><td>Waller et al. 1993</td></tr>
<tr><td>Bivalve, Zebra mussel (Dreissena polymorpha), 5-8 mm</td><td><u>Rotenone formulation</u>: Noxfish (5% a.i.) in static tests</td><td>48-hour LC_{50} = 0.165 mg/L
(95% CI = 0.147-0.185 mg/L)

48- hour post-exposure* LC_{50} = 0.149 mg/L
(95% CI = 0.129-0.172 mg/L)

*mussels held in untreated (reference) water for an additional 48 hours.</td><td>Waller et al. 1993</td></tr>
<tr><td>Bivalve, Zebra mussel (Dreissena polymorpha), four larval stages: pre veliger (no shell or velum); D-stage veliger (NOS); post D-stage (umbonal); and planti grade (shell length <0.5 mm with siphons retracted) and two adult stages: (5-8 mm) and (20-25 mm)</td><td><u>Rotenone formulation</u>: Noxfish (5% a.i.)</td><td>Rotenone Toxicity to Zebra Mussel Life Stages
<table><tr><th>Life Stage</th><th>24-hour LC_{50}</th></tr><tr><td>PreVeliger</td><td>232.0 µg/L</td></tr><tr><td>D-Stage</td><td>230.0 µg/L</td></tr><tr><td>Post D-Stage</td><td>264.0 µg/L</td></tr><tr><td>Plantigrade</td><td>275.0 µg/L</td></tr><tr><td>Adult (5-8 mm)</td><td>161.0 µg/L</td></tr><tr><td>Adult (20-25 mm)</td><td>155.0 µg/L</td></tr></table></td><td>Fisher et al. 1994</td></tr>
<tr><td>Cladoceran (Daphnia magna) <24 hours old, 20/1600 mL water</td><td>0.5-10.0 µg/L analytical grade rotenone (96.47% pure)</td><td>48-hour EC_{50} = 3.7 µg/L</td><td>Rach et al. 1988</td></tr>
<tr><td>Cladoceran (Daphnia magna), 0-24 hours, 20/test concentration</td><td>Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS)

Rotenone dissolved in DMF

Exposure period: 48 hours</td><td>48-hour EC_{50} = 0.008 mg/L
(95% CI = 0.007-0.010 mg/L)</td><td>Holcombe et al. 1987</td></tr>
<tr><td>Cladoceran (Daphnia pulex)</td><td>Rotenone, technical grade</td><td>3-hour LC_{50} = 0.57 ppm</td><td>Hashimoto and Nishiuchi 1981</td></tr>
</table>

Species	Exposure	Effects	Reference
Cladoceran (*Daphnia pulex*), 10/test concentration	Rotenone (95.0%), 0.009-3.3 ppm (at approximately 10% intervals), 48 hour exposure.	48-hour LC_{50} ≈0.065 ppm 70.0% mortality at 0.1 ppm Microscopic examination revealed no change in structure or deterioration of organs.	Claffey and Costa 1974
Cladoceran (*Moina macropcopa*)	Rotenone, technical grade	3-hour LC_{50} = 2.0 ppm	Hashimoto and Nishiuchi 1981
Cladoceran (*Simocephalus serrulatus*), first star, 10/test beaker	Rotenone (NOS) Ethanol solvent	Immobilization 48-hour EC_{50} = 190 µg/L (95% CL = 140-260 µg/L)	Sanders and Cope 1966
Cladoceran, *Daphnia magna*, first star, 10/test beaker	Rotenone (NOS) Ethanol solvent	Immobilization 48-hour EC_{50} = 100 µg/L (95% CL = 75-130 µg/L)	Sanders and Cope 1966
Cladoceran, *Daphnia pulex*, 1st instar	Rotenone (44.00% technical material) Static exposure	48-hour EC_{50} = 100 µg/L (95% CI = 74-134 µg/L)	Mayer and Ellersieck 1986
Crayfish (*Cambarus bartoni*), 10/test concentration	Rotenone (95.0%), 0.009-3.3 ppm (at approximately 10% intervals), 48 hour exposure.	48-hour LC_{50} ≈ 2.0 ppm Microscopic examination revealed no change in structure or deterioration of gills.	Claffey and Costa 1974
Cyclopoid (*Cyclops vernalis*), 10/test concentration	Rotenone (95.0%), 0.009-3.3 ppm (at approximately 10% intervals), 48 hour exposure.	48-hour LC_{50} ≈ 0.085 ppm 60. 75% mortality at 0.1 ppm Microscopic examination revealed no change in structure or deterioration of organs.	Claffey and Costa 1974
Dragonflies (*Basiaeschna janata*), naids, 33.0-46.5 mm body length, 10/test	0.05, 0.1, or 0.5 mg/L rotenone (NOS) in aerated water	96-hour LC_{50} = 0.22 mg/L	Watkins and Tartar 1975
Mayfly (*Cloeon dipterum*)	Rotenone, formulated product (NOS)	48-hour LC_{50} = 0.056 ppm	Hashimoto and Nishiuchi 1981
Midges, 3rd & 4th instar, 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in DMF Exposure period: 48 hours	48-hour LC_{50} >0.040	Holcombe et al. 1987
Mosquito larvae (*Aedes aegypti*)	Rotenone (NOS), 3 hour exposure period	EC_{50} (inhibition of phototaxis): > 10 ppm	Burchfield and Storrs 1954

