**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON, D.C. 20460**

**OFFICE OF**
**PREVENTION, PESTICIDES**
**AND TOXIC SUBSTANCES**

June 28, 2006

MEMORANDUM

SUBJECT: ROTENONE: Final HED Chapter of the Reregistration Eligibility Decision Document (RED). PC Code: 071003. DP Barcode: D328478

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The attached Human Health Risk Assessment for the rotenone RED document was generated as part of Phase 4 of the public participation process. The Health Effects Division's (HED) Final chapter reflects the comments received during the Phase 3 public comment period. In separate memos the rotenone technical registrants (Prentiss, Inc. 3/7/06; Foreign Domestic Chemicals Corporation 3/17/06; and Tifa Limited 4/5/06) voluntarily cancelled all residential and food crop uses of rotenone leaving only the piscicidal use pattern. In this document, EPA presents the results of its review of the potential human health effects resulting from the use of rotenone as a piscicide. The cancelled uses of rotenone were previously assessed in the January 24, 2006 risk assessment (DP barcode D307385), which can be found on EPA's website. This chapter includes a summary of the product chemistry review from Yvonne Barnes, plant and ruminant metabolism review from Sherrie Kinard, dietary risk assessment from Toiya Goodlow, toxicology review from Elissa Reaves, occupational exposure and risk assessment from Charles Smith, incidence review from Monica Hawkins, environmental fate and drinking water

exposures from R. David Jones [Environmental Fate and Effects Division (EFED)], as well as risk assessment and characterization from Elissa Reaves, and Charles Smith.

This risk assessment relies in part on data from studies in which adult human subjects were intentionally exposed to a pesticide. These studies, listed below, have been determined to require a review of their ethical conduct. The listed studies have either received the appropriate review or are in the process of being ethically reviewed.

Clark NWE, Scott RC, Blain PG, Williams FM (1993). Fate of fluazifop-butyl in rat and human skin in vitro. Arch Toxicol. 67:44-48.

The PHED Task Force, 1995. The Pesticide Handler Exposure Database (PHED), Version 1.1. Task Force members Health Canada, U.S. Environmental Protection Agency, and the National Agricultural Chemicals Association, released February 1995.

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# 1.0 Executive Summary

Rotenone labels currently contain uses on home gardens for insect control and on pets for lice and tick control. Rotenone is also currently registered for use on 91 food crops and on livestock and was exempt from the need to establish tolerances until the passage of the Food Quality Protection Act (FQPA). However, these uses are no longer being supported. The Agency received requests dated March 7, 2006; March 17, 2006; and April 5, 2006 from the technical registrants Prentiss Incorporated, Foreign Domestic Chemicals Corporation, and Tifa International, LLC, respectively, to cancel registrations of the following rotenone products: EPA Reg. Nos. 655-3, 655-69, 655-421, 655-422, 655-691, 655-795, 655-803, 655-804, 655-805, 655-806, 655-807, 655-808, 6458-1, 6458-5, 6458-6, 82397-1, 82397-2, 82397-3, 82397-4, and 82397-5. Rotenone is a non-specific botanical insecticide/miticide/piscicide used to control flying and crawling insects and fish. Specifically, the rotenone registrants request termination of rotenone uses that include formulations for livestock use, residential and homeowner uses, domestic pet uses, and all other uses, **except for the piscicide uses,** because they choose not to support these uses. Foreign Domestic Chemicals Corporation conditioned their request upon the allowance for use of existing stocks until March 11, 2008. The cancelled uses of rotenone were previously assessed in the January 24, 2006 risk assessment (DP barcode D307385), which can be found on EPA's website. The piscicidal use of rotenone is the subject of this risk assessment.

Rotenone is a naturally occurring compound that is present in a number of plants. This botanical pesticide is derived from the roots of *Derris* spp., *Lonchocarpus* spp., and *Tephrosia* spp., found primarily in Malaysia, South America, and East Africa, respectively. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Federal government has registered rotenone, a List A chemical, since 1947. Rotenone is a General Use Pesticide (GUP), but use for fish control, is a restricted use and applications must be made by certified applicators only. It can be rapidly degraded in soil, water, and by sunlight, usually within 2-5 days. However, limited environmental fate data show that, under some conditions (particularly in cold water), rotenone and/or its metabolites may persist for as long as 6 months.

Most current Chemical Statements of Formula (CSFs) either do not list, or do not quantify, the impurities. There are risk concerns for impurities, in general. **Batch analysis of the various formulations is required**, with the specific impurities identified, and levels quantified. When new CSFs, in compliance with item 10 of Form 8570-4[1], are submitted, the risk assessment will be revised to include any impurities of concern.

The toxicology database for rotenone is not complete with data gaps existing for chronic dog, developmental toxicity (non-rodent), dermal penetration, and repeated-dose dermal toxicity studies. In special studies, on one strain of rat, by the intravenous and subcutaneous routes, exposure to rotenone produced behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease in humans. While neither route of exposure is relevant to humans, the intravenous route may mimic inhalation exposure, which is a route of concern with rotenone. The only available inhalation toxicity study is an acute $LC_{50}$ in the rat (Category I). The current database includes guideline studies via the oral route of exposure that typically do not include the Functional Observational Battery (FOB) or neurotoxicity parameters. In addition, inhalation exposure can be a major route of exposure for some uses and no subchronic inhalation toxicity

[1] Form 8570-4. Item 10: Components of Formulation. "Each component that may have toxic effects must be listed separately, even if present at less than 0.1% by weight." Guidelines on estimating % limits on components can be found in 40 CFR 158.175.

studies are available for rotenone. The agricultural and residential uses for which inhalation exposure is greatest, and of potential concern, are no longer being supported. A data call-in (DCI) was issued (02/09/2004) requiring a 21-day inhalation neurotoxicity study in the Lewis rat. That DCI remains unfulfilled and a submitted data waiver was denied. The toxicity database for rotenone is marginally adequate for selecting toxicity endpoints for the risk assessment. However, none of the available studies suitable for risk assessment purposes are adequate to address the neurotoxicity concerns. The endpoints selected are based on decreased pup body weight and body weight gain, and increased resorptions. Rotenone is classified as Group E carcinogen (evidence of non-carcinogenicity for humans). The supported piscicide use is expected to reduce potential exposures by all routes though additional mitigation measures and monitoring are still needed. Therefore, the inhalation neurotoxicity study and all other toxicity data requirements are held in reserve (may be called in later) pending the outcome of monitoring and further mitigation measures.

Residue chemistry data have not been addressed because all food uses have been cancelled. Environmental fate data, reviewed by EFED, show that though the parent rotenone is photolytic and not persistent, several degradates can be formed. The only major degradate identified is rotenolone, or specific rotenolone isomers (> 10%). Structure Activity Relationship (SAR) analysis indicates that all of the metabolites identified in the environmental fate data (see Section 3.0) are expected to be of equal or less toxicity than the parent rotenone.

The fate and transport properties of rotenone in the environment are not well understood. Rotenone does degrade rapidly by aqueous photolysis and the photolysis half-life is less than one day. There are no reliable data on the microbial degradation of rotenone. There is evidence however that the metabolite rotenolone forms on plant surfaces by hydrolysis. Uncharacterized residues in unacceptable soil and aquatic metabolism studies suggest that other degradates are formed, but the identities and amounts are unknown. Rotenone does not appear to bioaccumulate in animals.

EFED provided HED with an estimated drinking water concentration (EDWC) of 200 ppb in surface water based on the solubility of rotenone in water. It is also worth noting that the maximum application rate for the piscicidal use of rotenone (250 ppb) exceeds the solubility of rotenone. The remaining rotenone above the solubility limit is likely either suspended or in an emulsion. In either case, the suspended/emulsified rotenone will be less available for metabolism or hydrolysis than that in the dissolved phase.

Based on the registrants' proposed support of the piscicide use only, a dietary risk assessment was conducted that estimates acute dietary risks resulting from direct applications of rotenone to, or adjacent to, bodies of water and drinking water consumption. An acute deterministic assessment was conducted using the Dietary Exposure Evaluation Model (DEEM), which uses drinking water consumption data from the United States Department of Agriculture (USDA) surveys and incorporated estimated drinking water concentrations (EDWC) provided by EFED. Food uses of rotenone are not being supported, therefore, dietary (food) exposure is not expected; and the Food Quality Protection Act (FQPA) of 1996 does not apply.

Conservative acute dietary (drinking water alone) exposure analyses were performed in order to determine the potential exposure and risks resulting from the piscicide use of rotenone. For acute exposures, HED is concerned when estimated dietary risk exceeds 100% of the reference dose (RfD). An appropriate acute endpoint for the general population, including infants and children, was not identified in the available toxicity studies; however, an acute analysis was performed for the population subgroup females 13-49 years of age as increased incidence of

resorptions in a mouse developmental study is applicable to women of childbearing age. Acute dietary (drinking water alone) risk estimates calculated for females 13-49 years of age are 65% of the aRfD and are below the HED's level of concern (100% aRfD) at the 95$^{th}$ exposure percentile. It is appropriate to consider the 95$^{th}$ percentile because the analysis is deterministic and unrefined.

The chronic dietary risk analysis was complicated by the variability of the degradation of rotenone under differing environmental conditions; consequently, chronic risk estimates were not quantified using DEEM-FCID. Under all conditions, it was assumed that rotenone could reach drinking water intakes (within 1 day) and potentially pose risks from consumption of rotenone contaminated drinking water. Rotenone degrades more rapidly under warm water conditions and less rapidly under cold water (4-5$^{o}$ C) conditions. Using the chronic dietary endpoint and conservative assumptions concerning drinking water consumption (1 L/day for infants and children; 2 L/day for adults) by all population subgroups, HED determined that chronic drinking water exposures greater than 40 ppb could pose a potential risk of concern (> 100% cRFD) to the most highly exposed population subgroup, children 1-2 years of age. Information provided by EFED shows that chronic EDWCs are expected to exceed 40 ppb for 4 days under warm water conditions, and for 53 days under cold water conditions. Data collected in association with piscicidal application to Lake Davis in California show that 40 ppb would be exceeded for up to 27 days.

The classification of carcinogenic potential for rotenone is "not likely to be carcinogenic in humans," based on the lack of evidence of carcinogenicity in rats and mice; therefore, a cancer dietary analysis was not performed.

Products containing rotenone are being supported for the piscicidal use only. As a result of the piscicidal use of rotenone, adults and children may be exposed to rotenone when contacting rotenone-treated waters through swimming. Risk assessments were conducted to reflect potential exposures to adult occupational handlers and potential recreational postapplication exposure to adults and children of varying ages.

The results of the recreational postapplication assessment indicate that some of the risks are of concern [i.e., Margins of Exposure (MOEs) are less than 1000]. Specifically, short-term MOEs exceed HED's level of concern for all toddler swimming scenarios (MOEs < 1000). The Environmental Fate and Effects Division (EFED) calculated the number of days it would take to reach a rotenone concentration which results in acceptable toddler MOEs (170 ppb of rotenone results in an MOE of 1000). This is done by assuming that the dissipation rate for rotenone in a warm water pond is 1.5 days, as seen in the aquatic dissipation study. The time it takes for the rotenone to dissipate (in 25$^{o}$C water) to 170 ppb from 200 ppb is 0.35 days and from 250 ppb is 0.89 days.

Food uses of rotenone are not being supported, therefore, dietary (food) exposure is not expected; and the Food Quality Protection Act (FQPA) of 1996 does not apply. Since FQPA does not apply, HED does not need to aggregate pesticide exposures and risks from other sources of exposure (drinking water and residential exposures).

It has been determined that exposure to pesticide handlers is likely during the occupational use of rotenone in aquatic environments. The anticipated use patterns and current labeling indicate several occupational exposure scenarios based on the types of equipment and techniques that can potentially be used for rotenone applications. For applications to aquatic sites (liquid applications), combined dermal and inhalation risks to mixers/loaders and aerial applicators,

generally did not exceed HED's level of concern at some level of risk mitigation. For applications to aquatic sites (wettable powder applications), combined dermal and inhalation risks to mixers/loaders, generally exceeded HED's level of concern even at maximum risk mitigation. Many of the mixer/loader/applicator scenarios for aquatic sites (liquid and wettable powder applications) also exceeded HED's level of concern even at maximum risk mitigation. In particular, HED has serious concerns for any scenario that involves open mixing/loading of wettable powder formulations of rotenone. HED also has concerns for mixing/loading/applying via backpack sprayers for both liquid and wettable powder formulations. Please see the January 24, 2006 risk assessment (DP barcode D307385), to see the occupational risks to the non-piscicidal uses of rotenone which are no longer being supported by the registrants but, remain on current labels to permit depletion of existing stocks.

HED expects minimal occupational postapplication exposure from the piscicidal use of rotenone. As a result, no quantitative assessment was completed for occupational postapplication exposure.

In summary, the toxicity database is incomplete and a potentially critical effect, neurotoxicity, cannot be addressed with the existing database. Identification and quantification of potential impurities in rotenone formulations remains unresolved. Though dietary (water only) risks are not of concern, based on reasonable but conservative assumptions, confirmatory data are and label amendments are also recommended. Recreational postapplication risks (i.e., swimming) are of concern for toddlers. There are also some occupational handler risks that exceed HED's level of concern.

# 2.0 Ingredient Profile

Rotenone is a non-specific botanical insecticide with some acaricidal properties. Rotenone is used for fish eradications as part of water body management. Rotenone is a rotenoid plant extract obtained from such species as barbasco, cube, haiari, nekoe, and timbo. These plants are members of the pea (Leguminosae) family. Rotenone-containing extracts are taken from the roots, seeds, and leaves of the various plants. Rotenone is only slightly insoluble in water. Rotenone is used either as a powder from ground-up plant roots or extracted from roots and formulated as a crystalline or liquid preparation. Formulations include crystalline preparations (approximately 95% pure), emulsified solutions (approximately 50% pure), and dusts (approximately 0.75% to 5% pure).

## 2.1 Summary of Registered/Proposed Uses

Rotenone is a widely used as a piscicide in the United States. Rotenone piscicide products are formulated as liquids and as wettable powders.

### 2.1.1 Registered Use Categories and Sites

Rotenone is currently registered for use in a variety of occupational and residential scenarios, however, in memos dated (March 7, 2006; March 17, 2006; and April 5, 2006) the technical registrants (Prentiss, Inc.; Foreign Domestic Chemicals Corporation; and Tifa Limited) for rotenone voluntarily cancelled all uses of rotenone except for the piscicidal uses. This assessment deals with occupational populations that could be potentially exposed while performing rotenone applications as well as residential populations that may be exposed to rotenone during postapplication time periods (i.e., swimming). The cancelled uses of rotenone were previously assessed in the January 24, 2006 risk assessment (DP barcode D307385), which can be found on EPA's website.

Currently, rotenone is used as a piscicide in two main areas. The first use is when rotenone is used in water body (lakes, ponds, streams, etc.) fish management strategies. Rotenone is typically used in this manner when a water body has an unbalanced fish population or a non-native introduced species threatens native fish populations. The second use is when rotenone is used in catfish aquaculture. The use of rotenone in catfish aquaculture is typically limited to treatment of the aquaculture ponds in the spring prior to stocking of a new "crop" of catfish fry. The purpose of this treatment is to eliminate undesirable fish species (i.e., shad, blue gills, and mud cats) that would compete with the catfish fry.

### 2.1.2 Application Methods and Rates

Piscicidal applications of rotenone are applied using several types of application equipment – including helicopters, closed system aspirators, boats with over-surface booms, boats with underwater hoses, drip bars (in rivers and streams) and backpack sprayers. Table 2.1 includes a description of application methods and rates that are currently being approved for use by the rotenone technical registrants. These rates and methods apply to use of rotenone in fish management strategies as well as catfish aquaculture.

The area treated per day throughout this assessment is described as acre-foot/day (A-ft/day) for lake, pond, and reservoir applications and as cubic feet/day ($ft^3$/day) for stream and river applications. Acre-foot/day numbers are calculated by taking the number of surface acres treated per day and multiplying by the depth of the lake being treated, which was assumed by HED to be 5 feet. For example, with helicopter applications, HED assumed the high end of the treatment range would be 10 surface acres and when this number is multiplied by the depth of 5 feet, a value of 50 A-ft/day is acquired. Similar calculations were performed for river and stream applications but for these applications HED used the length of stream treated, the depth of stream, and the width of stream. For example, with backpack applications, HED estimated that the length of stream that could be treated in one day is 10,560 ft (2 miles) and when this number is multiplied by the 2 foot depth of the stream and the 10 foot width of the stream, a value of 211,200 $ft^3$ is acquired.

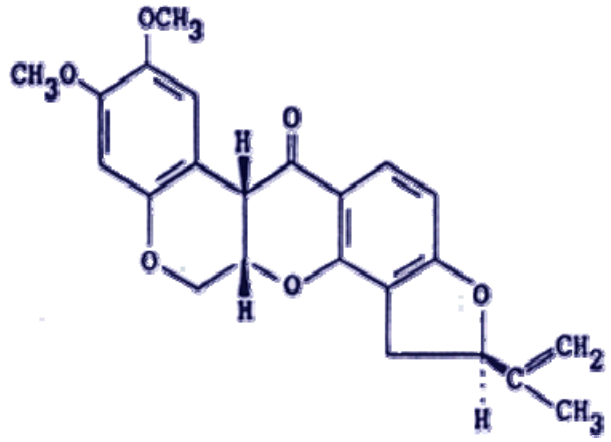
**Table 2.1. Summary of Maximum Application Rates for Registered Rotenone Aquatic Uses**

| Use Site | Target of Application | Maximum Application Rate [1] | Formulation | Application Equipment | Area Treated or Amount Handled Per Day [2] |
|---|---|---|---|---|---|
| Lakes, ponds, reservoirs | Fish | 0.68 lb ai/A-ft & 0.54 lb ai/A-ft | Liquid | Helicopter | 50 A-ft/day & 25 A-ft/day |
| | | | | Boat: over-surface boom | 500 A-ft/day & 250 A-ft/day |
| | | | | Boat: underwater hoses | 500 A-ft/day & 250 A-ft/day |
| | | | | Backpack | 10 A-ft/day |
| | | | | Closed system aspirator | 500 A-ft/day & 250 A-ft/day |
| | | 0.68 lb ai/A-ft & 0.54 lb ai/A-ft | WP | Boat: over-surface boom | 500 A-ft/day & 250 A-ft/day |
| | | | | Boat: underwater hoses | 500 A-ft/day & 250 A-ft/day |
| | | | | Backpack | 10 A-ft/day |
| | | | | Closed system aspirator | 500 A-ft/day & 250 A-ft/day |
| Moving water (streams) | | 0.000016 lb ai/$ft^3$ & 0.000013 lb ai/$ft^3$ | Liquid | Backpack | 211,200 $ft^3$ |
| | | | | Drip bar | |
| | | 0.000016 lb ai/$ft^3$ & 0.000013 lb ai/$ft^3$ | WP | Backpack | |
| | | | | Drip bar | |
| Seeps or Springs | | | WP | Volumetric container (powder/sand/gelatin paste) | |

[1] Maximum of two applications of rotenone per year.

[2] Area treated per day values for all application methods except boats are based on personal contact with Brian Finlayson, California Department of Fish and Game (1/9/06). Area treated per day values for boat application methods are based on HED professional judgment.

## 2.2 Structure and Nomenclature

| TABLE 2.2. Test Compound Nomenclature | |
|---|---|
| Chemical structure |  |
| Empirical formula | $C_{23}H_{22}O_6$ |
| Common name | Rotenone |
| Trade names | Chem-fish, Curex flea duster, Derrin, Cenol Garden Dust, Cuberol, Sinid, Tox-R, Noxfire, Cibe Extract, Rotacide, Fish Tox, Chem-Mite, Green Cross Warbler Powder |
| 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. |
| CAS name | (2*R*,6a*S*,12a*S*)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromeno [3,4-*b*]furo[2,3-*h*]chromen-6-one |
| CAS Registry Number | 83-79-4 |
| Chemical Class | Rotenoid |
| Known Impurities of Concern | Extraction compounds may exist as impurities in unspecified amounts[2] |

[2] Reported environmental incidents (Health & Safety Report; HS – 1772, 1998) have shown measurable concentrations of volatile organic compounds (trichloroethylene, toluene, xylene), semi-volatile organic compounds (2-methyl-naphthylene, 1-methyl naphthylene, naphthylene, ethyl benzene), and nonvolatile organic compounds (piperonyl butoxide, benzoic acid), after applications of rotenone to bodies of water for piscicidal use. See review in RDavid Jones 2006. Most current CSFs do not list and/or quantify the impurities. Updated CSFs that abide by EPA regulations are needed.

