

**DEMONSTATION OF A SYSTEM FOR REMOVING MALACHITE GREEN**

**Final Report**

by

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## CONTENTS

	<b>Page</b>
<b>Abstract</b> . . . . .	<b>2</b>
Introduction . . . . .	4
<b>Description of Study Area</b> . . . . .	<b>7</b>
<b>Methods and Materials</b> . . . . .	<b>9</b>
<b>Results and Discussion</b> . . . . .	<b>11</b>
<b>Summary and Conclusions</b> . . . . .	<b>17</b>
<b>Appendices</b> . . . . .	<b>35</b>

## Abstract

Malachite green is effective for the treatment of fungal infections on fish and fish eggs. Fungal infections are related to temperature, and treatments are generally needed for salmonids when water temperatures rise above 50°F. The U.S. Fish and Wildlife Service was granted an Investigational New Animal Drug permit (INAD #2573) by the U.S. Food and Drug Administration to allow the use of malachite green at selected state and federal fish hatcheries. However, the INAD requires that the fungicide be removed from all treated water after March 1989. Activated carbon has been used effectively to remove tastes, odors, and contaminants from public water supplies. The adsorption efficiency is influenced by the size of carbon granules, flow rate, column depth, and retention time. A study was designed to (1) determine the type of filter and kind of carbon that was most efficient and (2) demonstrate that carbon filters can be used to remove malachite green from water used for egg incubation or to hold adult salmon before spawning. Minicolumn simulation studies showed that 8 x 30 mesh granular carbon manufactured from bituminous coal was effective for continuously removing malachite green from water for 230 days at a flow rate of 500 gpm and for 62 days at a flow rate of 1,000 gpm. The removal capacity at the slower flow rate was 69 mg of malachite green per gram of carbon. A filter system that contained 20,000 pounds of activated carbon in each of two chambers was effective for removal of malachite green from treated water in adult salmon holding ponds at flows of 500 gpm and greater. The removal efficiency was 99.8% after 105 hours

of operation, and the adsorption capacity of the system was projected to be 20 or more years of routine hatchery operation. A filter system that contained 2,000 pounds of activated carbon in each of two chambers was effective for removal of malachite green from treated water in salmon egg incubation units at the designated flow rate of 50 gpm and also at faster flow rates. The removal efficiency was 96% after 114 hours of operation. Removal efficiency decreased only slightly for faster flows in both filter systems, and the efficiency improved when treated water was passed through two filter chambers in series.

## Introduction

Malachite green is an effective fungicide for the treatment of fish and fish eggs. It has been used for more than 50 years by fish culturists at federal, state, and private hatcheries. When this compound was identified as a potential teratogen, the U.S. Fish and Wildlife Service (FWS) restricted its use in national fish hatcheries. However, because there is no effective fungicide to replace malachite green, the U.S. Food and Drug Administration granted the FWS an Investigational New Animal Drug (INAD) permit (#2573) to allow the use of malachite green only at specified fish hatcheries that produce fish for restoration of depleted stocks of Atlantic salmon, Pacific salmon, striped bass, Atlantic sturgeon, and shortnose sturgeon. State hatcheries in Idaho, Oregon, and Washington were also allowed to use malachite green under the conditions of the INAD issued to FWS. The restricted use permit requires close monitoring of the malachite green used at fish culture facilities.

Specifically, the permit requires the keeping of accurate records of the success or failure of treatments, species and numbers treated, inventories and quantities used, assurance that workers are protected, and assurance that measures are taken to prevent releases of malachite green in the hatchery effluents. An annual report is required.

The continued use of malachite green for treating adults and eggs of important or endangered fish species is contingent on the development of systems to remove the therapeutic from hatchery effluents. Oxidizing and reducing agents are no longer acceptable for the treatment of water

containing malachite green because the basic structure of the molecule remains intact. The INAD permit requires that all hatcheries using malachite green for the treatment of eggs or adults must be in full compliance with conditions for malachite green removal by March 1, 1989.

Nelson (1974) reviewed the history of malachite green use in fish culture, its physical and chemical properties, its efficacy, toxicity to nontarget organisms, residues, application methodology, and registration status. Malachite green was dubbed the "fish-culturist's holy water" because of its extensive and effective use (Wood 1979). At that time, it was used primarily as a fungicide on fish eggs (Burrows 1949) but it was also effective for the prevention and control of fungal growths and for treating external parasites of fish, columnaris disease, fin rot, and certain algal growths. The basic procedures for use were described as dips, short exposures to high concentrations, and indefinite prolonged treatments at low concentrations. Exposures to a concentration of 1.0 ppm for 1 hour was a common treatment in salmon culture (Wood 1979).

Alderman (1985), in another review, concluded that there are numerous misunderstandings and much confusion about the use and safety of malachite green. Although many reports summarized the uses of the chemical, most did not discuss new treatment techniques or the efficacy of low treatment concentrations or of different exposure periods (Alderman 1982; Jenson and Windercher 1982; and Pickering and Pottinger 1985).

Researchers have demonstrated that activated carbon effectively removes tastes and odors and many organic chemicals from water (Davies et al. 1973). In laboratory tests, Dawson et al. (1976) showed that

activated carbon could be used to remove a variety of fish toxicants and anesthetics from water. Factors that influenced adsorption efficiency were the size of the carbon granules, flow rate, column depth, and retention time. Marking and Piper (1976) described a prototype carbon filter that effectively removed the drug Furanace from treated water in hatchery raceways or ponds.

