Appendix F. Ecological Effect Data and Ecotox Reviews

I. Categories of Acute Toxicity

In general, categories of acute toxicity ranging from "practically nontoxic" to "very highly toxic" have been established for aquatic organisms based on LC_{50} values (**Table F.1**), terrestrial organisms based on LD_{50} values (**Table F.2**), and avian species based on LD_{50} values (**Table F.3**). Subacute dietary toxicity for avian species is based on the LC_{50} values (**Table F.**4). and non-target insects based on LD_{50} values for honey bees (**Table F.4**) (EPA 2001).

Table F.1. Categories for aquatic animal acute toxicity based on median lethal concentration in mg per liter (parts per million).

LC_{50} (ppm)	Toxicity Category
<0.1	Very highly toxic
0.1–1	Highly toxic
>1–10	Moderately toxic
>10–100	Slightly toxic
>100	Practically nontoxic

Table F.2. Categories for mammalian acute toxicity based on median lethal dose in mg per kilogram body weight (parts per million).

LD_{50} (mg a.i./kg)	Toxicity Category
<10	Very highly toxic
10–50	Highly toxic
51–500	Moderately toxic
501–2000	Slightly toxic
>2000	Practically nontoxic

Table F.3. Categories of avian acute oral toxicity based on median lethal dose in milligrams per kilogram body weight (parts per million).	
LD_{50} (ppm)	**Toxicity Category**
<10	Very highly toxic
10-50	Highly toxic
51-500	Moderately toxic
501-2000	Slightly toxic
>2000	Practically nontoxic

Table F.4. Categories of avian subacute dietary toxicity based on median lethal concentration in milligrams per kilogram diet per day (parts per million).	
LC_{50} (ppm)	**Toxicity Category**
<50	Very highly toxic
50–500	Highly toxic
501–1000	Moderately toxic
1001–5000	Slightly toxic
>5000	Practically nontoxic

II. Toxicity to Aquatic Animals

a. Freshwater Fish, Acute

Two freshwater fish acute toxicity studies using the TGAI are required to establish the toxicity of rotenone to fish. The preferred test species are the bluegill sunfish, *Lepomis macrochirus* (a warmwater fish), and the rainbow trout, *Oncorhynchus mykiss* (a coldwater fish). Results of acute toxicity studies to freshwater fish exposed to rotenone are summarized in Table F-1.

Eighteen (18) studies of the acute toxicity of rotenone in bluegill sunfish have been submitted. Of these, 13 are classified as acceptable and 5 are classified as supplemental. Among the acceptable studies using bluegill sunfish, all 13 were at least highly toxic, and 7 of the 13 (54%) reported LC_{50} values were in the very highly toxic range (i.e., LC_{50} <100 µg a.i./L). Twenty (20) studies evaluating the acute toxicity of rotenone in rainbow trout have been submitted (16 acceptable and 4 supplemental studies). All of the acceptable studies using rainbow trout reported acute LC_{50} values in the very highly toxic range. Thus, based on the reported LC_{50} values in rainbow trout and bluegill sunfish from submitted studies, rotenone is somewhat more toxic to rainbow trout than bluegill sunfish.

To ensure the risk assessment is as protective as possible of non-target species, EFED uses the lowest scientifically defensible toxicity value available to evaluate acute risks to freshwater fish exposed to rotenone. The lowest acute toxicity value for fish resulted in an LC_{50} of 1.94 µg a.i./L (1.7-2.2; slope = 7.2), determined from a 96-hour acute toxicity study with juvenile rainbow trout (3.3–4.6 cm) in flow-through conditions (MRID 439751-02).

A number of acute toxicity studies using various fish species are also available from the open literature (ECOTOX). However, it was determined that no toxicity values obtained from the open literature were more sensitive than the submitted rainbow trout 96-h LC_{50} (1.94 •g a.i./L).

Table F.5. Freshwater Fish Acute Toxicity

Species	% ai	96-hour LC_{50} (•g a.i./L) (95%C.I.)	Toxicity Category	MRID/ Accession No.	Study Classification
Bluegill (*Lepomis macrochirus*)	98	4.9 (4.2-5.8)	very highly toxic	439751-01	Acceptable
Bluegill (*Lepomis macrochirus*)	98.6	5.5 (3.5-10)	very highly toxic	443829-02	Acceptable
Bluegill (*Lepomis macrochirus*)	2.5	138 (NS)	highly toxic	89909	Acceptable
Bluegill (*Lepomis macrochirus*)	5	157 (146-169)	highly toxic	90425	Acceptable
Bluegill (*Lepomis macrochirus*)	5	56 (51.9-60.5)	very highly toxic	121874	Acceptable
Bluegill (*Lepomis macrochirus*)	5	90 (NS)	very highly toxic	121877	Acceptable
Bluegill (*Lepomis macrochirus*)	5	72 (65.5-79.1)	very highly toxic	121880	Acceptable
Bluegill (*Lepomis macrochirus*)	5	105 (NS)	highly toxic	121881	Acceptable
Bluegill (*Lepomis macrochirus*)	5	157 (NS)	highly toxic	121883	Acceptable
Bluegill (*Lepomis macrochirus*)	5	165 (NS)	highly toxic	121885	Acceptable
Bluegill (*Lepomis macrochirus*)	5	127 (NS)	highly toxic	90288	Acceptable
Bluegill (*Lepomis macrochirus*)	7.67	80 (67.3-95.3)	very highly toxic	90366	Acceptable
Bluegill (*Lepomis macrochirus*)	5	76 (70.0-82.4)	very highly toxic	61296	Acceptable
Bluegill (*Lepomis macrochirus*)	5	168 (153.5-184.0)	highly toxic	90365	Supplemental
Bluegill (*Lepomis macrochirus*)	5	145 (NS)	highly toxic	121888	Supplemental