Species	Exposure	Effects	Reference
Several species	Static tests involving 1- to 96- hour exposure to Noxfish (emulsifiable concentrate containing 5% rotenone) Controls: acetone or untreated water	See Supplemental Table 1 below	Chandler and Marking 1982
Snail (*Indoplanorbis exustus*)	Rotenone, technical product (NOS)	48-hour LC_{50} = 27 ppm 48-hour exposure to a minimum concentration of 0.32 ppm caused the snails to contract their body muscle.	Nishiuchi and Yoshida 1972
Snail (*Physa acuta*)	Rotenone, technical product (NOS)	48-hour LC_{50} = 6.8 ppm	Nishiuchi and Yoshida 1972
Snail (*Semisulcospira libertine*)	Rotenone, technical product (NOS)	48-hour LC_{50} = 8.0 ppm 48-hour exposure to a minimum concentration of 0.32 ppm caused the snails to contract their body muscle.	Nishiuchi and Yoshida 1972
Snail, *Aplexa hypnorum*, adults, 20/test concentration	Exposure to measured water concentrations of 0.002-0.040 mg/L rotenone (NOS) Rotenone dissolved in dimethylformamide Exposure period: 96 hours	96-hour LC_{50} >0.040 mg/L This study involves the simultaneous exposure of multiple species in separate compartments of an individual exposure tank. Table 5 of the study ranks the tested species, including fish and amphibians, in order of their sensitivity.	Holcombe et al. 1987
Snail, Chinese mystery snail (*Cipangopaludina malleata*)	Rotenone, technical product (NOS)	48-hour LC_{50} = 15 ppm	Nishiuchi and Yoshida 1972
Snail, Marsh snail (*Semisulcospira libertine*)	Rotenone, technical grade	48-hour LC_{50} = 8.0 ppm	Hashimoto and Nishiuchi 1981
Snail, Pond snail (*Physa acuta*)	Rotenone, technical grade	48-hour LC_{50} = 6.8 ppm	Hashimoto and Nishiuchi 1981

Species	Exposure	Effects	Reference
Snail, Pond snails (*Lymnaesa stagnalis*), adults, 20/group	0.1-5 µM rotenone (stock solution containing a maximum of 0.01% DMSO) for up to 10 days.	4-day LC_{50} = 0.84 µM or about 330 µg/L water Exposure resulted in progressive and irreversible behavioral deficits that were dose and time dependent. 100% mortality occurred at 5 µM (1,900 µ/L) on day 4 of exposure. Minimal effects over 10 day exposures to 0.1 µM (39 µg/L).	Vehovsky et al. 2007
Snail, Red snail (*Indo planorbis exustus*)	Rotenone, technical grade	48-hour LC_{50} = 27 ppm	Hashimoto and Nishiuchi 1981
Stoneflies (*Pteronarcys californica*)	Rotenone (NOS)	24-hr LC_{50} = 2900 µg/L 48-hr LC_{50} = 900.0 µg/L 96-hr LC_{50} = 250.0 µg/L	Cope 1965
Stoneflies (*Pteronarcys californica*)	Rotenone (NOS)	24-hr LC_{50} = 2900 (2300-3600) µg/L 48-hr LC_{50} = 1100 (800-1500) µg/L 96-hr LC_{50} = 380 (280-520) µg/L	Sanders and Cope 1969
Stoneflies (*Pteronarcys californica*), immature	Rotenone formulation: *Cubic resin extract* (33.7% rotenone): 14.39% *Piperonyl butoxide*: 9.71% *Tergitol*: 24.90% *Aerosol OT*: 4.74% *Oil Yellow G Extra*: 4.74% *Xylene:* 31.52% Formulation contained 4.85% rotenone.	24-hr LC_{50} = 2900 (2300-3600) µg/L 48-hr LC_{50} = 900 (680-1200) µg/L 96-hr LC_{50} = 250 (200-310) µg/L The test results are expressed as the weight of the formulation in µg/L of test water.	Bridges and Cope 1965
Stoneflies (*Pteronarcys californica*), naids, last instar, 30-35 mm, 10/test concentration	Rotenone, technical grade. Test conducted under static conditions without aeration.	Estimated acute toxicity values: 24-hour LC_{50} = 2900 (2300-3600) µg/L 48-hour LC_{50} = 1100 (800-1500) µg/L 96-hour LC_{50} = 380 (280-520) µg/L	Sanders and Cope 1968