In laboratory studies, where activated carbon was used for the removal of malachite green, Bills et al. (1977) reported that an average of 23.4 mg of malachite green could be removed per gram of carbon. They concluded that carbon filtration would effectively remove malachite green from treated water and that the development of a carbon filtration system would be feasible for use in fish culture.

Most activated carbon is manufactured from bituminous coal that is processed at high temperatures in a controlled atmosphere. Other types of activated carbon made from animal and vegetable matter are also available (Hassler 1963). Each granule is highly porous with a structure that provides a huge surface area. One gram of high-quality granular activated carbon has a surface area equal to about 89,000 cm<sup>2</sup>. Chemical charges within the pores make it possible for the carbon to adsorb organic molecules. Adsorption capacity is thus a function of the amount of surface area contained in the pores and the efficiency of adsorptive forces. The present project was begun to develop and test carbon filters that would be feasible for removing malachite green from hatchery effluents.

### Description of Study Area

During the week of April 6-10, 1987, nine hatcheries in the Columbia River basin were visited to determine the magnitude of fungal problems on brood stock salmon and the need for development of removal systems. Information was collected on water supplies, species cultured, use of malachite green, and the effectiveness of treatments (Table 1).

Water sources at the various hatcheries included springs, creeks, or rivers in the Columbia River Basin. Some surface water sources were supplemented with groundwater from wells or springs. Typically, the surface waters were very soft (10-30 ppm total hardness as CaCO<sub>3</sub>) and nearly neutral pH (6.6-7.4).

Malachite green treatments for control of fungus or parasites were used primarily on incubating eggs or on adult salmon held for long periods at higher than optimal temperatures. Stations that collected spawning stocks of spring or summer chinook salmon in May or June and held them till spawning in August or September used 15 to 211 pounds of malachite green per year. The fish were usually treated when first trapped because they were often in poor condition as a result of the migration or the waiting period below collection sites. During holding, periodic treatments with malachite green were used as necessary to control diseases.

The need for malachite green treatments is correlated with the high temperatures that develop in surface waters during summer. During holding periods, the water temperature may rise to 60°F at Bonneville State Fish Hatchery (SFH), 70°F at Clackamas SFH, 55°F at Cowlitz SFH, or 65°F at

**Kalama Falls SFH Spring or fall chinook salmon held in cooler water at Carson National Fish Hatchery (NFH) (44-47°F), Little White Salmon NFH (35-52°F), and Spring Creek NFH (47°F) did not require the use of malachite green to maintain satisfactory conditions.**

**Mortalities of adult fish during holding also are correlated with the high temperatures of supply waters. The mortality of untreated salmon at stations where malachite green is not needed was low (2 to 20%). At stations where temperatures were higher, mortalities in untreated fish ranged up to 80%. However, losses decreased significantly (5 to 30%) when malachite green was used. The decrease in mortality of adults was most marked at Kalama Falls where it dropped from 75% in untreated fish to only 5% in treated fish. Clearly, malachite green treatments are very effective for reducing mortalities in adult fish.**

**Methods for treating the holding water differed at the various stations. Some stations used the "California flush" in which a calculated amount of malachite green (in a water solution) was dumped into the headbox of an egg incubator or into the inflow of the holding pond for adults and flushed through the pond (Wood 1979). Other methods involved dripping aqueous stock solutions of malachite green into the head end of a holding pond for a designated time. Hatchery personnel reported that they wanted to maintain a 1.0 ppm concentration for 1 hour and that the methods in use yielded satisfactory results.**

**Water flows in adult holding ponds during the period when it is necessary to treat adult fish ranged from 1,000 gpm at Kalama Falls to 10,000 gpm at Bonneville. Water exchange rates ranged from 2 volumes per**

hour to 1 volume per 1.5 hours. These high flow rates were considered necessary because of heavy loading rates (1,000 adults per pond) at higher than optimal temperatures.

#### Methods and Materials

The first phase of this work was conducted in the laboratory using simulation equipment. Minicolumn adsorption techniques are effective and rapid for determining representative carbon adsorption capacities and usage rates for specific organic components. These systems can simulate 30 days of actual use in a single day and provide information on flow dynamics for the removal of a specific compound (Bilello and Beaudet 1983). Two minicolumn tests were done by Calgon Carbon Corporation to identify optimal component characteristics for the removal of malachite green from treated water. In these tests, water was treated with malachite green at a concentration of 1 mg/L. Samples of treated waters and filter effluent were analyzed for malachite green concentrations using a calorimeter at 615 nm (Appendix 1). The malachite green breakthrough point was arbitrarily chosen to be 0.1 mg/L.

The second phase of the study involved the application of data generated in the laboratory study regarding type and amounts of activated carbon to be used in prototype filter units designed for full-scale hatchery use. A carbon filter system was installed at the Carson NFH to (1) remove malachite green from treated water during loading studies to determine the adsorption capacity at a flow rate of 500 gpm (2) monitor levels of malachite green in the adult holding ponds during actual

treatments and removal, (3) determine the adsorption capacity of the system for faster flow rates when chambers are used singly or in series, and (4) demonstrate the operation of this large-scale system in actual hatchery use.

Each of the two chambers in the unit were 10 feet in diameter and contained 20,000 lbs of 8 x 30 mesh activated carbon (Fig. 1). The malachite green treated and filtered water was analyzed colorimetrically at the hatchery about 2 hours after the samples were taken. Some of the samples were split; one portion was analyzed at the hatchery and the other portion was sent to the La Crosse, Wisconsin, National Fisheries Research Center, for confirmatory High Performance Liquid Chromatography analysis.