Table F.5. Freshwater Fish Acute Toxicity					
Species	**% ai**	**96-hour LC_{50} (•g a.i./L) (95%C.I.)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Bluegill (*Lepomis macrochirus*)	96.47	6.09 (5.39-6.88)	very highly toxic	400633-01	Supplemental
Bluegill (*Lepomis macrochirus*)	5	117 (86.9-157)	highly toxic	400633-01	Supplemental
Bluegill (*Lepomis macrochirus*)	2.5	122 (94.7-157)	highly toxic	400633-01	Supplemental
Rainbow Trout (*Oncorynchus mykiss)*	5	46.5 (NS)	very highly toxic	89905	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	38.5 (NS)	very highly toxic	89906	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	29 (NS)	very highly toxic	89908	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	22 (20.0-24.2)	very highly toxic	90367	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	35 (31.8-38.6)	very highly toxic	90420	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	35 (29.2-40.0)	very highly toxic	90421	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	48 (NS)	very highly toxic	121882	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	52 (NS)	very highly toxic	121884	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	38 (NS)	very highly toxic	121886	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	98	1.9 (1.7-2.2)	very highly toxic	439751-02	Acceptable

Table F.5. Freshwater Fish Acute Toxicity					
Species	**% ai**	**96-hour LC_{50} (•g a.i./L) (95%C.I.)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Rainbow Trout (*Oncorynchus mykiss)*	98.6	2.8 (2.0-3.4)	very highly toxic	443829-01	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	35 (NS)	very highly toxic	89907	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	11.5 (10.14-13.05)	very highly toxic	121873	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	48 (NS)	very highly toxic	121882	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	1.8 (1.59-2.04)	very highly toxic	121875	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	5	0.84 (NS)	very highly toxic	121876	Acceptable
Rainbow Trout (*Oncorynchus mykiss)*	96.47	2.82 (2.27-3.49)	very highly toxic	400633-01	Supplemental
Rainbow Trout (*Oncorynchus mykiss)*	5	45.0 (35.5-57.0)	very highly toxic	400633-01	Supplemental
Rainbow Trout (*Oncorynchus mykiss)*	2.5	36.2 (25.2-52.0)	very highly toxic	400633-01	Supplemental
Rainbow Trout (*Oncorynchus mykiss)*	6.8	45 (NS)	very highly toxic	89904	Supplemental

b. Freshwater Fish, Chronic

A freshwater fish early life-stage test using the TGAI is required for rotenone because the end-use product may be applied directly to water or it may contaminate surface water through drift or runoff events. Thus, rotenone may be transported to non-target water from the intended use site.

An early life-stage study on rotenone was conducted using rainbow trout (MRID 400633-02). This 32-day study demonstrated decreased growth and survival of rainbow trout embryo-larvae exposed to rotenone. The study established LOAEC and NOAEC values for larval growth were 2.21 µg/L and 1.01 µg/L, respectively. The purity of the test substance was 96.47%. This value was used to assess the risk of chronic exposure of freshwater fish to rotenone. A search of the open literature did not identify other studies that would provide additional information concerning the chronic toxicity of rotenone to fish.

c. Freshwater Invertebrates, Acute

A toxicity test using the TGAI is required to establish the acute toxicity of rotenone to aquatic invertebrates. The preferred test species is *D. magna*. An acute toxicity test measuring effects of rotenone TGAI to *D. magna* was submitted (MRID 400633-03). Although this study is classified as supplemental due to inadequate reporting, it will be used for risk assessment because it is the only study available to assess this endpoint. This study was conducted under static-renewal conditions and evaluated the following concentrations: 0, 0 (solvent control, acetone), 0.5, 2.5, 5.0, 7.5, or 10.0 µg a.i./L. Results of the study establish a 48-hour LC_{50} value of 3.7 µg/L. The purity of the test material was 96.47%. Thus, based on the reported LC_{50}, rotenone is classified as very highly toxic to freshwater invertebrates.

A search of the open literature did not identify other studies that would provide additional information concerning the acute toxicity of freshwater invertebrates exposed to rotenone.

d. Freshwater Invertebrate, Chronic

A freshwater aquatic invertebrate life-cycle test using the TGAI is required for rotenone because the end-use product may be applied directly to water or is expected to be transported to water from the intended use site due to recurrent application. Also, the aquatic invertebrate acute LC_{50} value is less than 1 mg/L. The preferred test is a 21-day life cycle study of *Daphnia magna*. A 21-day static renewal, life-cycle study testing the toxicity of the TGAI (96.47% pure rotenone) to *D. magna* was submitted (MRID 400633-04). This study was classified as supplemental due to inadequate reporting; the study will be used for risk assessment because it is the only study available to assess this endpoint for rotenone. The results of this study show that chronic exposure to rotenone TGAI produced adverse effects on survival and number of young produced. The test concentrations of the study were 0, 0 (solvent control, acetone), 0.312, 0.625, 1.25, or 2.5 µg a.i./L. The reproductive **NOAEC of 1.25 µg a.i./L** established by this study was used to assess chronic risk of rotenone TGAI in freshwater invertebrates.

A search of the open literature did not identify other studies that would provide additional information concerning the chronic toxicity of rotenone to fish.

e. Estuarine/marine Fish, Acute

No acute estuarine/marine fish toxicity data were submitted and no useable data were located in the open literature for rotenone; therefore, acute risks associated with estuarine/marine fish exposure to rotenone are unknown.

f. Estuarine/marine Fish, Chronic

No chronic estuarine/marine fish toxicity data were submitted and no useable data were located in the open literature for rotenone; therefore, chronic risks associated with estuarine/marine fish exposure to rotenone are unknown.

g. Estuarine/marine Invertebrates, Acute

No acute estuarine/marine invertebrate toxicity data were submitted and no useable data were located in the open literature for rotenone; therefore, acute risks associated with estuarine/marine invertebrate exposure to rotenone are unknown.

H. Estuarine/marine Invertebrates, Chronic

No chronic estuarine/marine invertebrate toxicity data were submitted and no useable data were located in the open literature for rotenone; therefore, chronic risks associated with estuarine/marine invertebrate exposure to rotenone are unknown.

III. Toxicity to Terrestrial Animals

a. Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of rotenone to birds. The preferred test species is either Mallard duck (a waterfowl) or Bobwhite quail (an upland gamebird). The available oral toxicity study in birds (MRID 143250) evaluated the toxicity of rotenone (32.38% cube resins) to mallard ducks and ring-necked pheasants (**Table F.6**) The authors reported that the LD_{50} exceeds 2,200 mg/kg BW in mallard ducks. The authors also reported an LD_{50} of 1,680 mg/kg bw (95% CI: 1,444-2,000) for ring-necked pheasant.

Table F.6. Avian acute oral toxicity.