Note on Cope Studies on Stoneflies: The Bridges and Cope (1965) paper clearly describes the toxicity values as pertaining to a 4.85% formulation. The other Cope publications do not note this.

Supplemental Table 1: Acute toxicity of Noxfish (5% a.i.) to aquatic invertebrates in limed water in static tests at 16 ±·1° C (taken from Chandler and Marking 1982)

NB: All values appear to be given as Formulation and not a.i. but this is not explicitly stated in the publication. This interpretation of the reported units is consistent with that by U.S. EPA/OPP 2006c, p. 148.

	LC_{50} and 95% confidence interval (mg/L) at				
Organism	**1 hour**	**3 hours**	**6 hours**	**24 hours**	**96 hours**
Flatworm (*Catenula* sp.)	-- --	8.95 8.27-9.68	6.40 4.72-8.68	5.10 3.70-7.03	1.72 1.15-2.57
Daphnid (*Daphnia pulex*)	0.118 0.102-0.137	0.0960 0.0807-0.114	0.0360 0.0317-0.0409	0.0275 0.0239-0.0316	-- --
Ostracod (*Cypridopsis* sp.)	2.80 2.35-3.34	2.55 2.11-3.08	2.15 1.80-2.56	0.490 0.299-0.803	0.340 0.280-0.557
Freshwater prawn (*Palaemonetes kadiakensis*)	28.3 22.8-35.0	24.0 19.9-28.9	6.35 5.43-7.43	5.15 4.44-6.00	1.12 0.760-1.65
Dragonfly naiad (*Macromia* sp.)	-- --	275 230-329	34.0 19.6-58.9	4.70 1.45-15.2	1.00 0.730-1.59
Backswimmer (*Notonecta* sp.)	105 86.5-128	21.0 17.7-25.0	9.00 6.79-11.9	3.42 2.27-5.15	1.58 0.727-3.44
Caddisfly larva (*Hydropsyche* sp.)	10.7 7.98-14.5	8.00 6.69-9.56	3.55 2.88-4.38	-- --	0.605 0.329-1.17
Whirligig beetle, adult (*Gyrinus* sp.)	47.5 32.6-69.2	8.30 5.42-12.7	8.00 5.51-11.6	3.55 2.05-6.15	0.700 0.400-1.21
Snail (*Physa pomilia*)	-- --	-- --	-- --	6.35 5.61-7.19	4.00 3.45-4.63
Snail (*Oxytrema catenaria*)	-- --	-- --	-- --	-- --	1.75 1.00-3.06
Snail (*Helisoma* sp.)	-- --	33.5 28.0-40.1	33.5 28.0-40.1	30.0 24.1-37.3	7.95 4.63-13.7
Buckley's filter clam (*Elliptio buckleyi*)	-- --	-- --	-- --	-- --	2.95 2.23-3.90
Flattened filter clam (*Elliptio complanata*)	-- --	-- --	-- --	-- --	2.00 1.53-2.61
Asiatic clam (*Corbicula manilensis*)	-- --	-- --	-- --	-- --	7.50 5.74-9.81