Test and evaluation procedures were chosen to simulate the use over a shorter duration to obtain a more realistic time frame for evaluation. The influent concentration of malachite green in loading tests was selected to be 10 ng/L rather than 1.0 ng/L and the test period was then shortened from 30 weeks to 3 weeks.

A second carbon filter system was installed at the Abernathy Salmon Culture Technology Center, Longview, Washington, to (1) remove malachite green from treated water during loading studies to determine the adsorption capacity at a flow rate of 50 gpm per chamber, (2) monitor levels of malachite green in the egg incubation unit during actual treatments and removal, (3) determine the adsorption capacity of the system for faster flow rates when chambers are used singly or in series, and (4) demonstrate operation of the equipment to the fish cultural community.

Each of the two chambers in the system were 4 feet in diameter and contained 2,000 lbs of 8 x 30 mesh activated carbon (Fig. 2). The procedures for operation and sampling and the rationale for concentrations and test duration was the same as for the 500 gpm flow system at Carson NFH.

### Results and Discussion

The simulation studies showed that 8 x 30 mesh activated carbon granules were effective for removing 1 mg/L of malachite green from water to a concentration less than 0.1 mg/L during 230 days of simulated operation at a flow of 500 gpm (Table 2). Operation at fish hatcheries for that duration would extend the life of the filter beyond 20 years in most instances. Accordingly, 1,417 lbs of malachite green could be removed from water with each chamber that contained 20,000 lbs of activated carbon. The effectiveness lasted only 62 days at a flow of 1,000 gpm. Concentrations in the fast flow effluent increased rapidly in the 1,000 gpm flow after the breakthrough at 0.1 mg/L, whereas, they increased slowly in the 500 gpm effluent. Accepting a breakthrough of 0.2 mg/L would have extended the effectiveness of the 500 gpm flow filter to nearly 400 days (Fig. 3 and Appendix 2 and 3).

The simulated optimum bed contact time was 9 minutes with a bed depth of 9 feet. The optimum surface loading rate was 70 gpm/ft<sup>2</sup>. Since the 500 gpm flow was the most efficient, in-place systems at fish hatcheries should be designed with similar flow characteristics and in modules to accommodate faster flows. The Filtrasorb 300 granules used in these tests

were durable and capable of withstanding the abrasion associated with repeated backwashing, air scouring, and hydraulic transport. Information obtained in the simulation studies was then used to design and construct in-place carbon filter systems for (1) the effluent from a holding pond for adult brood stock and (2) the effluent of egg incubation units.

The in-place carbon filter at Carson NFH was operated for 105 hours at a flow rate of about 500 gpm to demonstrate the loading capacity. During each week of operation over a million gallons of water treated with about 10 mg/L of malachite green were filtered through a single chamber. Samples of influent and effluent were taken about 1 hour after startup and 1 hour before shutdown each day. The first indication of detectable malachite green in the effluent was at the end of the second week when 0.02 mg/L was detected (Table 3). During the third week of operation, detectable concentrations averaged about 0.02 mg/L in the effluent or equal to about 99.8% removal efficiency.

The elapsed time of operation after 3 weeks was 105 hours for a 10.0 mg/L solution of malachite green; the calculated elapsed time for a 1.0 mg/L solution would be 10 times longer or over 1,000 hours. Few, if any, hatcheries contacted on the Columbia River system exceed 50 1-hour treatments per year. Therefore, on the basis on this loading experiment, the capacity of this filter system is sufficient to effectively remove malachite green from treated water for a projected 20 or more years.

Malachite green concentrations were monitored during and after 1.0 mg/L treatment of adult Pacific salmon. A stock solution of malachite

green was dripped into the inflow for a period of 1 hour using the amount known to be effective for fungus control. Concentrations were monitored every 10 minutes near the tail end of the holding pond (40' x 146' x 4' in depth). The mean concentrations and standard deviations for three applications are recorded in Appendix 4 and illustrated in Figure 4. As shown, the concentration peaked at about 0.6 mg/L halfway through the application. The concentrations did not result in a plateau as anticipated, nor did the measured concentration approach 1.0 mg/L, the assumed treatment rate. Water inflow created a circulating motion in the pond. Therefore, it is possible that concentrations may vary considerably in different areas, and that the concentration stretches out and dissipates over an additional hour. However, samples taken near the head end of the holding pond were generally higher in concentration than elsewhere in the pond. In two instances, the level tested over 1.0 mg/L. Removal efficiency was satisfactory and all of the filter effluent samples contained less than 0.01 mg/L of malachite green.

A separate experiment was undertaken to determine the impacts of faster flows and flows through both chambers of the system. The flow rate of malachite green treated water was first increased by 2x and then to a maximum flow of the pump through a single chamber. The concentration in the effluent was 0.03 mg/L at the 1,000 gpm flow rate and 0.04 mg/L at the rate over 1,250 gpm (Table 4). When the treated water was filtered through two chambers in a series, removal efficiency was slightly better. The carbon filters were slightly less efficient at higher flow rates. These results correlate well with the simulation studies. In actual water

treatment applications, carbon filters are generally used in a series so that the first bed serves to remove most of the compound, and the second serves as a finishing filter.

