

Chapter 6. Delivery Systems of Piscicides

by Michael A. Boogaard

Successful application of chemicals to control or eradicate invasive fishes depends on the system used to deliver the piscicide into the aquatic environment. Delivery systems can consist of combinations of various piscicide formulations and application techniques. This chapter highlights current formulations of piscicides and the techniques and equipment used to deliver them to the aquatic environment. Factors to consider when choosing a delivery system for a chemical control treatment include (1) the formulation and amount of the toxicant, (2) the objectives of the application, (3) the area and depth of waters to be treated, (4) the physicochemical characteristics of the waters to be treated, (5) treatment site accessibility, (6) the obstacles that prevent complete distribution and dispersion of the toxicant, (7) concerns of chemical toxicity to applicators and nontarget organisms, (8) the speed with which the application must be completed, (9) the time of year when the application must be made, and (10) the residual of the toxicant in water over time and distance (Lennon et al. 1970).

A piscicide is rarely applied in its pure form (i.e., as the active ingredient). Instead, it is mixed with inert ingredients to create a formulation that allows safe and effective application of the piscicide. Trade names, active and inert ingredients, and manufacturers of each formulation of piscicide registered by the EPA are given in Table 6-1. Piscicides are generally formulated as either liquids or solids. Liquids exist as emulsifiable and water soluble concentrates. Emulsifiable concentrates are formulated for active ingredients that are insoluble in water. The active ingredient is dissolved in an appropriate solvent and emulsifiers are added to allow the piscicide to be effectively and uniformly mixed with water. When applied to water, emulsifiable concentrates form a suspension or emulsion of the active ingredient in the water column. The emulsion allows the active ingredient to be delivered to the target organism. Water soluble concentrates are formulated with water or a water-soluble solvent that allows the active ingredient to dissolve in water. When applied, the result is a true solution of the piscicide in water.

Piscicides formulated as solids include bar formulations, wettable powders, and granules. Bar formulations contain the active ingredient incorporated into a matrix of one or more surfactants. The resulting bar, when applied to the treatment area, allows the piscicide to be released slowly and uniformly as the bar dissolves in water. Wettable powders consist of the active ingredient combined with a dry diluent, such as clay or talc. These formulations may also include wetting or dispersing agents that help keep the formulation in suspension when applied. Wettable powders are often mixed with a small amount of water to form a slurry before application. In granular formulations, the active ingredient is coated on inert particles, usually sand. When

Table 6-1. Chemicals, trade names, active and inert ingredients, and manufacturers of currently registered piscicide formulations.

Chemical	Formulation	Formulation active ingredient	Inert ingredients	Manufacturer(s)
TFM	Lampricid® TFM Sea Lamprey Larvicide	36-40% 3-trifluoromethyl-4-nitrophenol Other TFM-related ingredients: 1.5-4.0% 4-hydroxy-3-nitrobenzoic acid 3.0-8.0% 3-nitro-4-hydroxybenzoic acid 2.0-6.0% 5-trifluoromethyl-2-nitrophenol	35-43% water 11-13% isopropyl alcohol 6.4-7.8% sodium hydroxide	Clariant LSM (America), Inc. 3411 Silverside Road Wilmington, Delaware 19810 Kinetic Industries, Inc. 139-36 58th Avenue Flushing, New York 11355-5311
TFM	TFM Bar	22-24% 3-trifluoromethyl-4-nitrophenol	21.3% magnesium silicate <4.2% sodium lignosulfonate 0.9-1.0% amorphous silica <1.1% alkylated napthylene sulfonate, sodium salt <0.1% crystalline silica	Bell Laboratories, Inc. 3699 Kinsman Boulevard Madison, Wisconsin 53704
Niclosamide	Bayluscide® 70% Wettable Powder	69-74% niclosamide ethanolamine salt	21.3% magnesium silicate <4.2% sodium lignosulfonate 0.9-1.0% amorphous silica <1.1% alkylated napthylene sulfonate, sodium salt <0.1% crystalline silica	Pro-Serve 400 E. Brooks Road PO Box 161059 Memphis, Tennessee 38186-1059
Niclosamide	Bayluscide® 3.2% Granular Sea Lamprey Larvicide	3.0-3.6% niclosamide ethanolamine salt	68-72% amorphous silica 18-20% polyoxyethylene-polyoxypropylene block copolymer 4.0% ethyl cellulose 2.0% hydroxypropyl cellulose salt	The Coating Place, Inc. Box 930310 Verona, Wisconsin 53593
Niclosamide	Bayluscide® 20% Emulsifiable Concentrate	20% niclosamide ethanolamine salt	64-68% N-methyl-2-pyrrolidone 12-14% coconut oil diethanolamide 1.1-1.3% diethanolamide	Pro-Serve 400 E. Brooks Road PO Box 161059 Memphis, Tennessee 38186-1059
Rotenone	5% Rotenone-Liquid	5% rotenone	80% aromatic petroleum solvent 7.5% acetone 1.5% Emulsifier #1 4.5% Emulsifier #2	AgrEvo Environmental Health, Inc. 95 Chestnut Ridge Road Montvale, New Jersey 07645 Prentiss, Inc. C.B. 2000 Floral Park, New York 11001

Table 6-1. Continued

Chemical	Formulation	Formulation active ingredient	Inert ingredients	Manufacturer(s)
				Tifa Limited 50 Division Avenue Millington, New Jersey 07946
Rotenone	2.5% Synergized Rotenone-Liquid	2.5% rotenone 5.0% other cube resins 2.5% piperonyl butoxide	90% Xylene range aromatic solvent	AgrEvo Environmental Health, Inc. 95 Chestnut Ridge Road Montvale, New Jersey 07645 Prentiss, Inc. C.B. 2000 Floral Park, New York 11001 Tifa Limited 50 Division Avenue Millington, New Jersey 07946
Rotenone	5% Powdered Rotenone	7.4% rotenone 11.1% other cube resins	81.5% ingredients (not available)	AgrEvo Environmental Health, Inc. 95 Chestnut Ridge Road Montvale, New Jersey 07645 C.J. Martin Company PO Box 630009 Nacogdoches, Texas 75963 Drexel Chemical Company 1700 Channel Avenue Box 13327 Memphis, Tennessee 38113-0327 Foreign Domestic Chemicals Corp. 3 Post Road Oakland, New Jersey 07436 Prentiss, Inc. C.B. 2000 Floral Park, New York 11001 Sureco, Inc. 9555 James Avenue South Millington, New Jersey 07946

Table 6-1. Continued

Chemical	Formulation	Formulation active ingredient	Inert ingredients	Manufacturer(s)
				Tifa Limited 50 Division Avenue Millington, New Jersey 07946 Zeneca Agro 250-3115 12 th Street NE Calgary, Alberta T2E 7J2 Canada
Antimycin	Fintrol® Concentrate	23% Antimycin A	77% inert ingredients (not available)	Aquabiotics Corporation 10750 Arrow Point Drive Bainbridge Island, Washington 98110

applied, the piscicide slowly releases as the granule sinks through the water column. In addition, some granular formulations incorporate an outer coating of surfactant-like materials to allow the granule to sink to a certain depth or to the bottom before the piscicide is released.

As presented earlier, only four chemicals are currently registered by the EPA for use as piscicides: TFM, Bayluscide®, antimycin, and rotenone. Because the goal of the present chapter is to provide a thorough description of delivery systems used to apply piscicides, descriptions of delivery methods and formulations of all four registered piscicides will be presented. TFM and Bayluscide®, however, are registered only for use in the control of sea lamprey in tributaries to the Great Lakes with the exception of Bayluscide® that is also registered for use in snail control. Labels and Material Safety Data Sheets of all piscicides currently registered with the EPA are in Appendix F.

6.1 Lampricides

TFM Sea Lamprey Larvicide

The lampricide TFM is a primary management chemical tool used to control parasitic sea lampreys in the Great Lakes basin. A water soluble concentrate form is used in field operations and is formulated as a sodium salt of the active ingredient dissolved primarily in isopropanol and water. The formulation is about 36% active ingredient. The amount of TFM applied to water during a treatment depends on the flow rate of the water being treated and the target concentration. Target concentrations are predetermined through toxicity bioassays of larval sea lamprey and one or more nontarget organisms, sea lamprey minimum lethal concentration prediction models based on the chemical properties of the water to be treated, and a review of historical treatment records of the water body. Because the toxicity of TFM is influenced by the chemical and physical properties of water, accurate measurement of stream flow rates, pH, alkalinity, and lampricide application volumes are needed to assure treatment effectiveness while protecting nontarget biota.

The lampricide TFM is applied with either a 12-volt DC peristaltic pump for smaller applications (20-600 L) or a 120-volt AC peristaltic or centrifugal pump for larger applications (> 600 L; Klar and Schleen 2000). A spreader system is used to apply the lampricide evenly across the stream. First, a perforated hose or plastic tube is situated perpendicular to the stream. Then the metered lampricide is fed into a sump where it mixes with stream water (Figure 6-1). A pump is used to deliver the diluted lampricide through the perforated hose.

