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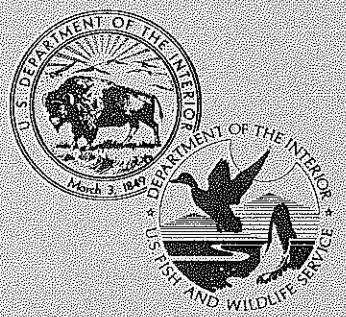

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Malachite Green: Its Toxicity to Aquatic Organisms, Persistence and Removal with Activated Carbon

by

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Abstract

The acute toxicity of malachite green was determined in standardized laboratory tests for chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout *(S. gairdneri)*, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass *(Micropterus salmoides)*, smallmouth bass *(M. dolomieui)*, bluegill *(Lepomis macrochirus)*, snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.). Bluegills were the most sensitive (96-h LC_{50},0.0305 mg/l), and coho salmon the most resistant (0.383 mg/l). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for rainbow trout was 0.0998 mg/l. The responses of frog and toad larvae (96-h LC_{50}, 0.173 and 0.0680 mg/l) were similar to those of fish, whereas adult newts were more resistant (1.03 mg/l). The invertebrates exposed were generally more resistant than the fish and amphibians; the 96-h LC_{50}'s ranged from 0.510 to 3.45 mg/l, except for the Asiatic clam, which was extremely resistant (122 mg/l), and the mayfly naiad, which was very sensitive (0.0790 mg/l). The toxicity of malachite green to fish was not affected by water hardness or pH, except bluegills, in which toxicity was about half as great at pH 6.5 as at pH 7.5 to 9.5, and was increased only slightly by increases in water temperatures. Malachite green was very persistent in aqueous solutions; it did not detoxify after 3 weeks of aging in glass containers. The chemical is readily absorbed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C) by filtration through activated carbon; the capacity was 23.4 mg of malachite green per gram of carbon.

Malachite green has been used in fish culture as a fungicide and parasiticide for about 40 years. It was first used as a dip treatment by Foster and Woodbury (1936) to treat fungal infections of four species of trout and largemouth bass *(Micropterus salmoides)*. More recently it has been used in combination with formalin to treat *Ichthyophthirius*, a serious parasite of catfishes (Leteux and Meyer 1972).

Although the use of malachite green as a therapeutant in fish culture has many advantages, it also poses various potential problems (Nelson 1974): toxicity to fishes (Willford 1967); possible teratogenic and mutagenic effects (Lieder 1961; Nelson 1974; T.D. Bills and L.L. Marking, in preparation); and stress induced during and after the treatment of fry of certain fishes (Glagoleva and Malikova 1968; Bills and Hunn 1976).

Malachite green is not registered for aquatic use by either the Food and Drug Administration or Environmental Protection Agency, because information required for registration—toxicity, efficacy, residues, metabolites, and counteraction—is incomplete. The purpose of the present study was to contribute laboratory data on (1) the toxicity of malachite green to nontarget aquatic organisms; (2) its toxicity to rainbow trout *(Salmo gairdneri)* and bluegills *(Lepomis macrochirus)* in extended exposures; (3) the effects of certain water characteristics on its toxicity to fish; (4) its persistence in water; and (5) its possible removal from water with activated carbon.

Materials and Methods

Concentrated stock solutions of commercial grade zinc-free malachite green (4-[P-(dimethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-l-ylidene dimethyl-ammonium chloride) manufactured by MCB Manufacturing Chemists, Norwood, Ohio, were prepared by mixing weighed portions with water. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into test vessels and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, the required amounts of the stock solution were delivered by a solenoid-activated pipette pump (Micromedic Systems Automatic Pipette Model 2500).

Tests were conducted according to the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975). Glass jars of 3.78 or 18.9 liters were used, depending on the size of the test organism. Reconstituted water was used in tests with fish (Marking 1969), and limed spring water (pH, 7.5 ± 0.1; total hardness, 20 mg/l as $CaCO_3$) in the tests with amphibians and invertebrates. Chemical buffers were added to soft water to adjust the pH (6.5–9.5), as described by Marking and Dawson (1973).

Flow-through tests were conducted in a proportional diluter similar to that of Mount and Brungs (1967). Test vessels were 45-liter glass aquariums supplied with a flow sufficient to replace the entire volume at least four times daily. Carbon-filtered, municipal well water (total hardness 300 mg/l, pH 7.5) was used in the flow-through system. Temperature was maintained by immersing test vessels in a water bath equipped with a chilling unit.

Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass, smallmouth bass *(Micropterus dolomieui)*, and bluegills. Test fishes weighed 0.5 to 1.5 g. Other aquatic organisms exposed were snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.).