## 2.3 Physical and Chemical Properties

| Table 2.3. Product Chemistry Data Summary Table for Rotenone | | | | |
|---|---|---|---|---|
| **OPPTS Guideline Numbers** | **Data Requirements: Rotenone [Technical Grade of Active Ingredient]**<br><br>CAS Number: 83-79-4<br>PC Code: 071003<br>Formula: $C_{23}H_{22}O_6$ | **Master Record Identification [MRID]** | **Status** | **Results or *Deficiency** |
| 830.1550 | Product Identity and Composition | 441115-01 | Acceptable | |
| 830.1600 | Description of Materials Used to Produce the Product | 441115-01 | Acceptable | |
| 830.1620 | Description of Production Process | 441115-01 | Acceptable | |
| 830.1650 | Description of Formulation Process | 446528-01 | Acceptable | |
| 830.1670 | Discussion of Formation of Impurities | 441115-01 | Acceptable | |
| 830.1700 | Preliminary Analysis | 441386-01 | Acceptable | |
| 830.1750 | Certified Limits | 443953-01 | Acceptable | |
| 830.1800 | Enforcement Analytical Method | 447265-01, 445108-01 | Acceptable | |
| 830.1900 | Submittal of Samples | | Acceptable | 44.2% Technical Grade Active Ingredient Expires: 02/22/2006, EPA Repository, Ft. Meade, MD |
| 830.6302 | Color | 438180-02 | Acceptable | Off White to Tan |
| 830.6303 | Physical State | 438180-02 | Acceptable | Powder |
| 830.6304 | Odor | 438180-02 | Acceptable | Wet chalk |
| 830.6313 | Stability to normal and elevated temperatures, metals and metal ions | 441237-05 | Acceptable | Temp(s) = No appearance change; the loss was less than 5%.<br>Metals = No appearance change, the loss was about 5%. |
| 830.7000 | pH | 441308-01, 446528-01 | Not Applicable | Insoluble in water |
| 830.7050 | UV/VIS absorption | | Acceptable | 480 at 235nm & 550 at 292 nm; Ref: Clarke's Analysis of Drugs and Poisons; London Pharmaceutical Press Electronic Version 2004 |
| 830.7200 | Melting Point/Melting Range | 441237-02 | Acceptable | 160 °C - 163 °C |
| 830.7220 | Boiling Point/Boiling Point Range | | Not Applicable | See 830.7200 |
| 830.7300 | Density/Relative Density/Bulk Density | 438180-02 | Acceptable | Fluffy -- 0.2400 g/cm$^3$; 14.70 lb/cu ft<br>Compacted -- 4500g/cm$^3$; 28.10 lb/cu ft |
| 830.7370 | Dissociation Constant | 447181-01 | Acceptable | None at pH 2-12 (OECD Method No. 112) |
| 830.7550 | Partition coefficient (n-octanol /water) shake flask method | 441237-04 | Acceptable | $K_{o/w}$ Log P = 4.16 |
| 830.7560 | Partition coefficient (n-octanol /water) generator column method | | | See Guideline 830.7550 |
| 830.7570 | Partition coefficient (n-octanol /water) estimation by liquid chromatography | | | See Guideline 830.7550 |
| 830.7840 | Water Solubility: Column Elution Method; Shake Flask Method | 441237-03 | Acceptable | Solvent = Water \| Temperature = 20 °C<br>Avg. Solubility = 0.142 µg/ml |
| 830.7860 | Water solubility, generator column method | | | See Guideline 830.7840 |
| 830.7950 | Vapor pressure | 446529-01 | Not Applicable | |

# 3.0 Metabolism Assessment

## 3.1 Comparative Metabolic Profile

The qualitative nature of the residue in living organisms is not understood. However, the rapidity of rotenone degradation in the environment may limit the potential for uptake by macroorganisms, and therefore may limit the potential for metabolism except by microbes. Enzymatic metabolism of the sort that might occur in living organisms may result in the same biotransformation pathway as other degradative processes. It is not known whether metabolism of rotenone by living organisms proceeds rapidly or whether it would give rise initially to rotenolone, and then to polar products as it occurs in the environment.

## 3.2 Nature of the Residue in Foods

Food uses are no longer being supported by the registrants (see section 2.1). HED's analysis of the available crop and livestock residue and metabolism data may be found in *ROTENONE: Phase 4 HED Chapter of the Reregistration Eligibility Decision Document (RED). PC Code: 071003. DP Barcode: D307385. January 24, 2006.*

## 3.3 Rat Metabolism

There are no acceptable guideline metabolism studies available for rotenone. However, an Acceptable/Non-guideline metabolism and pharmacokinetics study is available for rotenone (rat). The primary route of excretion was in the feces with polar metabolites being identified in the feces. Metabolic profiles for the seven metabolites found in the feces were not obtained. In conjunction with fecal elimination, rotenone underwent extensive enterohepatic circulation. Tissue accumulation was low, typically less than 1% of the administered dose.

A definitive target organ has not been identified although the mechanism of action is well known. Rotenone uncouples oxidative phosphorylation by blocking electron transport at complex I within the mitochondria. Numerous published literature studies conducted over the past ten years indicate rotenone inhibits the activity of complex I of the mitochondrial electron transfer chain but also reproduces features of Parkinson's disease (PD), including selective nigrostriatal dopaminergic degeneration and microglial activation [*Sherer TB, Betarbet R, Kim JH, Greenamyre JT (2003). Selective microglial activation in the rat rotenone model of Parkinson's disease. Neuroscience Letters 341 87-90*]. More recently, rotenone has been associated with features of Parkinson's disease by the development of a-synuclein-positive cytoplasmic inclusions [*Spillantini et al., 1997. Spillantini MG, Schmidt ML, Lee VM, Trojanawski JQ, Jakes R, Goedert M (1997). Alpha-synuclein in Lewy bodies. Nature 388:839-840.; Wooten, 1997 Wooten GF (1997). Neurochemistry and neuropharmacology of Parkinsons's disease. In: Movement disorders:neurologic principles and practice (Watts RL, Koller WC, eds), pp 153-160. New York: McGraw-Hill.; Sherer et al., 2003a Sherer TB, Kim JH, Betarbet R, Greenamyre JT (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggression. Exp Neurol 17:9-16., Sherer et al., 2003b Sherer TB, Betarbet R, Testa C, Byoung BS, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT (2003). Mechanism of toxicity in Rotenone models of Parkinson's disease. The Journal of Neuroscience 23(34):10756-10764.), another feature of PD.*]

### 3.4 Environmental Degradation

The primary degradate identified in the environmental fate studies is rotenolone. Rotenolone is believed to be a complex of related stereoisomers; at least four, and perhaps more occur. Rotenolone, or specific rotenolone isomers, are the only identified major (> 10%) degradates. Rotenolone occurs at up to 80% of the applied parent in the anaerobic aquatic metabolism study. Limited ecological test data show rotenolone to be one tenth as lethal to fish as rotenone (*CDFG 1991*). The other identified degradates, in addition to $CO_2$, are listed below. Many of these were identified only in a non-guideline photodegradation study on bean leaf.

**Table 3.4 Rotenone Degradation Products**

| Metabolite | Amount | Study |
|---|---|---|
| Rotenolone | 33-50% of applied | MRID# 00141409 (hydrolysis) |
| Rotenone | 13.5% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| 6a,12a-Dehydrorotenone | <0.2% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| 6',7'-Epoxyrotenone | 3.5% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| 6',7'-Epoxy-6ab,12ab-rotenolone | 4.8% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| 6ab,12ab-Rotenolone | 11% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| 6ab,12aa-rotenolone | 0.4% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| Rotenone | 0.7% of applied | MRID# 41125402 (photodegradation - bean leaf) |
| Rotenolone | 25% of applied | MRID# 00141274 (Aerobic aquatic metabolism) |
| Rotenolone | 80% of applied | MRID# 00141273 (Anaerobic aquatic metabolism) |
| Rotenolone | Identified, not quantified | MRID# 455801073 (Bioaccumulation in fish) |
| 6',7'-dihydro-6',7' -dihydroxyrotenolone | Identified, not quantified | MRID# 455801073 (Bioaccumulation in fish) |

# 4.0 Hazard Characterization/Assessment

## 4.1 Hazard Characterization

### 4.1.1 Database Summary

#### 4.1.1.1 Critical Studies (animal, human, general literature)

The acute toxicity profile for rotenone is complete. Rotenone is acutely toxic via the oral and inhalation routes of exposure (Toxicity Category I), with females more sensitive than males to acute oral toxicity. Rotenone was neither corrosive nor irritating to the skin or eye (Toxicity Category IV) and is not a dermal sensitizer.

The database for rotenone is not complete with data gaps existing for chronic dog, developmental toxicity (non-rodent), dermal penetration, and 21-day dermal toxicity studies. Acceptable oral studies for rotenone include an oral 90-day subchronic (dog), oral developmental toxicity (rat and mouse), reproduction (rat), carcinogenicity (rat and mouse), and combined chronic/cancer (rat) study. Rotenone was negative in several *in vitro* mutagenicity assays.

In a special continuous intravenous study (Betarbet et al., 2000[3], MRID# 45279501) with Lewis rats, exposure to rotenone (2.5-2.75 mg/kg/day) produced behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease in humans. Intravenous rotenone induced specific neurodegenerative lesions in nigrostriatal dopaminergic neurons. The microscopic lesions progressed over time and correlated with complex I inhibition. Clinical signs in affected animals included hypoactivity, unsteady gait, and hunched posture. Since the publication of the Betarbet study in 2000, several laboratories have published studies verifying the systemic complex I inhibition caused by rotenone, including dopaminergic neurotoxicity, and currently use rotenone as a model (*in vitro*[4] and *in vivo*) for Parkinson's disease. While the intravenous route of exposure is not relevant to humans, it may mimic inhalation exposure, which is a route of concern with rotenone. Inhalation is the most direct point-of-entry for absorption, similar to the intravenous route, with distribution prior to metabolism of the chemical and therefore most comparable to the intravenous route. Inhaled substances may pass directly into the bloodstream and circulate once through the body, including the brain, before they reach the liver, where most materials are substantially metabolized. The only available inhalation toxicity study is an acute $LC_{50}$ study in the rat. Therefore, a DCI (Data Call In) was issued (02/09/2004) requesting a 21-day inhalation neurotoxicity study in the Lewis rat.

#### 4.1.1.2 Metabolism, toxicokinetics, mode of action data

There are no guideline metabolism studies available for rotenone. However, an Acceptable/Non-guideline metabolism and pharmacokinetics study is available for rotenone (rat). The primary route of excretion was in the feces with polar metabolites being identified in the feces. Structural characterization of the seven metabolites found in the feces were not obtained. In conjunction

[3] Betarbet R., TB Sherer, G MacKenzie, M Garcia-Osuna, AV Panov, JT Greenamyre 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nature neuroscience 3(12) 1301-1306 (MRID 45279501).

[4] Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR, Greenamyre JT (2002). An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered α-synuclein metabolism and oxidative damage. J Neuroscience 22:7006-7015.

with fecal elimination, rotenone underwent extensive enterohepatic circulation. Tissue accumulation was low, typically less than 1% of the administered dose.

A definitive target organ has not been identified although it is known that rotenone uncouples oxidative phosphorylation by blocking electron transport at complex I within the mitochondria. Numerous published literature studies within the past ten years indicate rotenone inhibits the activity of complex I of the mitochondrial electron transfer chain and also reproduces features of Parkinson's disease (PD), including selective nigrostriatal dopaminergic degeneration and microglial activation[5]. More recently, rotenone has been associated with features of PD by the development of α-synuclein-positive cytoplasmic inclusions (Spillantini etal., 1997[6]; Wooten, 1997[7]; Sherer et al., 2003a[8], Sherer et al., 2003b[9]), another feature of PD. It is currently not understood how the mechanism for rotenone translates into the etiology of PD.

#### 4.1.1.3 Sufficiency of studies/data

The toxicity database for rotenone is adequate for selecting toxicity endpoints for the risk assessment. However, the available oral studies defining the hazard component of this risk assessment are not adequate to assess neurotoxicity. The current database includes guideline studies via the oral route of exposure that typically do not include the Functional Observational Battery (FOB) or neurotoxicity parameters. In addition, inhalation exposure can be a major route of exposure for some uses and no subchronic inhalation toxicity studies are available for rotenone. As stated earlier, published literature has reported behavioral effects and brain lesions in the Lewis rat, resembling PD in humans, after continuous intravenous, and recently subcutaneous[10], exposure to rotenone. In the subcutaneous study, decreased survival rate and behavioral impairment were observed in male Lewis rats beginning with subcutaneous administration of 2 mg/kg rotenone (in DMSO) for 21-days (lowest dose tested).

### 4.1.2 Toxicological Effects

As stated above, a definitive target organ has not been identified although rotenone uncouples oxidative phosphorylation by blocking electron transport at complex I within the mitochondria. The major toxicological concern that HED has with rotenone is the potential to cause PD in humans. PD is not normally seen in rats and yet studies in the Lewis rat clearly show PD-like effects that cannot be ignored. HED is uncertain whether these findings are applicable to humans and by what route. Minimal systemic toxicity has been observed in subchronic and chronic animal studies. The most common effect in animal studies from intermediate- or long-term oral exposure was a decrease in body weight or body weight gain. Rats were more sensitive than mice and in both species, females were more sensitive than males to effects on body weight.

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[5] Sherer TB, Betarbet R, Kim JH, Greenamyre JT (2003). Selective microglial activation in the rat rotenone model of Parkinson's disease. Neuroscience Letters 341 87-90.

[6] Spillantini MG, Schmidt ML, Lee VM, Trojanawski JQ, Jakes R, Goedert M (1997). Alpha-synuclein in Lewy bodies. Nature 388:839-840.

[7] Wooten GF (1997). Neurochemistry and neuropharmacology of Parkinsons's disease. In: Movement disorders:neurologic principles and practice (Watts RL, Koller WC, eds), pp 153-160. New York: McGraw-Hill.

[8] Sherer TB, Kim JH, Betarbet R, Greenamyre JT (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggression. Exp Neurol 17:9-16.

[9] Sherer TB, Betarbet R, Testa C, Byoung BS, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT (2003). Mechanism of toxicity in Rotenone models of Parkinson's disease. The Journal of Neuroscience 23(34):10756-10764.

[10] Fleming SM, Zhu C, Fernagut PO, Mehta A, DiCarlo CD, Seaman RL, and Chesselet MF (2004). Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Experimental Neurology 187 pp 418-429.

In chronic studies, the basis for the lowest observed adverse effect levels (LOAELs) was a decrease in body weight and body weight gain by female rats (1.88 mg/kg/day) and male and female mice (111 and 124 mg/kg/day, respectively). The no observed adverse effect level (NOAEL) for chronic toxicity in rats was 0.375 mg/kg/day but a NOAEL was not identified in mice.

Decreased maternal body weight gain was also observed in developmental toxicity studies with rats and mice (1.5 and 24 mg/kg/day, respectively). Additionally, rats showed clinical signs of toxicity (salivation and rubbing the face and paws after treatment) at maternal doses as low as 0.75 mg/kg/day. Developmental toxicity was observed as decreased fetal body weight (23%) in rats (maternal 6 mg/kg/day) and increased resorptions (3.8 vs. 0.5 controls) with correspondingly fewer live fetuses/litter in mice (8.2 vs. 10.8 controls, maternal 24 mg/kg/day). No treatment-related structural external, visceral, or skeletal abnormalities were found in fetuses from treated dams.

In a two-generation reproductive toxicity study (rat) with rotenone, adult and offspring toxicity were observed at doses greater than 3.0 mg/kg/day. The main effect in both parental animals and pups was decreased body weight and body weight gain. Females were more sensitive than males and the magnitude of effects was similar between generations. Parental toxicity was indicated by decreased absolute body weight and body weight gain for the high-dose males and females (4.8 and 6.2 mg/kg/day, respectively) and the mid-dose females (3.0 mg/kg/day) of both generations. Food consumption was only marginally affected and mainly in the high-dose groups. Decreased maternal weight gain by the 6.2-mg/kg/day $F_0$ and $F_1$ dams during gestation correlated with a decrease in the mean number of live pups/litter in the high-dose groups of both generations (9.7-9.9 vs. 11.4-11.8 for the controls). $F_1$ and $F_2$ offspring body weight was slightly or significantly less than that of controls for the 6.2-mg/kg/day pups beginning at birth and for the 3.0-mg/kg/day pups beginning on post natal day (PND) 4. Body weight gain was reduced in the mid- (20-26%) and high-dose (40-60%) pups of both generations throughout lactation beginning with the interval PND 0-4.

None of the results from the available studies, except the acute oral toxicity study, showed evidence of neurotoxicity. However, as discussed earlier, rotenone administered via the intravenous or subcutaneous route of exposure in male Lewis rats produced behavioral, biochemical, and neuropathological effects that resemble PD in humans. Rotenone administered intravenously at 2.5-2.75 mg/kg/day induced specific neurodegenerative lesions in nigrostriatal dopaminergic neurons. The microscopic lesions progressed over time and correlated with complex I inhibition. Clinical signs in affected animals included hypoactivity, unsteady gait, and hunched posture. Inhalation is the most direct point-of-entry for absorption similar to the intravenous route with distribution prior to metabolism of the chemical and therefore, most comparable to the intravenous route. Inhaled substances may pass directly into the bloodstream and circulate once through the body, including the brain, before they reach the liver, where most materials are substantially metabolized.

Rotenone is classified as Group E (evidence of non-carcinogenicity for humans) (CARC 10/05/1988). No evidence for carcinogenicity was seen in mice or rats from available carcinogenicity studies. Administration of rotenone to both species for up to two years did not result in an increase in overall tumor incidence or increase the incidence of any specific type of tumor. The chemical was negative for gene mutation in two studies with *Salmonella typhimurium* and for mitotic gene conversion with *Saccharomyces cerevisiae*. Micronucleus formation was not induced in the bone marrow of mice. Rotenone also did not cause chromosomal aberrations in CHO cells *in vitro* with or without activation or in bone marrow

cells from rats administered up to 7 mg/kg orally. However, both the rat and mouse micronucleus and bone marrow assays are classified unacceptable/non-guideline since a maximum tolerated dose (MTD) was not achieved in either speicies. Positive results for gene mutation were obtained only in mouse lymphoma cells, without metabolic activation, at concentrations equal to and below those which also caused significant cytotoxicity.

### 4.1.3 Dose-response

Based on the registrants' proposed support of the piscicide use only, a dietary risk assessment was conducted that estimates acute and chronic dietary risks resulting from direct applications of rotenone to, or adjacent to, bodies of water and drinking water consumption. Food uses are not supported, therefore, the Food Quality Protection Act (FQPA) of 1996 does not apply.

A dose related decrease in weight gain was shown in the parental, reproductive, and offspring endpoints in the two-generation reproduction study. Decreased fetal body weight was also evident at the highest dose tested in the developmental toxicity study. Both the cancer studies in the mouse and rat showed a good dose-response in body weight decreases. The body weight decrement of the offspring at birth from the rat reproduction study was selected for the acute reference dose (RfD) for females aged 13-49 years. Parental body weight decrement from the reproduction study was the selected adverse effect for the intermediate-term oral and incidental oral, and short- and intermediate-term inhalation exposure. Decreased body weight and food consumption from the chronic/oncogenicity study supports the chronic RfD and long-term inhalation exposure endpoints.

**Table 4.1.3a: Acute Toxicity Data on Rotenone**

| Guideline No. | Study Type | MRID#(s) | Results | Toxicity Category |
|---|---|---|---|---|
| 870.1100<br>81-1 | Acute oral [rat]<br>99.23% a.i. | 00145496 | $LD_{50}$ = 102 mg/kg (M)<br>$LD_{50}$ = 39.5 mg/kg (F) | I |
| 870.1200<br>81-2 | Acute dermal [rabbit]<br>97.9% a.i. | 43907501 | $LD_{50}$ = >5000 mg/kg | IV |
| 870.1300<br>81-3 | Acute inhalation [rat]<br>98% a.i. | 43882601 | $LC_{50}$ = 0.0212 mg/L combined<br>$LC_{50}$ = 0.0235 mg/L (M)<br>$LC_{50}$ = 0.0194 mg/L (F) | I |
| 870.2400<br>81-4 | Acute eye irritation [rabbit]<br>97.9% a.i. | 43907503 | minimal, in unwashed eyes conjunctival irritation, PIS 3.3 at 1 hr, cleared less than 24 hrs. | IV |
| 870.2500.<br>81-5 | Acute dermal irritation [rabbit]<br>97.9% a.i. | 43907504 | PIS 0.08 at 1 hour which decreased to 0 at 72 hours | IV |
| 870.2600<br>81-6 | Skin sensitization [guinea pig]<br>98% a.i. | 43817903 | Not a dermal sensitizer | NA |

| Table 4.1.3b: Subchronic, Chronic, Developmental, Reproductive and Other Toxicity Profile on Rotenone | | |
|---|---|---|
| **Guideline#/ Study Type** | **MRID# (year)/ Classification /Doses** | **Results** |
| 870.3100<br>82-1a<br>90-Day oral toxicity (rat) | | **Satisfied by MRID 00156739, 41657101 (83-5)** |
| 870.3150<br>82-1b<br>90-Day oral toxicity (dog) | 00141406 (1980)<br>Acceptable/guideline<br>M&F: 0, 0.4, 2, 10 mg/kg/day | NOAEL = 0.4 mg/kg/day<br>LOAEL = 2 mg/kg/day based decreased body weight in mid-dose females (20%) and high-dose males and females (30%) and treatment-related inanition.<br>(duration of treatment was 26 weeks) |
| 870.3200<br>82-2<br>21/28-Day dermal toxicity | | **Not Available/Data Gap** |
| 870.3250<br>82-3<br>90-Day dermal toxicity | | Not Required |
| 870.3465<br>82-4<br>90-Day inhalation toxicity | | Not Required. **21/28-Day study with neurological parameters is required.** |
| 870.3700a<br>83-3a<br>Developmental Toxicity (rat) | 00144294 (1982)<br>Acceptable/guideline<br>F: 0, 0.75, 1.5, 3, 6 mg/kg/day (GD 6-19) | **Maternal** NOAEL = not identified<br>LOAEL = 0.75 mg/kg/day, based on clinical signs of toxicity (salivation, rubbing of face and paws on cage in all groups).<br>**Developmental** NOAEL = 3 mg/kg/day<br>LOAEL = 6 mg/kg/day based on decreased fetal body weight (23%). |
| 870.3700a<br>83-3a<br>Developmental Toxicity (mouse) | 00141707 (1981) (main)<br>00145049 (1981) (range-finding)<br>Acceptable/guideline<br>F: 0, 3, 9, 15, 24 mg/kg/day (GD 6-17) | **Maternal** NOAEL = 15 mg/kg/day<br>LOAEL = 24 mg/kg/day, based on decreased body weight (10%) and body weight gain (41%), from range-finding study.<br>**Developmental** NOAEL = 15 mg/kg/day<br>LOAEL = 24 mg/kg/day, based on increased resorptions (3.8 vs. 0.5 controls), from range-finding study.<br>**Acceptable when main and range-finding study considered together.** |
| 870.3700b<br>83-3b<br>Developmental Toxicity (non-rodent/rabbit) | | **Not Available/Data Gap** |