Lampricide concentrations are monitored spectrophotometrically from samples collected far enough from the application site to allow complete mixing. Metering adjustments are made to the TFM concentration as needed (Klar and Schleen 2000). Volumes less than 20 L are applied with adjustable gravity-fed drip systems and are used primarily on smaller creeks and tributaries that flow into the main treatment stream. Backpack sprayers can be used to apply the lampricide directly to backwater areas that are difficult to reach with the main treatment block (Klar and Schleen 2000).

TFM Bar

The TFM Bar is a water-soluble solid formulation containing about 22% active ingredient incorporated into a matrix of two or more non-ionic surfactants. Developed by Gilderhus (1985), the bars are used to treat small tributaries (generally <85 L/sec) that flow into the main

Figure 6-1. Lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) application apparatus showing treatment personnel loading Lampricid® into mixing tanks during the 1994 treatment of the Manistee River in northwestern lower Michigan.

treatment stream. When applied, the bar dissolves at a nearly constant rate over a period of 8 to 10 hours, depending on water temperature, and yields a TFM concentration of approximately 1 mg/L for every 7 L/sec of water discharge (Klar and Schleen 2000). With the development of the bar formulation, intensive stream monitoring of the lampricide concentration is no longer necessary and has thereby reduced the number of on-site personnel required to conduct a treatment. In addition, the bar formulation has allowed the successful treatment of smaller tributaries that once provided sea lampreys a refuge from the main treatment block.

Bayluscide® 70% Wettable Powder

Used in sea lamprey control operations, Bayluscide® 70% Wettable Powder (WP) is a powder formulation consisting of 70% Bayluscide® (59% active ingredient niclosamide) and 30% inert ingredients. Bayluscide® 70% WP is currently used in conjunction with TFM, primarily as a cost-saving measure to reduce the amount of TFM required to treat streams with high flows. When used in combination, the TFM:niclosamide ratio ranges from 98:2 to 99.5:0.5 (National Research Council of Canada 1985). An application of 1% niclosamide by weight of TFM reduces the amount of TFM required for efficacious treatment by up to 40%. To apply the formulation, stream water is drawn into a mixing tank where the powder is added (Figure 6-2). The resulting slurry is then metered into the stream at rates that achieve the desired percentage (0.5-2%) of the TFM concentration.

Niclosamide concentrations are monitored by high performance liquid chromatography from water samples collected at a site far enough downstream to allow complete mixing in the water. Adjustments in concentration are then made as needed (Klar and Schleen 2000).

Bayluscide® 3.2% Granular Sea Lamprey Larvicide

The granular formulation of Bayluscide® is used to control larval sea lamprey populations in lentic areas and also as a survey tool to assess larval abundance in deeper portions of streams not conducive to electrofishing. The formulation consists of Bayluscide® 70% WP coated onto sand granules with an outer coating of surfactant-like materials. The resulting formulation is

Figure 6-2. Bayluscide® 70% Wettable Powder mixing and application apparatus. The powder is mixed with fresh river water to form a slurry to facilitate application of the formulation to the river. Note the apparatus is self-contained to minimize applicator exposure to the lampricide formulation.

about 3.2% Bayluscide® by weight, and the formulation is applied over the surface of the target waters. The surfactant coating allows the granules to sink to the bottom of the water column before the active ingredient is released. The formulation has proven to be effective at killing larval sea lamprey at depths of up to 30 m. When applied according to label instructions, the result is a niclosamide concentration of about 9 mg/L in the bottom 5 cm of the water column and is effective at killing larvae within 30 min of application. A broadcast spreader mounted on the back of a boat is normally used to apply the granular formulation. Aerial applications have also been conducted, however, special permits must be obtained because this formulation is not registered for this method of application. See Chapter 11 for an example of aerial application of granular Bayluscide®.

Bayluscide® 20% Emulsifiable Concentrate

A new formulation of the lampricide Bayluscide® was recently developed for application in conjunction with TFM to control larval sea lampreys. The new liquid formulation, consisting of about 20% Bayluscide® (16% active ingredient niclosamide) dissolved in petroleum based solvents, emulsifiers, and other inert ingredients, was developed to improve the ability to apply the chemical uniformly and to eliminate the formation of dust encountered with the 70% WP during slurry formation. Because the new liquid formulation has only recently been registered

for use, application techniques have not been fully developed but will probably follow those used to apply TFM.

6.2 Antimycin

Fintrol® Concentrate

The only formulation of antimycin currently registered with the EPA is a concentrate of about 23% active ingredient. The formulation is registered as a general piscicide and is primarily used as a nonselective fish toxicant for partial or total reclamation of ponds, lakes, and streams although it has been shown to be selective for scaled fishes and is used in the aquaculture industry to rid undesirable fish species from catfish ponds. Fintrol® concentrate comes in a kit containing crystalline antimycin along with a diluent consisting mostly of acetone with other inert ingredients. Because antimycin degrades in acetone, application procedures require on-site mixing of the crystalline form and the diluent before addition to treated waters. Once the concentrate is formed, application techniques are similar to those of the lampricide TFM. Metering pumps, sprayers, and gravity-fed drip systems have all been successfully used to apply the concentrate to streams and shallow waters (Gilderhus et al. 1969, Lennon and Berger 1970, Engstrom-Heg 1971, Stefferud and Propst 1996). Fintrol® concentrate is applied to lakes and ponds with metering pumps or sprayers attached to motorized boats. The concentrate is applied to the propeller wash to aid in mixing. Deeper water can be treated using metering pumps connected to weighted perforated tubing that is lowered to the desired depth. Fintrol® concentrate has also been applied aerially. The formulation was originally applied using fixed-wing aircraft with spray booms at relatively fast air speeds. However, under these conditions the acetone carrier evaporated before the formulation reached the water causing the piscicide to precipitate and float resulting in an ineffective application. Since 1968, slower-moving helicopters have been successfully used to distribute Fintrol® concentrate to target areas (Selbig 1974).

Lethality of antimycin to fish varies from <1.0 µg/L for most salmonids to 25 to 200 µg/L for ictalurids; cyprinids and centrarchids are susceptible to concentrations of 5.0 to 10 µg/L (Berger et al. 1969). The relative resistance of ictalurids to antimycin makes it ideal for use in removing scaled fish from catfish ponds before restocking and in live-haul tanks to remove unwanted species, particularly green sunfish, from shipments of catfish fingerlings (Lloyd 1987). Gilderhus (1972) noted that at a concentration of 5 µg/L the effective exposure time to eliminate trout was 2 hours and 6 hours for common carp. Antimycin is typically applied at concentrations of ≤10 µg/L, although higher concentrations are needed in alkaline waters. Antimycin has been shown to be less toxic in waters of high pH (> 8.0; Berger et al. 1969, Schnick 1974), probably because of its rapid degradation in alkaline waters (Walker et al. 1964), and requires significantly longer contact time at lower water temperatures (< 5°C) to maintain effectiveness.

Although antimycin degrades rapidly in water (Gilderhus et al. 1969), detoxification of treated waters is sometimes necessary for partial stream reclamations or where the treated water could enter municipal water supplies. Detoxification of 5 µg/L of antimycin can be achieved with 300 µg/L potassium permanganate within 6 hours (Berger et al. 1969). Potassium permanganate, however, can be toxic to aquatic organisms (Marking and Bills 1975), and detoxification must be conducted with calibrated equipment to assure that metering rates are not excessive. Marking and Bills (1977) also found that 500 µg/L chorine detoxified 10 µg/L antimycin in 2 hours.

Fintrol® Granular Antimycin

In the late 1960s, two granular formulations (Fintrol®-5 and Fintrol®-15) of antimycin were developed for use in pond, lake, and reservoir reclamations. Developed as alternatives to the liquid formulation, these granular formulations were effective in several reclamation projects and particularly effective for removing undesirable fish species from catfish ponds. Although the formulations are no longer registered for use (annual registration renewal fees have not been paid since 1991), they do merit consideration. Fintrol®-5 consisted of a 1% by weight formulation of antimycin coated on sand. This formulation was designed to release the toxicant within the first 1.5 m of the water column and was particularly useful in shallow waters. Fintrol®-15 was a 5% formulation designed to deliver the toxicant within the first 4.5 m of the water column and was used to treat deeper waters. Both formulations were effectively delivered to target waters with broadcast spreaders mounted on boats or by aerial application. Helicopters were preferred over fixed-wing aircraft because of their maneuverability and adaptability to use varied equipment (Selbig 1974). The helicopter was equipped with a remote bucket consisting of a hopper, spreader, and a power source. Before being added to the hopper, one part granular Fintrol® was mixed with 10 parts of similar size sand because aerial application of the formulation alone could not be conducted at sufficiently low rates to achieve the desired antimycin concentration. The granular mix was routed from the hopper through an adjustable control box that delivered a specific quantity of the total mixture per unit time or surface area. From the control box, the mixture entered a powered spreader device that applied a uniform swath (Selbig 1974). In addition, granules could be applied by hand while the helicopter was hovering. This method used the wind turbulence from the helicopter rotors to uniformly disperse the granules and was particularly useful when treating small ponds or ditches or areas that were difficult to access by land (Selbig 1974).