Saltwater Acute

Species	Exposure	Effects	Reference
Oysters (*C. virginica*), yearlings, approximately 1.5" long, shells filed with iron rasp to remove all soft new shell growth on valve edges, 50 oysters/test	Rotenone (98%) for 24 hours	Minimum effective concentration for the inhibition of shell growth = 0.01 ppm.	Butler et al. 1960
Oysters (*Ostrea edulis*)	Rotenone, 4 aquaria with nominal 0.75 ppm to 1.00 ppm treatments.	Measured concentrations of about 9 ppb to 23 ppb in two aquaria and 8 ppb to 31 ppb in a third aquarium. No mortality in the two aquaria with lower concentrations (N=94 per aquaria). In the two aquaria with higher measured concentrations, mortality of 21/150 (14%) and 7/50 (14%) by day 22. Mortality attributed to oxygen depletion.	Samuelsen et al. 1988
Tiger shrimp (*Penaeus monodon*), juveniles, average weight: 14 ± 3.6 g,	Static toxicity tests with 0.001, 0.01, 1.0, 10 or 50 ppm rotenone (95-95%) for 96 hours.	No mortality at any test concentration; statistically significant ($p<0.05$) percentages of soft-shelling, relative to controls, was observed in shrimp exposed to rotenone test concentrations ≥1.0 ppm; concentrations ≥1.0 caused shrimp to be passive (i.e., easily handled) within 15 minutes of exposure, and the effect was reversible within 24 hours after exposure.	Cruz-Lacierda 1993
Copepod (*Acartia clausi)*, males and females	Exposure to 0.01, 0.05, 0.10, 0.50, or 1.00 ppm rotenone (mixture manufactured by Gullvik in Sweden and *almost identical with Pro-Noxfish*) **NOTE**: Gullvik is an emulsifiable concentrate containing 2.65% pure rotenone and an equal amount of a synergist, sulfoxide.	Adult males significantly less tolerant to rotenone: 50% mortality occurred at 0.05 ppm after 4 hours for males, 18 hours for females, and 16 hours for copepodids. At 0.50 ppm, 100% mortality occurred within 2 hours for all stages.	Naess 1991

Species	Exposure	Effects	Reference
Chameleon shrimp (*Praunus flexuous*), larvae	Exposure to 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, or 10.0 ppm rotenone (mixture manufactured by Gullvik in Sweden and *almost identical with Pro-Noxfish*) **NOTE**: Gullvik is an emulsifiable concentrate containing 2.65% pure rotenone and an equal amount of a synergist, sulfoxide	>48-hour LC_{50} = 1.0 ppm >48-hour LC_{50} = 2.0 ppm 27-hour LC_{50} = 5.0 ppm	Naess et al. 1991
Carid shrimp (*Leander squilla*), larvae	Exposure to 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, or 10.0 ppm rotenone (mixture manufactured by Gullvik in Sweden and *almost identical with Pro-Noxfish*) **NOTE**: Gullvik is an emulsifiable concentrate containing 2.65% pure rotenone and an equal amount of a synergist, sulfoxide	>48-hour LC_{50} = 2.0 ppm >48-hour LC_{50} = 5.0 ppm 19-hour LC_{50} = 10.0 ppm	Naess et al. 1991

Freshwater Chronic

Species	Exposure	Effects	Reference
Daphnia magna, <24 hours old, 20/1600 mL water	0.312-5.0 µg/L analytical grade rotenone (96.47% pure) for 21 days.	21-day EC_{50} = 2.1 µg/L NOEC = 1.25 µg/L	Rach et al. 1988
Pond snails (*Lymnaesa stagnalis*), adults, 12 treated and 12 controls	5 µM rotenone (stock solution containing a maximum of 0.01% DMSO) for up to 10 days.	On days 2 and 3 of exposure, signs of toxicity included severe postural and behavioral abnormalities which led to cessation of movement and feeding by day 7 and eventually death.	Vehovsky et al. 2007
Vehovsky et al. 2007 (*continued*): The investigators indicate that pond snails are exposed to rotenone via dermal absorption and oral ingestion (while feeding) and that the LC_{50} of 0.8 µM or 0.34 mg/L water indicates that pond snails are more sensitive that aquatic mollusks but less sensitive than fish to rotenone exposure.			

