

Rotenone. A Brief Review of its Chemistry, Environmental Fate, and the Toxicity of Rotenone Formulations.

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Background on the Chemistry of Rotenone. Rotenone is a natural product isolated from certain subtropical and tropical members of the pea family found in South America and Southeast Asia. The use of rotenone by man may be ancient, as early explorers noted Peruvian natives using crude extracts of the Cubé plant to stun fish for eating. In the early 1900's, botanists exploring Peruvian jungles searching for new and useful plants of potential commercial value exported large quantities of plant roots and extracts to the US for use as insecticides on food crops, with the major active ingredient of the extracts being rotenone. Rotenone has been used commercially as a garden insecticide since the middle 1800's, primarily in the UK. Rotenone is still currently widely used as a garden insecticide, and in organic farming as a natural, botanical insecticide. In California, about 200 kg of rotenone is used per year as an insecticide for organically grown lettuce and tomatoes.¹ Rotenone has also found significant use as a highly selective piscicide for the control or eradication of non-native fish in streams and lakes across the United States.² Rotenone has been used successfully for native trout reintroduction in the United States since the 1960's.

In the late 1920's, the chemical structure of rotenone was determined. Rotenone is a member of a class of compounds of related molecular structure referred to as isoflavones. Many will recognize the term 'isoflavone', as these compounds are components in a variety of foods thought to be beneficial to health obtained from the pea family such as soybeans (edamame) and its products – tofu, tempeh, and soybean oil, the major component of vegetable oil.

When ingested, isoflavones are poorly absorbed because they are decomposed to more excretable fragments by a variety of oxidases as well as by bacterial fauna that thrive in our gut. Another efficient route of excretion is by way of molecular conjugation to sugar molecules. The resulting conjugates are then readily excreted from the body. For several isoflavone compounds, the biological half-life in humans (the time at which half of the original dose remains) is on the order of 8 hours. Rotenone has a similar biological half-life.

While rotenone is not highly toxic to humans, mammals, and birds if ingested orally, rotenone is highly toxic to fish. The toxicity of rotenone is because of its efficacy in interrupting mitochondrial electron transport which hinders the utilization of oxygen in respiratory organisms, leading to cell death and eventually to the death of the organism if the dose is high

¹ M. B. Islam, "Botanical Insecticides, Deterrents, and Repellents in Modern Agriculture and an Increasingly Regulated World", *Annual Review of Entomology*, **51**, 45-66, (2006).

² W. McClay, "Rotenone Use in North America", p. 15-27 in "Rotenone Use in Fisheries Management. Administrative and Technical Guidelines Manual", B. J. Finlayson *et al*, editors. American Fisheries Society, 2000.

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enough. The mechanism of interrupting electron transfer is similar to other well-studied respiratory inhibitors. Because the respiratory mechanism of fish is directly linked to water through the gills, rotenone may pass directly into the bloodstream of fish, leading to death. Rotenone is much less toxic to mammals and birds because the route of ingestion is through the gut where much of the compound is broken down to less toxic components before toxic quantities can enter the bloodstream. Rotenone ingestion through inhalation results in significantly higher toxicity, as there is a more direct pathway into the bloodstream. This is why professionals must use appropriate respiratory protection while handling concentrated formulations containing rotenone during preparation of materials for piscicidal applications. Once the compound is diluted in the water column, the risk of ingestion via inhalation is insignificant because of the very low concentrations of rotenone added to the water, and the remote chance of humans, mammals, or birds aspirating treated water in huge quantities into the lungs. The amount of water that would have to be aspirated into the lungs would cause drowning far before any toxic dose could be delivered.

Even though a great deal of literature exists that describe the safe use of rotenone, a link between rotenone use and Parkinson's Disease has been proposed and has received a good deal of coverage in the popular press. The route of ingestion in these studies was directly into the bloodstream, and not through oral administration. This topic will be discussed in more detail below.

Because rotenone is so highly specific and highly toxic to fish, only very small quantities need be added to a stream or a lake to kill undesired fish. The amount of rotenone administered to a water column to achieve the desired degree of toxicity to fish depends on several factors: temperature, hardness, pH, the amount of organic detritus in the stream, and the amount of sunlight that penetrates the water. Typically, the concentration of rotenone for piscicidal application ranges from 20-40 parts per billion (ppb). To put this into context, 40 ppb of sugar solution would result from the addition of about a teaspoon of sugar to an Olympic size swimming pool.