In addition to Fintrol®-5 and -15, an experimental timed-release granular formulation of antimycin was developed by Gilderhus (1979) for controlling larval sea lampreys in lentic habitats. The 1% formulation proved effective in three of four lake trials, killing about 90% of larvae in 0.74- to 1.5-ha plots applied at 75 g/ha. In a similar study, a 0.25% granular formulation of antimycin was applied to the mouth of the Falls River, Baraga County, Michigan, resulting in a treatment effectiveness similar to that observed with the 1% formulation (Terry D. Bills, Great Lakes Fishery Commission, unpublished data). Although successful, registration of the timed-release formulations were not pursued because of the uncertain registration status of the parent compound.

6.3 Rotenone

Rotenone 5% and Rotenone 2.5% Synergized Liquid

Two liquid formulations of rotenone are currently registered by the EPA for use as general piscicides. Rotenone 5% consists of 5% active ingredient rotenone, emulsifiers, petroleum-based solvents, and other inert ingredients. Rotenone 2.5% Synergized is a synergistic formulation containing 2.5% active ingredient rotenone and 2.5% of the synergist piperonyl butoxide (PB), a derivative of piperic acid. Although Rotenone 2.5% Synergized contains only half of the active ingredient of its counterpart, its toxic effects are similar when applied at the same rates. Rotenone 2.5% Synergized was at least twice as toxic to rainbow trout as rotenone 5%, based on the amount of active ingredient (Marking and Bills 1976). Although the EPA does not recognize the effect of the synergist on the label instructions, the Canadian (Health Canada) label does and most applicators apply Rotenone 2.5% Synergized and Rotenone 5% at the same rates (Finlayson et al. 2000). It is unlikely, however, that PB will remain as a synergist in the 2.5% formulation for long. The registrants are no longer willing to support its aquatic use

because of the extensive data requirements for registration. A third liquid formulation of rotenone is currently being developed that does not contain the petroleum-based solvent that fish are suspected of avoiding.

Treatment concentrations range from 0.005 to 0.25 mg/L rotenone. The degradation rate of rotenone is affected primarily by temperature and sunlight (Gilderhus et al. 1986, Finlayson et al. 2000). The half-life of rotenone in water at 24°C was 13.9 hours compared to 83.9 hours in water at 0°C (Gilderhus et al. 1986). Alkalinity and pH also influence rotenone degradation. Waters with high alkalinity and pH degrade rotenone faster than waters of low alkalinity and pH (Finlayson et al. 2000). Additional rotenone is required in waters with high pH, alkalinity, sunlight penetration, and in waters organically rich with high volumes of suspended solids. Gilderhus (1982) noted that suspended clay particles reduced rotenone efficacy. Dawson et al. (1991) found that some rotenone was bound to suspended material in the water and that rotenone in the bottom sediments could take up to 14 days to decay below detection limits.

Several techniques have been developed to apply liquid rotenone to a variety of aquatic systems. Finlayson et al. (2000) described these techniques and recommended application rates based on the physicochemical characteristics of the water to be treated and the fish species targeted for removal. Treatment of smaller ponds was accomplished from shore or small boats with conventional commercial pesticide sprayers. Sprayers can be hand pumped, electric, or gas-powered equipped with 10- to 300-L tanks and can be mounted on backpacks, pick-ups, all terrain vehicles, or in small boats. Larger ponds, lakes, and reservoirs required gas-powered pumps using a venturi boat bailer system to deliver the liquid formulations to the water surface. Once applied, the liquid formulations readily disperse horizontally and vertically in shallow waters. Extended discharge hoses are weighted to prevent the hose from surfacing when treating deeper waters with a strong thermocline. Vertical mixing can be further facilitated by extending the water pump suction line near the bottom to draw cold, dense water to the surface where the rotenone is mixed (Finlayson et al. 2000). In addition, deep lakes were successfully treated in Michigan and Minnesota just before or during ice cover using lower concentrations of rotenone. Rotenone remains toxic longer in cold water providing longer exposure time. Concentrations of rotenone remained toxic for up to 2 months when applied to waters at these temperatures (Finlayson et al. 2000).

Aerial applications by fixed-wing aircraft or helicopters have been used for rotenone treatments when application by boat cannot be completed in a timely manner. In aerial applications, large droplets or streams of dilute rotenone are the preferred method of application over mist or small droplets. Mist or small droplet applications may result in drift that can reduce treatment efficacy and increase the risk of detrimental effects on nontarget organisms because of uncontrolled dispersion (Finlayson et al. 2000).

Application of liquid rotenone to rivers and streams is accomplished using techniques similar to those used to apply the lampricide TFM. Continuous drip systems (smaller streams) or metering pumps (larger streams and rivers) are used to deliver the chemical.

Depending on access, application sites are spaced at intervals sufficient to maintain the desired treatment concentration. For remote streams, liquid rotenone can be applied using a lightweight, constant-flow drip system that is easily portable. Originally developed by Stefferud and Propst (1996) for applying antimycin, this drip system can apply rotenone with better precision and consistency than drip systems used previously.

Powdered Rotenone 5-7.5%

Powdered rotenone is a formulation consisting of 5-7.5% active ingredient rotenone. The powdered formulation has been used extensively since the 1960s in reclamation and survey operations. Problems associated with powder applications, such as the inability to apply uniformly, human health considerations from accidental inhalation, and the subsequent development of the liquid formulations, significantly reduced the demand for powdered rotenone through the years. In 1990, the Utah Division of Wildlife Resources treated more than 4,900 ha of the Strawberry Reservoir with the powdered formulation (Lentsch et al. 2001). Spateholts and Lentsch (2001) developed a rotenone sand mix that comprised powdered rotenone, sand, and gelatin for use on smaller seeps and springs. The mixture successfully released rotenone for up to 12 hours after application and was applied in more than 450 situations where conventional treatments using drip systems were not normally possible.

A recent survey of rotenone use (1988-97) by McClay (2000) indicates that the preferred formulation of rotenone has shifted back to the powder formulation. This trend is probably because of reduced costs and improved application techniques of the formulation. In addition, increased environmental and public health concerns over the inert ingredients in the liquid formulations may contribute to the shift back to the powered formulation. Although the liquid formulations are proven safe and effective when applied according to label directions, some agencies find it difficult to plan and execute treatments using these formulations because they require environmental monitoring studies not normally needed for the powder formulation (McClay 2000).

6.4 Toxic Baits

Toxic baits are yet another system of delivering a known piscicide to a target organism. By formulating the piscicide into an edible bait, this method allows the compound to be delivered directly to the target organism thereby significantly reducing the amount of toxicant required compared to more conventional total water column piscicide applications and also avoids exposure of nontarget species. Although most, if not all, attempts to successfully formulate a toxic bait have ended in failure, some have resulted in marketable products.

Antimycin Impregnated Bait

Developed by Rach et al. (1994) as a control method for common carp, antimycin impregnated bait consists of a formulation of about 0.1% antimycin in fish meal, binder, and water. Trials of the formulation in 0.04-ha earthen ponds resulted in a 19% to 74% reduction in the abundance of common carp. The authors strongly caution, however, that this strategy should only be used in conjunction with other management techniques and under specific conditions, such as when common carp congregate to feed or when few other nontarget bottom-feeding species are present.

Rotenone Impregnated Bait

A similar study by Bonneau and Scarnecchia (2001) investigated the use of a rotenone impregnated bait for control of common carp. They conducted field trials where common carp were fed a nontoxic bait for 2 to 3 weeks followed by one feeding of rotenone impregnated bait. Common carp ceased feeding on the toxic bait within minutes and most did not eat enough bait to receive a fatal dose. Although the study was unsuccessful, the idea showed promise, especially if a more palatable pellet could be developed. Prentiss, Inc. (Floral Park, New York), has since developed and was marketing two formulations of a rotenone impregnated toxic bait

for controlling carp. The first, Prentox® Prenfish Grass Carp Management Bait (EPA Reg. No. 655-795), is a specially formulated bait containing 2.64% active ingredient and is specifically designed to control grass carp. Field trials show that the formulation is more palatable to the plant-eating grass carp than other fish species. Success is contingent upon training the grass carp to eat the pellets. Therefore, bait stations are placed in areas of known infestation, and nontoxic pellets (Prentox® Prenfish Grass Carp Management Trainer, typically 0.5-1 kg daily) are dispersed for up to 14 days. Feed retention rings are used to limit feed pellets from spreading and to concentrate fish at the bait station. Field trials have shown that grass carp will begin feeding within 1 to 14 days. Once routine feeding has been established, the toxic form of the pellet is distributed. Oral toxicity studies by Prentiss, Inc., have shown that a single pellet of Prentox® Prenfish Grass Carp Management Bait contains enough rotenone to kill a 1-2 kg grass carp.

The second rotenone bait formulation developed by Prentiss, Inc., targets common carp. Prentox® Prenfish Common Carp Management Bait contains the same amount of active ingredient (2.64% rotenone) as its counterpart but is formulated to be more palatable to common carp. Application procedures follow those for the grass carp bait with 14 days of training (Prentox® Prenfish Common Carp Management Trainer) followed by application of the toxic form. Prentiss, Inc., claims that in one experimental trial, 3,000 common carp were removed from Crooked Lake near Chicago, Illinois, using only 13.6 kg of the poison bait. Although both bait formulations have demonstrated the potential for use in carp control, stability problems have forced Prentiss, Inc., to pull their registrations. The company has no immediate plans to pursue development of new bait formulations because of low demand for the product.