The egg incubation carbon filter at Abernathy Salmon Culture Technology Center was operated for about 8 hours/day for a total of 114 hours over 3 weeks at a flow rate of 50 or more gpm. During each week of operation, about 120,000 gallons of water treated with 10 mg/L of malachite green were filtered through a single chamber. Concentrations in the effluent from the 10 mg/L application ranged from 0.03 to 0.10 mg/L during the study (Table 5). Removal efficiency was about 99%.

Fall chinook salmon eggs were treated with 1.0 mg/L of malachite green for 1 hour, three times a week, for 8 weeks. A stock solution of malachite green was metered into the water entering the egg incubation unit to produce calculated 1 mg/L treatments. Concentrations in the treated and filtered water were monitored during three of the applications to establish the exposure levels and removal efficiency. Mean concentrations and standard deviations for the three applications indicate that there was a rapid increase in concentration during the first 30 minutes, a gradual increase thereafter to about 0.9 mg/L at 70 minutes, and a rapid decline after the malachite green was no longer administered (Fig. 5 and Appendix 5). This application procedure produced concentrations of 0.8 or 0.9 mg/L for 40 minutes. Thus, exposure levels were closer to calculated values than those observed in the tail end of the brood stock pond. Concentrations of malachite green in the treated

effluent from the incubator units ranged from <0.01 to 0.04 ng/L; a removal efficiency of 96%.

In tests to determine the efficiency of the carbon filters to treat faster flows of malachite green treated water, the 50 gpm flow rates were doubled (2x) and then tripled (3x). The concentration in the effluent following an application (about 8.75 ng/L) was decreased to 0.04 ng/L at a flow of about 100 gpm (Table 6). However, when the flow was tripled to about 150 gpm, the concentration in the effluent was higher--the average concentration for three samples was 0.13 ng/L. The mean treatment concentration of three unfiltered samples was 11.58 ng/L so the removal efficiency was 98.9%. These data indicate that the filter was as efficient at the 2x flow as for the 50 gpm flow but that the efficiency decreased markedly at the 3x flow rate. However, when the treated water was passed through two filter chambers in a series at the 150 gpm rate, the removal efficiency of the system was as good as with 1x or 2x flow rates through a single chamber (Table 6).

A discrepancy was noted on September 3, when results of colorimetric and HPLC determinations were compared. HPLC results were consistently higher than the calculated colorimetric values. Upon review of the entire analytical process, it became apparent that the problem was related to acidification of the standards. The higher results achieved by HPLC may have been caused by the use of a weakly acid mobile phase that apparently was not strong enough to convert the leuco or carbinol form to parent malachite green in the standards (Appendix 6 and 7). That standard curve

gave values that were lower than calculated and this resulted in higher than calculated readings for the unknown samples. Since all samples were acidified when they were taken, the standards were also acidified after the September 2nd sample. Also, at that time, the effluent samples were not quantitated at concentrations  $<0.1$  ng/L by the HPLC method, even though lower levels were detectable. The acidification process was also important in the colorimetric procedure because the maximum color of parent malachite green will not occur until about 2 hours after the acidification. Consequently, all samples were allowed to develop maximum color before they were analyzed on the spectrophotometer.

After the September 2nd sample, the analytical methods yielded results that were in close agreement. The tendency for slightly lower values in the HPLC analyses may be related to slight deterioration of the sample during shipment and storage, generally about 1 week. All values derived from the colorimetric procedures were rounded to two decimal places to reflect limitations on the accuracy of the colorimetric procedure.

Costs of the prototype filter systems, including the carbon, was about \$170,000 for the large unit and about \$30,000 for the small one. These filters were designed for operating in numerous modes; each has a control panel with nine flow options, 12 pneumatic valves for control of flows, and an air compressor to drive the pneumatic system. These optional accessories were useful in the test models, but they would not be required for production models at hatchery sites. Other factors, such as volume purchases, may also reduce the costs of these units.

Because the cost of carbon filter removal systems is substantial, alternatives to treating the large flows through adult holding ponds should be considered. If possible, retention lagoons could be used to store treated water so that it could be treated over a longer time period at a more practical flow rate. Recirculation of treated water in adult holding ponds would decrease the amount of malachite green needed for treatment and for removal. Only the replacement water (i.e., 10%) would then have to be treated after the initial application. During the recirculation, oxygen deficiencies could be partly alleviated by installing simple, packed column aerators or oxygen injection units on the return line for recycled water.

#### Summary and Conclusions

Malachite green is highly effective for the treatment of fungal infections on fish and fish eggs. Fungal infections of salmonids are often related to temperature, and treatments are generally needed when water temperatures rise above 50°F. The U.S. Fish and Wildlife Service was granted an Investigational New Animal Drug permit (INAD #2573) by the U.S. Food and Drug Administration to allow data gathering on the use of malachite green at selected state and federal fish hatcheries. The current INAD requires that the fungicide be removed from all treated water after March 1989.

The adsorption efficiency of activated carbon is influenced by the kind and size of carbon granules, flow rate, column depth, and retention time. A study was designed to (1) determine the type of filter and kind

of carbon that was most efficient and (2) demonstrate that operational sized activated carbon filters can be used to remove malachite green from water used for egg incubation or for holding adult salmon before spawning. Minicolumn simulation studies showed that 8 x 30 mesh granular carbon manufactured from bituminous coal was effective for continuous removal of malachite green from water to a level  $<0.1$  mg/L for 230 days at a flow rate of 500 gpm and for only 62 days at a flow rate of 1,000 gpm. The adsorption capacity at the slower flow rate was 69 mg of malachite green per gram of carbon.