| Table 4.1.3b: Subchronic, Chronic, Developmental, Reproductive and Other Toxicity Profile on Rotenone | | |
|---|---|---|
| **Guideline#/ Study Type** | **MRID# (year)/ Classification /Doses** | **Results** |
| 870.3800<br>83-4<br>Reproduction<br>(rat) | 00141408 (1983)<br>Acceptable/guideline<br>F0: M: 0, 0.5, 2.4, 4.8 mg/kg/d<br>F0: F: 0, 0.6, 3.0, 6.2 mg/kg/day<br>F1 (M):0, 0.6, 3.1, 7 mg/kg/day<br>F1 (F): 0, 0.7, 3.7, 8.1 mg/kg/day | **Parental/Systemic** NOAEL (M/F) = 0.5/0.6 mg/kg/day<br>LOAEL (M/F) = 2.4/3.0 mg/kg/day based on decreased body weight (10-13%) and body weight gain (16-25%).<br>**Reproductive** NOAEL (M/F) = 2.4/3.0 mg/kg/day<br>LOAEL (M/F) = 4.8/6.2 mg/kg/day based on decreased live pups/litter in both generations (9.7-9.9 vs. 11.4-11.8 controls).<br>**Offspring** NOAEL (M/F) = 0.5/0.6 mg/kg/day<br>LOAEL (M/F) = 2.4/3.0 mg/kg/day based on decreased $F_1$ and $F_2$ pup body weight (8-18%) and body weight gain (mid 20-26%; high 40-60%). |
| 870.4100a<br>83-1a<br>Chronic toxicity<br>(rat) | | **Satisfied by MRID# 00156739, 41657101 (83-5)** |
| 870.4100b<br>83-1b<br>Chronic toxicity<br>(dog) | | **Not Available/Data Gap** |
| 870.4200<br>83-2a<br>Carcinogenicity<br>(rat) | 40179801b/46274301<br>(1986) NTP<br>**Unacceptable/guideline**<br>0, 38, 75 ppm<br>M: 0, 1.7, 3.4 mg/kg/day<br>F: 0, 1.8, 3.6 mg/kg/day | NOAEL (M/F) = 3.4/3.8 mg/kg/day<br>LOAEL = not identified<br>Animals could have tolerated a higher dose, MTD not achieved<br>**No evidence of carcinogenicity** |
| 870.4200<br>83-2a<br>Carcinogenicity<br>(rat) | 00143257 (1979)<br>**Unacceptable/non-guideline**<br>M&F: 0, 1.7, 3.0 mg/kg/day (i.p., 42 days, observed for 17 months)<br>M&F: 0, 1.7, 3.0 mg/kg/d (gavage, 42 days) | NOAEL = 3.0 mg/kg/day<br>LOAEL = not identified<br>Animals could have tolerated a higher dose, MTD not achieved<br>**No evidence of carcinogenicity** |
| 870.4200<br>83-2b<br>Carcinogenicity<br>(mouse) | 40179801a/46274301 (1986)<br>NTP<br>Acceptable/guideline<br>0, 600, 1200 ppm<br>M: 0, 111, 242 mg/kg/day<br>F: 0, 124, 265 mg/kg/day | NOAEL = not identified<br>LOAEL (M/F) = 111/124mg/kg/day based on decreased body weight (low: (M) 6-12%, (F) 12-20%, high: (M) 12-17%, (F) 17-26%.<br>**No evidence of carcinogenicity** |
| 870.4200<br>83-2b<br>Carcinogenicity<br>(hamster) | 00143256 (1979)<br>**Unacceptable/non-guideline**<br>0, 125, 250, 500, 1000 ppm<br>M&F: 0, 10, 21, 42, 83 mg/kg/day (food factor of 0.083) | NOAEL = 42 mg/kg/day<br>LOAEL = 83 mg/kg/day based on decreased weight gain.<br>**No evidence of carcinogenicity**<br>Excessive mortality due to secondary infection; additional groups administered 500 and 1000 ppm for 3 or 4 months were mated resulting in no viable offspring at 1000 ppm and maternal neglect and cannibalization at 500 ppm |

| Table 4.1.3b: Subchronic, Chronic, Developmental, Reproductive and Other Toxicity Profile on Rotenone | | |
|---|---|---|
| **Guideline#/ Study Type** | **MRID# (year)/ Classification /Doses** | **Results** |
| 870.4300<br>83-5<br>Chronic/Oncogenicity (rat) | 00156739 (1985)<br>41657101 (1989 amendment)<br>Acceptable/guideline<br>0, 7.5, 37.5, 75 ppm<br>M&F: 0, 0.375, 1.88, 3.75 mg/kg/day (food factor of 0.05) | NOAEL = 0.375 mg/kg/day<br>LOAEL = 1.88 mg/kg/day, based on decreased body weight at termination [M:7% (mid) and 15% (high); F: 24% (mid) and 42% (high)] and cumulative weight gain [M: 10% (mid) and 20% (high); F: 31% (mid) and 55% (high)] and food consumption in females [9% and 21% in mid and high, respectively]<br><br>**No evidence of carcinogenicity** |
| Gene Mutation<br>84-2<br>870.5100<br>(*Salmonella typhimurium*) | 40170506 (1988)<br>NTP study<br>Acceptable/guideline | No evidence of induced mutant colonies over background for any tester strain at any concentration up to 10,000 µg/plate with and without metabolic activation; strains TA98, TA100, TA1535, TA1537. |
| Gene Mutation<br>84-2<br>870.5100<br>(*Salmonella typhimurium*) | 40170502 (1978)<br>Acceptable/guideline | No evidence of induced mutant over background for any test strain at any concentration up to 10,000 µg/disk with and without metabolic activation; strains TA98, TA100, TA1535, TA1537, TA1538. |
| Gene Mutation<br>870.5300<br>84-2<br>(mouse lymphoma cells) | 40170505 (1984)<br>Acceptable/guideline | Evidence of a concentration-related positive response of induced mutant colonies over background at 0.25-8.0 µg/mL without metabolic activation; significant cytotoxicity at 4 and 8 µg/mL. |
| Cytogenetics<br>870.5375<br>84-2<br>(Chinese hamster ovary) | 40179801c (1986)<br>Acceptable/guideline | No evidence of chromosome aberrations up to 100 µg/mL without metabolic activation and 250 µg/mL with activation. |
| Cytogenetics<br>870.5385<br>84-2<br>(rat and mouse) | 00093702 (1981)<br>**Unacceptable/non-guideline** | Maximum tolerated dose (MTD) was not achieved in either the rat or mouse assays. No evidence of induced chromatid/chromosome aberrations in rat bone marrow cells up to 7.0 mg/kg; no significant increase in frequency of micronuclei in erythrocytes from bone marrow of mice up to 80 mg/kg. |
| Micronucleus<br>870.5395<br>84-2<br>(mouse) | 00093702 (1981)<br>Acceptable/guideline | Negative at oral doses of 0, 10, or 80 mg/kg |
| Mitotic gene conversion<br>870.5575<br>84-2<br>(*Saccharomyces cerevisiae*) | 00144292 (1981)<br>Acceptable/guideline | No evidence of induced mutant colonies over background for any test concentration up to 10,000 ug/plate with and without metabolic activation. Limit dose 5000 µg/plate. |

| Table 4.1.3b: Subchronic, Chronic, Developmental, Reproductive and Other Toxicity Profile on Rotenone | | |
|---|---|---|
| **Guideline#/ Study Type** | **MRID# (year)/ Classification /Doses** | **Results** |
| 870.6200a<br>81-8<br>Acute neurotoxicity screening battery | | Pending results of the subchronic inhalation neurotoxicity study. |
| 870.6200b<br>82-7<br>Subchronic neurotoxicity screening battery | | **Requested, DCI 2/9/04 (GDCI-071003-20980) -inhalation (rat)** |
| 870.6300<br>83-6<br>Developmental neurotoxicity | | Study required pending results of the subchronic inhalation neurotoxicity study. |
| 870.7485<br>85-1<br>Metabolism and pharmacokinetics (rat) | 00145496 (1984)<br>Acceptable/non-guideline<br>0.01, 0.1, 5 mg/kg (oral and iv) | Primary route of excretion is in feces; extensive enterohepatic circulation; some urinary excretion with females greater than males; polar metabolites reported in feces but metabolites not identified |
| 870.7600<br>85-2<br>Dermal penetration (rat) | | Not Available/Data Gap |
| Special studies<br>Subacute neurotoxicity (rat) | 45279501 (Betarbet et al., 2000)<br>Acceptable/nonguideline<br>M: 2.5-2.75 mg/kg/day by i.v. infusion for 1-5 weeks | Behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease in humans; induction of specific neurodegenerative lesions in nigrostriatal dopaminergic neurons |

## 4.2 Hazard Considerations

### 4.2.1 Adequacy of the Toxicity Data Base

Data are adequate for evaluation of effects resulting from *in utero* and postnatal exposure in rodents only. Two acceptable developmental toxicity studies have been conducted in rodents (mice and rats) and a reproductive toxicity study in rodents (rats) is available. It is noted that a developmental toxicity study in nonrodents (rabbit) has not been submitted. In the available studies, developmental toxicity was observed in both rats and mice at doses greater than or equal to those resulting in maternal toxicity. At the same dose that resulted in adult toxicity, offspring growth was decreased during the first four days of lactation, prior to direct contact with rotenone by the pups.

### 4.2.2 Evidence of Neurotoxicity

Evidence of neurotoxicity was not observed in available toxicity tests. In acute lethality studies, clinical signs included tremors, prostration, labored breathing, and soft feces following oral dosing and decreased activity, gasping, piloerection, ptosis, and sensitivity to touch after inhalation exposure. These clinical signs of toxicity are likely the result of the known

mechanism of action of rotenone, which is uncoupling of oxidative phosphorylation via blocking electron transport at complex I within the mitochondria. No clinical signs of toxicity were noted in subchronic or chronic studies in dogs, rats, mice, or hamsters. Histopathology of the nervous system is not typically evaluated in these subchronic or chronic studies.

In a special study with rats, continuous intravenous exposure for up to 5 weeks produced behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease in humans. Rotenone administered at 2.5-2.75 mg/kg/day was shown to induce specific neurodegenerative lesions in nigrostriatal dopaminergic neurons. The microscopic lesions progressed over time and correlated with complex I inhibition. Clinical signs in affected animals included hypoactivity, unsteady gait, and hunched posture. While this route of exposure is not relevant to humans, it does somewhat mimic inhalation exposure, which is a route of concern with rotenone. Except for one $LC_{50}$ study, no inhalation studies have been conducted.

### 4.2.3 Developmental Toxicity Studies

#### 4.2.3.1 Developmental Toxicity Study Conclusions

Developmental toxicity studies have been conducted with rotenone in the rat and mouse. A study in rabbits has not been submitted. Developmental toxicity was found in both species at a dose similar to or greater than that resulting in maternal toxicity. Rats were administered 0, 0.75, 1.5, 3, or 6 mg/kg/day by gavage on gestation days (GDs) 6-19. Mice were administered 0, 3, 9, 15, or 24 mg/kg/day by gavage on GDs 6-17. Maternal toxicity was evident in both species as decreased body weight gain during the treatment interval at doses of 1.5 mg/kg/day for rats and 24 mg/kg/day for mice. In addition, rats in all treated groups had clinical signs of toxicity including salivation and rubbing of the face and paws on the cage bottom after treatment.

Developmental toxicity was observed at the highest dose tested in both species. Fetal body weight was decreased in rats following maternal administration of 6 mg/kg/day. In mice, maternal treatment with 24 mg/kg/day resulted in increased resorptions with correspondingly fewer live fetuses/litter. No treatment-related structural external, visceral, or skeletal abnormalities were found in fetuses from treated dams.

#### 4.2.3.2 Rotenone - COBS® CD® Rats

In a developmental toxicity study (MRID# 00144294), 25 presumed pregnant COBS® CD® rats per group were administered 0, 0.75, 1.5, 3, or 6 mg/kg/day of Rotenone (97-98% a.i.; Lot No. not given) by gavage on gestation days (GD) 6-19, inclusive. Corn oil was used as the vehicle and negative control. On GD 20, all surviving dams were sacrificed and cesarian sectioned. All fetuses were weighed, sexed, and examined for external malformations/variations. Approximately one-half of the fetuses were examined for visceral malformation/variations by the Wilson technique. The remaining fetuses were processed for skeletal examination.

Two animals in the 6 mg/kg/day group were found dead, one each on GDs 10 and 17; another dam in this group was sacrificed moribund on GD 18. One animal in the 1.5 mg/kg/day group died on GD 11 probably due to a dosing error. All remaining animals survived to scheduled termination. Clinical signs of toxicity were observed in all treated groups and in the premature decedents of the 6 mg/kg/day group. The most frequent observations were salivation and rubbing of face and paws on the bottom of the cage after treatment which were seen in 11-14, 12-14, 15-22, and 21-24 animals of the 0.75, 1.5, 3, and 6 mg/kg/day groups, respectively. At necropsy, eight animals in the 6 mg/kg/day group had stomachs distended and filled with food.

Maternal body weight gain was significantly decreased for animals administered 1.5 mg/kg/day compared with the control group. Weight change for GDs 0-20 was 95, 94, and 58% of the control level for the 1.5, 3, and 6 mg/kg/day groups, respectively. Body weight and body weight gain corrected for gravid uterine weight was also significantly less than that of the controls for these treated groups. Lower weight gain resulted in GD 20 absolute body weight 83% of control for the high-dose group, although statistical significance was not attained. Maternal food consumption was not measured.

**Therefore, the maternal toxicity LOAEL for rotenone in rats is 0.75 mg/kg/day based on clinical signs of toxicity. The maternal toxicity NOAEL is not identified.**

At cesarean section, the pregnancy rates, number of corpora lutea, number of implantations per dam, live fetuses per litter, and fetal sex ratios were similar between the treated and control groups. Mean fetal body weight was significantly less in the 6 mg/kg/day group than that of the controls. No dose- or treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus.

**Therefore, the developmental toxicity LOAEL for rotenone in rats is 6 mg/kg/day based on decreased fetal body weight. The developmental toxicity NOAEL is 3 mg/kg/day.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study [OPPTS 870.3700 (83-3a)] in rats. This study was conducted prior to implementation of current guidelines.

#### 4.2.3.3 Rotenone - CD-1 Mice

In a developmental toxicity study (MRID# 00141407 main, 00145049 range-finding), 30 presumed pregnant CD-1 mice per group were administered 0, 3, 9, or 15 mg/kg/day of Rotenone (98.2% a.i.; Lot No. 100287) in corn oil by gavage on GDs 6-17, inclusive. Doses were selected on the basis of a range-finding study (MRID# 00145049) in which 7 pregnant mice/group were administered up to 24 mg/kg/day. On GD 18, all surviving dams were sacrificed and cesarian sectioned. All fetuses were weighed, sexed, and examined for external malformations/variations. Approximately one-half of the fetuses were examined for visceral malformation/variations. The remaining fetuses were eviscerated and processed for skeletal examination. In the main study, a total of 26 (278), 23 (268), 24 (271), and 25 (299) litters (fetuses) were examined in the control, low-, mid-, and high-dose groups, respectively.

Several intercurrent deaths of control and treated animals were considered incidental to treatment. No treatment-related clinical signs of toxicity were observed in any animal. Maternal body weight and body weight gain was similar between the treated and control groups. Food consumption was not measured and gross necropsy was unremarkable. In the range-finding study for animals administered 24 mg/kg/day, maternal body weight on GD 18 was 90% of the control level and weight gain for GD 0-18 was 59% of the controls.

**Therefore, the maternal toxicity LOAEL for rotenone in mice is 24 mg/kg/day based on decreased body weight and body weight gain during gestation. The maternal toxicity NOAEL is 15 mg/kg/day.**

At cesarean section in the main study, the pregnancy rates, number of implantations per dam, live fetuses per litter, mean fetal weight, and fetal sex ratios were similar between the treated and

control groups. The 24 mg/kg/day group of the range-finding study had a greater number of resorptions per dam as compared with the control group (3.8 vs. 0.5 for the controls) resulting in fewer numbers of live fetuses/litter (8.2 vs. 10.8 for controls). No dose- or treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus.

**Therefore, the developmental toxicity LOAEL for rotenone in mice is 24 mg/kg/day based on increased resorptions and the developmental toxicity NOAEL is 15 mg/kg/day.**

This study is classified as **Acceptable/Guideline** when considered with the range-finding study and together these satisfy the guideline requirements for a developmental toxicity study [OPPTS 870.3700 (§83-3a)] in mice.

### 4.2.4 Reproductive Toxicity Study

In a two-generation reproduction study Rotenone (97.9% a.i., Lot Nos. 091947 and 100287) was administered to groups of 15 male and 25 female rats in the diet at concentrations of 0, 7.5, 37.5, or 75 ppm (MRID# 00141408). Premating doses for the $F_0$ parental animals were 0, 0.5, 2.4, and 4.8 mg/kg/day, respectively, for males and 0, 0.6, 3.0, and 6.2 mg/kg/day, respectively, for females. Premating doses for the $F_1$ parental animals were 0, 0.6, 3.1, and 7.0 mg/kg/day, respectively, for males and 0, 0.7, 3.7, and 8.1 mg/kg/day, respectively, for females. $F_0$ and $F_1$ parental animals were administered test or control diet for 15 or 17 weeks, respectively, prior to mating and throughout mating, gestation, and lactation, until sacrifice. One litter was produced in each generation.

No treatment-related deaths or clinical signs of toxicity were observed in adults of either generation.

Body weight gain during premating weeks 13-15 was significantly less ($p \leq 0.05$ or 0.01) for the high-dose $F_0$ males and females and the mid-dose $F_0$ females compared with that of the controls. The most pronounced effect was an overall weight loss for these groups during week 13 resulting in premating weight gain 75-87% of the control group level. Lower weight gain resulted in significantly reduced ($p \leq 0.05$ or 0.01) absolute body weight for the high-dose $F_0$ males after the premating phase until termination (weeks 16-32), for the mid-dose $F_0$ females on premating weeks 14 and 15, and for the high-dose $F_0$ females on premating weeks 13, 14, and 15. Premating food consumption was decreased only for the high-dose $F_0$ males during week 15 compared with the controls. Absolute body weight of the $F_1$ adults was significantly less ($p \leq 0.01$) than controls throughout premating for the high-dose males and females and for the mid-dose females. Premating weight gain for these groups was 80-85% of the control level. Mid-dose $F_1$ males had lower body weight on weeks 0-10 of the premating interval with premating weight gain similar to the control level. Food consumption by the $F_1$ adults was significantly less ($p \leq 0.05$ or 0.01) than that of controls throughout premating for the high-dose males and females and occasionally for the mid-dose groups. Significantly lower body weights for the mid- and high-dose $F_0$ and $F_1$ females continued throughout mating, gestation, and lactation.

At necropsy, organ weight was not affected in the $F_0$ animals. In the $F_1$ mid- and high-dose groups, organ weight was decreased similar to body weight. No treatment-related microscopic lesions were found in the reproductive organs of parental animals of either generation. **The parental systemic LOAEL for rotenone in male and female rats was 37.5 ppm (2.4 and 3.0 mg/kg/day for males and females, respectively) based on decreased body weight and body**

**weight gain. The parental systemic NOAEL was 7.5 ppm (0.5 and 0.6 mg/kg/day for males and females, respectively).**

The mean number of days to mate, mating index, mean gestation length, and number of live litters were similar between the treated and control groups of both generations. Maternal weight gain by the high-dose $F_0$ and $F_1$ dams during gestation was 66% and 77%, respectively of the controls, while maternal weight changes during lactation were highly variable. The mean number of live pups/litter was significantly decreased ($p \leq 0.05$) in the high-dose groups of both generations (9.7-9.9 vs. 11.4-11.8 for the controls). **The reproductive toxicity LOAEL for rotenone in male and female rats was 75 ppm (4.8 and 6.2 mg/kg/day for males and females, respectively) based on decreased mean number of live pups/litter in both generations. The reproductive toxicity NOAEL was 37.5 ppm (2.4 and 3.0 mg/kg/day for males and females, respectively).**

$F_1$ and $F_2$ offspring body weight was slightly or significantly less than that of controls for the high-dose pups beginning at birth and for the mid-dose pups beginning on PND 4. Body weight gain was severely reduced in the mid- and high-dose pups of both generations throughout lactation beginning with the interval PND 0-4. Weight gain at all intervals during lactation by the mid- and high-dose groups was 72-79% and 43-52%, respectively, of control for the $F_1$ pups and 74-80% and 41-60%, respectively, for the $F_2$ pups. Offspring viability during lactation days 0-4 was slightly decreased for the high-dose $F_2$ pups (86.1% vs. 98.8% for controls). **The offspring toxicity LOAEL for rotenone in male and female rats was 37.5 ppm (2.4 and 3.0 mg/kg/day for males and females, respectively) based on decreased body weight and body weight gain in both generations. The offspring toxicity NOAEL was 7.5 ppm (0.5 and 0.6 mg/kg/day for males and females, respectively).**

This study is classified as **Acceptable/Guideline** and does satisfy the guideline requirement for a reproductive toxicity study [OPPTS 870.3800 (83-4)] in rats.

#### 4.2.5 Additional Information from Literature Sources

Since the original published study that suggested rotenone as a dopaminergic neurotoxin and a link to Parkinson's disease (2000), hundreds of studies have been published with rotenone as an *in vitro* and *in vivo* model. A recent literature search on the Entrez PubMed[11] website and using keywords "rotenone and Parkinson's Disease" identified approximately 200 studies related to rotenone as a model for understanding Parkinson's disease.

#### 4.2.6 Pre-and/or Postnatal Toxicity

A literature search identified several studies related to neurotoxicant exposure during development which may lead to susceptibility to chemical insult later during adulthood (*Melamed et al., 1990; Eriksson et al., 1993; Eriksson et al., 1996; Gupta et al., 1999; Thiruchelvam et al., 2002; Barlow et al., 2004*).

##### 4.2.6.1 Determination of Susceptibility

No quantitative or qualitative evidence supports increased susceptibility of rat or mouse fetuses or rat offspring. Fetuses were affected from *in utero* exposure to rotenone in the developmental toxicity studies at the same dose that resulted in maternal toxicity. Likewise, post-natal growth

[11] Entrez PubMed website available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi

and survival were reduced prior to direct exposure to the test material at the same or higher doses, respectively, that caused adult systemic toxicity. Similar doses also resulted in systemic toxicity in rats following chronic exposure. In rats, the same endpoint of toxicity, reduced body weight, was the main effect in adults, fetuses, and offspring.

It is currently unknown whether *in utero* exposure to rotenone in the developing rabbit results in toxicity below or at the same dose resulting in maternal toxicity. Likewise, post-natal growth and survival is unknown for the developing rabbit.

#### 4.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Post-natal Susceptibility

A moderate degree of concern exists for protection of infants and children. A non-rodent developmental toxicity study is currently not available. It is possible that toxicity not observed in the available rodent developmental toxicity studies would be identified in the non-rodent developmental toxicity study. However, in available rat studies, developmental and offspring toxicity occurred at doses that also caused parental/adult toxicity; qualitatively the effect in all ages was the same, i.e., reduced body weight and weight gain. For the relevant studies in rats, well defined NOAELs were identified as 3 and 0.6 mg/kg/day for developmental and offspring effects, respectively. A NOAEL of 2.0 mg/kg/day was identified in dogs following 6-month administration. A long-term study in adult rats has yielded a NOAEL similar to the dose affecting pup growth, e.g., 0.375 mg/kg/day.