Calcium Carbide Impregnated Bait

Huston (1955) described the selective poisoning of common carp with calcium carbide. Pellets of the compound were coated with beef tallow, paraffin, liquid plastic, or placed in gelatin capsules to make them waterproof and attractive to common carp. After the pellets are ingested, the coating material dissolves, and carbide reacts with liquid in the gut to form a large quantity of acetylene gas. Inflation of the gut leads to death of the fish. Results were inconsistent and not always selective. Because of the inconsistent results, efforts to register the formulation with EPA were never attempted.

Chapter 7. Identification of New Candidate Piscicides

by William H. Gingerich

Pesticides occupy a unique position in the array of chemicals in that they are used specifically to kill, disable, or injure pests of humans. In an ideal situation, the actions of such chemicals would be highly specific for a target species, however, most chemicals used as pesticides today are not highly selective in causing their effects and target and nontarget animals are generally affected (Murphy 1975). The selectivity of the pesticides then must be enhanced to maximize the effectiveness for their intended target animal by operational factors such as (1) selective and timed applications, (2) regulating the rate and proximity of pesticide release, and (3) extending or reducing the time of release. These operational procedures also apply to the use of piscicides to produce selective toxicity. Potential mechanisms of action for candidate piscicides will be identified from the several general classes of toxicants now available. Also, a focused evaluation of promising new candidate fishery piscicides will be presented on the basis of relative potencies of the candidate toxicants that have been identified by structure-activity testing of enzyme complex receptors and in some instances structure-toxicity testing of the chemical to aquatic species including fish.

7.1 Overview of Potential Piscicides from General Classes of Pesticides

Pesticides in general can be grouped into one of two broad categories based on their mechanism of action. First are those chemicals termed nerve poisons because they act by disrupting the nerve-facilitated integration of biological function. Pesticides in this category broadly include (1) central nervous system disrupting agents, (2) ganglionic blocking agents, and (3) neuromuscular blocking agents (Hayes and Laws 1991*a*,*b*,*c*). Susceptibility of the nervous system to chemical disruptors has been aggressively exploited by agrochemical companies in the production of a variety of agricultural pesticides, particularly insecticides. Included in this broad category of pesticides are synthetic organochlorine and organophosphate pesticides as well as derivatives of natural products, such as the permethrins. These pesticides have proven effective during the time that they have been used but have traditionally suffered from problems. Control failures, lack of sufficient selectivity, and resistance problems experienced with the use of nerve poisons have caused agrochemical companies to look elsewhere for potential pesticides (Wood et al. 1996). Moreover, most of the pesticides in this category are particularly toxic to the majority of aquatic organisms.

A second category of pesticides have the general mode of action of disrupting energy production by the mitochondria, thereby reducing the amount of cellular energy available within the animal to perform biochemical or physiological work. These pesticides include a large number of natural product and synthetic chemicals (Ray 1991, Nicolaou et al. 2000). Many of the natural product chemicals that have been discovered are derived from plants, molds, fungi, or yeast. The origin of these natural products are probably defensive chemicals used to fend off or discourage attack from natural predators.

Included in the category of cellular energy disruptors are specific inhibitors of the electron transport system (ETS) as well as inhibitors of adenosine triphosphate (ATP) synthesis and production. Inhibitors of ATP production are also known as oxidative phosphorylation (OP)

inhibitors or uncouplers. The mode of action of this broad class of pesticides is to block the biochemical pathways associated with ATP production. Because energy generation and storage is a basic requirement of all living organisms, selectively blocking the system has important implications in a number of areas including pesticide research and development. Candidate inhibitory chemicals of the energy generating pathways are currently being investigated for potential use as anti-tumor drugs, antibiotic agents, as well as pesticide uses including arachnicides, insecticides, parasiticides, fungicides, and piscicides (Schuler et al. 1999, Schuler and Casida 2001). This class of chemicals potentially provides a rich source of new candidate toxicants.

7.2 Energy Production Pathway Receptors as a Target for Inhibitory Ligands

The pathway of aerobic energy production in animals is a suitable target for development of pesticides because it is broadly similar among most obligate aerobic invertebrate and vertebrate animals. The generation and use of ATP are vital to support all life processes in prokaryotic and eukaryotic organisms. For most animals, energy in the form of ATP is derived from carbohydrate and lipid resources consumed by the organism, broken down to similar products of intermediary metabolism, and routed into common pathways for energy synthesis. The biochemical complex of enzymes required to synthesize ATP in eukaryotic cells is universally located within specific internal membranes of the mitochondria (Nelson and Cox 2000).

Energy production in eukaryotic cells occurs in the mitochondria and is carried out through the biochemical coupling of two integrated and complementary subsystems, an ETS and a system of OP. The ETS collects energy in the form of electrons from reduction products of intermediary metabolism and passes the captured energy from a state of higher to lower electromotive force by the controlled oxidation/reduction of a series of specific quinone or cytochrome substrates. In synchrony with the movement of electrons down this electrochemical gradient, protons are transported across the inner mitochondrial membrane thereby generating an electrochemical proton gradient across the inner mitochondrial membrane between the intermembrane space and the mitochondrial matrix space. It is the generation of the proton gradient between the inner mitochondrial membrane separating the matrix and intermembrane spaces that drives ATP synthesis (Boyer 1997).

The integrated ETS and OP systems are composed of five identified complexes located within and on either the matrix or intermembrane space side of the inner mitochondrial membrane (Nelson and Cox 2000). The mitochondrial energy production system showing known receptors and general sites of activity for known inhibitor ligands is depicted in Figure 7-1. Simultaneous oxidation/reduction events and proton translocations occur at three critical receptor sites in the electron transport chain, within complex I, complex III, and complex IV. Each receptor complex is unique and generally consists of a protein complex on the inner mitochondrial membrane termed an oxidoreductase and an associated water or lipid soluble coenzyme, generally ubiquinones and/or cytochromes (b,c_1,c, aa_3). The ATP formation in mitochondria is accomplished by ATP synthase (sometimes referred to as complex V) that is located on the matrix side of the inner mitochondrial membrane.

Because depletion of ATP in the animal cell is nearly always fatal, many inhibitors of energy production have been used successfully as general toxicants (Hollingworth and Gadelhak 1998). Five potential receptors exist within the integrated mitochondrial energy production system, each particular receptor being potentially sensitive to different inhibitory ligands. Critical receptor sites in the complexes have been identified and are generally associated with competitive binding by inhibitor ligands on sites of complexes I and III occupied by the cofactor ubiquinone (Xia et al. 1997, Darrouzet et al. 1998, Tormo et al. 2000, 2001), or on the heme A of