In tests for the determination of persistence of malachite green, aqueous solutions were aged for 1, 2, and 3 weeks in glass containers. Rainbow trout were introduced concurrently to these and a freshly prepared reference solution for comparison of mortality. Deactivation indices were computed from these data according to the method of Marking (1972).

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method published by Green (1965) for computing $TILC_{50}$'s (lethal concentration producing 50% mortality independent of time).

To determine if malachite green could be removed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C), we filtered a concentrated solution (2 mg/l) at a flow rate of 100 ml/min through a glass 2.7 cm ID column containing 15 cm (35.5 g dry weight) of activated carbon (Darco 20 × 40 mesh). Samples were taken periodically and concentrations in the effluent determined colorimetrically (620 nm). The carbon bed was considered saturated when the concentration in the effluent reached 10% of that in the original stock solution (0.2 mg/l). The capacity of activated carbon for the chemical was determined by the following formula:

$$\text{Milligrams of malachite green adsorbed per gram of carbon} = \frac{\text{Concentration (mg/l)} \times \text{liters passed through filter}}{\text{Grams of carbon (dry weight)}}$$

Results

Toxicity to Fish

Malachite green was toxic to all species of fish exposed; LC_{50}'s ranged from 0.0305 to 0.383 mg/l in 96-h exposures in soft water at 12 C (Table 1). Centrarchids were 1.5 to 3.5 times more sensitive to the chemical than the ictalurids and 3 to 7 times more sensitive than the salmonids. The bluegill was the most sensitive species (96-h LC_{50}, 0.0305 mg/l) and the coho salmon the most resistant (0.383 mg/l). The toxicity of the chemical increased as exposures lengthened in all species; for bluegills the LC_{50} was 6.00 mg/l at 3 h and 0.0305 mg/l at 96 h.

Toxicity to Other Aquatic Organisms

In 96-h exposures, the LC_{50}'s for malachite green to frog larvae (0.173 mg/l) and toad larvae (0.0680 mg/l) were similar to those for fish (Table 2). Adult newts were more resistant than frog or toad larvae (96-h LC_{50}, 1.03 mg/l), but about equally or less resistant than most of the invertebrates exposed. Mayfly naiads were the most sensitive invertebrate

Table 1. *Toxicity of malachite green to fingerling fish in soft water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at			
	3 h	6 h	24 h	96 h
Chinook salmon	1.72	1.38	0.292	0.224
(Oncorhynchus tshawytscha)	1.22–2.42	1.04–1.82	0.245–0.348	0.209–0.240
Coho salmon	—	>3.00	0.569	0.383
(O. kisutch)	—		0.486–0.662	0.327–0.449
Atlantic salmon	3.56	1.09	0.497	0.283
(Salmo salar)	2.77–4.58	0.929–1.28	0.415–0.595	0.229–0.350
Brown trout	1.73	1.27	0.352	0.237
(S. trutta)	1.23–2.43	0.991–1.63	0.280–0.443	0.209–0.268
Rainbow trout	1.41	0.760	0.360	0.248
(S. gairdneri)	1.14–1.74	0.649–0.890	0.305–0.425	0.193–0.319
Brook trout	3.00	1.44	0.300	0.220
(Salvelinus fontinalis)	2.06–4.37	1.05–1.98	0.259–0.348	0.188–0.257
Channel catfish	>3.00	1.10	0.181	0.112
(Ictalurus punctatus)		0.904–1.34	0.123–0.266	0.0893–0.140
Largemouth bass	—	—	0.282	0.0728
(Micropterus salmoides)	—	—	0.211–0.376	0.0604–0.0877
Smallmouth bass	1.36	—	0.154	0.0453
(M. dolomieui)	1.09–1.70	—	0.117–0.202	0.0366–0.0561
Bluegill	6.00	2.19	0.231	0.0305
(Lepomis macrochirus)	4.41–8.17	1.66–2.89	0.184–0.290	0.0218–0.0427

Table 2. *Toxicity of malachite green to selected nontarget aquatic organisms in limed water at 16 C.*

Organism	LC_{50} and 95% confidence interval (mg/l) at		
	6 h	24 h	96 h
Snail	—	—	0.720
(Pleurocera sp.*)*	—	—	0.483–1.07
Asiatic clam	—	—	122
(Corbicula leana)	—	—	93.8–159
Ostracod	5.85	5.85	3.45
(Cypridopsis sp.*)*	4.00–8.57	4.29–7.97	2.49–4.80
Freshwater prawn	—	9.10	1.90
(Palaemonetes kadiakensis)	—	7.29–11.3	1.76–2.06
Midge (larvae)	5.00	1.00	0.510
(Tanytarsus dissimilis)	3.13–7.99	0.636–1.57	0.295–1.10
Mayfly naiads	5.75	2.75	0.0790
(Callibaetis sp.*)*	4.95–6.69	2.07–3.65	0.0442–0.141
Newts (adult)	—	3.90	1.03
(Notophthalmus viridiscens)	—	3.47–4.38	0.672–1.58
Leopard frog (larvae)	1.00	0.380	0.173
(Rana pipiens)	0.875–1.14	0.351–0.412	0.149–0.200
Toad (larvae)	1.70	0.355	0.0680
(Bufo sp.*)*	1.54–1.87	0.235–0.276	0.0530–0.0860