Species	% a.i. *	Toxicity Value	Toxicity Category	MRID No. Author, Year	Study Classification
Mallard duck *(Anas platyrhynchos)*	32.38	LD_{50}: >2,200 mg/kg	practically nontoxic	143250 (Tucker, 1968);	Supplemental
Ring-necked pheasant *(Phasianus colchicus)* ‡	32.38	LD_{50}: 1,680 mg/kg	slightly toxic	143250 (Tucker, 1968);	Supplemental

* The administered material is described as having a concentration of 32.38% cubé resins.
‡ Not a recommended surrogate species.

Two subacute dietary studies using the TGAI are required to establish the toxicity of rotenone to birds. The preferred test species are mallard duck and bobwhite quail; however, Japanese quail and ring-necked pheasant are acceptable species. The data submitted (ACC#: 248788) show that the LC_{50}s for rotenone (administered to the animals as 34.5% rotenone and 65.5% cubé resins) were approximately 2,600 ppm in mallard duck (95% CI could not be determined), 1,608 ppm (95% CI: 1,365-1,875 ppm) in ring-necked pheasants, and 1,882 ppm (95% CI: 1,418-2,497 ppm) in Japanese quail (**Table F.7**). Analytical confirmation of the rotenone concentrations in the feed was not provided. The concurrence of the LC_{50}s suggests that rotenone can be categorized as slightly toxic to avian species on a subacute dietary basis.

Table F.7. Avian subacute dietary studies.					
Species	**% a.i. ***	**LC_{50} (ppm a.i.)**	**Toxicity Category**	**MRID No. Author, Year**	**Study Classification**
Mallard duck *(Anas platyrhynchos)*	34.5	2600	Slightly toxic	ACC#:248788 (Hill et al., 1975)	Supplemental
Ring-necked pheasant *(Phasianus colchicus)*	34.5	1608	Slightly toxic	ACC#:248788 (Hill et al., 1975)	Supplemental
Japanese quail *(Coturnix japonica)*	34.5	1882	Slightly toxic	ACC#:248788 (Hill et al., 1975)	Supplemental

* 34.5% rotenone and 65.5% cubé resins.

b. Birds, Chronic

Avian reproduction studies using the TGAI are required for rotenone because, given their environmental fate characteristics, birds may be subject to repeated or continuous exposure to this class of pesticides, especially preceding or during the breeding season. Currently, there are no available avian reproduction studies.

c. Mammals, Acute

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In most cases, rat or mouse toxicity values obtained from the Agency's Health Effects Division (HED) substitute for wild mammal testing. An acute oral LD_{50} for male and female is 102 mg/kg and 39.5 mg/kg, respectively (MRID 00145496).

d. Terrestrial Insects, Acute

A honey bee acute contact study using the TGAI is required for rotenone because its use outdoors may result in exposure of honey bees. The acute contact LD_{50}, using the honey bee, *Apis mellifera*, is an acute contact, single-dose laboratory study designed to estimate the quantity of toxicant required to cause 50% mortality in a test population of bees. The TGAI is administered by one of two methods: whole body exposure to technical pesticide in a nontoxic dust diluent or topical exposure to technical pesticide via micro-applicator. The median LD_{50} is expressed in micrograms of active ingredient per bee (•g a.i./bee). Results of this study are tabulated below (**Table F.8**). The toxicity category descriptions for honey bee acute contact toxicity are the following (Atkins, 1981):

If the LD_{50} is *less than 2* µg a.i./bee, then the test substance is *highly toxic*.
If the LD_{50} is *2 to less than 11* µg a.i./bee, then the test substance is *moderately toxic.*
If the LD_{50} is *11* µg a.i./bee or *greater,* then the test substance is practically nontoxic

Rotenone can be classified as *practically nontoxic to honeybees* based on a contact LD_{50} (> 60 µg/bee) (MRID 05001991) that is at least six time greater than the risk presumption for this category (11 µg a.i./bee). The results of an acute oral toxicity test in the honey bee are also included in **Table F.8**.

Table F.8. Honey bee acute contact and oral toxicity.					
Species/ Study Duration	**% ai**	**Toxicity Value**	**Toxicity Category**	**MRID No, Author, Year**	**Study Classification**
Honey bee Acute Contact Toxicity					
Honey bee (*Apis mellifera*)	> 95	LD_{50}: > 60 µg a.i./bee	practically nontoxic	Stevenson JH, 1978 (MRID 05001991)	Acceptable
Honey bee (*Apis mellifera*)/ 48 hour	Technical	2.4 µg a.i./bee elicited 12% mortality	–	Atkins EL et al., 1975 (MRID 00036935)	Supplemental
Honey bee Acute Oral Toxicity					
Honey bee (*Apis mellifera*)	> 95	LD_{50}: > 30 µg a.i./bee	–	Stevenson JH, 1978 (MRID 05001991)	Supplemental

APPENDIX F2. Rationale for Use or Non-use of Rotenone Open Literature (ECOTOX) with a More Sensitive Endpoint than the Registrant-submitted Data

Table F1. Open Literature References

Citation (Reference #)	Species	Toxicity Endpoint	Used In Risk Assessment? Yes (Quantitatively / Qualitatively) or No	Rationale
Birds				
Hill *et al.* (1975) (Ref #: 35243)	Ring-necked pheasant; Japanese quail; Mallard duck	Ring-necked Pheasant 8-d LC50 = 1608 mg/kg bw Japanese quail 8-d LC50 = 1882 mg/kg bw Mallard duck 8-d LC50 = 2600 mg/kg bw	Yes - Quantitatively	Already captured by EFED - assigned Accession number 248788
Mammals				
No studies more sensitive				
Terrestrial Plants				
No studies that meet guidelines to enable quantitative use				
Terrestrial Invertebrates				
No studies that meet guidelines to enable quantitative use				
Freshwater Fish				

Table F1. Open Literature References				
Citation (Reference #)	**Species**	**Toxicity Endpoint**	**Used In Risk Assessment? Yes (Quantitatively / Qualitatively) or No**	**Rationale**
Olson and Marking (1975) (Ref #: 525)	Chinook salmon, Brook trout, Lake trout	Chinook salmon: 96-h LC50 (eggs) >150 ppb 96-h LC50 (juveniles) = 2.5 ppb Brook trout: 96-h LC50 (eggs) = 170 ppb 96-h LC50 (juveniles) = 2.4 ppb Lake trout: 96-h LC50 (eggs) >50 ppb 96-h LC50 (juveniles) = 1.4 ppb	No	Primarily focused on toxicity of NoxFish (5% rotenone) to green eggs of salmonids. All toxicity values determined for eggs are less sensitive than value used to calculate RQs. Juveniles toxicity data also presented and 96-h LC50 for lake trout is slightly more sensitive (1.4 ug/L) compared to the value used to calculate RQs (1.94). However, this value was not used for the following reasons: 1) Although authors refer to "standardized laboratory toxicity test", no reference is provided to show which standardized method was followed. 2) No control data are provided 3) formulated product was used compared to technical grade 4) only nominal concentrations were reported versus measured concentrations