Relative toxicity of rotenone. While 40 ppb (40 micrograms of rotenone per liter of water) is more than adequate to kill most species of fish, other animals are significantly less sensitive. The US Environmental Protection Agency (EPA) has set a reference dose for humans (the daily dose that the general population, including sensitive subgroups, could tolerate over a lifetime of exposure) of 4 micrograms per kilogram.³ This dose includes a safety factor of 100, meaning that the data supports that there are no harmful effects observed below a dose of 400 micrograms per kilogram. The lower value with the safety factor corresponds to a dose of 280 micrograms for a 70 kg adult (178 lb). To obtain this dose from piscicidal concentrations of rotenone (40 micrograms per liter), this adult could safely ingest 7 liters of 40 ppb rotenone treated water *every day, for life*. The average adult consumes around 2 liters of water per day up to 4 liters per day during the hot summer months. For a lethal dose, it is estimated that an average adult would have to consume 300 – 500 milligrams per kg of body weight, equivalent to drinking 500,000 liters of the water or 1/5 of the water in an Olympic sized pool – clearly impossible. Also, to compare against a point of reference, the lethal dose of caffeine is estimated to be 192 mg/kg in

³ EPA web site: <http://www.epa.gov/iris/subst/0344.htm>

humans; this would be like drinking 80 – 100 cups of coffee in rapid succession. It is probably more feasible to die from caffeine poisoning from drinking too much coffee than from drinking rotenone-treated water at piscicidal concentrations.

The toxicity of orally administered rotenone in rats and dogs has been well studied. Marking administered 75 mg/kg (nearly 20,000 times that of the safe reference dose for humans) to rats for a period 2 years.⁴ Gross and microscopic examination of tissues of the rats indicated no abnormalities. According to data compiled in ExToxNet⁵ and EPA's Integrated Risk Information System (IRIS)³ database, there is no conclusive evidence for rotenone having reproductive, teratogenic, mutagenic, or carcinogenic effects, particularly at piscicidal concentrations. There is no data that suggests that rotenone is an endocrine disruptor.

Environmental fate of rotenone in natural water systems. Rotenone is difficult to handle in the pure state, as it is sensitive to light and oxygen, quickly decomposing to less toxic products. This feature carries over to its use in natural water systems as water solutions of rotenone exposed to sunlight decompose readily to less toxic products. Rotenone is poorly soluble in water. Because of this hydrophobicity, rotenone absorbs to soil particles strongly, removing rotenone from solution until other chemical actions occur. The major route of photochemical oxidation is through the intermediate compound rotenolone, which is approximately an order of magnitude less toxic than rotenone.⁶ Also, in the presence of water, hydrolysis of side chain groups occurs, reducing the toxicity while also increasing the water solubility of the product, increasing the rates of desorption from soils and particulates in the water column. Retention of rotenone on streambed soils depends upon temperature. The concentration of absorbed rotenone on soils has been measured to drop below the limits of detection in under 3 days during the summer months when water temperatures are at their peak.⁷

In natural water systems, the rate of destruction of rotenone by way of hydrolysis, oxidation, and photochemical conversion to compounds and fragments of lower toxicity is dependent upon several factors such as temperature, pH, water hardness, and sunlight. In 'typical' natural systems of ponds and streams, the half life of rotenone in natural waters ranges from around a half a day at 24 °C (75 °F) to 3.5 days at 0 °C (32 °F).^{6,8}

⁴ L.L. Marking, 1988. "Oral Toxicity of Rotenone to Mammals". U.S. Fish and Wildlife Service Investigations in Fish Control **94**. 5 pp.

⁵ ExToxNet: <http://extoxnet.orst.edu/pips/rotenone.htm>

⁶ B. J. Finlayson, S. Siepmann, and J. Trumbo, "Chemical Residues in Surface and Ground Waters Following Rotenone Application to California Lakes and Streams", pp. 37-54 in "Rotenone in fisheries science: are the rewards worth the risks?" R.L. Cailteux, R.L. DeMong, Finlayson, B.J., W.D. Horton, W. McClay, R.A. Schnick, and C.W. Thompson, eds. American Fisheries Society, Trends in Fisheries Science and Management 1, Bethesda Maryland, 2001.

⁷ V. K. Dawson *et al*, "Rotenone Persistence in Freshwater Ponds: Effects of Temperature and Sediment Adsorption", *North American Journal of Fisheries Management*, **11**, 226 (1991).

⁸ P. A. Gilderhus J. L. Allen, and V. K. Dawson, "Persistence of Rotenone in Ponds at Different Temperatures", *North American Journal of Fisheries Management*, **6**, 129, (1986).