### 4.3 Recommendation for a Developmental Neurotoxicity (DNT) Study

#### 4.3.1 Evidence that supports requiring a DNT study

None of the results from the tests conducted to date support the recommendation for a developmental neurotoxicity study. However, in a special study with rats, continuous intravenous exposure for up to 5 weeks produced behavioral, biochemical, and neuropathological effects that resemble Parkinson's disease in humans. Based on these findings, an inhalation neurotoxicity study is recommended. The requirement for a developmental neurotoxicity study is pending until the results from the inhalation study in adults are available.

#### 4.3.2 Evidence that supports not requiring a DNT study

The currently available data on the toxicity of rotenone (via oral route) do not support the recommendation for a developmental neurotoxicity study. Prenatal exposure has not resulted in central nervous system malformations. While offspring growth was affected at a dose which also affected parental animals, no functional or behavioral changes were reported in adults or pre- and post-weaning pups (complete neurotoxicity evaluation not done). Clinical signs suggestive of neurotoxicity were not observed in any study at doses that caused systemic toxicity, such as decreased body weight gain.

#### 4.3.3 Rationale for a Database Uncertainty Factor ($UF_{db}$)

No increased offspring sensitivity over parent was observed in the available rat or mouse pre-natal developmental studies or the post-natal reproduction study. However, a data gap does exist for a non-rodent (rabbit) developmental toxicity study. In addition, a recent review of the published literature indicates prenatal exposure to neurotoxicants (such as maneb and paraquat)

may result in selective, permanent alterations of the nigrostriatal dopaminergic system, which would enhance susceptibility to chemical exposure later in life (adulthood) (Barlow et al., 2004[12]). In essence, the current literature suggests that exposure to a neurotoxicant during development may produce a biological system that is more vulnerable to chemical insult later in life (Melamed et al., 1990[13]; Eriksson et al., 1993[14]; Eriksson, 1996[15]; Gupta et al., 1999[16]; Thiruchelvam et al., 2002[17]). A DCI for a subchronic inhalation study with neurotoxicity parameters was issued due to available literature indicating Parkinson's disease in rotenone (intravenous) exposed Lewis rats. A DNT study, as well as a subchronic oral neurotoxicity study, are reserved pending the results of the inhalation neurotoxicity study. Additional toxicity data gaps remain for a chronic toxicity study in dogs, dermal penetration study, and a 21-day dermal study. HED concluded that an $UF_{db}$ of 10X is warranted since significant data gaps exist.

The registrants are no longer supporting agricultural or residential uses, where the greatest potential for inhalation, dietary, and dermal exposure could occur. The potential for inhalation or dermal exposure by certified applicators using required PPE during the piscicide use is negligible. Therefore, the inhalation neurotoxicity study and all other toxicity data requirements are held in reserve (may be called in later) pending the outcome of monitoring and further mitigation measures. The $UF_{db}$ will remain in place until data deficiencies are satisfied.

### 4.4 Hazard Identification and Toxicity Endpoint Selection

Based on the registrants' proposed support of the piscicide use only, a dietary risk assessment was conducted that estimates acute and chronic dietary risks resulting from direct applications of rotenone to, or adjacent to, bodies of water and drinking water consumption. Dietary (food) exposure is not expected from the piscicidal use. Likewise, the 10x factor provided by the Food Quality Protection Act of 1996 does not apply.

#### 4.4.1 Acute Reference Dose (aRfD) - Females 13-49 years old

See section 4.2.3.3 for a descriptive summary of the developmental toxicity study in mice (MRID# 00141407 and 00145049).

Dose and Endpoint for establishing aRfD: The developmental toxicity NOAEL of 15 mg/kg/day based on increased resorptions at 24 mg/kg/day.

Uncertainty Factor (UF): 1000; includes 10X for interspecies extrapolation, 10X for intraspecies extrapolation, and 10X for database uncertainty.

Comments about Study/Endpoint/UF: At the LOAEL, increased resorptions resulted in fewer numbers of live fetuses/litter. This effect could have resulted from one or two exposures during development. Therefore, this developmental effect has implications for women of childbearing

[12] Barlow B.K., Richfield E.K., Cory-Slechta D.A., and Thirsuchelvam M. 2004. A fetal risk factor for Parkinson's disease. Dev. Neurosci. 4(26): 11-23.
[13] Melamed E., Rosenthal J., and Youdim M.B.H. 1990. Immunity of fetal mice to prenatal administration of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J. Neurochem. 55: 1427-1431.
[14] Eriksson P., Johansson U., Ahlbom J., and Fredriksson A 1993. Neonatal exposure to DDT induces increased susceptibility to pyrethroid (bioallethrin) exposure at adult age- Changes in cholinergic muscarinic receptor and behavioral variables. Toxicology. 77:21-30.
[15] Eriksson P. 1996. Developmental neurotoxicology in the neonate- Effects of pesticides and polychlorinated substances. Arch. Toxicol. Suppl. 18: 81-88.
[16] Gupta a., Agarwal R., and Shukla G.S., 1993. Functional impairment of the blood-brain barrier following pesticide exposure during early development in rats. Hum. Exp. Toxicol. 18: 174-179.
[17] Thiruchelvam M., Richfield E.K., Goodman B.M., Baggs R.B., and Cory-Slechta D.A., 2002. Developmental exposure to pesticides paraquat and maneb and the Parkinson's disease phenotype. Neurotox. 33: 621-633.

age. Since the effect occurred during development from one or two exposures, the duration is appropriate for this scenario. Application of a 10X $UF_{db}$ is recommended based on the lack of several studies.

| **Acute RfD (Females 13-49 years)** = $\frac{15 \text{ mg/kg/day}}{1000}$ = **0.015 mg/kg/day** |
|---|

### 4.4.2 Acute Reference Dose (aRfD) - General Population

A dose and endpoint are not proposed because, based on the available data, a single dose endpoint was not identified for this population subgroup. Clinical signs were reported in rats following a single oral dose, but a NOAEL could not be identified and a dose-response assessment could not be made from the data provided.

### 4.4.3 Chronic Reference Dose (cRfD)

In the chronic/oncogenicity study (MRID# 00156739 and 41657101), rotenone (>95%, a.i.) was administered in feed to 40 male and 40 female Charles River Fischer 344 rats per group at concentrations of 0, 7.5, 37.5 or 75 ppm for two years. Based on the standard food factor of 0.05 for rats, dietary concentrations of 7.5, 37.5 and 75 ppm resulted in doses of 0.375, 1.88 and 3.75 mg/kg/day, respectively.

No significant effect on mortality was noted in the control or treated groups. Male rats showed no statistically significant difference in body weight or cumulative weight gain until approximately week 68 in the mid-dose and high-dose groups, when compared to the controls. At termination, males showed a 7% decrease in body weight in the mid-dose group and a 15% decrease in the high-dose group, compared to the control group. Males also exhibited a 10% and 20% decrease in the cumulative weight gain at week 104 for the mid- and high-dose groups, respectively. Females in the mid- and high-dose groups had statistically significant decreases in body weight throughout the study. For terminal body weights, females had decreases of 24% in the mid-dose and 42% in the high-dose group, compared to control group. Cumulative weight gain was also significantly lower between control and treated females ranging from a 31% decrease in the mid-dose group to a 55% decrease in the high-dose group. While no significant difference in food consumption was noted in the male rats, females in the mid- and high-dose groups exhibited statistically significant decreases compared to the control group throughout the study. This decrease was on average 9% and 21% less, respectively.

No statistically significant or consistent differences were noted in the hematological parameters in either the male or female rats in any group. Urinalysis results in all rats were unremarkable. The only clinical chemistries and organ weights affected in the rats correlated with the low terminal body weights. The only macroscopic finding was thinness reported in 1/40 of the controls, 3/40 of the low-dose, 10/40 of the mid-dose and 25/40 of the high dose females upon necropsy.

No tumors were found of any treatment-related significance. The only non-neoplastic microscopic finding was an increased incidence of angiectasis and hemorrhage in the adrenals in the high-dose males and females. In MRID# 00156739, histopathological exam of the adrenals was not performed on the low- and mid-dose rats; however, an amendment providing this

additional histopathological information was provided (MRID# 41657101). In males, angiectasis was observed in 1/40 in the control and low-dose groups, 3/39 in the mid-dose group and 13/40 in the high-dose. Similar numbers were reported with adrenal hemorrhage: 3/40 controls and low-dose, 3/39 mid-dose and 14/40 in high-dose. Females exhibited adrenal angiectasis in 6/40 of controls, 5/40 low-dose, 4/40 mid-dose and 13/40 high-dose. The incidence in females of adrenal hemorrhage was 7/40 in controls and low-dose, 10/40 in mid-dose and 14/40 in high-dose. When the reviewer ran a Fischer Exact test on the data, results in high-dose male rats were statistically significant, suggesting the number of adrenal changes were treatment-related; however, based on the individual microscopic observations, the severity and distribution of the lesions were not different among any of the groups, control or treated. The results were less defined in females because of a higher number of incidences in the control group, and the increase in high-dose females was not statistically significant. As in males, the severity and distribution of lesions seen in microscopic observations in females were similar among all groups.

**The LOAEL for rotenone is 37.5 ppm (1.88 mg/kg/day), based on decreased body weight. The corresponding NOAEL was 7.5 ppm (0.375 mg/kg/day).**

At the doses tested, no treatment-related increases in tumor incidences were observed in male or female rats receiving any dose when compared to the controls.

Dose and Endpoint for establishing cRfD: Chronic NOAEL of 0.375 mg/kg/day based on decreased body weight and food consumption in females at 1.88 mg/kg/day.

Uncertainty Factor (UF): 1000; includes 10X for interspecies extrapolation, 10X for intraspecies extrapolation, and 10X for database uncertainty.

Comments about Study/Endpoint/UF: The duration of dosing and the endpoint are appropriate for this scenario. Application of the $UF_{db}$ is required due to the lack of several studies.

$$\textbf{Chronic RfD} = \frac{0.375 \text{ mg/kg/day}}{1000} = \textbf{0.004 mg/kg/day}$$

### 4.4.4 Incidental Oral Exposure (Short- and Intermediate-term)

See Section 4.2.4 for a descriptive summary of the reproductive toxicity study in rats (MRID# 00141408).

Dose and Endpoint: The parental toxicity NOAEL of 0.5 mg/kg/day based on decreased body weight and body weight gain at 2.4 and 3.0 mg/kg/day for males and females, respectively.

Uncertainty Factor (UF): 1000; includes 10x for interspecies extrapolation, 10X for intraspecies extrapolation, and 10X for database uncertainty.

Comments about Study/Endpoint: Reductions in offspring body weight began as early as PND 4 indicating that the effect began before the pups had direct contact with the food. Since the immediate effect on weight gain between PNDs 0 and 4 suggests that the effect could have been due to one or two exposures, the duration is appropriate for the short-term scenario. Sustained

lower weight gain throughout lactation indicates that the duration is appropriate for the intermediate-term scenario and included direct exposure to the offspring.

### 4.4.5 Dermal Absorption

Little information on the dermal absorption of rotenone is available and a dermal penetration study has not been submitted. Two suitable acute dermal toxicity studies in the rabbit are available for examination. In a dermal study with rotenone technical (97% a.i.), rotenone was applied as a single dose (5 g/kg) as light yellow crystals with no vehicle (not moistened). No mortalities or evidence of systemic toxicity were observed in rabbits at doses up to 5 g/kg (MRID# 43908501). Slight erythema is seen at the application site cleared within 24 hours. These results suggest negligible dermal absorption of rotenone. However, if rotenone was applied with a vehicle there may have been more absorption. In the second acute dermal study (MRID# 44336402) with rotenone brittle extract (rotenone 44.2%, other associated resins 44.2%, inerts 11.6%) the test material was applied moistened with deionized water (0.952 mL/2020 mg of test material). There were no deaths with the $LD_{50}$ >5.0 g/kg for both sexes. The potential toxicity from repeated dermal exposure is unknown.

No acute oral toxicity studies exist for rotenone in the rabbit to make a comparison of oral/dermal toxicity. Early studies found in the literature (Haag, 1931[18]; Lehman, 1954[19]; Soloway 1976[20]) contained oral/dermal toxicity data for the rabbit. However, these studies had multiple deficiencies including uncertainties as to purity and concentration of material tested, vehicle, and duration, and thus, were not considered.

If the acute oral toxicity study in the rat (MRID# 00145496) is considered in which the $LD_{50}$ for male and female rats is 102 mg/kg and 39.5 mg/kg, respectively, and assuming the rabbit is not unusually less sensitive than the rat, the comparison would indicate that the dermal absorption of rotenone (as crystals or from a water-wetted suspension) is less than 100% in the rabbit.

It should be noted that the concentration of rotenone that may be absorbed dermally under actual conditions will depend on the nature of the exposure. More absorption is likely to result from the emulsified solid than from the dry solid. However, if a structure activity relationship (SAR) search is considered, fluazifop-butyl is the compound most structurally similar to rotenone. The log P and molecular weight of fluazifop-butyl are 4.5 and 383.4 respectively. The log P and molecular weight of rotenone are 4.1 and 394.4, respectively. A dermal absorption study is available in humans for fluazifop-butyl, which indicated a dermal absorption factor of 9% (HIARC report 2004 fluazifop-butyl, Clark et al., 1993[21]). Based on relevant physical and chemical characteristics and dermal information in the human, the estimated dermal absorption of rotenone in humans is likely 10%[22].

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[18] Haag HB (1931). Toxicological studies of *Derris elliptica* and its constituents. I. Rotenone. J. Pharmacol. Exp. Ther. 43, 193-208.

[19] Lehman AJ (1954). A toxicological evaluation of household insecticides. Q. Bull.-Assoc. Food Drug Off. 18, 3-13.

[20] Soloway SB (1976). Naturally occurring insecticides. Environmental Health Perspectives Vol. 14, pp 109-117.

[21] Clark NWE, Scott RC, Blain PG, Williams FM (1993). Fate of fluazifop-butyl in rat and human skin in vitro. Arch Toxicol. 67:44-48.

[22] The dermal absorption human study for fluzifop-butyl is not subject to review by the Human Studies Review Board (HSRB).

### 4.4.6 Dermal Exposure (Short-, Intermediate- and Long- term)

Based on the rationale provided earlier, the dermal absorption of rotenone is 10% for short-, intermediate-, and long-term scenarios. See Section 4.2.4 for a descriptive summary of the reproductive toxicity study in rats (MRID# 00141408). Note – no long-term exposures are expected.

Dose and Endpoint: The parental toxicity NOAEL of 0.5 mg/kg/day based on decreased body weight and body weight gain at 2.4 and 3.0 mg/kg/day in males and females, respectively.

Uncertainty Factor (UF): 1000; includes 10X for interspecies extrapolation, 10X for intraspecies extrapolation, and 10X for database uncertainty.

Comments about Study/Endpoint/UF: Because effects from repeated dermal exposure are unknown, quantitative risk assessment for the short-, and intermediate-term exposure scenarios is recommended.

### 4.4.7 Inhalation Exposure (Short-, Intermediate- and Long-term)

See Section 4.2.4 and Section 4.4.3 for a descriptive summary of the two-generation reproduction study in rats and the chronic/oncogenicity study in rats, respectively.

Study Selected: reproduction study for short- and intermediate- term and chronic/oncogenicity study for long-term exposure. MRID# 00141408 and 00156739, 41657101. Note - currently, no long-term exposures are expected.

Short- and Intermediate-term:

The parental toxicity NOAEL of 0.5 mg/kg/day in rats based on decreased body weight and body weight gain in adults at 2.4 and 3.0 mg/kg/day M/F is recommended for short- and intermediate-term inhalation exposure scenarios.

Long-term:

The chronic oral toxicity NOAEL of 0.375 mg/kg/day in rats based on decreased body weight and food consumption in females at 1.88 mg/kg/day is recommended for use in long-term inhalation exposure scenarios.

Comments about Study/Endpoint/UF: Appropriate inhalation toxicity studies were not available for any exposure scenario. Reductions in offspring body weight began as early as PND 4 indicating that the effect began before the pups had direct contact with the food. Since the immediate effect on weight gain between PNDs 0 and 4 suggests that the effect could have been due to one or two exposures, the duration is appropriate for the short-term scenario. Sustained lower weight gain throughout lactation indicates that the duration is appropriate for the intermediate-term scenario and included direct exposure to the offspring. For long-term inhalation exposure, the chronic toxicity NOAEL is appropriate for this duration.

### 4.4.8 HED's Level of Concern (LOC)

Summary of HED's LOCs for risk assessment. Margins of exposure (MOEs) that are less than the LOCs are of concern.

**TABLE 4.4.8**

| Route / Duration | Short-term (1-30 Days) | Intermediate-term (1 - 6 Months) | Long-term (> 6 Months) |
|---|---|---|---|
| **Dietary Exposure** | | | |
| **Drinking Water** | Acute 1000 | NA | Chronic 1000 |
| **Occupational (Worker) Exposure** | | | |
| **Dermal** | 1000 | 1000 | 1000 |
| **Inhalation** | 1000 | 1000 | 1000 |
| **Residential (Non-Dietary) Exposure** | | | |
| **Oral** | 1000 | 1000 | N/A |
| **Dermal (All Populations)** | 1000 | 1000 | 1000 |
| **Inhalation (All Populations** | 1000 | 1000 | 1000 |

**For occupational exposure:** This is based on the 10X for interspecies extrapolation, 10X for intraspecies variation, and an additional 10X for database uncertainty (1000X).

**For residential exposure:** This is based on the 10X for interspecies extrapolation, 10X for intraspecies variation, and an additional 10X for database uncertainty (1000X).

### 4.4.9 Recommendation for Aggregate Exposure Risk Assessments

In accordance with the Food Quality Protection Act (FQPA) of 1996, for chemicals having tolerances in food, HED must consider and aggregate pesticide exposures and risks from three major sources: food, drinking water, and residential exposures (oral, dermal, and inhalation). All uses of rotenone on food crops have been proposed to be cancelled and thus the requirements of FQPA are not applicable and aggregate risk assessments have not been conducted.

### 4.4.10 Classification of Carcinogenic Potential

The Science Advisory Panel (SAP) met on September 7, 1988 to review the weight-of-the-evidence considerations and classification of the oncogenic potential of rotenone. The SAP panel endorsed the classification of rotenone in Group E because of lack of evidence of carcinogenicity in life-time studies in rats and mice. The Cancer Assessment Review Committee (CARC) then met on September 29, 1988 to examine the review presented by the SAP for rotenone. The CARC agreed with the classification recommended by the SAP and classified rotenone as Group E.

In summary, no evidence of carcinogenicity was seen in mice or rats at doses that caused systemic toxicity. Administration of rotenone to both species for up to two years did not result in an increase in overall tumor incidence or increase the incidence of any specific type of tumor. The chemical was negative for gene mutation in two studies with *Salmonella typhimurium* and

for mitotic gene conversion with *Saccharomyces cerevisiae*. Micronucleus formation was not induced in the mouse. Rotenone did not cause chromosomal aberrations in CHO cells *in vitro* with or without activation or in bone marrow cells from rats administered up to 7 mg/kg orally. Positive results for gene mutation were obtained in mouse lymphoma cells without metabolic activation at concentrations equal to and below those which also caused significant cytotoxicity.

### 4.4.10.1 Carcinogenic Potential in Rats

**1.** In a combined chronic/oncogenicity study (MRID# 00156739, 41657101), rotenone (>95%, a.i.) was administered in feed to 40 male and 40 female Charles River Fischer 344 rats per group at concentrations of 0, 7.5, 37.5 or 75 ppm for two years. Based on the standard food factor of 0.05 for rats, dietary concentrations of 7.5, 37.5 and 75 ppm resulted in doses of 0.375, 1.88 and 3.75 mg/kg/day, respectively.

No significant effect on mortality was noted in the control or treated groups. Male rats showed no statistically significant difference in body weight or cumulative weight gain until approximately week 68 in the mid-dose and high-dose groups when compared to the controls. At termination, males showed a 7% decrease in body weight in the mid-dose group and a 15% decrease in the high-dose group compared to the control group. Males also exhibited a 10% and 20% decrease in the cumulative weight gain at week 104 for the mid- and high-dose groups, respectively. Females in the mid- and high-dose groups had statistically significant decreases in body weight throughout the study. For terminal body weights, females had decreases of 24% in the mid-dose and 42% in the high-dose group compared to control group. Cumulative weight gain was also significantly lower between control and treated females ranging from a 31% decrease in the mid-dose group to a 55% decrease in the high-dose group. While no significant difference in food consumption was noted in the male rats, females in the mid- and high-dose groups exhibited statistically significant decreases compared to the control group throughout the study. This decrease was on average 9% and 21% less, respectively.

No statistically significant or consistent differences were noted in the hematological parameters in either the male or female rats in any group. Urinalysis results in all rats were unremarkable. The only clinical chemistries and organ weights affected in the rats correlated with the low terminal body weights. The only macroscopic finding was thinness reported in 1/40 of the controls, 3/40 of the low-dose, 10/40 of the mid-dose and 25/40 of the high dose females upon necropsy.

No tumors were found of any treatment-related significance. The only non-neoplastic microscopic finding was an increased incidence of angiectasis and hemorrhage in the adrenals in the high-dose males and females. In MRID# 00156739, histopathological exam of the adrenals was not performed on the low- and mid-dose rats; however, an amendment providing this additional histopathological information was provided (MRID# 41657101). In males, angiectasis was observed in 1/40 in the control and low-dose groups, 3/39 in the mid-dose group and 13/40 in the high-dose. Similar numbers were reported with adrenal hemorrhage: 3/40 controls and low-dose, 3/39 mid-dose and 14/40 in high-dose. Females exhibited adrenal angiectasis in 6/40 of controls, 5/40 low-dose, 4/40 mid-dose and 13/40 high-dose. The incidence in females of adrenal hemorrhage was 7/40 in controls and low-dose, 10/40 in mid-dose and 14/40 in high-dose. When the reviewer ran a Fischer Exact test on the data, results in high-dose male rats were statistically significant suggesting the number of adrenal changes were treatment-related; however, based on the individual microscopic observations, the severity and distribution of the lesions were not different among any of the groups, control or treated. The results were less defined in females because of a higher number of incidences in the control

group, and the increase in high-dose females was not statistically significant. As in males, the severity and distribution of lesions seen in microscopic observations in females were similar among all groups.