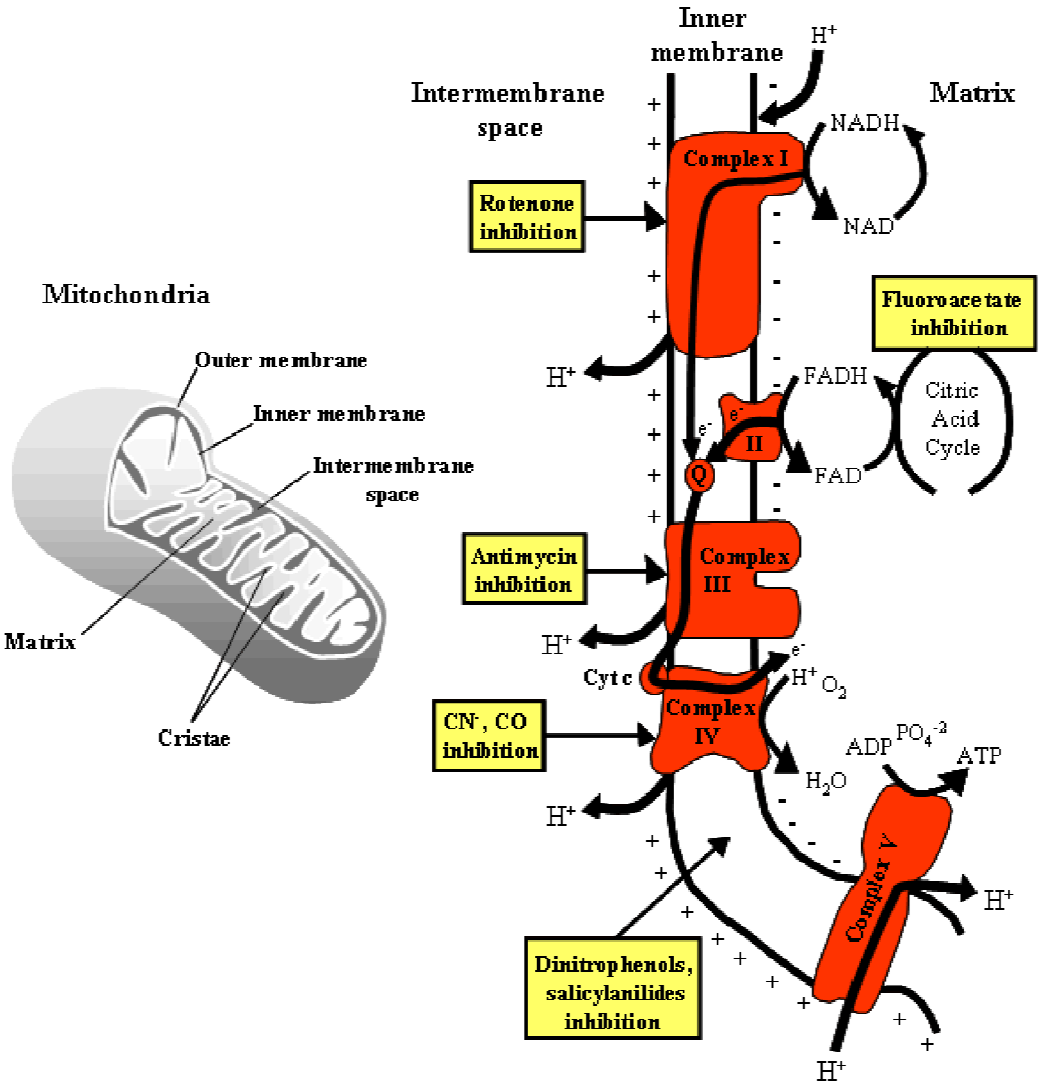


Figure 7-1. A representation of the inner membrane of a mitochondria and the relative positions of the inner membrane and matrix spaces. Electron transport complexes are embedded within the inner mitochondrial membrane and pass electrons from a state of higher to lower electromotive energies while capturing reductive energies from metabolic precursors. Hydrogen ions are simultaneously pumped from the matrix space to the intermembrane space by complexes I, III, and IV to create a proton gradient across the inner membrane. Adenosine triphosphate (ATP) synthase uses the proton gradient to synthesize ATP. Familiar electron transport system inhibitors and oxidative phosphorylation uncoupling agents used in fisheries block at various sites in the system. Rotenone blocks at complex I, antimycin A at complex III, and cyanide and carbon monoxide block at complex IV. Weakly acidic organic molecules, such as dinitrophenols and salicylanilides, act as protonophores to shuttle protons across the inner membrane and degrade the proton gradient required for ATP synthesis.

cytochrome c of complex IV (Tsukihara et al. 1996). Two broad classes of energy production inhibitors are ETS inhibitors and OP inhibitors/uncouplers. A listing of classes of energy production inhibitors and structures of representative chemicals in the class are given in Table 7-1.

Table 7-1. Identification of classes of naturally derived and synthetic electron transport and oxidative phosphorylation inhibitors as potential candidate fishery management chemicals.

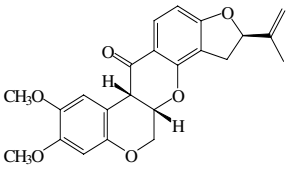
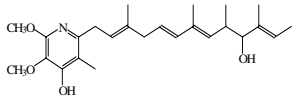
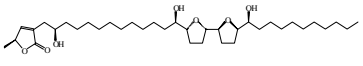
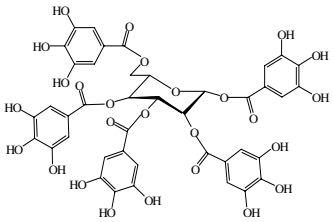
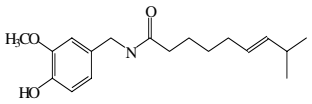
Classes of compounds (references)	Source	Representative compound(s)/ reference structure	Type of inhibition
Rotenoids (Fang et al. 1997; Fang and Casida 1997, 1999; Degli-Esposti 1998; Lummen 1998; Nicolaou et al. 2000; Schuler and Casida 2001)	Natural: plant species—*Derris* sp., *Lonchocarpus utilis*, and *L. urucu*	degulin, **rotenone**, and tephrosin 	Complex I - semiquinone antagonist
Piercidins (Tamura et al. 1963; Takahashi et al. 1968)	Natural: *Streptomyces* fermentations	**piercidin A** 	Complex I - quinone antagonist
Annonaceous acetogenins (Degli-Esposti et al. 1994; Gu et al. 1995; Landolt et al. 1995; Ye et al. 1996; He et al. 1997; Tormo et al. 1999, 2001)	Natural: plant family Annonaceae	**rolliniastatin-1**, cherimolin-1, itrabin, laherradurin, squamocin, otivarin 	Complex I - quinone antagonist
Tannins (Konishi and Tanaka 1999)	Natural: plant species *Sanguisorba officinale*	sanguiin H-11, **pentgalloylglucose**, oolonghomobisflavin A 	Complex I - NADH antagonist
Vanilloids (Shimomura et al. 1989; Yagi 1990; Satoh et al. 1996)	Natural: plants (e.g., capsicum)	**capcacin** 	Complex I - NADH antagonist

Table 7-1. Continued

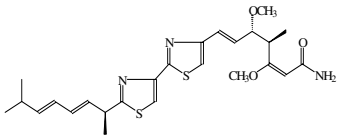
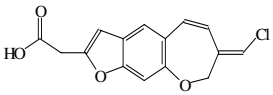
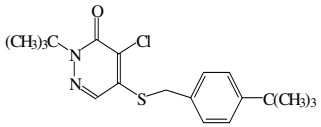
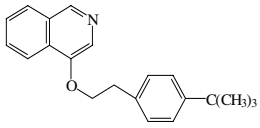
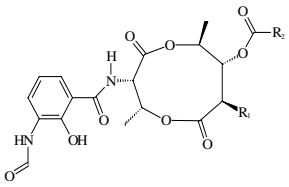
Classes of compounds (references)	Source	Representative compound(s)/ reference structure	Type of inhibition
Myxobacterial antibiotics (Degli-Esposti et al. 1993; Friedrich et al. 1994; Degli-Esposti 1998)	Natural: *Myxococcus*, *Stigmatella*	**myxothiazol**, aurachin A 	Complex I - quinol antagonist
Pterulinic acid (Engler et al. 1997*a*,*b*)	Fungal species *Pterula* sp. (basidiomycete)	**pterulinic acid**, pterulone 	Complex I - quinol antagonist
Pyridazinones (Degli-Esposti 1998)	Synthetic	**pyribaden** 	Complex I - quinone antagonist
Quinazolines (Hollingworth et al. 1994)	Synthetic	**fenazaquin** 	Complex I - quinone antagonist
Antimycin (Degli-Esposti 1998; Matsuno-Yagi and Hatefi 1999, 2001)	Yeast fermentations *Streptomyces* sp.	**antimycin A**, funiculosin, 2-nonyl-4-hydroxyquinoline-N-oxide 	Complex III - Q_i site inhibitor
Myxobacterial antibiotics (Degli-Esposti 1998; Matsuno-Yagi and Hatefi 1999, 2001)	*Myxococcus*, *Stigmatella*	**myxothiazol** (see above)	Complex III - Q_o site inhibitor

Table 7-1. Continued

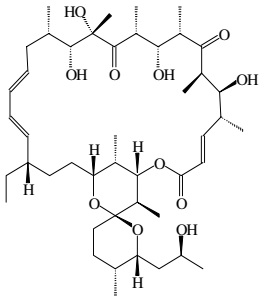
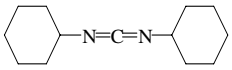
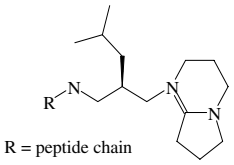
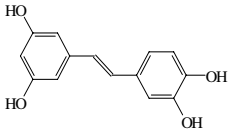
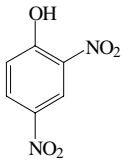
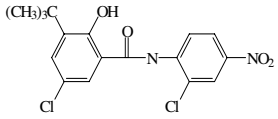
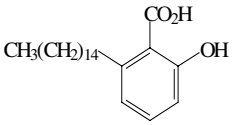
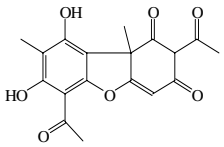
Classes of compounds (references)	Source	Representative compound(s)/ reference structure	Type of inhibition
Oligomycin (Matsuno-Yagi and Hatefi 1993; Vuorinen et al. 1995)		**oligomycin** 	F_0 transmembrane sector of F_0F_1-ATP
Dicyclohexylcarbo-diimide (Matsuno-Yagi and Hatefi 1993; Vuorinen et al. 1995)	Synthetic	**dicyclohexylcarbodiimide** 	F_0 transmembrane sector of F_0F_1-ATP synthase
Efrapeptins (Gupta et al. 1991; Krasnoff et al. 1991; Krasnoff and Gupta 1992; Abrahams et al. 1996; Bandani et al. 2000; Strasser et al. 2000)	Fungus of the genera *Tolypocladium*	 R = peptide chain	F_1 globular domain sector of F_0F_1-ATP synthase
Polyphenolic phytochemicals (Zheng and Ramirez 2000)	Plants	**piceatannol**, resveratrol, isoflavones, tannic acid 	F_1 globular domain sector of F_0F_1-ATP synthase
Nitrophenols (Toyomizu et al. 2000)	Synthetic	**2,4-dinitrophenol** 	oxidative phosphorylation uncoupler