Table F1. Open Literature References				
Citation (Reference #)	**Species**	**Toxicity Endpoint**	**Used In Risk Assessment? Yes (Quantitatively / Qualitatively) or No**	**Rationale**
Rowe-Rowe (1971) (Ref #: 9423)	7 African species and rainbow trout and largemouth bass	Most sensitive species tested was rainbow trout (24-h LC50 = 1.6 ug a.i./L)	No	The only species tested that was more sensitive than the value used in this assessment to calculate RQs was for the rainbow trout. This study was not used for the following reasons: 1) Fish were collected from ponds and streams (by seining or electrofishing) and no information regarding water quality of the ponds and streams was provided. 2) Tap water was used and no water quality values are reported for any of the LC50 tests. 3) Treatment concentrations tested were not provided and LC50 concentrations are assumed to be nominal versus measured. 4) Derris powder (6.5% rotenone) was used instead of technical grade. 5) Authors state that no control mortality occurred, but data are not provided to verify this.
Bretaud *et al.* (2004) (Ref #: 76023)	Zebrafish	Locomotor activity, dopaminergic neurons, and development	Yes - Qualitatively	This is the only non-mammalian study that we are aware of that addresses the Parkinson's disease issue as it is related to rotenone. However, there are several issues related to this study such as: 1) dechorionating the embryos, 2) addition of methylene blue to water that larval fish were raised in, and 3) the effects of DMSO as the solvent (DMSO is not one of EFED's suggested solvents).
Freshwater Invertebrates				

Table F1. Open Literature References				
Citation (Reference #)	**Species**	**Toxicity Endpoint**	**Used In Risk Assessment? Yes (Quantitatively / Qualitatively) or No**	**Rationale**
Rach *et al.* (1988) (Ref #: 2370)	Daphnia magna	48-h EC50 = 3.7 ug/L 21-d NOAEC = 1.38 ug/L based on number of young produced	Yes - Quantitatively	Already captured by EFED - assigned MRID 40063303
Chandler and Marking (1982) (Ref # 10211)	Various aquatic invertebrates and frog larvae	48-h EC50 for Daphnia pulex = 28 ug/L	Not used	All organisms tested are less sensitive than value used to calculate RQ values. However, if concentrations are presented as a formulation, then this study would represent the most sensitive species, but there is no way to tell if the values are reported as a.i. or formulation. This study was not used in the study because no control data are discussed or presented. Also, concentrations are assumed to be reported as nominal and not measured.
Estuarine/Marine Fish				
Naess *et al.* (1991) (Ref #: 7105)	Carid shrimp Mysid Goby	Carid shrimp - 50% mortality after 9 h in 10 ppm Mysid - 50% mortality after 27 hours at 5.0 ppm Goby - 50% mortality occurred after 5, 15, and 36 h in 0.5, 0.25, and 0.1 ppm	Not used	Time to death (LT50) instead of LC50s were determined. Also, a formulation containing rotenone (2.65%) and a synergist, sulfoxide (2.65%) was used. As a result, the information presented in this study is not very useful to be used in this risk assessment.

Table F1. Open Literature References				
Citation (Reference #)	**Species**	**Toxicity Endpoint**	**Used In Risk Assessment? Yes (Quantitatively / Qualitatively) or No**	**Rationale**
Cruz-Lacierda (1992) (Ref. #: 18762)	Milkfish (*Chanos chanos*) Tilapia (Freshwater) (*Oreochromus mossambicus*)	Milkfish 96-h LC50 = 25 ug/L Tilapia 96-h LC50 = 80 ug/L	Not used	Although the authors state that controls were run, no data is presented. In addition, there were very high ammonia concentrations at the end of each study. Although the study authors state that this was not a cause of mortality, it cannot be ruled out. In addition, test water was aerated during the static tests. It is also assumed that concentrations reported are nominal and not measured.
Estuarine/Marine Invertebrates				
Naess (1991) (Ref. #: 8623)	Copepod (*Acartia clausi*)	Time to 50% mortality	No	Time to death (LT50) instead of LC50s were determined. Also, a formulation containing rotenone (2.65%) and a synergist, sulfoxide (2.65%) was used. As a result, the information presented in this study is not very useful to be used in this risk assessment.
Cruz-Lacierda (1993) (Ref #: 4275)	Tiger shrimp (*Penaeus monodon*)	Survival and Shell softening	No	No LC50 was established. Concentrations are assumed to be nominal, not measured. Effects on shell quality were observed; however, solvent controls were not conducted in this experiment so it is not possible to determine what effects the solvent (acetone) may have had on the shrimp. Also, it is difficult to extrapolate shell quality effects to the assessment endpoints of reduced survival, growth and reproduction.

ECOTOX Record Number and Citation: 525

Olson, L.E., and L.L. Marking. 1975. Toxicity of four toxicants to green eggs of salmonids. The Progressive Fish Culturist 37(3):143-147.

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: The objective of this study was to determine the toxicity of four piscicides (TFM, Bay 73, Antimycin, and Noxfish®) to salmonid green eggs and to compare the sensitivity of eggs and fingerlings of the same species. This review focuses only on the results using NoxFish®. Static toxicity tests were conducted. Green eggs of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), brown trout (*Salmo trutta*), brook trout (*Salvelinus namaycush*), and lake trout (*Salvelinus namaycush*) were obtained from Federal hatcheries within 24 hours after fertilization. 25 eggs of a selected species were placed in each 3.8 L test jar containing 2.5 L of test water; NoxFish® was added the next day. Dead eggs were counted and removed each day. Tests were discontinued when significant mortalities occurred in control vessels. Test waters were prepared by adding reagent grade chemicals to deionized water to make the following hardnesses (mg/L $CaCO_3$): very soft (10-12), soft (40-44), hard (160-180), and very hard (290-310). Data were analyzed to determine LC50 values and 95% confidence intervals. Data for NoxFish® against fingerlings were determined in standardized laboratory toxicity tests.