**The LOAEL for rotenone is 37.5 ppm (1.88 mg/kg/day), based on decreased body weight. The corresponding NOAEL was 7.5 ppm (0.375 mg/kg/day).**

At the doses tested, no treatment-related increases in tumor incidences were observed in male or female rats receiving any dose when compared to the controls.

This chronic toxicity/oncogenicity study in the rat is **Acceptable/Guideline** with the addition of the amendment providing more comprehensive histopathological data.

**2.** In a carcinogenicity study (MRID# 46274301, 40179801) from the National Toxicology Program (NTP) rotenone (lot no. 735-RAP-1502, purity >98% a.i.) was administered in diets at 0, 38, or 75 ppm to 50 F344/N rats/sex/dose for 103 weeks. The average daily dose for males and females in the low dose group was 1.7 and 1.8 mg/kg/day, respectively.

Survival of controls and dosed rats was similar (M: 22/50, 31/50, 30/50 and F: 27/50, 32/50, 31/50 for control, low, high dose, respectively). Mean body weights of dosed and control male rats were comparable. Mean body weights of high dose female rats were 5%-9% lower than control rats between weeks 58 and 88.

Neoplastic examination revealed parathyroid gland adenomas in 1/41 control, 0/44 low, and 4/44 high dose male rats. The historical incidence of this uncommon tumor in untreated control male rats in NTP studies is 4/1,314 (0.3%). However, since a tumor was identified in the control group out of 41 animals, the increased incidence in the high dose male rats cannot be specifically related to rotenone administration.

No significant dose-related trend was observed in the incidence of subcutaneous tissue fibromas, fibrosarcomas, sarcomas, myxosarcomas, or neurofibrosarcomas in the low dose females. Statistical significance ($p<0.05$) was only attained by combining tumors of differing morphology. Therefore, the subcutaneous tissue tumors in female rats were not considered to be chemically related. The incidences of these tumors in dosed male rats were not significantly different from that in the controls.

**The LOAEL for rotenone in rats was not established. The NOAEL is >75 ppm.**

This study is classified as **Unacceptable/Guideline** and satisfies the guideline requirement for oncogenicity studies [OPPTS 870.4200a] in rats.

### 4.4.10.2 Carcinogenic Potential in Mice

In a carcinogenicity study performed by NTP (MRID# 40179801) rotenone was administered to 50 male and 50 female $B6C3F_1$ mice (lot no. 735-RAP-1502, purity >98% a.i.) at dietary concentrations of 0, 600 or 1200 ppm for 103 weeks. The average daily dose for males and females in the low dose group was 111 and 124 mg/kg/day respectively, and the average daily dose for males and females in the high dose group was 242 and 265 mg/kg/day, respectively.

The only treatment-related effect noted on mortality was an increase in survival of the low and high dose male mice compared to the control group. The animals surviving the study were 29/50

for control group, 36/50 for 600 ppm group and 47/50 for 1200 ppm group ($p<0.001$). No change in survival occurred in the treated female mice.

Mean body weight was depressed in the male and female mice fed the 600 and 1200 ppm concentrations. Weight was measured weekly through week eight and monthly thereafter. The low dose male and female mice did not show significant differences in weight compared to the control group until approximately week 37. At that time, the mean body weight for low dose males was 6- 12% lower and for low dose females was 12- 20% lower than controls until the end of the study. The high dose males and females also did not show a significant difference in weight until week 37. Mean weight was then 12-17% below that of the control group in the high dose males and 17-26% lower in the high dose females. Final mean body weight was decreased by 6 and 13% compared to controls for the low and high dose males and 17 and 24% in low and high dose females. Body weight gain was reduced by 12 and 29% compared to the control group in low and high dose males, and 29 and 40% in low and high dose females, respectively. Feed consumption was not decreased in any groups compared to controls and feed efficiency was not reported.

Histopathological findings at necropsy in the male mice revealed a significant negative trend for combined hepatocellular adenomas and carcinomas with dose. Incidences were 12/47 (26%); 12/49 (24%); and 1/50 (2%) for controls, low- and high-dose groups, respectively. Fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas counts were combined as evidence of subcutaneous tissue tumors and were also observed in male mice with a negative ($p< 0.05$) trend with dosing. Control, low- and high-dose groups had incidences of 8/49 (16%); 4/50 (8%) and 2/50 (4%), respectively. Historical evidence suggests that decreased body weight is associated with decreased subcutaneous tumors in mice. No significant histopathological changes were observed in the female mice.

**The LOAEL for rotenone is 600 ppm for male and female mice (111 and 124 mg/kg/day, respectively) based on decreased body weight. The NOAEL was not determined.**

Dosing appeared to be adequate based on the decreased body weight in both male and female groups and there was not an increase in treatment-related tumor incidence.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for oncogenicity studies [OPPTS 870.4200b] in mice.

### 4.4.10.3 Classification of Carcinogenic Potential

The classification of carcinogenic potential for rotenone is “not likely to be carcinogenic in humans,” based on the lack of evidence of carcinogenicity in rats and mice.

| Table 4.4. Summary of Toxicological Doses and Endpoints for Rotenone for Use in Human Risk Assessments | | | |
|---|---|---|---|
| **Exposure Scenario** | **Dose Used in Risk Assessment, UF** | **Endpoint and Level of Concern for Risk Assessment** | **Study and Toxicological Effects** |
| Acute Dietary (females 13-49) | NOAEL = 15 mg/kg/day<br>UF = 1000<br>**Acute RfD** = 0.015 mg/kg/day | **aRFD** =<br><br>= 0.015 mg/kg/day | Developmental toxicity - mouse<br>LOAEL = 24 mg/kg/day based on increased resorptions |
| Acute Dietary (general population including infants and children) | An appropriate endpoint attributable to a single dose was not identified in the available studies, including the developmental toxicity studies. | | |
| Chronic Dietary (all populations) | NOAEL = 0.375 mg/kg/day<br>UF = 1000<br>**Chronic RfD** = 0.0004 mg/kg/day | **cRFD** =<br><br>= 0.0004 mg/kg/day | Chronic/oncogenicity - rat<br>LOAEL = 1.88 mg/kg/day based on decreased body weight and food consumption in both males and females |
| Incidental Oral Short-term (1 - 30 days) | NOAEL = 0.5 mg/kg/day | Recreational LOC for MOE = 1000 | Reproductive toxicity - rat<br>LOAEL = 2.4/3.0 mg/kg/day [M/F] based on decreased parental (male and female) body weight and body weight gain |
| Incidental Oral Intermediate-term (1 - 6 months) | NOAEL = 0.5 mg/kg/day | Recreational LOC for MOE = 1000 | Reproductive toxicity - rat<br>LOAEL = 2.4/3.0 mg/kg/day [M/F] based on decreased parental (male and female) body weight and body weight gain |
| Dermal All Durations | NOAEL = 0.5 mg/kg/day<br>10% dermal absorption factor | Recreational and Occupational LOC<br>MOE = 1000 | Reproductive toxicity - rat<br>LOAEL = 2.4/3.0 mg/kg/day [M/F] based on decreased parental (male and female) body weight and body weight gain |
| Inhalation Short- and Intermediate-term (1 - 30 days) | NOAEL = 0.5 mg/kg/day<br>100% inhalation absorption factor | Recreational and Occupational LOC<br>MOE = 1000 | Reproductive toxicity - rat<br>LOAEL = 2.4/3.0 mg/kg/day [M/F] based on decreased parental (male and female) body weight and body weight gain |
| Inhalation Long-term (> 6 months) | NOAEL = 0.375 mg/kg/day<br>100% inhalation absorption factor | Recreational and Occupational LOC<br>MOE = 1000 | Chronic/oncogenicity - rat<br>LOAEL = 1.88 mg/kg/day based on decreased body weight and food consumption in both males and females |
| Cancer (oral, dermal, inhalation) | **Classification:** no evidence of carcinogenicity | | |

UF = uncertainty factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, RfD = reference dose (a = acute, c = chronic), MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

* Refer to Section 4.1.4

### 4.5 Endocrine Disruption

EPA is required under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (FQPA), to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

In the available toxicity studies on rotenone, there was no estrogen, androgen, and/or thyroid mediated toxicity shown. When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, rotenone may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

# 5.0 Public Health Data

### 5.1 Incident Reports

The following databases were consulted for the poisoning incident data on the active ingredient rotenone (071003):

1) OPP Incident Data System (IDS) - reports of incidents from various sources, including registrants, other federal and state health and environmental agencies and individual consumers, submitted to OPP since 1992. Reports submitted to the Incident Data System represent anecdotal reports or allegations only, unless otherwise stated. Typically no conclusions can be drawn implicating the pesticide as a cause of any of the reported health effects. Nevertheless, sometimes with enough cases and/or documentation risk mitigation measures may be suggested.

2) Poison Control Centers - as the result of a data purchase by EPA, OPP received Poison Control Center data covering the years 1993 through 1998 for all pesticides. Most of the national Poison Control Centers (PCCs) participate in a national data collection system, the Toxic Exposure Surveillance System which obtains data from about 65-70 centers at hospitals and universities. PCCs provide telephone consultation for individuals and health care providers on suspected poisonings, involving drugs, household products, pesticides, etc.

3) California Department of Pesticide Regulation - California has collected uniform data on suspected pesticide poisonings since 1982. Physicians are required, by statute, to report to their local health officer all occurrences of illness suspected of being related to exposure to pesticides. The majority of the incidents involve workers. Information on exposure (worker activity), type of illness (systemic, eye, skin, eye/skin and respiratory), likelihood of a causal relationship, and number of days off work and in the hospital are provided.

4) National Pesticide Information Center (NPIC) - NPIC is a toll-free information service supported by OPP. A ranking of the top 200 active ingredients for which telephone calls were received during calendar years 1984-1991, inclusive has been prepared. The total number of calls was tabulated for the categories human incidents, animal incidents, calls for information, and others.

5) National Institute of Occupational Safety and Health's Sentinel Event Notification System for Occupational Risks (NIOSH SENSOR) performs standardized surveillance in seven states from 1998 through 2002. States included in this reporting system are Arizona, California, Florida, Louisiana, Michigan, New York, Oregon, Texas, and Washington. Reporting is very uneven from state to state because of the varying cooperation from different sources of reporting (e.g., workers compensation, Poison Control Centers, emergency departments and hospitals, enforcement investigations, private physicians, etc.). Therefore, these reports should not be characterized as estimating the total magnitude of poisoning. The focus is on occupationlly-related cases not residential or other non-occupational exposures. However, the information collected on each case is standardized and categorized according to the certainty of the information collected and the severity of the case.

A comparison (expressed in percent of cases,) between rotenone and all other pesticides reported to Poison Control Centers between 1993-2003 with either symptomatic outcome (SYM), moderate or more severe outcome (MOD), life-threatening or fatal outcome (LIFE-TH), seen in a health care facility (HCF), hospitalized (HOSP), or seen in an intensive care unit (ICU), showed that for occupational exposure cases, as well as for non-occupational cases involving adults, older children, and children under six years old, rotenone had a similar or higher percentage of poisoning incidents reported than other pesticides (*Hawkins 2005*).

In general, the most common symptom reported was eye irritation, which was four times more prevalent than any other symptom. Other symptoms reported included dermal irritation, throat irritation, nausea, and cough/choke. This supports the finding that rotenone's main effect is due to its irritant properties. Few neurological symptoms, other than headache and dizziness, were reported, though there were a few reports of peripheral neuropathy, numbness, or tremor.

Neither fatalities nor systemic poisonings have been reported in relation to "ordinary use." There were reports of fatalities from intentional ingestion of rotenone.

### 5.2 Other

No scientific literature pertinent to additional health effects of rotenone in humans was located.

# 6.0 Exposure Characterization/Assessment

### 6.1 Dietary Exposure/Risk Pathway

Food crop uses are no longer being supported by the registrants (see section 1.0) and are not included in this assessment. Details of the dietary assessment may be found in HED's earlier risk assessment, *ROTENONE: Phase 3 HED Chapter of the Reregistration Eligibility Decision Document (RED). PC Code: 071003. DP Barcode: D307385. January 24, 2006.*

### 6.1.1 Residue Profile

Rotenone ((2*R*,6a*S*,12a*S*)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromeno [3,4-*b*]furo[2,3-*h*]chromen-6-one) is a botanical acaricide, insecticide, and piscicide. Rotenone, cube resins other than rotenone, and derris resins are currently registered for foliar pre-harvest applications to food/feed crops and are also registered for direct treatment to livestock, use on lakes, ponds, and reservoirs, and livestock premises. However, in memos dated (March 7, 2006; March 17, 2006; and April 5, 2006) the technical registrants (Prentiss, Inc.; Foreign Domestic Chemicals Corporation; and Tifa Limited) for rotenone voluntarily cancelled all uses of rotenone except for the piscicidal uses.

No acceptable studies were submitted by the registrant(s) to support the nature of the residue guideline requirements; therefore, the nature of the residue in raw agricultural commodities and animal commodities is not adequately understood. Residues of concern could not be adequately assessed. Additionally, an acceptable analytical method was not provided. These studies and method are no longer needed as all food uses have been proposed to be cancelled by the rotenone technical registrants.

An exemption from tolerances was originally granted under 40 CFR §180.1001 (b) for residues of rotenone ((2*R*,6a*S*,12a*S*)-1,2,6,6a,12,12a-hexahydro-2-isopropenyl-8,9-dimethoxychromeno [3,4-*b*]furo[2,3-*h*]chromen-6-one) in/on raw agricultural commodities. The exemption from tolerances is currently listed under 40 CFR §180.905. As all food uses have been proposed to be cancelled by the rotenone technical registrants, HED recommends that this exemption be revoked.

### 6.1.2 Acute and Chronic Dietary Exposure and Risk

An acute dietary risk analysis (drinking water only) was conducted using the Dietary Exposure Evaluation Model (DEEM-FCID™, Version 2.03) which uses food and drinking water consumption data from the USDA's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. The analysis was performed to support the reregistration eligibility decision of rotenone.

Dietary risk analyses incorporate both the exposure and toxicity of a given pesticide. For acute and chronic analyses, the risk is expressed as a percentage of a maximum acceptable dose (i.e., the dose which HED has concluded will result in no unreasonable adverse health effects). This dose is the Reference Dose (RfD) which is the NOAEL divided by the sum total of all uncertainty factors.

For acute and non-cancer chronic exposures, HED is concerned when estimated dietary risk exceeds 100% of the RfD. References which discuss the acute and chronic risk assessments in more detail are available on the EPA/pesticides web site: "Available Information on Assessing Exposure from Pesticides, A User's Guide," 6/21/2000, web link: http://www.epa.gov/fedrgstr/EPA_PEST/2000/July/Day_12/6061.pdf ; or see SOP 99.6 (08/20/99).

### 6.1.2.1 Acute Dietary Exposure Results and Characterization

An acute dietary risk assessment (drinking water only) was conducted using the Dietary Exposure Evaluation Model (DEEM-FCID™), Version 2.03, which uses food and drinking water consumption data from the United States Department of Agriculture's (USDA's) Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. The analysis was performed to support the Revised HED Human Health Risk Assessment for rotenone.

No appropriate acute dietary toxicity endpoint could be identified for the general population based on the toxicology data currently available for rotenone. Therefore, the acute (drinking water only) assessment was conducted only for the 'females 13-49 years old' population subgroup.

An acute dietary exposure assessment was performed for rotenone considering exposure from surface water only, as all food uses for this chemical are no longer supported. An estimated drinking water concentration (EDWC) for rotenone surface water provided by the Environmental Fate and Effects Division (EFED) was used in this assessment (see section 6.2.2). The dietary exposure analysis results in dietary risk estimates that are below the Agency's level of concern for acute dietary exposure. Generally, HED is concerned when risk estimates exceed 100% of the aRfD. The exposure for the 'females 13-49 years old' population subgroup was 0.009735 mg/kg/day, which utilized 65% of the acute reference dose (aRfD) at the 95$^{th}$ percentile, see Table 6.1.2.1 below. It is appropriate to consider the 95$^{th}$ percentile because the analysis is deterministic and unrefined.

**Table 6.1.2.1. Acute Dietary Exposure and Risk for Rotenone at the 95$^{th}$ Percentile**

| Population Subgroup | aRfD (mg/kg/day) | EDWC (ppb) | Exposure (mg/kg/day) | %aRfD |
|---|---|---|---|---|
| Females 13-49 years old | 0.015 | 200 | 0.009735 | 65 |

### 6.1.2.2 Chronic Dietary Exposure Results and Characterization

HED believes the likelihood of chronic drinking water exposure is very low for most piscicidal applications of rotenone. However, HED does feel that the possibility for extended drinking water exposure (from a few days to a few months) resulting from rotenone piscicide applications does exist. This fact along with the lack of any application temperature restrictions on current rotenone labels, the fact that rotenone degradation varies greatly depending on water temperature, and the limited rotenone monitoring data currently available led HED to produce a drinking water only chronic dietary exposure analysis (see Table 6.1.2.2). Using the DWLOC approach, HED determined that chronic drinking water exposures greater than 40 ppb could pose a potential risk of concern (> 100% cRfD) to the most highly exposed population subgroups, infants and children.

Information provided by EFED shows that chronic EDWCs are expected to exceed 40 ppb for varying numbers of days, depending on the water temperature and other environmental factors. Rotenone degradation in 4 to 5$^{o}$C water was the worst case where HED had actual monitoring data and under these conditions, rotenone exceeded 40 ppb for 53 days. Under all conditions,

HED assumed that rotenone could reach drinking water intakes (within 1 day) and potentially pose risks from consumption of rotenone-contaminated drinking water. As a result, of this analysis, HED believes that 40 ppb is a conservative threshold level for drinking water exposure when rotenone is applied to bodies of water containing drinking water intakes.

**Table 6.1.2.2**
**Results of the Drinking Water Only Chronic Dietary Exposure Analysis for Rotenone**

| Population Subgroup | cRfD (mg/kg/day) | Exposure of Concern (ppb) | Number of Days that Exceed Exposure of Concern |
|---|---|---|---|
| General U.S. Population | 0.0004 | 140 | |
| All Infants (< 1 year old) | 0.0004 | 40 | |
| Children 1-2 years old | 0.0004 | 40 | |
| Children 3-6 years old | 0.0004 | 40 | 4 in warm water |
| | | | 53 in cold (4-5$^{o}$ C) water |
| | | | 27 in Lake Davis, CA |
| Females 13-49 years old | 0.0004 | 120 | |

### 6.1.2.3 Cancer Dietary Exposure Results and Characterization

The classification of carcinogenic potential for rotenone is "not likely to be carcinogenic in humans," based on the lack of evidence of carcinogenicity in rats and mice; therefore, a cancer dietary analysis was not performed.

## 6.2 Water Exposure/Risk Pathway

### 6.2.1 Environmental Fate

The fate and transport properties of rotenone in the environment are not well understood. In the past, rotenone has been characterized as immobile and non-persistent. This characterization is true only in some circumstances (*R. David Jones, 2006*). Rotenone does degrade rapidly by aqueous photolysis, the photolysis half-life is less than one day. Thus degradation would be expected to be rapid on sunny days in clear water. Degradation by hydrolysis is also moderately rapid at 25°C with half-lives of 12.6 days at a pH of 5 and 2 days at a pH of 9. However, aquatic field studies show that rotenone can persist in cold water at sufficient concentrations to cause fish mortality for at least 25 days, even in alkaline conditions. Rotenone does not appear to bioaccumulate.

Using Quantitative SAR estimation methods, rotenone does not appear to be volatile. Rotenone bonds sufficiently strongly to soils and sediments that it is unlikely to leach in most circumstances as $K_d$s range from 4.2 to 122 L kg $^{-1}$. Binding is well correlated to specific surfaces $K_{ss} = 0.29$ with an $R^2$ of 93%. Rotenone binding is not well correlated with organic carbon content alone. There is expected to be some propensity to leach in very sandy soils with low organic carbon, but ground water is unlikely to be affected as hydrolysis occurs too quickly at all pHs to allow contamination of groundwater to occur except for the briefest periods. Rotenone should be mobile in runoff to surface water.

As noted above, there is little information on rotenone degradates. Rotenolone is known to form by hydrolysis and on bean leaves (available data), probably by photolysis. It appears to be more

persistent than the parent rotenone on bean leaves with apparent half-lives of 4 to 5 days. A few other degradates were identified, but none formed at above 10% of the nominal concentration of rotenone. Additional data are needed for potential metabolites of rotenone, particularly for aquatic sites.

It is worth noting that potassium permanganate, $KMnO_4$, is recommended (not required) on the labels to 'detoxify' rotenone in streams and rivers downstream of the use site (piscicide use). Recommended concentrations of $KMnO_4$ are 2 to 4 mg $L^{-1}$, depending upon stream conditions and the rotenone concentration. Labels also note that rotenone toxicity may continue downstream as far as the water moves in 30 minutes. Water temperatures less than 50° F can result in longer times (and distances required for detoxification). While this advice appears to be based on a body of practical experience, there are currently no data to identify the degradation rate of rotenone in the presence of $KMNO_4$, or how the rate changes with permanganate concentration.

### 6.2.2 Drinking Water Estimates

EFED provided HED with an estimated drinking water concentration (EDWC) of 200 ppb for surface water (*R. David Jones, 2006*) based on the solubility of rotenone in water. It is also worth noting that the maximum application rate for the piscicidal use of rotenone (250 ppb) exceeds the solubility of rotenone. The remaining rotenone above the solubility limit is likely either suspended or in an emulsion. In either case, the suspended/emulsified rotenone will be less available for metabolism or hydrolysis than that in the dissolved phase.

### 6.2.3 Monitoring Data and Piscicide Use

*Monitoring Data.* There are limited monitoring data for rotenone. An aquatic field dissipation study, and data collected in association with a piscicidal application to Lake Davis in California are informative but not useful for quantitative risk assessment purposes (*R. David Jones, 2006*).

Rotenone can persist in water bodies for at least several weeks. In California's Lake Davis, rotenone was shown to have a half-life of 10.3 days. Concentrations were initially 45 $\mu g/L^{-1}$ and had decreased to about 7 $\mu g/L^{-1}$ on a mean basis across the Lake after 26 days (*see review in R. David Jones 2006*). Conversely, application to a warm water pond in an aquatic field dissipation study showed rotenone to have a half-life of 1.5 days. The difference in dissipation rates was likely due to differences in temperature.