A filter system that contained 20,000 pounds of activated carbon in each of two chambers effectively removed malachite green from treated water in adult salmon holding ponds at flows of 500 gpm. The removal efficiency was 99.8% after 105 hours of operation. At this rate, the adsorption capacity of the system was projected to accommodate 20 or more years of routine hatchery operation.

Adult Pacific salmon were treated with malachite green at 1.0 mg/L by adding it to the water supply of a holding pond. Concentrations peaked at 0.6 mg/L at the tail end of the holding pond and filter effluents contained less than 0.01 mg/L of malachite green. When the flow rate through the filter was increased to 1,000 gpm through a single chamber, the effluent concentration reached 0.04 mg/L. However, when the treated water was filtered through two chambers in a series at the fast flow rate, the effluent contained 0.03 mg/L of malachite green.

A filter system that contained 2,000 pounds of activated carbon in each of two chambers effectively removed malachite green from treated

water from salmon egg incubation units at a flow rate of 50 gpm. Removal efficiency was about 99%. The mean concentration of malachite green applied in three egg treatments was about 0.9 mg/L for 30 minutes with lower levels present on each end of the 1-hour application of 1 mg/L. Concentrations of malachite green in the filter effluent ranged from <0.01 to 0.03 mg/L for a removal efficiency of 97%. This filter was as efficient for 2x flows through a single chamber, but the efficiency decreased markedly at a 3x flow. However, when treated water was passed through two filter chambers in a series at the 150 gpm rate, the removal efficiency was as good as with the 50 gpm flow rate through a single chamber.

The methods for application of malachite green were found to be inconsistent and imprecise; studies are needed to verify the concentrations and durations of treatments that are found to be safe and effective. Quantities of malachite green used could possibly be decreased if exposure times were optimized and concentrations were kept at the minimum effective concentration.

Removal of malachite green from treated water with activated carbon is feasible at most hatcheries. Because of restrictions on the space available for installing equipment and the great variability in existing flows, alternatives to conventional water treatment should also be considered. Treated water might be collected and stored in a large reservoir and treated by carbon filtration at a slower, more practical flow rate. Recirculation of malachite green-treated water through ponds

used to hold adults would decrease the amount of malachite green required for treatment and facilitate easier removal.

Filter efficiency at a hatchery may be influenced by the presence of organic compounds in the water supply or in retention ponds. These molecules would compete with malachite green for active sites on the carbon granules, thus, the adsorption capacity for malachite green would be diminished.

In summary, filter units on adult salmon holding ponds can accommodate flows up to 1,000 gpm and units on the egg incubation units can accommodate flows of 100 gpm or greater. If greater flows are necessary, multiples of these units or larger units could be installed. The carbon filtration systems are readily available from commercial sources.

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Table 1. Water, species, and results of malachite green treatments at salmonid hatcheries in the Columbia River basin.

Station <sup>a</sup>	Water supply		Temperature (°F)	Species	Malachite green usage (lbs.)	Flow treated <sup>b</sup> (gpm)	Mortality (0/0)	
	Source	Usage (gpm)					Treated	Untreated
Abernathy NFH Longview, WA	Abernathy Creek	3,000	55-60	Fall Chinook	None	None	NA	15
Carson NFH Carson, WA	Tyee spring Wind River	21,000 2,500	44-47 61	Spring Chinook	None	None	2.5	7.2
Little White Salmon NFH Cook, WA	Little White Salmon River Well	23,000 150	39-52 50	Spring Chinook Fall Chinook Coho	None	None	NA	2.0
Spring Creek NFH Underwood, WA	Spring creeks (6)	2,500 <sup>c</sup>	47	Fall Chinook	None	None	NA	2.5
Bonneville SFH Cascade Locks, OR	Tanner Creek Well	16,000 18,000	32-60 48-52	Summer Chinook Fall Chinook	211	10,000	11-20	50-80
Clackamas SFH Estacada, OR	Clackamas River Well	20,000 170	62-70 53	Spring Chinook	15	1,350	15	20-25
South Santiam SFH Sweet Home, OR	Santiam River (Foster Reservoir)	8,500	50	Spring Chinook Steelhead Coho	75	2,700	25	60-75
Cowlitz SFH Solukum, WA	Cowlitz River Well	75,000 1,750	44-55 48-50	Spring Chinook Fall Chinook Coho	150	3,500	30	50
Kalama Falls, SFH Kalama, WA	Kalama River	6,000	61-65	Spring Chinook Fall Chinook Coho	52	1,000	5	75

<sup>a</sup>NFH=National fish hatcheries; SFH+=state fish hatcheries.

<sup>b</sup>Flow through individual adult-holding ponds.

<sup>c</sup>Ninety percent of water is recycled at this station.