NoxFish® is less toxic to green eggs than to fingerlings in soft water at 12•C. At 96 h, LC50s for NoxFish® against green eggs are more than 50 times greater than LC50s for NoxFish® against fingerlings (**Table F.2**). The 96-h LC_{50}s are >150 µg a.i./L, 170 µg a.i./L, and >50 µg a.i./L for green eggs of chinook salmon, brook trout, and lake trout, respectively.

Table F.2. Summary of toxicity of Noxfish (5% rotenone) to fingerlings and green eggs in standard laboratory tests at 12•C.

	LC50 (mg Noxfish/L) 95% confidence interval		
	Fingerlings	Green Eggs	
Species	96-h	96-h	192-h
Chinook salmon	0.049 NA	>3.00 NA	>3.00 NA
Brook trout	0.047 0.042 - 0.052	3.40 2.74 - 4.22	NA
Lake trout	0.027 0.020 - 0.037	>1.00 NA	>0.250 NA

Description of Use in Document: Not Used
Rationale for Use: See "Limitations of Study"
Limitations of Study: This study focused primarily on toxicity to green eggs of salmonids. Although data are presented showing toxicity to juveniles, essentially no information is provided on how these tests were conducted, the size of the fish used, or where the fish were collected from. Although the study authors refer to "standardized laboratory toxicity test," no reference is provided to show which "standardized" method was followed. Other issues with this study include (1) the use of formulated product vs. technical product, (2) only nominal concentrations were reported versus measured concentrations, (3) concentrations that the fish were exposed to were not provided, (4) survival data in

each treatment were not provided, including the controls. Finally, the information reported in the ECOTOX output Table 1 for aquatic animals incorrectly reported all values in µg/L instead of mg/L. This error makes it appear that the species tested in this study were more sensitive when in fact the only species more sensitive was the juvenile lake trout (1.34 µg a.i./L). However, because of the lack of information provided in this study, this value is not able to be used quantitatively.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 2370 (See MRID 400633-03)

Rach, J.J., T.D. Bills, L.L. Marking. 1988. Acute and chronic toxicity of rotenone to Daphnia magna. Invest. Fish Control No. 92, Fish Wildl. Serv., Bur. Sport Fish. Wildl., USDI, Washington, DC.

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: This study is assigned MRID 400633-03 and is used quantitatively in the rotenone risk assessment. It was conducted by the National Fisheries Research Center, U.S. Fish and Wildlife Service, La Crosse, Wisconsin.

Description of Use in Document: Quantitatively

Rationale for Use: Toxicity values have already been captured by EFED - assigned MRID 400633-03.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 4275

Cruz-Lacierda, E.R. 1993. Effect of rotenone and saponin on the shell quality of juvenile tiger shrimp, Penaeus monodon. The Israeli Journal of Aquaculture - Bamidgeh 45(3):126-130.

DP Barcode: Not applicable

Date of Assessment: 7/25/2005

Brief Summary of Study Methods and Findings: Tiger shrimp juveniles were exposed to varying concentrations of rotenone and saponin to determine their effects on survival and shell quality. This review focuses only on rotenone. The highest concentration tested, 50 ppm rotenone, was not lethal to shrimp. Shrimps exposed to 0.001 to 50 ppm rotenone had 7.4 - 66.6% shell softening. Shell softening rates were significantly higher in 1.0 to 50 ppm rotenone compared to controls.

Normal, hard-shelled shrimp juveniles with an average weight of 14 • 3.6 g were obtained from brackish water ponds of the University of the Philippines. The shrimps were acclimatized for one week under laboratory conditions (26-28°C; 32-34 ppt). Shrimp were not fed during the test. Static tests were conducted in glass aquaria with 5 L of filtered and aerated sea water for 96 hours. Shrimps were exposed to technical grade rotenone at the following concentrations: 0.001, 0.01, 0.10, 1.0, 10, and 50 ppm rotenone. Three experimental runs were conducted with 5 and 3 replications for the first two runs and the third run, respectively. A control treatment (no pesticide) was also evaluated.

During the tests, water temperature was 27 • 1°C; salinity, 33 • 1 ppt; pH, 8.33 to 9.0; total ammonia-nitrogen, less than 2.0 ppm; nitrite-nitrogen, not detectable; and hardness, 5664 to 6853 ppm $CaCO_3$. There were no significant differences in water quality parameters between treatments or between the control and treatments. The tested concentrations did not cause any mortality. Rotenone was not letal to juvenile shrimps even when exposed to concentrations as high as 50 ppm rotenone for 96 hours. During the testing period, some shrimps molted and the post-molt shell remained soft even after 3 days. Softening of the normal hard-shelled shrimps which did not molt also occurred within the period of exposure to the pesticides. However, no association exists between pesticide-exposed, soft-shelled shrimps that molted and pesticide-exposed, soft-shelled shrimps which did not molt. The rates of soft shelling after 96 hours ranged from 7.4 to 66.6% for shrimps exposed to 0.001 to 50 ppm (**Table F.3**). The percentage of soft-shelling was significantly different from the control in 1.0 to 50 ppm.

Table F.3. Response of *Penaeus monodon* juveniles exposed to rotenone for 96 hours.	
Concentration (ppm)	**% Soft-shelling***
0.0	0.0[a]
0.001	14.4[ab]
0.01	14.4[ab]
0.10	7.4[a]
1.0	55.6[bc]
10.0	55.6[bc]
50.0	66.6[c]

*Values with the same superscript are not significantly different.

Description of Use in Document: Not Used
Rationale for Use: See "Limitations of Study"

Limitations of Study: An LC_{50} value was not established. It does not appear that solvent controls were run in this experiment. No data on survival are provided, including the controls, although it was stated that no mortality occurred in the experiments. Concentrations are assumed to be nominal, not measured. Effects on shell quality were observed. Although this effect appears to be caused by rotenone, extrapolation of this effect to the assessment endpoints of reduced survival, growth and reproduction of individuals in the wild is difficult.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/25/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 7105

Naess, T., K.E. Naas, and O.B. Samuelsen. 1991. Toxicity of rotenone to some potential predators on marine fish larvae - An experimental study. Aquacultural Engineering 10 (3):149-159.