Application	Observations	Reference
Derris root powder (5% rotenone) equivalent to 0.75 ppm applied by boat over the surfaces of Patricia Lake (Sept 7, 1966 between 7 am and 6 pm) and Celestine Lake (Sept 26, 1967 between 9:30 am and 1:30 pm) under calm weather conditions and comparatively high hypoilimnetic oxygen concentrations in both lakes. [Eradication of fish complete in both lakes.]	Very little effect on phytoplankton and only temporary suppression of rotifers. Reappearance of some species within 6 months of initial devastation of crustacean zooplankton, with most reappearing after 10 months in numbers that exceeded those before treatment. *Daphnia galeata mendotate* did not appear in post-treatment samples from Celestine Lake; however investigators speculate that single or immature specimens may have been undetected among numerous immature *Daphnia pulex*. Study indicates that 3 years is the minimum time required for zooplankton to recover to pretreatment levels of species diversity and abundance.	Anderson 1970
6 µL/L of 2.5% rotenone applied to a pond with a surface area of 0.48 ha, a volume of 247,000 L, and a center depth of 2 meters located on the golf course of Western Illinois Univ. Bottom substrate of the experimental pound was silt-clay with some gravel areas. The control pond, located approximately 150 meters away from the experimental pond, was slightly smaller (0.32 ha, 165,000 L, and maximum depth of 1.8 meters). Both ponds are nutrient enriched by the fertilizer runoff from the golf course.	Within 48 hours, treatment eliminated all zooplankton from the water column, and onset of recovery ranged from 1 to 6 months, with full recovery taking from 6 to 8 months. The first of the zooplankton community to recover were the copepods, followed by the rotifers, and finally the cladocerans, which were not present until 6 months after treatment.	Beal and Anderson 1993
18 orchard ponds. Treated versus untreated ponds in Motueka, New Zealand. Selected ponds in 5 groups: rotenone-free but with pest fish present (n = 4); rotenone free without pest fish (n = 4); and treated with rotenone 6 months (n = 2), 1 year (n = 4), and 3 years (n = 4) prior to population sampling	Few remarkable differences in invertebrate composition. Rotenone treated ponds had higher abundance of some Diptera larvae – i.e., Chironominae and Orthocladiinae. Rotenone free ponds had greater abundance of some diving beetles and flatworms. Rotenone treated ponds may have favored Cladocerans relative to Copepods.	Blakely et al. 2005

Application	Observations	Reference
Formulation: Pro-Noxfish (synergized emulsifiable concentrate containing 2.5% rotenone. Ponds: surface areas about 0.05 ha; maximum depths about 1.1 and 1.5 meters w/clay bottoms. Populations: low populations of zooplankton; moderate populations of phytoplankton and benthic vertebrates; few aquatic plants; no fish. Pretreatment: Applications of 20-20-5 fertilizer and hydrated lime to each pond to stimulate plankton production, increase pH, and increase total hardness to about 20 mg/L. Applications: Pond I: 2 µL/L formulation (0.05 µL/L a.i.); Pond II: 5 µL/L formulation (0.125 µL/L a.i.) on August 24 by outboard motor with a boat bailer. Sampling: 3, 7, 14, 37, and 69 days after treatment. Sampling terminated after 69 days when most groups of benthic organisms had recovered from the treatments.	At both application rates, there was a temporary reduction in total numbers and diversity of benthic invertebrates and complete mortality of caged Asiatic clams (*Corbicula manilensis*). Treatment with 5 µL/L, there was partial mortality of resident population of larval leopard frogs (*Rana pipiens*). At 7 days: benthic organisms (no./m^2) decreased 67% at 2 µL/L concentration and 96% at 5 µL/L concentration. Diversity index decreased sharply in both treated ponds between days 3 and 7 and equitability index decreased from day 3 to day 37. By day 69, 121% increase in benthic organisms at 2 µL/L and 223% increase in benthic organisms at 5 µL/L; increase in control pond virtually unchanged (2% increase). Zooplankton populations remained consistently low throughout the study in both treated and control ponds.	Burress 1982
Treatment: Pronoxfish "at a concentration level of not less than 0.050 active ingredient rotenone." Units are not specified but are presumably in ppm (mg/L). Duration not specified.	Explosive increase in invertebrate drift after application. A trend toward recovery apparent after about 6 months for many groups of invertebrates. An exception is black fly larvae in which no recovery was observed.	Cook and Moore 1969
Treatment: Application rate and formulation not specified. *Rotenone… was administered until* [fish] *mortality.. was observed.*	Increase in invertebrate drift, typically by 2 orders of magnitude (see Fig. 2, p. 41 in paper). Substantial impact on mayflies (Ephemeroptera). Very little impact on benthics (subsurface habitat).	Dudgeon 1990