exposed (96-h LC_{50}, 0.0790 mg/l), and the Asiatic clam was by far the most resistant to the chemical; it tolerated concentrations in excess of 100 mg/l. The other invertebrates exposed were more resistant than fish or amphibians, but less resistant than the Asiatic clam. The 96-h LC_{50}'s for these organisms were between 0.510 and 3.45 mg/l.

Effects of Temperature, Water Hardness, and pH on Toxicity

In short exposures of 3 or 6 h, malachite green was more toxic to rainbow trout, channel catfish, and bluegills in warm water (17 and 22 C) than in cool water (7 and 12 C), but at 96 h the LC_{50}'s at different temperatures were not significantly different, except for channel catfish (Tables 3, 4, 5). Neither water hardness nor pH influenced the toxicity of the chemical to any species except bluegills, in which toxicity was about half as great as pH 6.5 as at pH 7.5 to 9.5.

Chronic Toxicity

Rainbow trout and bluegills were exposed simultaneously to the chemical in a flow-through toxicity test to determine the $TILC_{50}$. Mortality increased with time in both species. The 24-h LC_{50} for bluegills was 0.151 mg/l. A $TILC_{50}$ could not be calculated because mortality continued until all organisms succumbed at the lowest concentration (0.0316 mg/l) after 16 days of exposure. The 24-h LC_{50} was 0.230 mg/l for rainbow trout, and mortality continued through 30 days. A $TILC_{50}$ of 0.0998 mg/l was determined after 36 days of exposure.

Persistence of Malachite Green in Water

Bioassays with rainbow trout of aqueous solutions of malachite green aged for 1, 2, and 3 weeks in glass jars indicated no significant loss of activity. The LC_{50} was 0.173 mg/l for the freshly prepared reference solution and 0.179 mg/l for the solution aged for 3 weeks.

Table 3. *Toxicity of malachite green to fingerling rainbow trout at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 3 h	6 h	24 h	96 h
7	Soft	7.5	>2.00	2.30 1.71–3.10	0.400 0.330–0.486	0.168 0.137–0.206
12	Soft	7.5	1.41 1.14–1.74	0.760 0.649–0.890	0.360 0.305–0.425	0.248 0.193–0.319
17	Soft	7.5	1.42 1.15–1.76	0.567 0.517–0.621	0.569 0.516–0.627	0.284 0.229–0.353
12	Very soft	8.0	2.00 1.55–2.58	0.780 0.726–0.838	0.362 0.307–0.426	0.286 0.230–0.355
12	Soft	8.0	2.31 1.72–3.10	0.800 0.659–0.971	0.280 0.226–0.347	0.234 0.179–0.305
12	Hard	8.0	2.30 1.43–3.71	1.40 1.13–1.73	0.345 0.296–0.403	0.288 0.233–0.356
12	Very hard	8.0	2.35 1.74–3.17	0.820 0.701–0.959	0.280 0.226–0.347	0.249 0.195–0.318
12	Soft	6.5	>2.00	1.01 0.764–1.34	0.279 0.207–0.375	0.280 0.227–0.345
12	Soft	8.5	2.60 1.86–3.63	0.980 0.851–1.13	0.284 0.229–0.351	0.212 0.172–0.262
12	Soft	9.5	>2.00	1.26 0.978–1.62	0.367 0.311–0.434	0.173 0.136–0.220

Table 4. *Toxicity of malachite green to fingerling channel catfish at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l at 6 h	24 h	96 h
12	Soft	7.5	1.10 0.904–1.34	0.181 0.123–0.266	0.112 0.0893–0.140
17	Soft	7.5	0.552 0.499–0.610	0.222 0.168–0.293	0.0940 0.0860–0.103
22	Soft	7.5	0.400 0.331–0.483	0.0691 0.0576–0.0831	0.0535 0.0442–0.0647
12	Very soft	8.0	0.600 0.440–0.818	0.106 0.0935–0.120	0.0750 0.0555–0.101
12	Soft	8.0	1.30 1.01–1.67	0.285 0.232–0.350	0.117 0.0972–0.140
12	Hard	8.0	1.72 1.23–2.41	0.284 0.229–0.351	0.142 0.115–0.176
12	Very hard	8.0	1.71 1.22–2.40	0.286 0.232–0.353	0.142 0.115–0.176
12	Soft	6.5	0.960 0.717–1.29	0.236 0.181–0.308	0.0975 0.0937–0.101
12	Soft	8.5	0.835 0.665–1.05	0.835 0.665–1.05	0.237 0.182–0.309
12	Soft	9.5	0.519 0.377–0.714	0.191 0.155–0.236	0.162 0.135–0.194