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: The rotenone mixture used in this experiment (an emulsifiable concentrate containing 2.65% rotenone and an equal amount of a synergist, sulfoxide) is almost identical with Pro-Noxfish. Specimens of the carid shrimp (*Leander squilla* (L.)), the mysid (*Praunus flexuosus*) and the small goby (Gobiusculus flavescens) were collected from the littoral zone of an enclosed lagoon in Western Norway. The experiments were conducted in a laboratory under controlled light (50 lux at the surface) and temperature (10 • 0.5°C) conditions during all studies. The experiments were run for 48 hours and oxygen was monitored throughout the study. Water samples were collected from each test unit at 0, 27, and 48 hours and measured using HPLC. Each test species was exposed to **3** concentrations of rotenone. Ten specimens of each species were transferred to separate 5 L cylindrical plexiglass containers and exposed to the following rotenone concentrations: gobies: 0.10, 0.25, and 0.50 ppm rotenone mixture; the mysids: 1.0, 2.0, and 5.0 ppm; and the carid shrimps: 2.0, 5.0, 10.0 ppm. In addition, a control group of each species was performed.

Concentrations of active rotenone in the test units decreased 0.57%/hour on average. All concentrations were below the expected calculated values. The lowest concentrations of 0.10 and 0.25 ppm were below the detection level for the present analyses.

Effects on tested animals

Gobiusculus flavescens

The weakest solution, 0.10 ppm, was sufficient to cause total moribundity within 9 hours and 70% mortality within 48 hours. 50% mortality occurred after 5, 15, and 36 h in 0.50, 0.25, and 0.10 ppm, respectively. In the control groups, no organisms showed evidence of moribundity or death after 48 h.

Praunus flexuosus

Mysids showed a much higher tolerance to rotenone than the gobids, and a concentration as high as 5.0 ppm was needed to kill all specimens. 50% mortality occurred within 27 hours at this concentration. Only 10% of the mysids became moribund after having been exposed to 2.0 ppm, and in both 1.0 ppm concentration and the control group no organisms died or were moribund.

Leanaer squilla

The shrimps showed the highest tolerance, and 10 ppm was needed to kill all specimens within 48 hours. However, all organisms were moribund after 9 hours, and 50% mortality occurred within 19 h. At 5.0 and 2.0 ppm respectively, 20 and 30% mortalities were recorded at the end of the experiment. No individuals were affected in the control group.

Description of Use in Document: Not Used

Rationale for Use: See "Limitations of Study"

Limitations of Study: Two major issues: (1) L**T**$_{50}$s are calculated, but not L**C**$_{50}$s and (2) a rotenone mixture manufactured by Gullvik AB, Malmø, Sweden containing 2.65% rotenone and 2.65% of a synergist, sulfoxide, was used.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 8623

Naess, T. 1991. Ontogenetic and sex dependent rotenone tolerance of a marine copepod, Acartia clausi Giesbrecht. Sarsia 76:29-32.

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: The rotenone mixture used in this experiment (an emulsifiable concentrate containing 2.65% rotenone and an equal amount of a synergist, sulfoxide) is almost identical with Pro-Noxfish. Zooplankton were collected from an enclosed lagoon in western

Norway. The majority of zooplankton consisted of the copepod *Acartia clausi* (>90%). Experiments were carried out in a laboratory under controlled light (3 lux) and temperature (9.5 • 0.5°C) conditions. Experiments were initially carried out at 0.10, 0.50, and 1.0 ppm of the rotenone mixture. Due to very rapid mortalities observed at all concentrations, experiments with 0.05 and 0.01 ppm were initiated. The experiments were run for 48 hours and oxygen levels were monitored throughout the study. Adult males had significantly lower tolerance to rotenone than copepodids and adult females. 50% mortality occurred at 0.05 ppm after 16, 18, and 4 hours for copepodids, females and males, respectively. At 0.50 ppm, total mortality of all stages occurred within two hours.

Description of Use in Document: Not Used

Rationale for Use: See "Limitations of Study"

Limitations of Study: Two major issues: (1) L**T**$_{50}$s are calculated, instead of L**C**$_{50}$s and (2) a rotenone mixture manufactured by Gullvik AB, Malmø, Sweden containing 2.65% rotenone and 2.65% of a synergist, sulfoxide, was used.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 9423

Rowe-Rowe, D.T. 1971. Rotenone tolerances of some freshwater fishes of Natal. Progressive Fish Culturist 33(4):206-209

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: The objective of this study was to determine whether the tolerances of nine species of freshwater fishes differed appreciably. Derris powder containing 6.5% rotenone was used in all the experiments in a dilution of waters of pH 7.2 or 9.0. Fish used in experiments were either taken by seine net from ponds or collected from streams with a 220-volt electric shocker. All fish collected were acclimated for 24 hours before being tested. The 9 species tested are listed in Table 1. Tests were carried out in enameled aquariums, each containing 40 L of **tap water** and 10 fish. Each aquarium was fitted with one 75-watt heater and thermostat to maintain a constant temperature of approximately 22•C, except rainbow trout which were conducted at 15°C. At least four simultaneous replicate tests were made at each concentration, except in those using *T. sparrmanii* (FL = 77 mm) and in rainbow trout tests. One control was used for each concentration.

No losses occurred in the control. Tolerances at the 50% level at approximately 22°C ranged from 0.035 mg/L Derris (0.0023 mg/L rotenone) to 0.794 mg/L (0.0516 mg/L rotenone) for B. gurneyi, after 24 hours (**Table F.4**). The LC50 for the rainbow trout was 0.184 mg/L Derris (0.0016 mg/L rotenone)

Table F.4. Summary of 24-h LC50 values of Derris (6.5% rotenone) to nine freshwater fish species.