Application	Observations	Reference
Formulation: Noxfish (5% a.i.) Ponds: three similar ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri with average dimensions: ***standing water mass***: 21.4 x 15.6 x 0.6 m ***surface area***: 0.03 ha ***volume***: 251.3 m^3 ***pond bottom***: sloped ***water depth***: 0.3-1.2 m Soil type: Mexico silt loam Ponds contained large beds of vegetation and no fish. Applications: 0.5 or 2.0 mg/L Noxfish formulation in late August	No effects at either concentration on species diversity, emergence, seasonal dynamics, abundance, or relative numbers of invertebrate macrobenthos. The investigators conclude that the environmental factors influencing the results of the study were the large beds of vegetation, which increased the number of available niches, and the absence of fish to prey upon the benthic organisms.	Houf and Campbell 1977
Application: 2.5 ppm dosage achieved with forty-five 55- gallons of 5% liquid emulsifiable synergized rotenone (provided by Roussel Bio Corp). Backpack application requiring 15-20 personnel in four boats and two barges to Hyatt Lake (reservoir) of Jackson County. Oregon on October 12, 1989. Purpose of application: to eradicate undesirable fish (brown bullheads). Characteristics of Hyatt Lake: eutrophic lake, covers 987 acres with a volume of 16,900 acre feet, and average depth of 18 feet when full. Bottom >99% silt w/trace of clay and fine-rained sand.	Treatment caused a reduction in the number and diversity of live invertebrates for up to a couple of weeks, with little improvement observed by 28 days when the number of live organisms increased. 1 year after treatment, nontarget organisms were present in greater diversity and equivalent abundance, relative to pre-treatment conditions. Investigator concludes that there were no long-term adverse effects of rotenone treatment on the nontarget organism collected in the study.	Linn 2002
Application: Stream treatments. Target concentration of 50 ppb. 300-350 mL of 5% rotenone every 15 minutes along with tracer dye. Followed by potassium permanganate once rotenone had reached the end of the area to be treated.	"*Catastrophic*" drift of macroinvertebrates during treatment. Decrease in benthic abundance after treatment. Greatest impact on dipterans with recovery in 7 weeks. Also substantial effects on Ephemeroptera and Trichoptera. Little impact on chironomids (midges) probably due to subsurface habitat.	Lintermans and Raadik 2001

Application	Observations	Reference
Application: Rotenone (Noxfish w/5% by volume a.i.) applied by drip barrels to achieve 3 mg/L Noxfish on the streams. Rotenone applied twice: early to mid-August and from September 25 through October 16. Target Concentration: To maintain a 0.15 mg/L a.i. concentration in stream channels for 48 hours. Application site: Entire Strawberry River watershed in Utah. Sampling: Pretreatment quantitative sampling of benthic invertebrate communities; Post treatment, samples were taken monthly at each of four Strawberry River stations during spring, summer, and fall for 5 years.	Treatment significantly affected species density of mayflies, stoneflies and caddisflies (*Cinygmula sp*., *Pteronarcella badia*, *Hesperoperla pacifica*, *Hydropsyche* sp., and *Brachycentrus americanus*); and 100% of mayflies, stoneflies, and caddisflies were missing after the second rotenone application. Resistance to rotenone was observed in 33% of the benthic invertebrate taxa at the four stations. 46% of the affected benthic invertebrates recovered within 1 year; however, 21% of the taxa were still missing after 5 years. Of the 19 taxa still missing, 47% were Trichoptera (caddisflies), 21% were Ephemeroptera (mayflies), 16% were Plecoptera (stoneflies), 11% were Coleoptera (beetles and weevils), and 5% were Megaloptera (Alderflies, dobsonflies, and fishflie**s).**	Magnum and Madrigal 1999
Application: Rotenone (Bugges Liquid Derris (5% rotenone by volume.) Application site: Streams in Scotland. Target Concentration: 0.5 mg/L a.i. concentration in stream for 30 minutes. Additional amounts applied to pools in streams.	Short term increase in drift of many invertebrate species during and immediately after treatment. Full recovery within one year.	Morrison 1977
Application site: Cove of South Branch Lake in north-central Maine. Cove (located on the west side of the lake: 4.52 ha; medium depth of 1.6 m; sparse vegetation; mud bottom; dissolved oxygen 9.1 ppm; alkalinity 8 ppm; pH 6.5; and temperature 16.0°C. Application: Noxfish (5% rotenone) applied by boat to attain a concentration of approximately 0.6 ppm on the afternoon of June 2nd. Sampling: Zooplankton samples collected during the afternoon of each sampling date (NOS).	Treatment greatly decreased the abundance of most zooplankton species. Within 24 hours after treatment, net plankton volume decreased to 3% of pretreatment levels. After 2 days, copepod and cladoceran populations were nearly exterminated. There was also a general decline in rotifer populations except for *Keratella* and C*onochilus*, which produced minor blooms in the study cove during the recovery period and peaked in abundance on June 9. By June 8th, species composition was similar to that of the control cove; moreover, zooplankton abundance returned to normal in less than 1 week after treatment.	Neves 1975