*Piscicide Use.* An important use of rotenone is as a piscicide. Peak concentrations that could occur in water used for drinking water from the piscicide use of rotenone are 250 $\mu g \bullet L^{-1}$ from the use in static water bodies, and 50 $\mu g \bullet L^{-1}$ in flowing waters. In the general directions for the piscicide use of rotenone, labels state' "Do not use water treated with rotenone to irrigate crops or release within ½ mile upstream of a potable water or irrigation water intake in a standing body of water such as a lake, pond, or reservoir." In addition, in the sections labeled "For Use in Streams and Rivers" the labels state "Contact the local water department to determine if any water intakes are (within one mile) down flow of the section of stream, river or canal to be treated. If so, coordinate with the water department to make sure that the intakes are closed during treatment and detoxification." While it is clear that these instructions are intended to prevent the contamination of drinking water with rotenone, it is not clear to what extent they are able to keep rotenone from reaching the intake of drinking water facilities. As noted above, temperature can strongly influence the persistence of rotenone in water - the half life of rotenone

in a 25° C pond was 1.5 days, increased to 10 days at Lake Davis (9° C), and 20 days in a cold pond (5° C). Based on the available fate and transport data, it is not clear that a half-mile restriction around the intake in lakes and reservoirs would be sufficient to keep rotenone from reaching the intake, particularly for colder bodies of water such as Lake Davis. Justification for the efficacy of this restriction has not been provided. This is also true for the one-mile buffer for streams and rivers. Since the efficacy of permanganate detoxification is not known, it is not clear that even the one mile restriction would be sufficient to ensure that drinking water would not be contaminated. Given that rotenone can persist for days to weeks in water, rotenone would be likely to move many miles downstream before degradation and dilution would result significantly to reduce exposure at drinking water intakes, particularly if the water is cold. Also, the dissipation of rotenone in streams will be dependent upon the flow rate of the water body. While potassium permanganate treatment may significantly reduce concentrations, data showing the rate at which this occurs were not identified for use in this exposure assessment.

#### 6.2.4. Drinking Water Treatment

OPP does not have direct information on the removal of rotenone during drinking water treatment. However, hydrolysis of rotenone is relatively fast under alkaline conditions, about 2 days at pH 9. Some processes used to treat drinking water, such as softening may raise the pH as high as 11 during treatment. These processes would be expected to substantially reduce the rotenone concentration, though it is unclear at this time what degradates might form and what their persistence might be. In some cases, strong ultraviolet light is used for disinfection. Because rotenone is so susceptible to aqueous photolysis, this treatment may also be expected to substantially reduce rotenone parent concentration present in the source water. However, because neither of these processes can currently be quantified in the context of drinking water treatment of rotenone, nor can the locations where these processes are used be identified, it is not possible at this time to assess how they might reduce rotenone in drinking water quantitatively. Softening is used only where water is high in calcium and magnesium. UV treatment is considered an advanced treatment technique and has yet to be widely adopted as a practice in the United States.

### 6.3 Residential (Non-Occupational) Exposure/Risk Pathway

#### 6.3.1 Residential Handler Exposures and Risks

Rotenone is currently registered for use in a variety of residential scenarios, however, the rotenone technical registrants (Foreign Domestic Chemicals Corporation (3/17/06); Prentiss, Inc. (3/7/06); and Tifa Limited (4/5/06) voluntarily cancelled all uses of rotenone except for the piscicidal uses. The cancelled uses of rotenone were previously assessed in the January 24, 2006 risk assessment (DP barcode D307385), which can be found on EPA's website.

#### 6.3.2 Residential (Recreational) Postapplication Exposures and Risks

HED uses the term "postapplication" to describe exposures to individuals that occur as a result of being in an environment that has been previously treated with a pesticide. Rotenone can be used in various types of water bodies that can be frequented by the general public. As a result, individuals can be exposed by swimming in the rotenone treated water.

The *Standard Operating Procedures (R-SOPs) For Residential Exposure Assessment* define several scenarios that apply to uses specified on current rotenone labels. These scenarios served as the basis for the residential postapplication assessment. The assumptions and factors used in the risk calculations are consistent with current Agency policy for completing residential exposure assessments (i.e., R-SOPs) and can be found in detail in section 3.2.2 of *Rotenone: Phase 5 Occupational and Residential Exposure Assessment for the Reregistration Eligibility Decision Document. Charles W. Smith. May 30, 2006.*

**Adults:** For all adult postapplication scenarios, short-term risks for swimming do not exceed HED's level of concern (i.e., the MOEs are greater than 1000) on the day of application. Table 6.3.2a presents the postapplication MOEs for adults following applications of rotenone.

**Table 6.3.2a: Adult Residential (Recreational) Risk Estimates for Postapplication Exposure to Rotenone**

| Exposure Scenario | Route of Exposure | Application Rate | MOE at Day 0 |
|---|---|---|---|
| Swimming - Dermal | Dermal | 0.25 ppm | 1,300 |
| | | 0.20 ppm | 1,600 |
| Swimming – Incidental Ingestion | Oral | 0.25 ppm | 5,600 |
| | | 0.20 ppm | 7,000 |

**Toddler (3 year old):** For all toddler postapplication scenarios, short-term risks for swimming exceed HED's level of concern (i.e., the MOEs are less than 1000) on the day of application. Table 6.3.2b presents a summary of the MOE estimates for toddlers.

**Table 6.3.2b: Toddler Residential (Recreational) Risk Estimates for Postapplication Exposure to Rotenone**

| Exposure Scenario | Route of Exposure | Application Rate | MOE at Day 0 |
|---|---|---|---|
| Swimming - Dermal | Dermal | 0.25 ppm | 770 |
| | | 0.20 ppm | 970 |
| Swimming – Incidental Ingestion | Oral | 0.25 ppm | 680 |
| | | 0.20 ppm | 850 |

The Environmental Fate and Effects Division (EFED) calculated the number of days it would take to reach a rotenone concentration that results in acceptable toddler MOEs (170 ppb of rotenone results in an oral MOE of 1000 and a dermal MOE of 1100). This is done by assuming that the dissipation rate for rotenone in a warm water pond is 1.5 days, as seen in the aquatic dissipation study. The time it takes for the rotenone to dissipate (in 25$^{o}$C water) to 170 ppb from 200 ppb is 0.35 days and from 250 ppb is 0.89 days. EFED assumed first order degradation below 200 ppb and zero order degradation above. Zero order degradation assumes that the degradation rate is constant with time. This includes the assumption that more rotenone dissolves to keep the concentration constant at 200 ppb until all the rotenone is in solution, and then first order kinetics occurs after that. The temperature in the "warm water" pond in the aquatic dissipation study was 25$^{o}$C which EFED and HED consider to be a temperature at which swimming by the general public could reasonably occur.

Combined Risk Assessment for Residential (Recreational) Scenarios

HED combines risk values resulting from separate postapplication exposure scenarios when it is likely they can occur simultaneously based on the use-pattern and the behavior associated with the exposed population. Table 6.3.2c presents a summary of the combined MOE estimates.

**Table 6.3.2c: Rotenone Residential (Recreational) Scenarios for Combined Risk Estimates**

| Postapplication Exposure Scenario | | | Margins of Exposure (MOEs) (UF=1000) | |
|---|---|---|---|---|
| | | | Short-Term Oral (Non-Dietary) | Total Non-Dietary Risk |
| Toddler | Swimming (0.25 ppm) | Dermal | 770 | 360 |
| | | Incidental Ingestion | 680 | |
| | Swimming (0.20 ppm) | Dermal | 970 | 450 |
| | | Incidental Ingestion | 850 | |

HED calculated the number of days it would take to reach a rotenone concentration that results in acceptable toddler combined MOEs (90 ppb of rotenone results in an oral MOE of 1900 and a dermal MOE of 2100, which results in a combined MOE of 1000). The time it takes for the rotenone to dissipate to 90 ppb from 200 ppb is approximately 2 days and from 250 ppb is approximately 3 days. HED believes that swimming in rotenone treated waters should be prohibited for at least 2 days after completion of a 200 ppb rotenone application and at least 3 days after completion of a 250 ppb rotenone application.

In residential settings, HED does not use restricted-entry intervals or other mitigation approaches to limit postapplication exposures, because they are viewed as impractical and not enforceable. As such, risk estimates on the day of application are the key concern. However, in the case of rotenone, HED believes that swimming in rotenone treated waters should be prohibited for at least 2 days after completion of a 200 ppb rotenone application and at least 3 days after completion of a 250 ppb rotenone application.

### 6.3.3 Spray Drift

Spray drift is always a potential source of exposure to residents nearby to spraying operations. This is particularly the case with aerial application. The Agency has been working with the Spray Drift Task Force, EPA Regional Offices and State Lead Agencies for pesticide regulation and other parties to develop the best spray drift management practices. The Agency is now requiring interim mitigation measures for aerial applications that must be placed on product labels/labeling. The Agency has completed its evaluation of the new data base submitted by the Spray Drift Task Force, a membership of the U.S. pesticide registrants, and is developing a policy on how to appropriately apply the data and the AgDRIFT computer model to its risk assessments for pesticides applied by air, orchard airblast, and ground hydraulic methods. After the policy is in place, the Agency may impose further refinements in spray drift management practices to reduce off-target drift and risks associated with aerial, as well as other application types, where appropriate.

# 7.0 Aggregate Risk Assessments and Risk Characterization

In accordance with the Food Quality Protection Act (FQPA) of 1996, for chemicals having tolerances in food, HED must consider and aggregate pesticide exposures and risks from three major sources: food, drinking water, and residential exposures (oral, dermal, and inhalation). All uses of rotenone on food crops have been proposed to be cancelled and thus the requirements of FQPA are not applicable and aggregate risk assessments have not been conducted.

# 8.0 Cumulative Risk Characterization/Assessment

FQPA stipulates that when determining the safety of a pesticide chemical, EPA shall base its assessment of the risk posed by the chemical on, among other things, available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances that have a common mechanism of toxicity. All uses of rotenone on food crops have been proposed to be cancelled and thus the requirements of FQPA are not applicable and a cumulative risk assessment has not been conducted.

# 9.0 Occupational Exposure/Risk Pathway

Rotenone is currently registered for use in a variety of residential scenarios, however, the rotenone technical registrants (Foreign Domestic Chemicals Corporation (3/17/06); Prentiss, Inc. (3/7/06); and Tifa Limited (4/5/06) voluntarily cancelled all uses of rotenone except for the piscicidal uses. This assessment deals with occupational populations that could be potentially exposed while performing rotenone piscicide applications. Occupational risks associated with the cancelled uses of rotenone were previously assessed in the January 24, 2006 risk assessment (DP barcode D307385), which can be found on EPA's website.

## 9.1 Short/Intermediate-term Noncancer Handler Exposure and Risk

Exposure scenarios categorize the exposures that occur during the use of a chemical. The commonly used scenarios in exposure assessments are described in the *U.S. EPA Guidelines for Exposure Assessment* (U.S. EPA; Federal Register Volume 57, Number 104; May 29, 1992). Information from the current labels, use and usage information, toxicology data, and exposure data were all key components in developing the exposure scenarios. For exposure and risk assessment purposes, tasks of pesticide handlers associated with occupational pesticide use are categorized as one of the following:

- **Mixers and/or Loaders:** these individuals perform tasks in preparation for an application. For example, prior to application, mixer/loaders would mix the rotenone and load it into the holding tank of the helicopter or boat.

- **Applicators:** these individuals operate application equipment during the release of a pesticide product into the environment. These individuals can make applications using equipment such as helicopters or boat-boom sprayers.

- **Mixer/Loader/Applicators and or Loader/Applicators:** these individuals are involved in the entire pesticide application process (i.e., they do all job functions related to a

pesticide application event). These individuals would transfer rotenone into the application equipment and then also apply it.

It is important to understand how exposures to rotenone occur (i.e., frequency and duration) and how the patterns of these occurrences can cause the effects of the chemical to differ (referred to as dose response). Wherever possible, use and usage data determine the appropriateness of certain types of risk assessments. Other parameters are also defined from use and usage data such as application rates and application frequency. HED always completes non-cancer risk assessments using maximum application rates for each scenario because what is possible under the label (the legal means of controlling pesticide use) must be evaluated in order to ensure there are no concerns for each specific use.

The frequency and duration of pesticide handlers' exposures must also be estimated in order to determine which toxicological endpoints are applicable to a handler exposure scenario. HED believes that occupational rotenone exposures may occur over a few days for many use-patterns and that intermittent exposure over several weeks also may occur. Custom or commercial applicators may apply rotenone over a period of weeks, completing applications for a number of different clients. HED classifies exposures up to 30 days as short-term and exposures greater than 30 days up to several months as intermediate-term. HED completes both short- and intermediate-term assessments for occupational scenarios in essentially all cases, because these kinds of exposures are likely, and often reliable use/usage data are not available to justify deleting intermediate-term scenarios. Long-term handler exposures are not expected to occur for rotenone. The same toxicological endpoint (0.5 mg/kg/day from an oral study) of concern was selected for short- and intermediate-term dermal exposures to rotenone, therefore the risk results for all dermal durations of exposure are numerically identical. The HazSPoC report, dated June 28, 2005, states that a dermal absorption factor of 10% should be used to assess dermal risks, since the dermal endpoint for rotenone is from an oral study. The same toxicological endpoint (0.5 mg/kg/day from an oral study) has been selected for short- and intermediate-term inhalation exposures to rotenone, therefore the risk results for all inhalation durations of exposure are numerically identical. A default inhalation absorption factor of 100% was used to assess inhalation risks, since the inhalation endpoint for rotenone is from an oral study.

Occupational handler exposure assessments are completed by HED using different levels of personal protection. HED typically evaluates all exposures with a tiered approach. The lowest tier is represented by the baseline exposure scenario (i.e., long-sleeve shirt, long pants, shoes, socks, and no respirator) followed by increasing the levels of personal protective equipment or PPE (e.g., gloves, double-layer body protection, and respirators), and then by engineering controls (e.g., enclosed cabs and closed mixing/loading systems). This approach is always used by HED in order to be able to define label language using a risk-based approach. In addition, the minimal level of adequate protection for a chemical is generally considered by HED to be the most practical option for risk reduction (i.e., over-burdensome risk mitigation measures are not considered a practical alternative).

### 9.1.1 Short/Intermediate-Term Handler Risks

The anticipated use patterns and current labeling indicate several likely occupational handler exposure scenarios, based on the types of equipment and techniques that can potentially be used to apply rotenone to aquatic use sites. Anticipated use pattern and current labeling indicate 12 likely occupational exposure scenarios. Scenarios in this document include:

**Mixer/Loaders:**
(1a) Liquid Formulations for Helicopter Applications
(1b) Liquid Formulations for Boat Applications (boom and underwater weighted hose applications)
(2a) Wettable Powder Formulations for Boat Applications (boom and underwater weighted hose applications)

**Applicators:**
(3) Helicopter Spray Applications (using PHED fixed wing aerial spray application data)
(4) Boat Boom Spray Applications (using PHED groundboom spray application data)

**Mixer/Loader/Applicators:**
(5) Liquid Formulations: Backpack Sprayer (using PHED liquid low pressure handwand data)
(6) Liquid Formulations: Closed System Aspirators (using PHED closed system mixing/loading liquids) – no contact should occur once liquid rotenone is loaded
(7) Liquid Formulations: Drip Bars (using PHED mixing/loading liquids) – no contact should occur once liquid rotenone is loaded
(8) Wettable Powder: Backpack Sprayer (using PHED wettable powder low pressure handwand data)
(9) Wettable Powder Formulations: Closed System Aspirators (using PHED closed system mixing/loading wettable powders) - no contact should occur once wettable powder rotenone is loaded
(10) Wettable Powder Formulations: Drip Bars (using PHED mixing/loading wettable powders) - no contact should occur once wettable powder rotenone is loaded
(11) Wettable Powder Formulations: Powder/Sand/Gelatin Pastes

The following assumptions and factors were used in order to complete this exposure assessment:

- Average body weight of an adult handler is 70 kg. This body weight is used in the short- and intermediate-term assessments, since the endpoint of concern is not gender-specific.

- The number of acres treated or volume of spray solution applied per day are specific to each equipment type addressed in the exposure assessment and are representative of the amount that can be treated/applied in a single 8 hour workday for each exposure scenario.

- Various exposure factors used in the calculations (e.g., acres treated or gallons handled per day for each application method) are based on the best professional judgment of EPA due to a lack of extensive pertinent data.

- Daily areas and volumes (as appropriate) to be treated in each occupational exposure scenario include: 5 to 10 acres with a water body depth of 5 feet for aerial applications to

stationary water bodies; 2 acres with a water body depth of 5 feet for backpack sprayer applications to stationary water bodies; 211200 $ft^3$ (10560 feet long with a water body depth of 2 feet and a water body width of 10 feet) for backpack sprayer and drip bar applications to moving water bodies (i.e., streams, rivers, etc.); and 50 to 100 acres with a water body depth of 5 feet for closed system aspirator, boat-boom, and boat-weighted hose applications to stationary water bodies (personal contact with Brian Finlayson, California Department of Fish and Game on 1/9/06).

- Occupational handler exposure estimates were based on surrogate data from the Pesticide Handlers Exposure Database (PHED) as no chemical or application equipment specific exposure data were available. PHED consists of data that were produced for the purposes of assessing land-based agricultural and residential application scenarios. In the case of rotenone, applications occur over and to water bodies. There are clearly limitations and uncertainties regarding the use of PHED to assess rotenone occupational handler exposure because of the distinct differences in application sites (land vs. water), however, HED can not currently define the extent of these limitations and uncertainties. Specific examples of surrogate scenarios used in this assessment are explained below:
  - To assess exposure from applying sprays via helicopter, the exposure scenario for applying via fixed wing aircraft was used.
  - To assess exposure from applying sprays via boat-mounted spray equipment, the exposure scenario for applying via ground boom equipment was used.
  - To assess exposure from mixing/loading/applying liquid formulations via closed system aspirators, the exposure scenario for liquid formulation closed system mixing/loading equipment was used.
  - To assess exposure from mixing/loading/applying wettable powder formulations via closed system aspirators, the exposure scenario for wettable powder formulation closed system mixing/loading equipment was used.
  - To assess exposure from mixing/loading/applying liquid formulations via drip bars (in moving waters), the exposure scenario for liquid formulation mixing/loading equipment was used.
  - To assess exposure from mixing/loading/applying wettable powder formulations via drip bars (in moving waters), the exposure scenario for wettable powder formulation mixing/loading equipment was used.

- Due to a lack of scenario-specific data, EPA sometimes calculates unit exposure values using generic protection factors that are applied to represent various risk mitigation options (i.e., the use of PPE and engineering controls). PPE protection factors include those representing double layers of clothing (50%) and respiratory protection (90%). Engineering controls are generally assigned a protection factor of 90% or higher. Engineering controls may include closed mixing/loading systems and enclosed cabs and enclosed cockpits.

The noncancer occupational handler exposure and risk calculations are included in Table 9.1.1 (see Appendix A Tables A2 & A3 in *Smith 2006* for complete aquatic handler exposure and risk calculations). The results indicate that many of the occupational aquatic-use handler risks are of concern [i.e., MOEs < LOC of 1000].