Because the half-life of rotenone in the water column may be longer than what is needed to remove undesired fish, rotenone is often ‘neutralized’ after the desired fish kill has been accomplished by the addition of potassium permanganate solution to the stream or lake. Potassium permanganate is a powerful oxidant that oxidizes rotenone to less toxic, more water soluble products, rotenolone being a major product. Potassium permanganate is commonly used as a municipal water purification agent in instances where the addition of chlorine is not practical to disinfect water for drinking, because the byproducts are safe and non-toxic at the concentrations of intended use. The permanganate ion, when reduced upon reaction with organic matter, results in the precipitation of harmless manganese oxides. The concentration of permanganate added to streams to neutralize the rotenone is on the order of 1-5 parts per million (ppm), but the concentration used by professional practitioners depends on factors such as temperature and other organics that are in the water that will compete in being oxidized along with rotenone. In high altitude lakes of California with cold waters, the oxidation of rotenone with potassium permanganate occurs within 30 minutes, rendering the resulting water non-toxic to fish.⁶

Toxicity of Rotenone to Aquatic Invertebrates and Amphibians. Rotenone effects on aquatic invertebrates and amphibians have been reviewed recently.⁹ Additional information on the ecotoxicity of rotenone may be found in the Pesticide Information Database.¹⁰ To broadly summarize the information available in these two sources, aquatic invertebrates are sensitive to rotenone at piscicidal concentrations. Fatal concentrations for the invertebrates studied are a factor of 10 to 100 higher than the piscicidal concentration used. Certain mayflies may be more sensitive.

As for amphibians, lethal concentrations of rotenone are species dependent, but in general amphibians are less sensitive than fish or invertebrates.

The proposed link between rotenone use and Parkinson’s Disease. Following the publication of a scientific paper prepared by J. T. Greenamyre’s group at Emory University titled “Chronic Systemic Pesticide Exposure Reproduces Features of Parkinson’s Disease”,¹¹ the news media picked up on this as there being a direct link between the development of Parkinson’s Disease (PD) and rotenone and other pesticides. The stated goal of Greenamyre’s research was not to study health effects of pesticides, but rather “To develop a more accurate *in vivo* model of PD” to enable a method to induce Parkinsonism in lab animals to further the study of the onset and development of the disease. Because mitochondrial electron transport defects have been implicated in the onset of PD, and rotenone and a variety of other compounds are electron transport inhibitors, it was felt that rotenone might be useful in stimulating the onset of PD in laboratory animals under controlled conditions. To achieve the onset of PD in rats, Greenamyre’s group infused rotenone directly into the jugular veins of test animals at a dose of 2-3 mg/kg. Why did they choose to use direct injection rather than feed the animals rotenone, an

⁹ N. Ling “Rotenone – a Review of its Toxicity and Use in Fisheries Management”, *Science for Conservation* **211**, Published by the Department of Conservation, Wellington NZ, 2003.

¹⁰ http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC35133

¹¹ R. Betarbet *et al.*, “Chronic Systemic Pesticide Exposure Reproduces Features of Parkinson’s Disease”, *Nature Neuroscience* **3**, 1301 (2000).

experimentally more simple approach? Their paper states the answer, an answer that was well-known in the literature prior to their publication – “Rotenone seems to have little toxicity when administered orally”. Feeding 75 mg/kg of rotenone for two years (versus 3 mg/kg for 5 weeks in Greenamyre’s study) resulted in no observed tissue or brain abnormalities. As discussed above, oral administration results in efficient metabolism of rotenone in the gut, and so little, if any, gets into the bloodstream and into the brain. These observations argue against the oral introduction of rotenone being a risk for the development of Parkinson’s Disease, especially at piscicidal concentrations and the corresponding small dose to humans that might result from drinking a substantial quantity of rotenone-treated water. Also important to note, only a small fraction of people that die from PD have shown abnormal electron transport chemistries in their brain tissues – the majority have normal function. This observation argues against a direct link with rotenone in PD in humans.¹²

In piscicidal applications of rotenone, it is extremely unlikely that the route of ingestion would be anything other than ingestion through the drinking of rotenone-treated water. Under those conditions of use, it is highly unlikely, and indeed, not observed, that the Parkinsonism that Greenamyre detected from direct injection of rotenone into the bloodstream could occur under the conditions of the piscicidal use of rotenone.