Table 7-1. Continued

Classes of compounds (references)	Source	Representative compound(s)/ reference structure	Type of inhibition
Salicylanalides (Toyomizu et al. 2000)	Synthetic	**5-chloro-3-tert-butyl-2'-chloro-4'-nitrosalicylanilide** 	oxidative phosphorylation uncoupler
Anacardic acids (Kubo et al. 1986; Toyomizu et al. 2000)	Cashew nutshell liquid	**6-pentadecylsalicylic acid** 	oxidative phosphorylation uncoupler
Lichen acids (Abo-Khatwa et al. 1996)	Lichens (genera of *Usnea*, *Letharia*, *Parmelia*)	**usnic acid**, vulpinic acid 	oxidative phosphorylation uncoupler

A number of piscicides currently or formerly used by fishery managers are characterized as ETS inhibitors or OP uncouplers. The success of these agents in fishery management applications suggests both that ETS/OP inhibitors can be truly efficacious as piscicides and that additional and perhaps more effective fishery management agents can be found in this category of toxicants. Of the five receptors associated with the ETS inhibitors/OP system, the largest and most diverse group of inhibitor ligands have been identified for complex I, commonly referred to as Reduced Nicotinamide Adenine Diphosphate (NADH):ubiquinone oxidoreductase. Moreover, insects and fish seem particularly sensitive to complex I inhibitors (Fang et al. 1997, Fang and Casida 1999) suggesting the possibility that additional new candidate piscicides could be identified in this complex. For that reason, additional effort has been devoted to characterizing complex I and discussing the variety of its potential inhibitor ligands.

Complex I - NADH:ubiqinone oxidoreductase (EC 1.6.5.3)[1]

Complex I is the first energy transducing complex of the electron transport chain and also the first site of OP (Scheide et al. 2002). It is considered the largest, most complicated, most

[1]Unique enzyme complex number assigned by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology

studied, and probably the least understood of the four oxidation/reduction complexes in the ETS (Brandt 1997). Most characterization studies of complex I derived from eukaryotic species have been conducted with extracts of bovine heart mitochondria; characterization studies of complex I from fish mitochondria were not found in the literature. Because of its unique location at the beginning of the ETS and because of its function to transport electrons and translocate protons, complex I continues to be a preferred target receptor for those seeking to develop commercial insecticides, miticides, arachnicides (Degli-Esposti 1998, Lummen 1998), and conceivably piscicides.

Complex I appears to have the greatest numbers and diversity of natural and synthetic inhibitors of the five receptor systems in the energy producing ETS (Degli-Esposti 1998, Tormo et al. 2001). Complex I ligands include a variety of naturally derived and synthetic inhibitors (Degli-Esposti 1998). Among the natural inhibitors, four representative classes are recognized and include rotenoids (Fang et al. 1997, Fang and Casida 1997, 1999), annonaceous acetogenins (He et al. 1997), piericidins (Friedrich et al. 1994), and vanilloids (Shimomura et al. 1989). Additionally, several representative types of synthetic inhibitors have been developed: quinazolines and pyrimidines represented by the compound fenazaquin (Hollingworth et al. 1994, Hollingworth and Gadelhak 1998, Schuler et al. 1999), pyrazoles (fenpyroximate and tebufenpyrad; Hollingworth and Gadelhak 1998), and pyridazinones represented by the compound pyridaben (Hollingworth et al. 1994, Hollingworth and Gadelhak 1998, Lummen 1998, Schuler et al. 1999). Interest in developing inhibitors of complex I is derived from the potential for these inhibitors to serve as insecticides (Wood et al. 1996, Fang et al. 1997, He et al. 1997, Lummen 1998, Fang and Casida 1999, Jewess and Devonshire 1999, Schuler and Casida 2001), aracacides/miticides (Wood et al. 1996, Lummen 1998), and piscicides (Fang et al. 1997, Fang and Casida 1999). On the basis of a variety of physical observations of the receptor and on the diversity of structures of known inhibitors, Darrouzet et al. (1998) proposed a general structure for the complex I receptor (Figure 7-2). Armed with this information, it has been possible to devise computing algorithms to predict small chemical structures from combinatorial chemical libraries that optimize inhibition of complex I (Nicolaou et al. 2000).

Complex II - Succinate:ubiquinone oxidoreductase (EC1.3.5.1)

Complex II represents the second step in the energy transducing complex of the electron transport chain. It differs from the other three complexes in the electron transport chain in that it transfers an electron through the system without simultaneously generating and translocating a proton for ATP synthesis (Scheide et al. 2002). In addition, it is linked directly to the citric acid cycle of intermediary metabolism where the electron derived from the oxidation of succinate to fumarate is captured by Flavin Adenine Diphosphate (FAD) to form Reduced Flavin Adenine Diphosphate (FADH2).

Thenoyltrifluoroacetone and carboxanilides are potent inhibitors of mammalian complex II. However, these chemicals only weakly inhibit the prokaryotic form of the enzyme (Maklashina and Cecchini 1999). Certain 2-alkyl-4,6-dinitrophenols and pentachlorophenols are potent inhibitors of eukaryotic and prokaryotic enzyme systems (Tan et al. 1993, Yankovskaya et al. 1996). A survey of the recent literature suggests that broad phylogenetic similarities in complex II make the likelihood of phylogenetic differences small and that little work has been done to recognize additional inhibitors of this complex. It is unlikely that selective piscicides could be efficiently developed against this receptor.

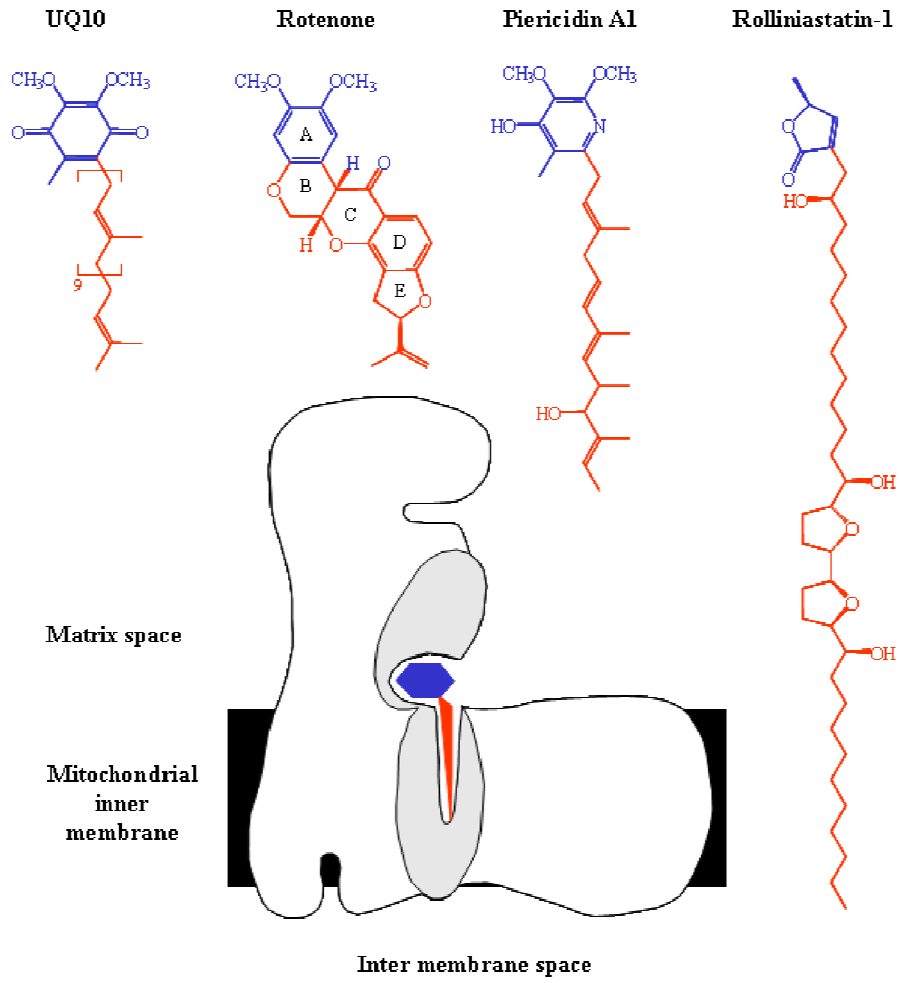


Figure 7-2. A representation of the complex I receptor for the electron transport cofactor ubiquinone (UQ10) and representatives of several classes of complex I inhibitor ligands. The membranous domain associated with quinone binding appears to be composed of two distinct subregions, an open and relatively hydrophilic region on the matrix side of the mitochondrial inner membrane surface of the complex (*blue*) and a more hydrophobic narrow cleft subregion that penetrates the membranous portion of the complex (*red*; after Darrouzet et al. 1998).

Complex III - Ubiquinol:ferrocytochrome c oxidoreductase (EC1.10.2.2)

Complex III or bc_1 complex represents the third complex in the energy transducing system of the electron transport chain in eukaryotic and prokaryotic cells. The complex catalyzes the transfer of electrons from ubiquinol to cytochrome c, an event that is coupled with the translocation of a proton from the mitochondrial matrix space to the intermembrane space.