	Fish length (Fork)		**Toxicity (mg/L)**		
Species	Mean (mm)	Range (mm)	LC10	LC50	LC90

Table F.4. Summary of 24-h LC50 values of Derris (6.5% rotenone) to nine freshwater fish species.					
	Fish length (Fork)		Toxicity (mg/L)		
Species	Mean (mm)	Range (mm)	LC10	LC50	LC90
Tilapia mossambica	67	50-90	0.0080	0.0103	0.0136
Tilapia sparrmanii	41	32-50	0.0054	0.0073	0.0098
Tilapia sparrmanii	77	51-84	0.0073	0.0098	0.0130
Tilapia melanopleura	68	55-100	0.0110	0.0120	0.0132
Pseudocrenilabrus philander	65	40-105	0.0059	0.0088	0.0127
Barbus natalensis	47	35-55	0.0027	0.0036	0.0047
Barbus anoplus	40	30-50	0.0013	0.0023	0.0039
Barbus gurneyi	51	35-60	0.0380	0.0516	0.0695
Micropterus salmoides	60	55-65	0.0029	0.0036	0.0044
Oncorhynchus mykiss (formerly *Salmo gairdneri*)	81	69-102	0.0012	0.0016	0.0026

Description of Use in Document: Not Used

Rationale for Use: Although the 24-h LC50 for rainbow trout is slightly lower (1.6 µg/L) than the registrant submitted 96-h LC50 value (1.94 µg/L), the registrant-submitted value will be used in calculating RQ for the reasons mentioned below in the "Limitations of Study" section.

Limitations of Study: Because fish were collected from ponds and stream, it cannot be determined if the fish used in these studies were previously exposed to contaminants. No description of the ponds or streams are provided. This study reported only nominal concentrations and not measured concentrations and Derris powder was used instead of technical grade rotenone. Tap water was used in this experiment. No water quality values are reported for any of the LC50 tests. Treatment concentrations used in each experiment for each species are not reported. **Important Note**: **The values reported in the ECOTOX Table 1 for Aquatic Animals corrects the original value; however, the original values have already been corrected for % active ingredient.**

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 10211

Chandler, J.H., Jr., and L.L. Marking. 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. The Progressive Fish-Culturist 44(2):78-80.

Date of Assessment: 8/4/2005
Brief Summary of Study Methods and Findings:

The purpose of this study was to determine the toxicity of Noxfish to aquatic non-target organisms in support of its continued registration as a piscicidal chemical.

Methods

Noxfish, an emulsifiable concentrate containing 5% rotenone was used for all toxicity tests. Stock solutions were prepared in acetone daily as needed. Static tests were performed in a manner similar to that outlined by Lennon and Walker (1964) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Most of the test organisms were collected from the wild, but several species came from laboratory or pond cultures. All organisms were held under laboratory conditions for 5 to 14 days before being tested. Ten organisms were added to each of a series of 3.7-L glass jars containing 3-L of limed water (20 mg/L total hardness; pH 6.6). Test organisms were acclimated for 16 h before test began. Two controls, on with acetone and the other with untreated water, were included for each test. Temperature of the tests was maintained at 16 • 1°C.

Results

The results are presented in **Table F.5**.

Table F.5. Toxicity of rotenone to selected aquatic invertebrates and frog larvae.

Organism	LC_{50} and 95% confidence interval (mg/L)				
	1-h	3-h	6-h	24-h	96-h
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	0.096 0.081-0.114	0.036 0.032-0.041	0.028 0.024-0.032	---
Ostracod *Cypridopsis* sp.	2.80 2.35-3.34	2.55 2.11-3.08	2.15 1.80-2.56	0.49 0.30-0.80	0.34 0.28-0.56
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 0.76-1.65
Dragonfly naiad *Macromia* sp.	—	275 230-329	34.0 19.6-58.9	4.70 1.45-15.2	1.00 0.73-1.59
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 0.73-3.44
Caddisfly larva *Hydropsyche* sp.	10.7 7.98-14.5	8.00 6.69-9.56	3.55 2.88-4.38	---	0.61 0.33-1.17
Whirligig beetle, adult *Gyrinus* sp.	47.5 32.6-69.2	8.30* 5.42-12.7	8.00* 5.51-11.6	* 2.05-6.15	0.70* 0.40-1.21

Table F.5. Toxicity of rotenone to selected aquatic invertebrates and frog larvae.					
Organism	**LC_{50} and 95% confidence interval (mg/L)**				
	1-h	**3-h**	**6-h**	**24-h**	**96-h**
Snail *Physa pomilia*	—	—	—	6.35 5.61-7.19	4.00 3.45-4.63
Snail *Oxytrema catenaria*	—	—	—	—	1.75 1.09-3.06*
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 2.23-3.99
Flattened filter crab *Elliptio complanata*	—	—	—	—	2.00 1.53-2.64*
Asiatic clam *Corbicula manilensis*	—	—	—	—	7.50* 5.74-9.81*
Southern leopard frog larvae *Rana sphenocephala*	0.830 0.795-0.867	0.775 0.740-0.812	0.635 0.596-0.677	0.580 0.494-0.680	0.500 0.423-0.591

*Values with as asterisk were difficult or unable to be read in the photocopy of the paper.

Description of Use in Document: Not Used

Rationale for Use: No control data are presented. Treatment concentrations are not provided and it is unclear as to whether the concentrations are reported as formulations or active ingredient. Concentrations are assumed to be reported as nominal and not measured.

Limitations of Study: See Rationale.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 8/4/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 18762

Cruz-Lacierda, E.R. 1992. Toxicity of rotenone to milkfish, *Chanos chanos*, and tilapia, *Oreochromis mossaambicus*. In: I.M. Shariff, R.P. Subasinghe, and J.R. Arthur (eds.), Diseases in Asian Aquaculture I, Fish Health Section, Asian Fisheries Society, Manila, Philippines 419-423.

DP Barcode: Not applicable

Date of Assessment: 7/21/2005

Brief Summary of Study Methods and Findings: Milkfish (Chanos chanos) and tilapia (Oreochromus mossambicus) with average weights of 4.0 and 36.0 g, respectively, were obtained from a commercial farm and acclimatized for at least 10 days under laboratory conditions (temperature, 27-29•C; salinity, 32-33 ppt for milkfish and 0 ppt for tilapia). Fish were not fed 24 h before and during the experiment. Analytical grade rotenone (95-98% pure) was used in this study. Test organisms were exposed to rotenone for 96 h at the following concentrations: 5, 10, 25, 50, 75, and 100 µg/L for milkfish; and 10, 25, 50, 75, 100, and 200 µg/L for tilapia. Rotenone was dissolved in analytical reagent grade acetone. 10 milkfish and 5 tilapia were separately exposed to each concentration of rotenone and a rotenone-free control that contained acetone equal to that used in dissolving the toxicant. Toxicity tests were carried out in static conditions in glass aquaria with 40 L of gently **aerated** test solutions. Three replicates per concentration per test. Test solutions were analyzed at the start and termination of the experiment for temperature, salinity, pH, ammonia, nitrite and hardness. LC50 were calculated using probit analyses.