Others agree with this assessment. The New York Times reports: “Dr. John Q. Trojanowski, an expert on neurodegenerative diseases at the University of Pennsylvania School of Medicine in Philadelphia and the moderator of [a recent] workshop, was quoted as saying, "This is the best model we have ever had for this disease being associated with an environmental agent." But Dr. Trojanowski was further quoted as cautioning that the findings "may not represent what anyone would experience in the real world." For one thing, the rats in the study were exposed to the chemical through their jugular veins, so it was not broken down or metabolized in the digestive tract.”¹³

It is also important to note that there is not a documented link between rotenone use for agricultural or piscicidal applications and the development of PD. PD predates the onset of use of rotenone. There is no documented increased occurrence of PD among native Peruvians that were clearly exposed to significant amounts of rotenone through the pounding of cubé roots to extract rotenone for their use in fishing, which included swimming in rotenone treated waters and consuming rotenone-killed fish. If ever there was a population that might exhibit rotenone-induced PD, the Peruvian natives would be one.

Toxicity of Other Ingredients in Rotenone Formulations. Rotenone does not dissolve in water. In order for practitioners to disperse it in water so that it can be effective at such low concentrations, rotenone must be formulated with solvents. Two commercial product

¹² P. Jenner, “Parkinson’s Disease, Pesticides, and Mitochondrial Dysfunction”, *TRENDS in Neuroscience*, **24**, 245 (2001). A response to Greenamyre’s paper, ref. 11.

¹³ From the New York Times Online:

<http://www.nytimes.com/2000/11/05/science/05PARK.html?ex=1139806800&en=4c66001db3168e11&ei=5070>

formulations containing rotenone as the active ingredient are considered here. One is called Nusyn-Noxfish[®], the other is called CFT Legumine[®].

Dispersing Nusyn-Noxfish[®] into water to yield a toxic dose to fish of 20 ppb rotenone is accompanied by 4 parts per trillion trichloroethylene, 120 parts per trillion ethylbenzene, 8 parts per billion isopropylbenzene, 43 parts per billion propylbenzene, 145 parts per billion trimethylbenzenes, 4 ppb sec-butylbenzene, 2 ppb butylbenzene, 1 ppb naphthalene and methylnaphthalene, 6 ppb isopropyl toluene, and 1 ppb of xylenes. In addition, 21 ppb of piperonyl butoxide is added to this formulation as a 'synergist' (more on this below).

The major solvent components added to the water being treated are alkylbenzenes. The EPA has set safe doses of a variety of alkylbenzenes either through establishing a safe reference dose, RfD, or by setting a drinking water standard. The drinking water standards refer to a Maximum Contaminant Level, MCL, that is safe for short term or long term ingestion by humans. Both of these standards include substantial safety factors. For ethylbenzene, the MCL is set at 700 ppb, far greater than the quantities that will be introduced during piscicidal applications of Nusyn-Noxfish[®]. For xylenes, MCL is set at 10 ppm. For isopropylbenzene, RfD is set to 0.1mg/kg/day, equivalent to drinking 4 liters per day containing 2 ppm isopropyl benzene as the lifetime safe dose in humans. sec-Butylbenzene has a drinking water standard of 280 ppb in Florida; this is 70 times the concentration anticipated for a typical piscicidal application of Nusyn-Noxfish[®]. In summary, all of the alkylbenzene components of Nusyn-Noxfish[®] at piscicidal concentrations are far below safe limits.

Naphthalene and methylnaphthalene have been assigned RfD's of .02mg/kg/day and .004mg/kg/day by the US EPA corresponding to concentrations of 350 ppb and 70 ppb, respectively, in 4 liters of water that could be consumed safely by an adult per day for life. The EPA also has a drinking water guideline that suggests naphthalene concentrations should not exceed 20 ppb for a safe, lifetime concentration level. The bottom line here is that the naphthalenes are the most problematic components of Nusyn-Noxfish[®] from a safety perspective. Even then, piscicidal concentrations from the use of Nusyn-Noxfish[®] are a factor of at least 20 below the most conservative safe dose for lifetime consumption of these components in water as determined by the EPA.

While there is a trace of trichloroethylene in the Nusyn-Noxfish formulation, the piscicidal concentration is greater than 1000 times below the drinking water standard for trichloroethylene of 5 ppb.

Nusyn-Noxfish[®] also contains 21 ppm of piperonyl butoxide at piscicidal concentrations. Piperonyl butoxide is a natural product originally obtained as a byproduct of safrole extraction. Piperonyl butoxide is known to interact with cytochrome P450, a potent enzyme that oxidizes cellular contaminants. The interaction of piperonyl butoxide with P450 allows lower concentrations of the piscicide to be used, as otherwise P450 would effectively oxidize rotenone, reducing the delivered dosage at the cellular level. In this regard, piperonyl butoxide is referred to as a synergist, hence the name 'Nusyn'.