Specific and potent inhibitors of the enzyme are known and include antimycin A (Bechmann et al. 1992, Matsuno-Yagi and Hatefi 1996, 1999, 2001), mucidin (Tokito and Daldal 1993), myxothiazol (Rauchova et al. 1992, Matsuno-Yagi and Hatefi 2001, Ouchane et al. 2002), and stigmatellin (Bechmann et al. 1992, Tokito and Daldal 1993, Matsuno-Yagi and Hatefi 1996, 1999, 2001). Only antimycin A has been developed for use as a piscicide (Morrison 1987, Finlayson et al. 2002). As with complex I, the function of complex III to simultaneously pass

electrons down the electron transport chain and translocate protons across the inner mitochondrial membrane make this complex a possible target for development of piscicides. Our review of the pertinent literature suggests that the number of candidate inhibitor ligands for this receptor complex is limited.

Complex IV - ferrocytochrome c:oxygen oxidoreductase (EC1.9.3.1)

Complex IV or cytochrome c oxidase represents the fourth and final complex in the electron translocating chain. The enzyme catalyzes the irreversible final step in the electron transfer chain, the transfer of reducing electrons to oxygen to form water.

Specific inhibitors of the enzyme system are known and include some familiar poisons, such as carbon monoxide (Miro et al. 1998), cyanide (Wilson et al. 1994, Ikegaya et al. 2001), hydrogen sulfide (Nicholson et al. 1998), and nitric oxide (Cleeter et al. 1994, Brown 2001, Shiva et al. 2001). These classic poisons inhibit cytochrome c oxidase mainly by interference with oxygen transfer to terminal cytochrome c. Less well known inhibitors of this enzyme have been identified and include dicarbanaborates (Drahota et al. 1996), valinomycin (Nicholls and He 1993), and N-retinyl-N-retinylidene ethanoloamine (Shaban et al. 2001). A cursory literature review of this enzyme did not reveal studies characterizing the enzyme system in eukaryotic and prokaryotic cells or studies emphasizing other phylogenetic comparisons. Because of the nature of the system, it is not likely that it represents a suitable target for development of either general or specific fishery management chemicals. Many of the identified inhibitor ligands for the complex, such as carbon monoxide, hydrogen sulfide, and cyanide could present major health problems for applicators.

Complex V - F_0F_1-ATP synthase (EC3.6.6.34)/Oxidative Phosphorylation Uncoupling Agents

Complex V catalyzes the production of ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i) in mitochondria and chloroplasts from eukaryotic cells, as well as in bacteria. The ATP production is tightly coupled to the mitochondrial proton electrochemical gradient developed across the membrane separating the mitochondrial intermembrane space and matrix (Walker 1994). The enzyme can also operate in the reverse direction, hydrolyzing ATP, and pumping protons in a retrograde manner against the normal proton gradient in the absence of a strong proton gradient (Walker 1994, Zheng and Ramirez 2000).

Two general classes of inhibitors block ATP production by F_0F_1-ATP synthase, those that directly inhibit F_0F_1-ATP synthase and those that act by degrading the transmembrane proton gradient and uncoupling ATP production from electron transport. Inhibitors of F_0F_1-ATP synthase at the F_0 moiety have been identified. Oligomycin, N,N'-dicyclohexylcarbodiimide, venturicidin, and tetracoordinate organotin compounds (R_3SnX) are potent inhibitors of the ATP synthase enzyme; all at the F_0 transmembrane sector (Matsuno-Yagi and Hatefi 1993). Efrapeptins, small polypeptides produced from fungus of the genus *Tolypocladium*, have been identified as potent inhibitors of the F_1 globular domain of complex V (Abrahams et al. 1996) and have been studied as candidate insecticides and fungicides (Krasnoff and Gupta 1992, Bandani et al. 2000, Strasser et al. 2000). A number of polyphenolic phytochemicals also have been purported to inhibit ATP synthase at high nanomolar to low micromolar concentrations; some inhibit the enzyme by binding to the F_1 subunit while others bind to the F_0 subunit (Zheng and Ramirez 2000).

Uncoupling agents function by increasing proton conductance across the inner mitochondrial membrane, thereby degrading the proton gradient and in the process reducing ATP formation

while allowing electron transport to continue in the mitochondria. That is, they uncouple the energy yielding reactions (i.e., electron transport) from energy conserving reactions (i.e., ATP formation). Lipid soluble weak acids, such as 2,4 dinitrophenol, carbonylcyanide, 5-chloro-3-tert-butyl-2'-chloro-4'-nitosalicylanilide, and 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, are recognized as uncoupling agents (Toyomizu et al. 2000). Similarly, lipid soluble weak bases also have been identified as uncoupling agents (Nagamune et al. 1993, Abo-Khatwa et al. 1996). It is assumed that the sea lamprey larvicides TFM and Bayluscide®, both lipid soluble, weakly acidic organic molecules, act in part by uncoupling OP (Lehninger 1975).

7.3 Identification of Energy Production Inhibitors as Candidate Piscicides

The properties of a chemical that confer selective toxicity are central to the issue of the development of a taxon-specific piscicide. The identification and development of chemicals are made challenging by the general requirements of the chemical to produce rapid toxicity to the target species while having little effect on nontarget species that may be residing in the same body of water. For a chemical to be toxic, it must rapidly produce effects such that the ability of the organism to persist is rapidly degraded. The rapid development of toxicity is generally desirable to reduce the chance that the animal could escape the toxicant field during treatment. Conversely, the toxicant may be effective at such low concentrations that it is undetectable by the animal. Because many of the physiological and particularly biochemical processes that are candidates for disruption by toxicants are highly conserved phylogenetically, many higher organisms share similar susceptible target sites for candidate toxicants thereby reducing the potential for selectivity.

Energy production inhibitors have been used extensively as general toxicants in fishery management applications. Examples of these general toxicants include rotenone (complex I inhibitor), fluoroacetate (citric acid cycle inhibitor linked to complex II), antimycin (complex III inhibitor), carbon monoxide and cyanide (complex IV inhibitors), and the lipophilic weakly acidic organic molecules TFM, niclosamide, and salicylanilide (purported OP uncouplers). It is significant that the only chemicals that are currently registered by the EPA for fishery management purposes belong to this class of diverse toxicants. These include antimycin, rotenone, and the sea lamprey larvicides—TFM and Bayluscide®. The sustained and successful use of these agents is due in large part to their general efficacy to fish, their relative safety to human applicators (Finlayson et al. 2002), and their safety to the environment because of their ability to degrade rapidly (Dawson et al. 1991, Dawson 2003, Hubert 2003). Much of the success enjoyed by energy production inhibitors in fishery management uses can be attributed to physicochemical properties that allow for their rapid uptake by fish across the relatively permeable water-blood barrier of the gills and subsequent rapid and ubiquitous distribution (Gingerich and Rach 1985, Rach and Gingerich 1986) and subsequent loss from the body (Dawson et al. 2002, Vue et al. 2002).

It is clear from the review of current literature that there are a number of new mitochondrial complex I inhibitor ligands that currently could be considered as potential general insecticide candidates (Nicolaou et al. 2000). Such chemicals generally have potency to target receptors that are equal to or greater than rotenone. Insect and fish mitochondria appear to be particularly sensitive to complex I inhibition (Jewess 1994, Degli-Esposti 1998). For this reason, complex I may also be a preferred receptor to target for the development of new piscicides. A number of these identified compounds appear to have complex I inhibition potency sufficient to allow them to be considered further as candidate general fish toxicants. A listing of potential complex I inhibitors and their relative *in vivo* and *in vitro* potencies is presented in Table 7-2.

Table 7-2. Identification of classes of complex I energy production inhibitors, *in vivo* or *in vitro* assay systems, and relative potency for potential candidate fishery management chemicals. LC = lethal concentration; IC = inhibitory concentration; ND = not determined; NA = not applicable.