Application	Observations	Reference
Application site: Southern arm of Lake Merced, CA. Northern and southern arms of the lakes have fairly firm and stabilized sand or muddy-sand bottoms. Application: 0.025 ppm rotenone *in a fish killing program* on October 26, 1963. Sampling: Anecdotal description: polychaete worm, *Nereis limnicola*, densities as high as 500 m^2 on sandy beach of eastern side of southern arm in summer and early fall of 1963.	Large numbers of the dead worms washed up on shore the day after rotenone treatment. By November 18, 1963, the populations of worms had nearly vanished; on December 4^{th} , and January 15^{th}, population densities were $\leq$10 m^2 at one location and *even scarcer or absent at all other sites*.	Oglesby 1964
Study site: Lake Wirbel, shallow, eutrophic lake in Poland. Lake Wirbel: 11 ha, 1.8 m mean depth, 4.4 m maximum depth. Rotenone (NOS) was applied in October 1991 to remove all of the fish in the lake. Sampling: Density, size, structure, fecundity, size at maturity, and vertical distribution of a dominant cladoceran and water quality were analyzed at 2- to 3-week intervals May-October 1991 (prior to rotenone application) and May-October 1992 (after rotenone application).	Summer months after rotenone application (June-August 1992), there was a 2.5-fold reduction in algal biomass in the "edible" fraction of the seston particles (<30 µm), relative to the previous summer. There was no significant increase in the total number of herbivorous plankton 1 year following the rotenone treatment. There was, however, a highly significant (p=0.001) increase in the density of herbivorous zooplankton, and *Bosmina longirostris*, which had been the dominant species, was replaced by *Daphnia cucullata*. In addition there was a simultaneous significant increase in *Daphnia* mean body size and a decrease in fecundity.	Pijanowska and Prejs 1997 Prejs et al. 1997 These two papers present largely the same data and are both concerned with food web manipulation.

Application	Observations	Reference
Study site: Lake Mosvatn, shallow, eutrophic lake situated in an urban park in Norway. Lake Mosvatn: surface area of 0.46 km^2, mean depth of 2.1 m and maximum depth of 3.2 m. Lake population: macrophytes restricted to narrow zones along the shores; stock of whitefish (*Coregonus lavaretus*) approximately 100 kg/ha; zooplankton community dominated by rotifers (high predation pressure and low grazing pressure). Rotenone application in September 1987 to whole water surface corresponded to 0.5 mg/L evenly disturbed in the total lake volume. Sampling: water samples taken weekly in 1986 and fortnightly (every 2 weeks) in 1987 and 1988; zooplankton sampled in 1987 and 1988/	The first summer after treatment there was a marked community change from rotifer dominance and a few grazers to grazer dominance and a few rotifers, with a 5-fold increase in the biomass of *Daphnia galeata*; adult females almost doubled in weight. Treatment also had a marked effect on the phytoplankton community manifested as an increase in the proportion of small and gelatinous algae (i.e., turbidity (Secchi depth) increased from 1.7 to >2.3 m) and a decrease in the mean chlorophyll concentration (i.e., from 23 to 7 µg/L). Treatment also resulted in fewer cyanobacterial blooms, which seemed to be an indirect effect of the increased grazing by zooplankton. Total nutrition concentrations were affected by treatment: total phosphate decreased from 44 µg/L (pre-treatment) to 20 µg/L (in the first summer after treatment), and total nitrogen decreased from 0.68 mg/L (pretreatment) to 0.32 mg/L (in the first summer after treatment). Phosphate loading was not affected. Investigators conclude that removing the planktivorous fish (mainly whitefish) resulted in a biomanipulation causing the more oligotrophic lake conditions.	Sanni and Waervagen 1990

Application	Observations	Reference
Study site: McHose Pond located in McHose park in Boone County, Iowa. Purpose of study: to report changes in fish community and population structure associated with eradication and restocking project and to assess whether eradication was justified and restocking improved angling opportunities and quality. McHose Pond: 0.25 ha impoundment, located in a small clearing in a mixed deciduous forest. In 1984, pond (filled with 1.4 m sediment) dredged to a maximum depth of 2.7 m. Fish Community: consisted of eight species, and although large numbers of small bluegills, green sunfish, and stunted crappies, dominated the pond numerically, seven large carp and 18 large bigmouth buffalo accounted for 80% of the total biomass. The only popular angling species were a few largemouth bass and channel catfish. Application/Eradication: On September 9, 1985 liquid formulation of rotenone (NOS) at a concentration of 2-3 mg/L water and mixed into the water with the propeller of a small motorboat. Fish stocking: 1985-1986 using the split stocking method: **October 1985**: pond stocked with 500 bluegills >2.5 cm long and 50 channel catfish 5-7.5 cm long. **June 1986**: pond stocked with 35 largemouth bass with a mean length of 2.5 cm.	By fall of 1987, an estimated 110 bluegills (95% CL = 72-235) were at least 80 mm long, and 25 largemouth bass (95%CL = 16-61) were in the pond. The quality of sport fishing opportunities were improved, and the biomass of bluegills, largemouth bass, crappies and green sunfish increased by about 50%.	Scarnecchia 1988