Another formulation for rotenone dispersal in water is CFT Legumine[®] which lacks the alkylbenzene solvents that Nusyn Noxfish[®] possesses. In place of the alkylbenzenes, CFT Legumine[®] contains N-methylpyrrolidone (NMP) and di(ethylene glycol) ethyl ether (DGEE) as solvents for the dispersal of rotenone in water. CFT Legumine[®] lacks the synergist piperonyl butoxide, and so the rotenone concentration to achieve toxicity in fish is approximately double that of Nusyn-Noxfish[®].

Typical piscicidal concentrations of CFT Legumine[®] result in concentrations of DGEE of 569 ppb; NMP, 90 ppb, and rotenone, 43 ppb. These values may go up or down by a small factor in concert depending on local conditions for the piscicidal application.

N-Methyl pyrrolidone is seeing increasing use as a solvent because of its low toxicity. It is used as a solvent for pharmaceuticals for oral ingestion, and sold under the tradename Pharmasolve[®].¹⁴ This latter use is supported by a large volume of short- and long-term toxicological effects data that indicates NMP is safe to use as a solubilizer for pharmaceutical ingredients in drugs. Consistent with this, a reference dose for NMP is not available on EPA's IRIS site, but literature data indicates that the no observable abnormal effects level in rats is 6,000 to 18,000 ppm (0.6-1.8%). In mice, the value is 2500 ppm.¹⁵ Taking a safety factor of 1000, this would translate to a safe reference dose concentration of 2-6 ppm, a factor of around 25 greater in concentration than what will occur from typical piscicidal application of CFT Legumine[®].

NMP is readily transformed and excreted from the body, and so does not bioaccumulate. The half-life of NMP in the body is between 3 and 7 hours. In natural water systems, NMP is not expected to bind to soils or clays, and biodegrades readily. 210 ppm of NMP in natural water systems biodegrades to greater than 98% within 24 hours.

The other solvent component in CFT Legumine[®] is DGEE. In rats given 10,000 ppm DGEE in drinking water over two years, slight, if any effects were noted. In mice, similar results were noted even at 50,000 ppm (5%) administered in the drinking water. These doses are nearly 90,000 times greater than the 569 ppb of DGEE anticipated for a typical piscicidal application of CFT Legumine[®].

Additional toxicology and health safety data are compiled in the ToxNet database that indicate the relatively low toxicity of even high concentrations of DGEE.¹⁵

DGEE is excreted readily through metabolic destruction or by conjugation to glucuronic acid. The conjugate is rapidly excreted. DGEE does not bioaccumulate.

In water, DGEE is not readily hydrolyzed or photolyzed. 400 ppm DGEE in water was observed to degrade to an extent greater than 90% after 28 days.¹⁵

The remainder of the components of CFT-Legumine[®] include minute traces of various alkylbenzenes and naphthalene and methylnaphthalene. When diluted to piscicidal

¹⁴ International Specialty Products, Pharmaceuticals Product Guide

¹⁵ Data from ToxNet: <http://toxnet.nlm.nih.gov>

concentrations intended for use, the concentrations of these compounds are in the part per trillion range, and far below either drinking water standards, or safe reference doses determined by the EPA as was discussed for the Nusyn-Noxfish[®] formulation. From a health safety standpoint, the naphthalene and methylnaphthalene concentrations are of no concern, as they are on the order of 350 parts per *trillion* and 140 parts per *trillion*, respectively. These are concentrations one to two orders of magnitude lower than the safe lifetime doses determined by the EPA.

To summarize, when rotenone formulations intended for piscicidal applications are administered to rivers and streams for the highly selective removal of invasive fishes, the resulting concentrations of ingredients, including rotenone, are of little concern regarding human health, or the welfare of other mammals and birds that may come in contact with the rotenone-treated water. Rotenone is fairly quickly detoxified by degradation pathways involving photolysis and hydrolysis, and has a short half life in the environment. Excess rotenone may be converted to products of lower toxicity by introduction of potassium permanganate. The connection between oral ingestion of rotenone and Parkinson's Disease is extremely tenuous, mainly because this route of ingestion effectively detoxifies rotenone by enzymatic, bacterial, and hydrolytic reactions in the gut that lead to rapid excretion of degradation byproducts. Other solvent components of rotenone formulations when diluted to the low concentrations intended for piscicidal applications, are substantially below the safe concentrations set for drinking water contaminants by the EPA.