**Table 2. Simulated effectiveness of 8 x 30 mesh granular activated carbon for removal of malachite green from treated water at two flow rates.**

<b>Flow rate (gpm)</b>	<b>Gallons treated</b>	<b>Operation days</b>	<b>Capacity ng/L</b>
<b>500</b>	<b>170,000,000</b>	<b>230</b>	<b>69</b>
<b>1,000</b>	<b>89,100,000</b>	<b>62</b>	<b>37</b>

**Table 3. Concentrations (mg/L) of malachite green in treated and filtered Carson NFH spring water at a flow rate of about 500 gpm**

Flow rate (gpm)	Elapsed time (h)	Cumulative volume filtered (gal )	Mean concentration (n=2)	
			Influent	Effluent
536	7	225, 300	10. 59	<0. 01
545	14	454, 110	10. 23	<0. 01
520	21	672, 460	10. 54	<0. 01
512	28	887, 570	11. 12a	<0. 01a
489	35	1, 092, 800	7. 29	<0. 01
494	42	1, 300, 210	8. 70a	<0. 01a
485	49	1, 505, 010	8. 65	<0. 01
486	56	1, 710, 270	9. 12	<0. 01
485	63	1, 914, 020	9. 28	<0. 01
489	70	2, 119, 270	9. 49	0. 02
485	77	2, 323, 100	9. 49	0. 02
479	84	2, 524, 230	9. 28	0. 02
479	91	2, 725, 370	9. 18a	0. 01a
478	98	2, 926, 210	9. 49	0. 02
478	105	3, 127, 960	9. 28	0. 02

<sup>a</sup>Concentrations checked by HPLC analysis at La Crosse National Fisheries Research Center.

**Table 4. Concentrations (mg/L) of malachite green in treated and filtered Carson NFH water at increased flow rates and at maximum flow through a single chamber and through two chambers in series.**

Flow type and rate (gpm)	Volume filtered (gal )	Mean concentration (n=2)	
		Infl uent	Effluent
<b>Single chamber</b>			
1,006	60,350	8.50	0.03
1,000	60,020	8.76	0.03
998	59,850	8.76	0.03
1,256	75,370	8.50	0.04
<b>1, 278</b>	76,720	8.39	0.04
1,243	74,580	8.44	0.04
<b>Series</b>			
<b>1082</b>	<b>64, 900</b>	<b>9. 49</b>	0.02
<b>1102</b>	66,100	<b>9. 44</b>	0.03
<b>1095</b>	65,720	<b>9. 39</b>	0.03

**Table 5. Concentrations (mg/L) of malachite green in treated and filtered Abernathy creek water at a flow rate of about 50 gpm**

Flow rate (gpm)	Elapsed time (h)	Cumulative volume filtered (gal)	Mean concentration (n=4)	
			Infl uent	Effluent
51	8.0	24,640	7.32	0.03
52	16.5	51,240	10.64	0.09
51	24.8	76,640	11.60	0.09
51	30.8	95,640	9.28	0.07
51	39.3	121,440	9.32	0.07
51	47.7	147,140	9.41	0.07
51	53.7	165,540	9.82 <sup>a</sup>	0.07a
50	61.3	188,440	9.64	0.10
51	69.8	214,540	9.78	0.09
53	78.0	240,740	9.64	0.09
53	84.2	260,440	9.46a	0.08a
53	92.5	287,040	10.28	0.08
54	100.2	312,240	8.87	0.07
54	108.0	337,740	9.05	0.07
54	114.2	357,840	8.92a	0.07a

<sup>a</sup>Concentrations checked by HPLC analysis at La Crosse National Fisheries Research Center.

**Table 6. Concentrations (mg/L) of malachite green in treated and filtered Abernathy Salmon Culture Technology Center creek water at increased flow rates and at maximum flow through a single chamber and through two chambers in series.**

Flow type and rate (gpm)	Volume filtered (gal )	Mean concentration (n=2)	
		Influent	Effluent
<b>Single chamber</b>			
98	5,850	9.28	0.04
94	5,650	8.53a	0.04a
107	6,440	8.44	0.04
162	9,700	11.51	0.09
133	8,000	12.12a	0.14a
145	8,700	11.13	0.15
<b>Series</b>			
145	8,700	10.84	0.04
140	8,380	11.04	0.03
142	8,500	11.08	0.03

<sup>a</sup>Concentrations checked by HPLC analysis at La Crosse National Fisheries Research Center.

**FIGURE CAPTIONS**

- Figure 1. Carbon filtration unit at Carson NFH that can be operated with water flow in series or through single chambers at flows up to 1,000 gpm. Each chamber contains 20,000 lbs of activated carbon.**
- Figure 2. Carbon filtration unit at Abernathy Salmon Culture Technology Center that can be operated with flow in series or through single chambers at flows up to 150 gpm. Each chamber contains 2,000 lbs of activated carbon.**
- Figure 3. Concentrations of malachite green in effluents of minicolumn simulation experiments at flow rates of 500 or 1,000 gpm.**
- Figure 4. Concentrations of malachite green in an adult-holding pond during and after a 1-hour application.**
- Figure 5. Concentrations of malachite green in an egg incubation unit during and after a 1-hour application.**