## Table 9.1.1. Combined Dermal plus Inhalation Aquatic-Use Occupational Handler Risks

| Exposure Scenario | Crop or Target | Application Rate[a] | Area Treated Daily[b] | Depth of Water Body[b] | Width of Water Body[b] | Combined MOEs[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Baseline | G + NR | G, DL + NR | G + 80% R | G, DL + 80% R | G + 90% R | G, DL + 90% R | Eng Cont |
| **Mixer/Loader** | | | | | | | | | | | | | |
| Mixing/Loading Liquid Concentrates for Helicopter Applications (1a) | Lakes, ponds | 0.68 lb ai/A-ft | 10 acres | 5 ft | NA | 3.5 | 290 | 350 | 410 | 530 | 430 | 570 | 1100 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 5 acres | 5 ft | NA | 7.1 | 590 | 710 | 810 | 1100 | 850 | 1100 | 2200 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 10 acres | 5 ft | NA | 4.5 | 370 | 450 | 510 | 670 | 540 | 710 | 1400 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 5 acres | 5 ft | NA | 8.9 | 740 | 890 | 1000 | 1300 | 1100 | 1400 | 2700 |
| Mixing/Loading Liquid Concentrates for Boat Applications (1b) | Lakes, ponds | 0.68 lb ai/A-ft | 100 acres | 5 ft | NA | 0.35 | 29 | 35 | 41 | 53 | 43 | 57 | 110 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 50 acres | 5 ft | NA | 0.71 | 59 | 71 | 81 | 110 | 85 | 110 | 220 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 100 acres | 5 ft | NA | 0.45 | 37 | 45 | 51 | 67 | 54 | 71 | 140 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 50 acres | 5 ft | NA | 0.89 | 74 | 89 | 100 | 130 | 110 | 140 | 270 |
| Mixing/Loading Wettable Powders for Boat Applications (2a) | Lakes, ponds | 0.68 lb ai/A-ft | 100 acres | 5 ft | NA | 0.25 | 1.7 | 1.8 | 4 | 4.8 | 4.8 | 6 | 84 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 50 acres | 5 ft | NA | 0.5 | 3.4 | 3.7 | 8 | 9.5 | 9.7 | 12 | 170 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 100 acres | 5 ft | NA | 0.31 | 2.2 | 2.3 | 5.1 | 6 | 6.1 | 7.5 | 110 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 50 acres | 5 ft | NA | 0.63 | 4.3 | 4.6 | 10 | 12 | 12 | 15 | 210 |
| **Applicator** | | | | | | | | | | | | | |
| Applying Sprays via Helicopter (3) | Lakes, ponds | 0.68 lb ai/A-ft | 10 acres | 5 ft | NA | ND | ND | ND | ND | ND | ND | ND | 1800 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 5 acres | 5 ft | NA | ND | ND | ND | ND | ND | ND | ND | 3600 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 10 acres | 5 ft | NA | ND | ND | ND | ND | ND | ND | ND | 2300 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 5 acres | 5 ft | NA | ND | ND | ND | ND | ND | ND | ND | 4600 |
| Applying Sprays via Boat Over-surface Boom Equipment (4) | Lakes, ponds | 0.68 lb ai/A-ft | 100 acres | 5 ft | NA | 48 | 48 | 56 | 66 | 82 | 70 | 88 | 190 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 50 acres | 5 ft | NA | 96 | 96 | 110 | 130 | 160 | 140 | 180 | 380 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 100 acres | 5 ft | NA | 61 | 61 | 70 | 84 | 100 | 88 | 110 | 240 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 50 acres | 5 ft | NA | 120 | 120 | 140 | 170 | 210 | 180 | 220 | 480 |
| **Mixer/Loader/Applicator** | | | | | | | | | | | | | |
| Mixing/Loading/Applying Liquids with a Backpack Sprayer (using PHED liquid low pressure handwand data) (5) | Lakes, ponds | 0.68 lb ai/A-ft | 2 acres | 5 ft | NA | 0.51 | 71 | 77 | 110 | 120 | 110 | 130 | NF |
| | Lakes, ponds | 0.54 lb ai/A-ft | 2 acres | 5 ft | NA | 0.51 | 71 | 77 | 110 | 120 | 110 | 130 | NF |
| | Moving water (streams) | 0.000016 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 10 | 1400 | 1500 | 2100 | 2400 | 2300 | 2600 | NF |
| | Moving water (streams) | 0.000013 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 13 | 1700 | 1900 | 2600 | 3000 | 2800 | 3200 | NF |
| Mixing/Loading/ Applying Liquids with Closed System Aspirators (PHED: mixing/loading liquid - closed system) (6) | Lakes, ponds | 0.68 lb ai/A-ft | 10 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 110 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 5 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 220 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 10 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 140 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 5 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 270 |
| Mixing/Loading/ Applying Liquids with Drip Bars (PHED: mixing/loading liquid) (7) | Moving water (streams) | 0.000016 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 360 | 30000 | 36000 | 41000 | 53000 | 43000 | 57000 | 110000 |
| | Moving water (streams) | 0.000013 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 440 | 36000 | 44000 | 50000 | 66000 | 53000 | 70000 | 140000 |

| Table 9.1.1. Combined Dermal plus Inhalation Aquatic-Use Occupational Handler Risks | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exposure Scenario | Crop or Target | Application Rate[a] | Area Treated Daily[b] | Depth of Water Body[b] | Width of Water Body[b] | Combined MOEs[c] | | | | | | | |
| | | | | | | Baseline | G + NR | G, DL + NR | G + 80% R | G, DL + 80% R | G + 90% R | G, DL + 90% R | Eng Cont |
| Mixing/Loading/ Applying Wettable Powders with a Backpack Sprayer (using PHED wettable powder low pressure handwand data) (8) | Lakes, ponds | 0.68 lb ai/A-ft | 2 acres | 5 ft | NA | ND | 2.6 | 3 | 4.8 | 6.1 | 5.3 | 7.1 | NF |
| | Lakes, ponds | 0.54 lb ai/A-ft | 2 acres | 5 ft | NA | ND | 2.6 | 3 | 4.8 | 6.1 | 5.3 | 7.1 | NF |
| | Moving water (streams) | 0.000016 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | ND | 53 | 60 | 96 | 120 | 110 | 140 | NF |
| | Moving water (streams) | 0.000013 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | ND | 65 | 74 | 120 | 150 | 130 | 170 | NF |
| Mixing/Loading/ Applying Wettable Powders with Closed System Aspirators (PHED: mixing/loading liquid - closed system) (9) | Lakes, ponds | 0.68 lb ai/A-ft | 10 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 84 |
| | Lakes, ponds | 0.68 lb ai/A-ft | 5 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 170 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 10 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 110 |
| | Lakes, ponds | 0.54 lb ai/A-ft | 5 acres | 5 ft | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 210 |
| Mixing/Loading/ Applying Wettable Powders with Drip Bars (PHED: mixing/loading liquid) (10) | Moving water (streams) | 0.000016 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 250 | 1700 | 1800 | 4000 | 4800 | 4900 | 6000 | 85000 |
| | Moving water (streams) | 0.000013 lb ai/ft3 | 10,560 ft long | 2 ft | 10 ft | 310 | 2100 | 2300 | 5000 | 5900 | 6000 | 7400 | 100000 |
| Mixing/Loading/ Applying Wettable Powders via Powder/Sand/Gelatin Paste (11) | Seeps and Springs | | | N/A | N/A | There is currently no data to assess this scenario. HED believes this scenario will result in minimal exposure due to the amount of rotenone used and the fact that this paste is typically mixed in either a lab under a fume hood or by an individual wearing a respirator. | | | | | | | |

a Application rates are the maximum application rates determined from EPA registered labels for rotenone

b Area treated per day values for all application methods except boats are based on personal contact with Brian Finlayson, California Department of Fish and Game (1/9/06). Area treated per day values for boat application methods are based on HED professional judgement.

c Baseline: Long-sleeve shirt, long pants, no gloves, and no respirator.
PPE-G-NR: Baseline plus chemical-resistant gloves, and no respirator.
PPE-G,DL-NR: Coveralls worn over long-sleeve shirt and long pants, chemical-resistant gloves, and no respirator.
PPE-G-80% R: Baseline plus chemical-resistant gloves and an 80% PF (quarter-face dust/mist) respirator.
PPE-G,DL-80% R: Coveralls worn over long-sleeve shirt and long pants, chemical-resistant gloves, and an 80% PF (quarter-face dust/mist) respirator.
PPE-G-90% R: Baseline plus chemical-resistant gloves and a 90% PF (half-face dust/mist) respirator.
PPE-G,DL-90% R: Coveralls worn over long-sleeve shirt and long pants, chemical-resistant gloves, and a 90% PF (half-face dust/mist) respirator.
Eng Controls: Closed mixing/loading system, enclosed cab, or enclosed cockpit.

### 9.2 Short- and Intermediate-term Noncancer Postapplication Risk

HED expects minimal occupational postapplication exposure from the pisicidal use of rotenone. As a result, no quantitative assessment was completed for occupational postapplication exposure.

# 10.0 Data Needs and Label Requirements

### 10.1 Toxicology

The registrants are no longer supporting agricultural, occupational, or residential uses, where the greatest potential for inhalation, dietary, and dermal exposure could occur. Therefore, the inhalation neurotoxicity study and all other toxicity data requirements discussed below will currently be held in reserve (may be called in later).

- Guideline metabolism study
- 21-Day neurotoxicity study in Lewis rats by the inhalation route
- Dermal absorption/penetration study
- Repeated-dose dermal toxicity study, pending the results of the dermal absorption/penetration study
- Developmental toxicity study in the rabbit
- Developmental neurotoxicity (DNT) study by the oral route in the Lewis rat, pending the results of the subchronic neurotoxicity study by the inhalation route
- Subchronic oral neurotoxicity study in the Lewis rat, pending the results of the subchronic neurotoxicity study by the inhalation route
- For further details, see Table A1. Toxicology Data Requirements for Rotenone in Appendix A.

### 10.2 Residue Chemistry

The following is a list of deficiencies and data gaps that are no longer required as long as there are no food uses for rotenone:

- Guideline requirements regarding plant and animal metabolism remain outstanding.
- Supporting analytical methods, appropriate validation data and storage stability data are still required to accompany any submitted data pertaining to the magnitude of the residue.
- Information regarding whether the registrant submitted data on the applicability of the FDA Multiresidue Protocols needs to be provided.
- Guideline requirements regarding magnitude of the residue data for root and tuber vegetables, cucurbit vegetables, citrus fruits, pome fruits, tree nuts, cereal grains, herbs and spices, and oilseeds are required.

- Guideline requirements regarding residue decline in broccoli, lettuce, peach, snap bean, and tomato are required.
- Guideline requirements regarding magnitude of residue in potable water, fish and irrigated crops remain outstanding.
- No data reflecting residues in food products resulting from registered uses are available.
- The data requirements for meat, milk, poultry and eggs remain reserved pending the results of acceptable ruminant and poultry metabolism studies.
- Data remain outstanding pertaining to residues of rotenone in or on any plant commodity following registered pre-harvest applications, processed food/feed stuffs, confined rotational crops and field accumulation in rotational crops.

### 10.3 Occupational/Residential Exposure

The following is a list of deficiencies and data gaps that need to be resolved:

- Occupational handler exposure estimates were based on surrogate data as no chemical or application equipment specific exposure data was available. There are clearly limitations and uncertainties regarding the use of the surrogate data to assess rotenone occupational handler exposure because of the distinct differences in application sites (land vs. water), however, HED can not currently define the extent of these limitations and uncertainties. Actual data for rotenone handler exposure scenarios would provide better worker risk estimates.

# 11.0 Attachments

Barnes 2005. Rotenone: Summary of Product Chemistry Data for Reregistration Eligibility Decision (RED) Document. DP Barcode: D307391. P. Yvonne Barnes. August 11, 2005.

Carter 2005. Usage Report in Support of Reregistration for the Insecticide Rotenone (074002). Jenna Carter. August 3, 2005.

Hawkins 2005. Review of Rotenone Incident Reports. DP Barcode D307408, Chemical #071003 and #071002. Monica S. Hawkins. August 9, 2005.

R. David Jones 2006. Drinking Water and Swimmer Exposure Assessment for Rotenone from the Piscicide Use. D307383. R. David Jones. May 10, 2006.

Rotenone: Decisions on Critical Effects and Endpoint Selection. Results of the Meeting of the HED Hazard Science Policy Council. PC Code: 071003. DP Barcode: D307370. TXR# 0053480. Diana Locke. June 28, 2005.

Smith 2006. Rotenone: Phase 5 Occupational and Residential Exposure Assessment for the Reregistration Eligibility Decision Document. PC Code: 071003. DP Barcode: 307387. Charles Smith. May 30, 2006.

# 12.0 References

CDFG 1991. California Department of Fish and Game. Pesticide investigations unit, aquatic toxicology laboratory 1990 annual progress report. CDFG, Environmental Services Division, Sacramento, CA.

Health & Safety Report; HS – 1772. A report on the illnesses related to the application of rotenone to Lake Davis. M. Verder-Carlos and M. O'Malley. California Environmental Protection Agency. Department of Pesticide Regulation. November 12, 1998.

# Appendix A: Executive Summaries for Studies not Highlighted in Document and Toxicological Profile

**1. Subacute Neurotoxicity Study (Rat) (MRID# 45279501):**

**EXECUTIVE SUMMARY:** In this special neurotoxicity study (MRID# 45279501), a group of 25 Lewis rats were given doses of 2.5-2.75 mg/kg/day of rotenone dissolved in polyethylene glycol and DMSO by chronic intravenous infusion for 1-5 weeks. A variety of immunocytochemical and neuropathological tests, and some behavioral observations were used to assess the impact of treatment. Among treated rats, 12/25 had lesions, while no vehicle control rats did. Complex I was inhibited [73%] throughout the brain, but Complex II and IV were unaffected. But the level of Complex I inhibition did not impair cellular respiration in the brain. Rotenone induced specific neurodegenerative lesions in nigrostriatal dopaminergic neurons as evidenced by immunocytochemical markers, silver staining, and Fluoro-B Jade staining. Lesions were dose dependent and typically began as focal lesions in the anterior striatum and spread to most of the motor striatum, and in some rats in the *pars compacta* substantia nigra cell bodies. Only pre-synaptic dopamineric nerve terminals were affected. GABA neurons, which comprise 90% of striatal neurons, and cholinergic neurons were unaffected. Rotenone treated rats with lesions also showed hypoactivity, unsteady gait, and hunched posture. Giasson and Lee (MRID# 45279502) discuss how this study provides further evidence that environmental factors may play a role in Parkinson's disease. It does not provide new information itself.

**2. Subchronic Dog (MRID# 00141406)**

**EXECUTIVE SUMMARY:** In a subchronic oral toxicity study (MRID# 00141406), rotenone (> 99% a.i., lot no. 578-RSP-1424, Sample No. 9244-RC) was administered daily in gelatin capsules to 6 beagle dogs/sex/dose at dose levels of 0, 0.4, 2.0, or 10.0 mg/kg bw/day for 26 weeks. Individual animal data were not included in the report and body weight and food consumption data were presented graphically.

One low-dose male was sacrificed on day 52 due to an injury; all remaining animals survived to scheduled sacrifice. The first clinical sign attributed to the treatment compound was emesis by dogs of the high-dose group (10.0 mg/kg), which began after the second dose. After the first week of treatment, the incidence declined to, and remained at, an incidence comparable to that of the control group. Soft stools and/or diarrhea were the second most common clinical sign among treated animals, occurring at the highest incidence in the 10.0 mg/kg animals with males more frequently affected than females.

Based on graphs, body weight, body weight gain, and food consumption in the low-dose groups paralleled those of the control groups. During the first two months, high-dose males (≈22%↓) and females (≈24%↓) lost weight; thereafter, body weight of these animals remained constant but well below that of the controls (M: 20-25%; F: 25-30%). Mid-dose animals gain less weight than the controls resulting in lower absolute body weight by the second month of the study (M:4%, F: 14%). Body weight for the remainder of the study was more pronounced in mid-dose females (15-20%) than males (3-6%). Final body weight of the low-, mid-, and high-dose animals was 96%, 94%, and

76% of controls, respectively, for males and 99%, 79%, and 68% of controls, respectively, for females. Food consumption was less than that of controls for the high-dose groups (M: 50-100 gm, F:≈100 gm) throughout the study and occasionally for the mid-dose females (25-50 gm). Data were not available to calculate food efficiency, however, qualitative evaluation of food consumption values and reductions in body weight gain at the mid- and high- dosages indicated reduced food efficiency, a toxicologically significant effect of the treatment compound.

Beginning at approximately the 8th week of treatment, the hemoglobin (M 7%, F15%), hematocrit (M 7%, F 13%), and erythrocyte count (M 7%, F 7%) were decreased in high-dose males and females. This effect was more pronounced in females. Because mean corpuscular volume, methemoglobin and reticulocyte counts were normal, and no hemosiderotic lesions were reported at any dose, the mild anemia was considered normocytic and normochromic. Reductions in cholesterol and glucose levels in high-dose males (93% and 92%, respectively, of control) and females (66% and 88%, respectively, of control) occurred at week 26. Combined with the body weight data, results of clinical pathology indicate a pronounced inanition in high-dose animals.

Absolute and relative (to brain) liver weights were reduced in high-dose males (83% and 88% of control) and females (82% and 84% of control, respectively). Absolute and relative (to brain) kidney weights were reduced in high-dose females (79% and 82% of control, respectively), as were absolute and relative (to brain) weights of the gonads in high-dose females (64% and 63% of the control). However, the absence of associated histopathology or clinical chemistry changes suggested that the reductions in weight of some organs were due to lower body weight.

**Under the conditions of this study, the LOAEL for rotenone in male and female beagle dogs is 2.0 mg/kg bw/day, based on treatment-related inanition. The NOAEL for rotenone in male and female beagle dogs 0.4 mg/kg bw/day.**

This subchronic oral toxicity study in dogs is **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic study in dogs [OPPTS 870.3150 (§82-1b)].

### 3. Oncogenicity Rat (MRID# 00143257):

**EXECUTIVE SUMMARY:** This study (MRID# 00143257) was conducted to verify previously published reports of mammary tumor incidence in rats dosed with rotenone. Rotenone (>95% a.i., S.S. Penick and Co., Orange, N.J.) suspended in corn oil was administered intraperitoneally or orally by gavage. Twenty-five male and 25 female Sprague-Dawley rats/group were dosed with 1.7 or 3.0 mg/kg/day by intraperitoneal injection 7 days/week for 42 days. Control groups of 15 males and 15 females were dosed intraperitoneally with the vehicle only (0.1 mL corn oil) with the same protocol. Rats were observed for 17 months post-dosing prior to necropsy. The second study dosed 25 male and 25 female Wistar rats by oral gavage 7 days/week for 42 days with 0, 1.7 or 3.0 mg/kg/day. The rotenone in corn oil was given in 0.25 mL volumes. Rats were then observed for 12 months post-dosing prior to necropsy.

Body weight in male and female rats was presented in graph form for the intraperitoneal study. Examination of the graph showed no significant difference in the treated groups. The only tumor noted was fibroadenoma of the mammary gland observed in both control and dosed animals at the same incidence. These were seen in 7/21 females in the 3 mg/kg group, 13/25 females in the 1.7 mg/kg group, 8/15 females and 3/14 males in the control group. This does not indicate a treatment-related increase in incidence.
Body weight from the oral study was also presented in graph form and again no significant difference in body weight could be observed. No increased incidence of mammary tumors was noted between the treated groups. Mammary ductal ectasia and cysts were seen at a slightly increased incidence in the treated females. Ectasia occurred in 1/25 of the controls, 4/24 in the 1.7 mg/kg group and 6/24 in the 3.0 mg/kg group. Cysts occurred in 4/25 of controls, 3/24 in the 1.7 mg/kg group and 6/24 in the 3.0 mg/kg group.

At the doses tested, there was no treatment related increase in mammary tumor incidence in any group. Dosing appeared to be inadequate based on the rat's ability to maintain body weight and there was no evidence of systemic toxicity.

This carcinogenicity study in the rat is **Unacceptable/Non-guideline** and does not satisfy the guideline requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in rats.

**4. Oncogenicity & Reproductive Toxicity Hamster (MRID# 00143256):**

**EXECUTIVE SUMMARY:** In both reproductive and carcinogenic studies (MRID# 00143256), rotenone ( >95 % a.i., S.S. Penick and Co., Orange, N.J.) suspended in 1% corn oil and mixed in chow meal was fed to groups of Syrian Golden hamsters. In the reproductive study, 25 male and 50 female hamsters were administered 1000 ppm for four months prior to and during mating and 50 male and 50 female hamsters were administered 500 ppm for 3 months prior to and during mating. A control group of 50 male and 50 females was fed 1% corn oil and chow meal. In the carcinogenicity study, 50 male and 50 female hamsters/group were dosed with 0, 125, 250, 500 or 1000 ppm rotenone and 1% corn oil in chow meal for 18 months. Based on a food factor of 0.083 for the hamster, dietary concentration of 125, 250, 500 or 1000 ppm results in doses of 10, 21, 42 and 83 mg/kg/day, respectively.

In the reproductive study, 3 male and 12 female hamsters treated with the 1000 ppm diet died during the first two months. Surviving hamsters in the 1000 ppm group exhibited temporary decreases in food consumption after week five and had rough hair coats and some weight loss although these trends reversed by week nine. No data on body weight or food consumption were provided. No specific details on the early deaths were reported. While mating was confirmed by the presence of vaginal plugs, no pregnancies occurred in the 1000 ppm treated group implying that one or both sexes were unfertile. Males were observed grossly to have decreased testicular size although no actual measurements were recorded. The 500 ppm treated group resulted in cannibalization or

neglect of the young by dams in both the $F_1$a and $F_1$b generations with all pups being reported as smaller than normal although weight was not recorded. For the 0 ppm group, healthy offspring were produced in the $F_1$a and $F_1$b generations. The study was terminated after 6 months for the treated groups and 10 months for the control group at the request of the EPA Project Officer.

In the carcinogenesis study, hamsters in each group were weighed weekly for the first 6 months, then bi- or tri-weekly thereafter. Feed consumption was measured weekly. Data were reported in graph form only. Based on the graphs, decreased weight gain can be seen in the 1000 ppm treated groups compared to the controls. Spontaneous death occurred with the same frequency in all groups including the controls during the first 12 months of the study. Necropsy was performed on all but five of the 177 early decendents. Enteritis/Typhlitis was the predominant findings on the spontaneous deaths. All animals were examined grossly upon death for evidence of tumors but only the 0, 125 and 1000 ppm groups had tissues fixed for histopathological examination. Adrenal cortical carcinomas in 1/32 males and 2/33 females were presented only in the 1000 ppm group. Adrenal cortical hyperplasia and adrenal cortex adenoma were seen in all groups with no treatment-related incidence.

Doses of rotenone ($\geq$500 ppm) demonstrated embyotoxic effects, however, lower levels were not tested thus a NOAEL could not be identified.

In the carcinogenicity study, 1000 ppm resulted in toxicity (depressed body weight compared to the controls) but gross and histopathological examination did not indicate any treatment-related increased incidence of tumors. However, diseased hamsters were used in the carcinogenicity study and thus caused excessive death in controls (96% in females) and LDT (86%), invalidating any comparison with dosed groups. Therefore, the validation of the adrenal tumors observed in the study is compromised by disease in the colony of animals tested.

These studies are classified as **Unacceptable/Non-guideline** and do not satisfy the guideline requirement for a carcinogenicity study (870.4200) or reproduction study (870.3800). This study(ies) is unacceptable since: a) the reproduction study was inadequately described for body weight s of parents and offspring, infertility, and testes weights; b) offspring from the first and second matings were inadequately described; c) only two dose levels were used with excessive toxicity at the HDT; d) mating the F1 generation apparently did not occur; and e) no NOAEL was shown.

Testing of rotenone levels below 500 ppm is recommended for the reproductive study and another carcinogenicity study without a significant number of early mortalities is recommended.

**5. Metabolism and Pharmacokinetics Rat (MRID# 00145496):**

**EXECUTIVE SUMMARY:** In a metabolism study (MRID# 00145496), rotenone ($^{14}$C-labeled in the 6α position, Lot Nos. 500507 and 801110, purity 94.64%; unlabeled rotenone purity 99.23%. Lot No. 100287) was administered to male and female Sprague Dawley rats. In a preliminary balance study, one male and one female rat/group received a single 0.1 mg/kg or 5 mg/kg dose administered by gavage or by i.v. For the main study, groups of 5 male and 5 female rats received a single i.v. dose of 0.01 mg/kg $^{14}$C-rotenone via the tail vein; groups of 5 male and 5 female rats received a single gavage dose of 0.01 mg/kg $^{14}$C-rotenone; groups of 5 males and 5 females received 14 daily 0.01 mg/kg gavage doses of unlabeled rotenone followed by a single gavage dose of 0.01 mg/kg $^{14}$C-rotenone; and groups of 5 male rats and 5 female rats received a single gavage dose of 5 mg/kg $^{14}$C-rotenone. In addition, groups of 6 male and 6 female rats received a single gavage dose of 5 mg/kg $^{14}$C-rotenone or a single i.v. dose of 0.01 mg/kg $^{14}$C-rotenone to investigate enterohepatic circulation.