Table 5. *Toxicity of malachite green to fingerling bluegill at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	6.00 4.41–8.17	2.19 1.66–2.89	0.231 0.184–0.290	0.0305 0.0218–0.0427
17	Soft	7.5	2.17 1.63–2.88	0.656 0.584–0.737	0.0920 0.0663–0.128	0.0340 0.0242–0.0477
22	Soft	7.5	0.860 0.737–1.00	0.238 0.184–0.308	0.0780 0.0594–0.102	0.0308 0.0221–0.0430
12	Very soft	8.0	2.30 1.72–3.08	2.00 1.54–2.59	0.117 0.0967–0.142	0.0413 0.0343–0.0497
12	Soft	8.0	>2.00	1.52 1.15–2.00	0.122 0.100–0.149	0.0400 0.0330–0.0486
12	Hard	8.0	>2.00	1.41 1.14–1.74	0.141 0.114–0.174	0.0450 0.0384–0.0528
12	Very hard	8.0	>2.00	1.42 1.10–1.83	0.141 0.114–0.174	0.0440 0.0370–0.0523
12	Soft	6.5	7.43 5.76–9.59	2.18 1.64–2.90	0.282 0.219–0.394	0.0780 0.0594–0.102
12	Soft	8.5	4.68 3.77–5.80	2.18 1.64–2.89	0.123 0.0955–0.158	0.0339 0.0241–0.0476
12	Soft	9.5	3.70 2.81–4.87	2.20 1.67–2.89	0.0810 0.0562–0.117	0.0340 0.0242–0.0477

Counteraction with Activated Carbon

Aqueous solutions of malachite green (2.0 mg/l) were filtered through a bed of activated carbon. In three runs the activated carbon adsorbed the chemical from 420, 401, and 425 liters of solution before the endpoint was reached (0.2 mg/l), an average of 23.4 mg of malachite green per gram of carbon. Activated carbon thus is an excellent means for removing this chemical from water.

References

Bills, T. D., and J. B. Hunn. 1976. Changes in the blood chemistry of coho salmon exposed to malachite green. Prog. Fish-Cult. 38(4):214-216.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series. EPA [Environ. Prot. Agency]-660/3-75-009. 61 pp.

Foster, F. J., and L. Woodbury. 1936. The use of malachite green as a fish fungicide and antiseptic. Prog. Fish-Cult. 18(4):7-9.

Glagoleva, T. P., and E. M. Malikova. 1968. The effect of malachite green on the blood compositon of young Baltic salmon. Rybn. Khoz. (USSR) 44(5):15-18. (Transl. from Russian by R. M. Howland)

Green, R. H. 1965. Estimation of tolerance over an indefinite time period. Ecology 46(6):887.

Leteux, F., and F. P. Meyer. 1972. Mixtures of malachite green and formalin for controlling *Ichthyophthirius* and other protozoan parasites of fish. Prog. Fish-Cult. 34(1):21-26.

Lieder, U. 1961. Zur Wirkung des Cancerogens and Mutagens Malachitgreen [*p*-dimethylamino-fuchson-dimethylimino-oxalat (sulfat)] auf Mitosen bei Fischen und Fischeier. [On the effect of the carcinogen and mutagen malachite green on mitosis in fish and fish eggs.] Naturwissenschaften 48(11):437-438.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96(2):99-113.

Marking, L. L. 1969. Toxicological assays with fish. Bull. Wildl. Dis. Assoc. 5:291-294.

Marking, L. L. 1972. Methods of estimating the half-life of biological activity of toxic chemicals in water. U.S. Fish Wildl. Serv. Invest. Fish Control 46. 9 pp.

Marking, L. L. 1975. Toxicological protocol for the development of piscicides. Pages 26-31 *in* P.H. Eschmeyer, ed. Rehabilitation of fish populations with toxicants: a symposium. North Cent. Div., Am. Fish. Soc., Spec. Publ. No. 4.

Marking, L. L., and V. K. Dawson. 1973. Toxicity of quinaldine sulfate to fish. U.S. Fish Wildl. Serv. Invest. Fish Control 48. 8 pp.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. Water Res. 1:21-29.

Nelson, N. C. 1974. A review of the literature on the use of malachite green in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 74-11. Natl. Tech. Inf. Serv. No. PB-235 450/AS. 79 pp.

Willford, W. A. 1967. Toxicity of 22 therapeutic compounds to six fishes. U.S. Fish Wildl. Serv. Invest. Fish Control 18. 10 pp.