A study was also carried out that determined the toxicity of rotenone that was allowed to age for different periods up to 24 hours.

Concentrations of water parameters remained within acceptable limits except that **ammonia levels were high at the end of 96 h (Initial = 0.15 ppm NH_3-N; 96-h = 6.5 - 8.4 ppm NH_3-N)**. Fish reacted immediately after addition of rotenone by increased swimming activity, rapid opercular movements, and gulping at the water surface. This was followed by loss of equilibrium and direction of movement. Rotenone was not toxic to milkfish exposed to 5 and 10 µg/L after 96 h. However, at 100 µg/L rotenone, milkfish had 80% mortality rate within 1 h of exposure. Rotenone was not toxic to tilapia exposed to 10-25 µg/L after 96 h. Fish exposed to 50 and 75 µg/L rotenone had 10 and 20% mortality, respectively after 96 h. The LC50 values of rotenone and their corresponding 95% confidence intervals at different exposure periods for milkfish and tilapia are presented in **Table F.**6.

Table F.6. LC50 values and 95% confidence intervals of rotenone to milkfish and tilapia exposed at 28 • 1˚C.

Exposure time (h)	Milkfish			Tilapia		
	LC50 (ug/L)	95% C.I.		LC50 (ug/L)	95% C.I.	
		Lower	Upper		Lower	Upper
1	64	57	72	172	79	371
6	36	32	42	123	88	193
12	36	31	41	91	78	108
24	30	26	37	86	73	102
48	25	22	32	86	72	102
96	25	22	32	80	65	98

Description of Use in Document: Not Used

Rationale for Use: See "Limitations of Study"

Limitations of Study: No control data are presented. The major issue with this study is the very high ammonia concentrations at the end of the study. Although the study authors state that this was not a cause of mortality, the effects of these concentrations of ammonia on the fish cannot be dismissed. In addition, the test water was aerated during the study and only nominal concentrations are presented. As a result of the above mentioned factors, this study was not deemed appropriate to use qualitatively or quantitatively in the risk assessment.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/21/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency

ECOTOX Record Number and Citation: 76023

Bretaud, S., S. Lee, and S. Guo. 2004. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. Neurotoxicology and Teratology 26:857-864.

Date of Assessment: 7/25/2005

Brief Summary of Study Methods and Findings:

This study explores the effect of MPTP, rotenone, and paraquat in both adult and larval zebrafish, which are highly amenable to genetic analysis that can lead to the identification of the underlying genes and pathways. This review will focus on the effects on rotenone only.

Methods

Adult zebrafish (approximately 0.3 g) were raised at the University of California, San Francisco, following standard fish care and maintenance protocols. All zebrafish were maintained in deionized water containing 200 mg/L Instant Ocean Salt. Two strains were used: AB, which originated from Oregon, USA, and EK, which was from Ekkwill, FL. Larval zebrafish were obtained from these strains through natural mating. **Larval fish were raised in blue egg water (0.2 g/L Instant Ocean Salt, 0.12 g/L CaSO4, and 10 uL/L methylene blue)** at 28•C from birth to 7 days postfertilization (dpf) as previously described. Rotenone was obtained from Sigma. **Rotenone was dissolved in DMSO at a concentration of 20 mg/ml**. Because rotenone is extremely toxic in fish, a very low concentration, such as 2 ug/L was used for 4 weeks of exposure. Rotenone-containing water was changed every other day. For the exposure of embryonic and larval zebrafish, embryos were collected and raised in petri dishes for 1 day. At 24 hours postfertilization (hpf), embryos were dechorionated manually and transferred to six-well plates containing 5 and 10 ug/L rotenone. The treatment lasted until 5 dpf. All waters were changed daily. As controls, both adult and larval zebrafish were exposed to a solution containing DMSO, at the same concentration used to dissolve the highest amount of rotenone.

The locomotor activity of adult zebrafish was assessed in a 2-L tank filled with 1 L system water. Three vertical lines were drawn on the tank at equal distances, dividing the tank into four zones. Locomotor activity was measured for 5 min by counting the number of lines that adult zebrafish crossed. At 7 days old, the behavior of larvae is more random than that of an adult. To determine their locomotor activity, an automated computer tracking system was used. Ten larval zebrafish were transferred to a view chamber containing **blue egg water**. Larvae were allowed to habituate to the new environment for 5 minutes and

then their behavior was recorded for 5 min using a video camera. The results represent the mean swim speed of 10 larvae and are expressed in mm/s.

The effect of the neurotoxins on ventilation of adult zebrafish was simply determined and scored as 0 for normal ventilation and 1 for fast opercular movements. Intermediate opercular movements were qualitatively determined and assigned an intermediate number between 0 and 1. The skin color of adult zebrafish in response to neurotoxin was visually rated. Three stages were recognized and scored as light (0) and dark (2). Intermediate pigmentation was qualitatively determined and assigned an intermediate number between 0 and 2. Digoxygenin-labeled antisense RNA probe for tyrosine hydroxylase (TH) was synthesized and hybridized to whole mount larval zebrafish at 5 dpf. Neurons were visualized and counted.

Results

After 4 weeks of exposure to 2 ug/L of rotenone, no discernible difference in locomotor activity was observed in adult zebrafish. However, exposure to a higher concentration, such as 10 ug/L, was lethal after a few days. Also, no differences in DA (Dopaminergic) neurons were observed between rotenone-treated versus control zebrafish. Larval zebrafish treated with rotenone did not display any change in their locomotor activity. Rotenone-treated larval zebrafish only showed a trend of reduction of diencephalic DA neurons, which did not reach significance.

Description of Use in Document: Qualitatively

Rationale for Use: This study does provide some information on the effects of rotenone on zebrafish at low concentrations over an extended period of time. It is also the only non-mammalian study that EFED is aware of that relates to rotenone's role in causing symptoms of Parkinson's Disease.

Limitations of Study: For larval fish, only two concentrations were tested: 5 and 10 ug/L, and for adult fish only 2 ug/L was tested. Larval zebrafish were raised in "blue egg water" containing methylene blue. Rotenone was dissolved in DMSO. This study is assumed to have used nominal concentrations, no measured concentrations are reported.

Reviewer: Todd A. Phillips, Ph.D., Biologist Date: 7/25/05
ERB4, Environmental Fate and Effects Division, U.S. Environmental Protection Agency