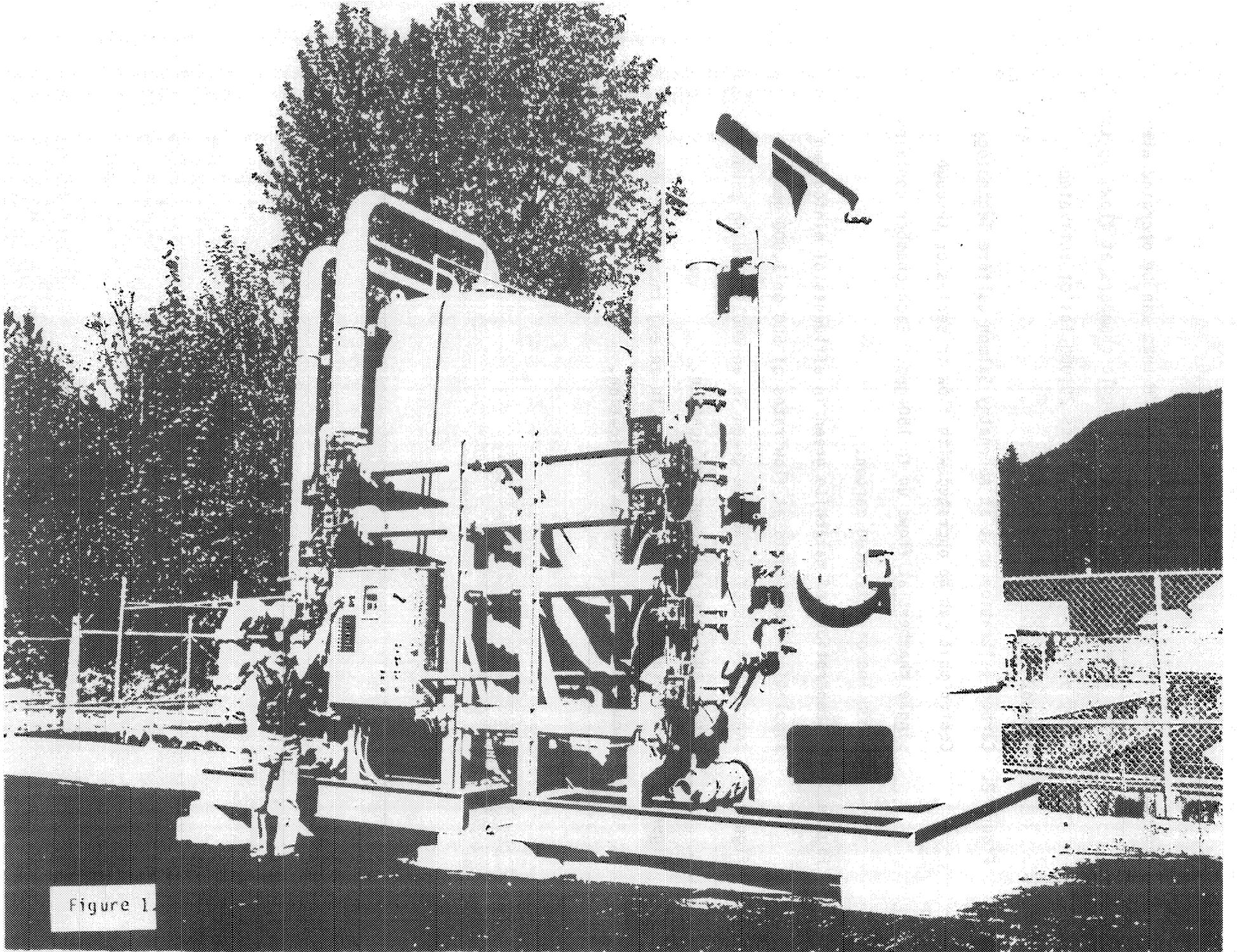


Figure 1.