Application	Observations	Reference
Study site: Bug Lake located in Forest County, Wisconsin. Bug Lake: seepage lake w/surface area of 4.5 ha and maximum depth of 13 m. Littoral zone approx. 65% sand, 15% gravel, and 15% much w/some rubble. Aquatic vegetation is sparse, except for some bur reed, spikerush, and water moss in deeper parts of the lake. Fish Community: consisted of golden shiners, bluenose minnows, blacknose shiners, largemouth bass, pumpkinseeds, and rock bass. Application/Eradication: 2.5 mg/L Pro-Noxfish (0.063 mg/L rotenone and 0.063 mg/L sulfoxide) on November 17, 1975. Sampling: **Benthic samples** taken on eight separate dates prior to treatment in 1975, seven dates in 1976, and five dates in 1977. **Zooplankton samples** collected on 51 separate dates from July 24 1975 (prior to treatment) and November 18, 1977 (2 years after treatment). Restocking: On May 24, 1976, 2725 yearling brook trout (≥150 mm), and on April 20, 1977, 1500 yearling brook trout were *planted*.	Treatment was immediately toxic to midges (*Chironomus*) and some zooplankton. The spring pulse of *Daphnia* and copepods (calanoids) was delayed in 1976 until after the detoxification of rotenone in mid-May. All benthic organisms and zooplankton survived the treatment, except *Pleuroxus dellticulatus* (water fleas), which were collected in only one sample prior to treatment. In addition, the levels of the major taxa of benthic organisms were comparable before and after treatment, except for a decrease in the mean densities of caddisflies (trichopterans) and dipterans. Most zooplankton were found at pretreatment levels within one or two years after treatment. The investigators conclude that the observed changes/variances in the data collected before and after treatment have been the result of factors other than rotenone toxicity (e.g., *the illegal introduction of fathead minnows sometime in late 1976 or early 1977*.)	Serns 1979

Application	Observations	Reference
Study site: Round Lake in Eden Prarie, Minnesota. Round Lake: small (12.6 ha) dimetic lake w/maxium depth of 10.5 m and mean depth of 2.9 m. Lake population: dominated by bluegills, black crappies, and black bullheads. Application: Rotenone (NOS) applied to Round Lake in the autumn of 1980 to eradicate predominantly planktivorous and benthivorous fish. Sampling: In 1980 prior to treatment and in 1981 and 1982 samples were taken every fortnight (every 2 weeks) between May and September at a single station at the deepest part of the basin. Restocking: undertaken in October 1980 to produce a *piscivore-dominated community*.	Treatment decreased the abundance of phytoplankton, which resulted in increased transparency. Zooplankton populations were fewer in number in 1981 and 1982; however, the decreases were offset by the significant increase in mean sizes of the zooplankton present. Accordingly, estimated grazing pressures in 1981 and 1982 were double, relative to 1980. *Daphnia*, which were not common in 1980 became the dominant genus 1981 and 1982, and the investigators observed a gradual shift to a *progressively larger-bodied Daphnia*.	Shapiro and Wright 1984
Study site: Eight small forest lakes in southwestern Sweden. Lake characteristics: all of the lakes are shallow (mean depths ranging from 1.6 to 3.2 m w/maximum depths ranging from 4.5 to 10 m). The individual areas of the lakes range from 1.0 to 4.3 ha. There were few differences among the lakes with regard to sediment composition and vegetation. Application: Four for the lakes were treated with rotenone (NOS) from 1957 to 1961, three of which were restocked with new fish species. In the lake that was not restocked (served as a control), the original fish species entered the water a few years after eradication via a ditch from another lake during an exceptionally high spring water.	In the lake treated with rotenone but not restocked, the composition of zooplankton species was the same as in the untreated lakes. Predation intensity in the treated lakes accounted for the clear difference in size distribution among the cladoceran communities: lakes that were not restocked had high predation intensity, relative to the stocked lakes. In the low predation lakes, larger species of zooplankton (*Bythotrephes longimanus* and *Daphnia longispina*) prevailed but were all but eliminated and replaced by the smaller species, *D. cristata*, in the high predation lakes. When, however, predation intensity decreased, the larger *Bosmina coregoni*, replaced the smaller *B. longirostris*.	Stenson 1973