		***In vivo* assays**			***In vitro* assays**			
Chemical class	**Chemical**	**Assay system[a]**	**Activity LC_{50} (µg/L)**	**Relative potency[b]**	**Assay system[c]**	**Activity IC_{50} (nM/mg)**	**Relative potency[d]**	**References**
Rotenoids	rotenone	GF	50	1	NADH-Q	4.4	1	Fang et al. 1997
	degulin	GF	30	0.6	NADH-Q	6.9	1.57	Fang et al. 1997
Oxadehydrdorotenoids	oxadehydrotenone	GF	≥1,000	≥200	NADH-Q	115	26.1	Fang et al. 1997
	oxadehydrodegulin	GF	≥3,000	≥600	NADH-Q	138	31.4	Fang et al. 1997
Dehydrorotenoids	dyhydrorotenone	GF	≥3,000	≥600	NADH-Q	8,630	1,960	Fang et al. 1997
	dehydrodegulin	GF	≥3,000	≥600	NADH-Q	1,590	361.4	Fang et al. 1997
Annonaceous acetogenins	bullatacin	BS	1.6	0.0327	ND	NA	NA	He et al. 1997
	trilobin	BS	9.7	0.198	ND	NA	NA	He et al. 1997
	trilobacin	BS	8.7	0.178	ND	NA	NA	He et al. 1997
	asiminacin	BS	5.7	0.116	ND	NA	NA	He et al. 1997
	asimicin	BS	26	0.531	ND	NA	NA	He et al. 1997
	motrilin	BS	10	0.204	ND	NA	NA	He et al. 1997
	bullatalicin	BS	150	3.06	ND	NA	NA	He et al. 1997
	rotenone (control)	BS	49	1	ND	NA	NA	He et al. 1997
	rolliniastatin-1	ND	NA	NS	NADH-Q	0.03	0.077	Degli-Esposti et al. 1994
	rolliniastatin-1	ND	NA	NA	NADH-Q	0.75	0.026	Tormo et al. 1999
	rolliniastatin-2	ND	NA	NA	NADH-Q	0.06	0.149	Degli-Esposti et al. 1994
	rolliniastatin-2	ND	NA	NA	NADH-Q	0.61	0.021	Tormo et al. 1999
	otivarian	ND	NA	NA	NADH-Q	0.9	≥1	Degli-Esposti et al. 1994
	corossolin	ND	NA	NA	NADH-Q	6.2	0.215	Tormo et al. 1999

Table 7.2. Continued

Chemical class	Chemical	*In vivo* assays			*In vitro* assays			References
		Assay system[a]	Activity LC_{50} (µg/L)	Relative potency[b]	Assay system[c]	Activity IC_{50} (nM/mg)	Relative potency[d]	
	corossolone	ND	NA	NA	NADH-Q	10.5	0.365	Tormo et al. 1999
	murisolin	ND	NA	NA	NADH-Q	5.3	0.184	Tormo et al. 1999
	annonacinone	ND	NA	NA	NADH-Q	3.7	0.128	Tormo et al. 1999
	rotenone reference	ND	NA	NA	NADH-Q	28.8	1	Tormo et al. 1999
	tripoxyrollin	ND	NA	NA	NADH-Q	19.3	0.67	Tormo et al. 2000
	membrarollin	ND	NA	NA	NADH-Q	0.83	0.029	Tormo et al. 2000
	annonin IV	ND	NA	NA	NADH-Q	0.06	0.857	Friedrich et al. 1994
Piercidin	piercidin-A	ND	NA	NA	NADH-Q	0.036	0.414	Degli-Esposti et al. 1994
	piercidin-A	ND	NA	NA	NADH-Q	0.02	0.286	Friedrich et al. 1994
Vanilloids	capsaicin	NAD	NA	NA	NADH-Q	15	3.67	Wood et al. 1996
Pyridazinones	pyridaben	ND	NA	NA	NADH-Q	77	0.714	Wood et al. 1996
Quinazolines	fenaziquin	ND	NA	NA	NADH-Q	67	0.821	Wood et al. 1996

[a]GF = goldfish; BS = brine shrimp
[b]Potency referenced to rotenone positive control (LC_{50} test chemical/LC_{50} rotenone); potency ratios less than 1 indicate chemicals more toxic than rotenone
[c]Assay system is mitochondrial membrane NADH-ubiquinone reductase extracted from bovine heart
[d]Potency referenced to rotenone positive control (IC_{50} test chemical/IC_{50} rotenone); potency ratios less than 1 indicate chemicals more toxic than rotenone

How could newly identified chemicals be exploited for use as piscicides? These chemicals would need to be obtained and tested against the various target and nontarget fish species. Considerations for candidates should be given to those chemicals with physicochemical properties similar to antimycin and rotenone. That is, they are sparingly soluble in water but readily taken up across the fish gills and rapidly distributed throughout the body. Once the efficacy of one or more of the chemicals is confirmed, the candidate chemical(s) would need to be more fully evaluated as a potential management tool by assessing other characteristics of the chemical including potential mammalian safety, human food safety, and environmental safety concerns. A number of promising candidate chemicals seem to be more toxic than rotenone (Table 7-2). These include certain of the acetogenins as well as the synthetic pyridazinone pyridaben (Nexter®, Sanmite®), and the quinazoline fenazaquin (Matador®). The latter synthetic compounds have been developed as commercial agricultural insecticides and miticides, but there are no current registrations for their use in fishery management. Generally, the development of these commercial products for fishery management purposes would need to be done in close cooperation with a chemical sponsor. Likewise, the development of natural products would need to be undertaken with a sponsor who would supply the chemical.

A second and less expensive initial option in looking for selective toxicants would be to evaluate whether existing, registered ETS/OP piscicides could be used in combination to enhance selectivity to certain problem species. The rationale for this is that the ETS/OP complex receptors for different species may be differentially sensitive to several of the inhibitory ligands. For example, the treatment of certain species with a combination of both a complex I inhibitor ligand and a complex III inhibitor ligand or a complex I inhibitor ligand and an OP uncoupler may be more selective for a certain species than using just a single type of complex inhibitor.

Our laboratory and field experience as well as the literature reviewed to support this report suggest that there is no precedence for the use of combinations of different ETS or ETS/OP inhibitor ligands. It is known that the toxicity of rotenone can be enhanced when it is applied with the mixed function oxygenase inhibitors piperonyl butoxide or sulfoxide (Marking 1977). However, metabolic inhibitors prevent the metabolic degradation of rotenone rather than to additionally selectively block a different receptor site within the ETS. Moreover, use of such metabolic inhibitors would probably reduce rather than enhance the selectivity of toxicants whose potency is reduced by metabolism to less toxic degradation products. The use of multiple OP inhibitor ligands has been used commonly in sea lamprey control applications to enhance the efficacy of the treatment. Bayluscide® is commonly applied in a proportion of 98%:2% (TFM:Bayluscide®) to enhance the toxicity of TFM during sea lamprey control treatments with the effect that it allows less TFM to be used during certain treatments (Howell et al. 1964, Dawson 2003). However, the use of two OP inhibitor ligands has not been successful in enhancing the selectivity of TFM treatments for sea lamprey.

At the present time, it is not clear whether there would be advantages from combining various proportions of different ETS/OP inhibitors to enhance selectivity in fishery management treatments. This is because basic data do not exist for this type of testing. It is known that some interspecific and intraspecific differences exist among some of the electron transport receptor systems for fish species (Chew and Ip 1993, Freund and Kadenbach 1994, Arnold et al. 1997). These differences also may be modified by physicochemical changes in the environment, particularly seasonal temperature changes (Hardewig et al. 1999*a*,*b*, Kikuchi et al. 1999). Ultimately, the sensitivity of the species to the toxicants will be as sensitive as their receptors are to the inhibitory ligands.

Application of multiple ETS/OP inhibitors is an untested and novel approach that may reveal differential sensitivities to target or nontarget species that are not evident when just one specific inhibitor is applied. Moreover, if mixtures of different ETS/OP inhibitor ligands are found to require less of each inhibitor ligand in combination than the concentration of individual ones

used separately, there are immediate advantages from a monetary and practical perspective. First, if less total piscicide is required to effect a successful treatment, substantial savings in costs per treatment could be realized. Second, if less chemical is used for each treatment, detection of each chemical in the mixture by the target species would be more difficult with the result that they would not attempt to avoid the toxicant field.

How would data be developed to confirm or refute such a hypothesis? By applying different proportions of one inhibitor ligand in the presence of a fixed concentration of a second ligand to critical species of interest, it should be clear in a relatively short time whether there is merit to this approach. Conceptually, the 24-hour lethal concentrations to 50% of the test individuals (LC_{50}) would be determined for each inhibitor ligand to appropriate target species as well as to nontarget species of interest. Once established, a fixed concentration of one inhibitor ligand, representing perhaps 50% of the LC_{50}, will be chosen to be mixed with differing proportions of a second inhibitor ligand under conditions of continuous exposure. The proportions of the second inhibitor ligand might range from 10% to 90% of the LC_{50} in 10% increments. Successful combinations of inhibitor ligands would be based on the demonstration of increased selectivity for target over nontarget species.

From the preceding discussion, one can deduce that it is not likely that selective toxicants exist that could be applied immediately to management strategies for the control of invasive species in the southwestern United States. This conclusion stems from a review of the current literature that reveals the lack of available data relating apparent susceptibility of potential target species to specific poisons. At a minimum, selectivity is likely to be based on at least two inter-specific differences, one in the differences of the biochemistry of different species related to their strategies for self-sustenance and a second related to differences in how successful different species are at tolerating potential poisons. Too little information currently exists on the biochemical and pharmacokinetic factors that would act or interact to produce selectively toxic treatments to the problem species of interest.

Science-based evaluations of newer ETS/OP inhibitory ligands suggest that newly discovered chemicals, particularly the annonaceous acetogenins may prove useful as candidate management chemicals for fish. A series of potential candidate electron transport inhibitor ligands have been identified that could serve as the basis for additional evaluation.

As an alternative to full development of a new piscicide, it may be possible to develop specific combinations of currently registered piscicides that would allow for some selective toxicity between target and nontarget fishes of concern. Again, the lack of data precludes identification of any specific candidate combination; however, the advantage to this approach is that all chemicals currently registered for use as piscicides with the EPA could potentially be used in combinations without the development of major sets of additional regulatory data.