Whether administered orally or by i.v., the preliminary study showed the primary route of elimination of rotenone was in the feces. None of the radiolabel was detected in the expired air and <5% of the radiolabel was recovered in the urine. Greater than 70% of the administered dose was eliminated within 48 hours of treatment.

In the main study, male and female rats excreted 79.67% and 85.88%, respectively, of a 0.01 mg/kg i.v. dose of radiolabeled rotenone in the feces. Of this, male rats excreted 46.8% and female rats excreted 53.8% within 48 hours of treatment. Urinary elimination accounted for 2.96% and 3.02% in males and females, respectively, while cage debris accounted for 2.53% and 7.71% respectively. Following oral administration of 0.01 mg/kg of radiolabeled rotenone, 95.88% of the administered dose was excreted into the feces of male rats with 86.5% eliminated within 48 hours. Female rats excreted 79.14% of the dose in the feces with 70.41% of the dose within 48 hours. Urinary excretion accounted for 2.41% and 4.22% of the administered dose in males and females respectively. Similar results were found in the multi-low dose study. Male rats that received 14 daily doses of 0.01 mg/kg unlabeled rotenone followed by a single 0.01 mg/kg labeled dose of rotenone excreted 89.86% of the dose in the feces with 85.1% of the dose excreted in 48 hours. Females excreted 94.15% of the dose in the feces with 88.59% within 48 hours. Males and females excreted 3.43% and 2.74% of the labeled rotenone dose in the urine. Male and female rats treated orally with a single 5 mg/kg of rotenone excreted 79.14% and 78.77% of the dose in the feces, respectively and 3.09% and 3.22% in the urine. These results show that elimination is rapid and fecal excretion is the primary route of elimination of rotenone. Results also suggest that female rats excrete slightly more rotenone in the urine than male rats.

In conjunction with fecal elimination, extensive enterohepatic circulation occurred. Hepatic portal plasma to cardiac plasma ratios shows a greater concentration of radiolabel in the portal vein whether the dose was administered i.v. (1.7 x) or orally (2.2x males, 1.6x females). Tissue accumulation was low for all dosing groups, typically being <1% of the administered dose. As would be expected, organs involved with elimination of the test material had the greatest concentrations of radiolabel up to 144 hours after treatment.

These included the liver and kidney with 0.7% and 0.15% of the administered dose, respectively.

Following i.v. administration, the distribution/elimination half-life was 1.1 hours with a biological half-life of 14 hours. After oral dosing, the distribution/elimination and biological half-lives were similar (2.4 hours and 18 hours, respectively).

Metabolic profiles could not be obtained from the feces of male and female rats treated with 0.01 mg/kg rotenone orally or by i.v. Seven metabolites were found in the feces of male and female rats treated with 5 mg/kg rotenone. Polar metabolites accounted for 40.82-72.99% of the metabolites from male rats and 33.48-65.76% from female rats. Pretreatment of the fecal extracts with glucuronidase and aryl sulfatase did not affect the metabolic profile. No parent compound was identified. In the urine of male rats, 69.67-93.97% of the metabolites were identified as polar while 43.51-94.88% was identified as polar in female rat urine.

This metabolism study in the rat is classified **Acceptable/Non-guideline** because it does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417]. However, sufficient data is provided to show the metabolic disposition of rotenone.

**6. Gene Mutation Salmonella typhimurium: (MRID# 40170506):**

**EXECUTIVE SUMMARY:** In a reverse gene mutation assay in bacteria (MRID# 40170506), strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* were exposed to rotenone ( Lot #. 735-RAP-1502 and purity >98%) [1], dissolved in dimethylsulfoxide (DMSO) or 95% ethanol at concentrations of 0, 100, 333, 1000, 3333 or 10,000 $\mu$g/plate in the presence and absence of microsomes from livers of Aroclor 1254-induced male Sprague-Dawley rats and Syrian hamsters, using a pre-incubation procedure. After the test chemical was incubated with the appropriate tester strain, microsomal fraction or buffer for 20 minutes at 37 °C, top agar was added and the mixtures were overlaid on minimal bottom agar. The plates were then incubated under unspecified conditions. Sodium azide, 2-aminoanthracene, 9-aminoacridine and 4-nitro-o-phenylenediamine were used as positive controls[1]. Each dose, vehicle control and positive control were tested in triplicate and two separate assays were conducted. The arithmetic mean of the revertants on the triplicate plates was determined and the results were considered positive if there was a reproducible, dose-related increase in revertant colonies. Precipitation of the test material was reported at 3333 and 10,000 $\mu$g/plate +/- S9. No cytotoxicity study was included in the report but the number of revertants observed for each dose and treatment condition gave no indication of cytotoxicity. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background for any tester strain at any concentration, either with or without metabolic activation with microsomes from rat or hamster liver.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity bacterial reverse gene mutation data.

**7. Gene Mutation Salmonella typhimurium (MRID# 40170502):**

**EXECUTIVE SUMMARY:** In a reverse gene mutation assay in bacteria (MRID# 40170502), strains TA98, TA100, TA1535, TA1537 and TA1538 of *Salmonella typhimurium* were exposed to rotenone (Code #864200/Lot No. 735-RAP-1502, >98%), dissolved in dimethylsulfoxide (DMSO) at concentrations of 0, 30, 100, 330, 1000, 3300 and 10,000 $\mu$g/plate in the presence and absence of Aroclor 1254-induced rat liver microsomes added along with the tester strain and the test chemical to top agar and overlaid on minimal bottom agar (plate incorporation procedure). The plates were incubated under unspecified conditions. Rotenone was tested up to concentrations of 10,000 $\mu$g/plate, although some precipitation became noticeable at 48 $\mu$g/plate and became heavier at 5000 ug/plate and above in a preliminary toxicity assay. No cytotoxicity of rotenone was noted at any dose or with any tester strain either with or without S9 activation. 1,3-propane sulfone, 2-nitrofluorene and 2-aminoanthracene were used as positive controls.

Each dose, vehicle control and positive control was tested in triplicate and two separate assays were conducted. The arithmetic mean of the revertants on the triplicate plates was determined and the results were considered to be positive if the number of revertants at any test concentration was at least double that of the vehicle control and a dose-related increase in the number of revertants/plate was observed.

The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background for any tester strain at any concentration, either with or without S9 activation with rat liver microsomes.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity bacterial reverse gene mutation data.

**8. Gene Mutation Mouse lymphoma cells (MRID# 40170505):**

**EXECUTIVE SUMMARY:** In a mammalian cell gene mutation assay (MRID# 40170505), L5178Y mouse lymphoma cells (Tk $^{+/-}$) cultured *in vitro* were exposed to rotenone ( Lot #. 735-RAP-1502 and purity >98%) [1], dissolved in acetone at concentrations of 0, 0.50, 1.0, 2.0, 4.0 and 8.0 $\mu$g/ml in the first experiment and at concentrations of 0, 0.25, 0.50, 1.0, 2.0 and 4.0 $\mu$g/ml in the second experiment, both in the absence of mammalian metabolic activation. Cultures (6 x $10^6$ cells) were incubated with the test material for 4 hours, then washed and resuspended in medium for 2 days to allow expression of mutants. Cultures were kept in log phase growth during the expression period. At the end of the expression period, samples of each culture were

plated to determine cloning efficiency and mutant counts. All plates were incubated for 10 to 12 days before they were scored. Methyl methanesulfonate was used as a positive control. All data were evaluated statistically for both trend and peak response. An experiment was considered positive if there was a statistically significant ($p<0.05$) increase in the mutation frequency at any of the three highest concentrations in comparison to the vehicle control and a significant positive trend ($p<0.05$). A chemical was considered positive only if the positive response was confirmed in a repeat test.

In the first experiment the mutation frequency ranged from 208 to 3,142 per $10^6$ cells as the rotenone concentration was increased from 0.5 to 4.0 $\mu$g/ml and the vehicle control had a mutation frequency of 59 per $10^6$ cells. In the repeat experiment the mutation frequency ranged from 116 to 268 per $10^6$ cells as the rotenone concentration was increased from 0.25 to 2.0 $\mu$g/ml and the vehicle control had a mutation frequency of 71 per $10^6$ cells. Concentrations of 8.0 and 4.0 $\mu$g/ml rotenone were lethal in the first and second experiments, respectively. All concentrations of the test chemical produced mutation frequencies significantly ($p<0.05$) above the vehicle control values. The positive controls in both experiments showed the appropriate response. **There was evidence that rotenone caused a concentration-related positive response of induced mutant colonies over background in both experiments.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

**9. Cytogenetics (Chinese hamster ovary) (MRID# 40179801c):**

**EXECUTIVE SUMMARY:** [23]Appendix E (page 172 of 189) of a NTP cancer report (MRID# 40179801) described a mammalian cell cytogenetics assay (chromosome aberrations), in which CHO cells in culture were exposed to rotenone (batch/lot # not given), dissolved in acetone at concentrations of 0, 10, 25, 50, 75 or 100 $\mu$g/ml without activation (repeat assays), and 0, 100, 150, 200 or 250 $\mu$g/ml with metabolic activation using rat liver microsomes induced with Arochlor 1254. Exposures of the cells to the test material, and positive and negative controls were for 8-10 hours (non-activated) and 2 hours (activated). A preliminary cytotoxicity assay of Rotenone was not reported. The protocol stated that the cytotoxicity assay was incorporated into the actual assay with a five-log range of concentrations of test material in a half-log series of concentrations. Because of significant chemical-induced cell cycle delay, incubation time before addition of colcemid was lengthened to 21.5 hours (Trial 1) and 20.5 hours (Trial 2) to provide sufficient metaphases at harvest. Positive controls (mitomycin C-S9; cyclophophamide +S9) were included in each trial.

The first test results without activation were equivocal. There was an increase in aberrations at 25 $\mu$g/ml but not at 50 $\mu$g/ml (highest dose tested in the first assay). The repeat study did not show any positive results at 25, 50 or 100 $\mu$g/ml. The positive controls indicated that the assay was working properly. Only one assay was conducted with activation and it was negative. Significant cell-cycle delay was induced by the test chemical, indicating that sufficient cytotoxicity was obtained. **There was no evidence of chromosome aberrations induced over background, either with or without activation.**

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[23] This study was performed at Litton Bionetics, Inc. A detailed presentation of the technique for detecting chromosomal aberrations was referenced as Galloway et al. (1985). The techniques were described as "(a) Chinese ovary cells were incubated with study compound or solvent, as indicated in (b) or (d). Cells were arrested in first metaphase by addition of cocemid and harvested by mitotic shake-off, fixed, and stained in 6% Gemsa. (b) In the absence of S9, cells were incubated with study compound or solvent (acetone) for 8-10 hours at 37°C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest. (c) Because of significant chemical-induced cell cycle delay, incubation time before addition of colcemid was lengthened to 21.5 hrs (Trial 1) and 20.5 hrs (Trial 2) to provide sufficient metaphases at harvest. (d) In the presence of S9, cells were incubated with study compound or solvent (acetone) for 2 hours are 37°C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats."

Galloway, S, Bloom A, Resnich M, Margolin B, Nakamura F, Archer P, Zeiger E (1985). Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51. The protocol fo this reference is Provided in TXR No. 009028

This study is classified as **Acceptable/Guideline**, and satisfies the guideline requirement for *in vitro* mammalian cytogenetics - chromosome aberrations in Chinese hamster ovary cells *OPPTS 870.5375; OECD 473*.

**10. Cytogenetics (rat) and Micronucleus (mouse) (MRID# 00093702):**

**EXECUTIVE SUMMARY:** In whole animal cytogenetics assays [Chromosome aberration study in rats (8-10 week old Sprague Dawley males) and micronucleus study in mice (8-10 week old ICR Swiss males and females)] (MRID# 00093702), animals were gavaged daily for 2 days with rotenone dissolved in corn oil. Groups of ten rats received doses of 0, 0.7, 2.5 or 7.0 mg/kg, of the test agent and ten positive control rats received 1.0 mg/kg of triethylene melamine (TEM) given intraperitoneally (i.p.). The highest test dose was ~1/10th of the estimated rat oral $LD_{50}$ based on a preliminary toxicity study done by the same lab. Groups of eight mice received doses of 0, 10, or 80 mg/kg of the test agent and eight received 1.0 mg/kg of TEM given i.p. The highest test dose was the estimated mouse oral $LD_{50}$ based on a preliminary toxicity study done by the same lab.

Forty-five hours after the second dosing, rats were treated with colchicine (4 mg/ml) to arrest cells in metaphase. Three hours later bone marrow cells were collected, hypotonically treated, fixed, stained and mounted. Cells (400/animal) were examined for chromatid/chromosome gaps, breaks, fragments, minutes or other aberrations. Cytotoxicity was determined by measuring the mitotic index (1000 cells/dose group). The report had no indication of overt toxicity at any dose. The frequency of chromatid/chromosome aberrations was 0.88, 0.35, 1.1, and 0.43% for the 0, 0.7, 2.5 and 7.0 mg/kg dose groups, respectively. Positive control data were not presented. The mitotic indices for the corresponding groups were 26.3, 23.3, 28.7 and 30.1%. Based on a statistical analysis using Student's t test, **there was no evidence that the test material induced chromatid/chromosome aberrations in rat bone marrow cells over background**.

Six hours after the second dosing of the mice, bone marrow cells were collected, smeared on glass slides, dried, fixed, stained and mounted. Polychromatic and normochromatic erythrocytes (1000 each) from each dose group and the positive control were scored for micronuclei. The frequencies of polychromatic erythrocytes with micronuclei were 0.043, 0.031 and 0.029% in the 0, 10 and 80 mg/kg dose groups, respectively; the frequencies of normochromatic erythrocytes with micronuclei were 0.044, 0.038 and 0.038% in the 0, 10 and 80 mg/kg dose groups, respectively. There was no indication that the test material was toxic to the test animals. Positive controls showed appropriate responses for both the polychromatic and normochromatic erythrocytes. Based on probit analysis, the test material **did not produce a significant increase in the frequency of micronuclei in polychromatic or normochromatic erythrocytes from bone marrow.**

Assays on chromosomal effects of the test material in fruit flies (*Drosophila melanogaster*) and horse beans (*Vicia fabia*) were also carried out by Biotech Research Laboratories but, because of the limited solubility of rotenone in aqueous solution (20

$\mu$g/ml), these assays were not likely to detect potential genotoxicity and have been previously classified as unacceptable (Reviewed by the Toxicology Branch (6-14-82): Memorandum to W. Miller, Registration Division. From R. Gardner. Subject: Review of Mutagenicity Assays with Rotenone. EPA Reg. No. 6704-Q. Acc. No. 246587, Tox. Chem. No. 725).

This study is classified as **Unacceptable/Non-Guideline**, and does not satisfy the guideline requirement for *in vivo* mammalian cytogenetics - OPPTS 870.5385 (§84-2) OECD 475 Bone Marrow Chromosomal Aberration Test in the Rat and OPPTS 870.5395 (§84-2) OECD 474 Erythrocyte Micronucleus Test in the Mouse. In addition, classification of the Drosophilia melongaster vicia fabia assays are Unacceptable. The primary reason for this classification of the rodent assay as also being unacceptable is because the maximum tolerated dose (MTD) was not achieved in either the rat or the mouse assays.

**11. Mitotic gene conversion (Saccharmomyces cerevisiae) (MRID# 00144292):**

**EXECUTIVE SUMMARY:** In a mitotic gene conversion assay in diploid yeast (MRID# 00144292), strain D4 of *Saccharomyces cerevisiae* was exposed to rotenone (Batch #. 100287 and purity >97%), dissolved in ethanol at concentrations of 0, 1, 10, 100, 500, 1000, 2500, 5000 and 10,000 $\mu$g/plate in the presence and absence of microsomes from livers of Aroclor 1254-induced Sprague-Dawley rats. The test material, yeast cells and buffer or microsomes were mixed with 0.6% agar and poured onto minimal agar plates and incubated at 30 °C for ~4 days before scoring for tryptophan convertant colonies. The D4 strain measures only mitotic gene conversions, which involve nonreciprocal crossover events. Toxicity tests showed that even at the highest tested dose, viability of the D4 strain was ~95%. Ethyl methanesulfonate was used as a positive control without activation, and. 2-anthramine was used as a positive control with activation. However, it was noted by the author that positive controls with activation were historically inconsistent.

Each dose, vehicle control, and positive control was tested on a single plate. The positive control, without activation, induced the appropriate response, but the positive control with activation showed no increase in gene conversion events above the vehicle control. **There was no evidence of induced mutant colonies over background for any test concentration, either with or without metabolic activation up to levels in excess of the limit dose (5000 $\mu$g/plate).**

This study is classified as **Acceptable/Guideline** and satisfies the requirement for OPPTS 870.5575 [§84-2]; OECD 481 for a yeast mitotic gene conversion assay. The final report also included 3 other genotoxicity studies not covered by OPPTS Guidelines: (1) A reverse mutation assay using *S. cerevisiae* haploid strains S138 (frameshift mutant) and S211 (base pair substitution mutant) at dose levels of the test material up to 10,000 $\mu$g/plate (in excess of the limit dose of 5000 $\mu$g/plate) gave no evidence of induced mutant colonies over background for either strain, either without or with activation. Positive controls without activation gave appropriate responses in both

strains but positive controls with activation showed no increase in revertants above the vehicle control. (2) A mitotic recombination assay, repeated twice, using *S. cerevisiae* diploid strain D5 at dose levels of the test material up to 10,000 $\mu$g/plate gave no evidence of induced mutant colonies over background, either without or with activation. Positive controls without activation gave appropriate responses, as did positive controls with activation, although the positive controls with activation gave widely different mutation frequencies in the repeated trials. (3) A mouse somatic cell mutation test (spot test) was carried out by treating pregnant females, by gavage, with the test material dissolved in corn oil or corn oil plus DMSO, on days 8 through 11 of gestation, with doses of 0.05, 0.17. 0.5 or 1.0 mg/kg. Seven females receiving 0.17 mg/kg rotenone in DMSO, 11 females dosed with 0.5 mg/kg rotenone in DMSO, and 8 females administered 1.0 mg/kg rotenone in DMSO were found dead versus 5 in the DMSO negative control group. Other signs of compound toxicity included lethargy and clamminess. When the test material was prepared in corn oil at 1000 mg/kg, 11 deaths were recorded as compared to no deaths in the vehicle control group. The treatment did not cause any melanocyte toxicity nor did it induce any somatic mutations in the embryonic melanocytes. Positive controls using ethyl nitrosourea induced the appropriate response. An additional treatment with 1000 mg/kg of the test material, administered to pregnant mice under the same conditions, caused melanocyte toxicity but did not produce any somatic mutations.

The results of all four studies support the conclusion that rotenone was not genotoxic under the limited conditions of the experiments. Inconsistent results in the yeast studies using known mutagens that require activation (e.g., dimethyl nitrosamine) cast doubt on the reliability of the findings. In the mouse spot test, death and other clinical signs noted at the highest dose tested (1000 mg/kg in corn oil). There was, however, no cytotoxic effect or mutagenic effect on the target cell (melanocyts).

## TOXICOLOGY DATA REQUIREMENTS

The requirements (40 CFR 158.340) for food use for rotenone are in Table A1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

**Table A1. Toxicology Data Requirements for Rotenone.**

| Test | | Technical | |
|---|---|---|---|
| | | **Required** | **Satisfied** |
| 870.1100 | Acute Oral Toxicity | Yes | Yes |
| 870.1200 | Acute Dermal Toxicity | Yes | Yes |
| 870.1300 | Acute Inhalation Toxicity | Yes | Yes |
| 870.2400 | Primary Eye Irritation | Yes | Yes |
| 870.2500 | Primary Dermal Irritation | Yes | Yes |
| 870.2600 | Dermal Sensitization | Yes | Yes |
| 870.3100 | Oral Subchronic (rodent) | Yes | Yes[1] |
| 870.3150 | Oral Subchronic (nonrodent) | Yes | Yes |
| 870.3200 | 21-Day Dermal | Yes | **No**[2] |
| 870.3250 | 90-Day Dermal | no | - |
| 870.3465 | 90-Day Inhalation | no | - |
| 870.3700a | Developmental Toxicity (rodent) | Yes | Yes |
| 870.3700b | Developmental Toxicity (nonrodent) | Yes | **No** |
| 870.3800 | Reproduction | Yes | Yes |
| 870.4100a | Chronic Toxicity (rodent) | Yes | Yes[1] |
| 870.4100b | Chronic Toxicity (nonrodent) | Yes | **No** |
| 870.4200a | Oncogenicity (rat) | Yes | Yes |
| 870.4200b | Oncogenicity (mouse) | Yes | Yes |
| 870.4300 | Chronic/Oncogenicity | Yes | Yes |
| 870.5100 | Mutagenicity—Gene Mutation - bacterial | Yes<br>Yes | |
| 870.5300 | Mutagenicity—Gene Mutation - mammalian | Yes | Yes<br>Yes |
| 870.5xxx | Mutagenicity—Structural Chromosomal Aberrations | No | Yes |
| 870.5xxx | Mutagenicity—Other Genotoxic Effects | | - |

| Table A1. Toxicology Data Requirements for Rotenone. | | | |
|---|---|---|---|
| | | Technical | |
| Test | | Required | Satisfied |
| 870.6100a | Acute Delayed Neurotox. (hen) | No | |
| 870.6100b | 90-Day Neurotoxicity (hen) | No | - |
| 870.6200a | Acute Neurotox. Screening Battery | No | - |
| | (rat) | Yes | - |
| 870.6200b | 90 Day Neuro. Screening Battery (rat) | No | **No**[3] |
| 870.6300 | Develop. Neurotoxicity | | - |
| 870.7485 | General Metabolism | Yes | Yes |
| 870.7600 | Dermal Penetration | Yes | **No**[4] |
| Special Studies for Ocular Effects | | | |
| | Acute Oral (rat) | No | - |
| | Subchronic Oral (rat) | No | - |
| | Six-month Oral (dog) | No | - |

[1]Requirements for this study are fulfilled by the chronic/oncogenicity rat feeding study.
[2] A dermal toxicity study with neurotoxicity parameters is recommended.
[3]An inhalation neurotoxicity (rat) study has been recommended.
[4]CFR 158.340 (24): Dermal absorption studies required for compounds having a serious toxic effect as identified by oral or inhalation studies, for which a significant route of human exposure is dermal and for which the assumption of 100 percent absorption does not produce an adequate margin of safety. Registrants should work closely with the Agency in developing an acceptable protocol and performing dermal absorption studies.