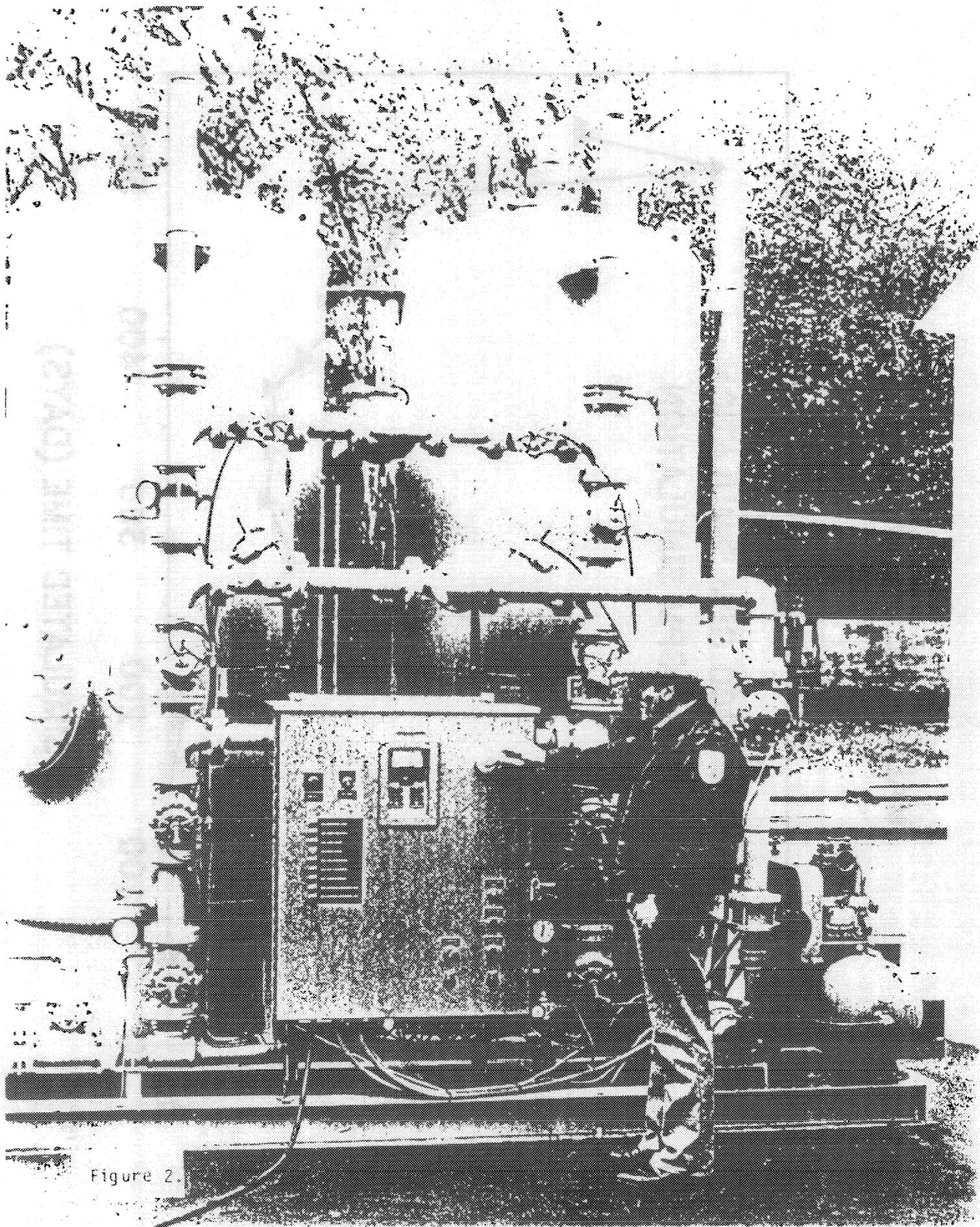


Figure 2.

# MALACHITE GREEN REMOVAL

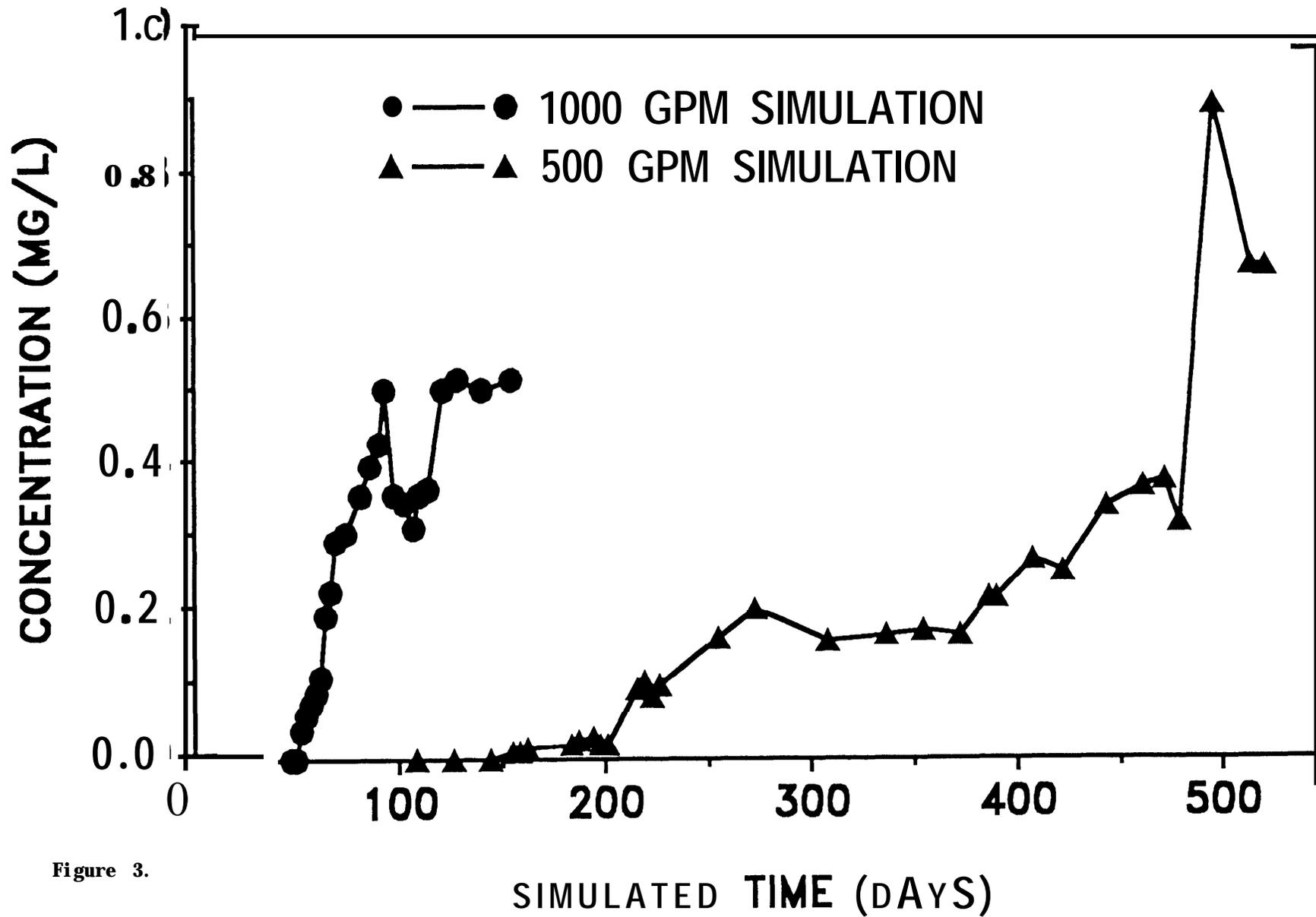


Figure 3.

# MALACHITE GREEN

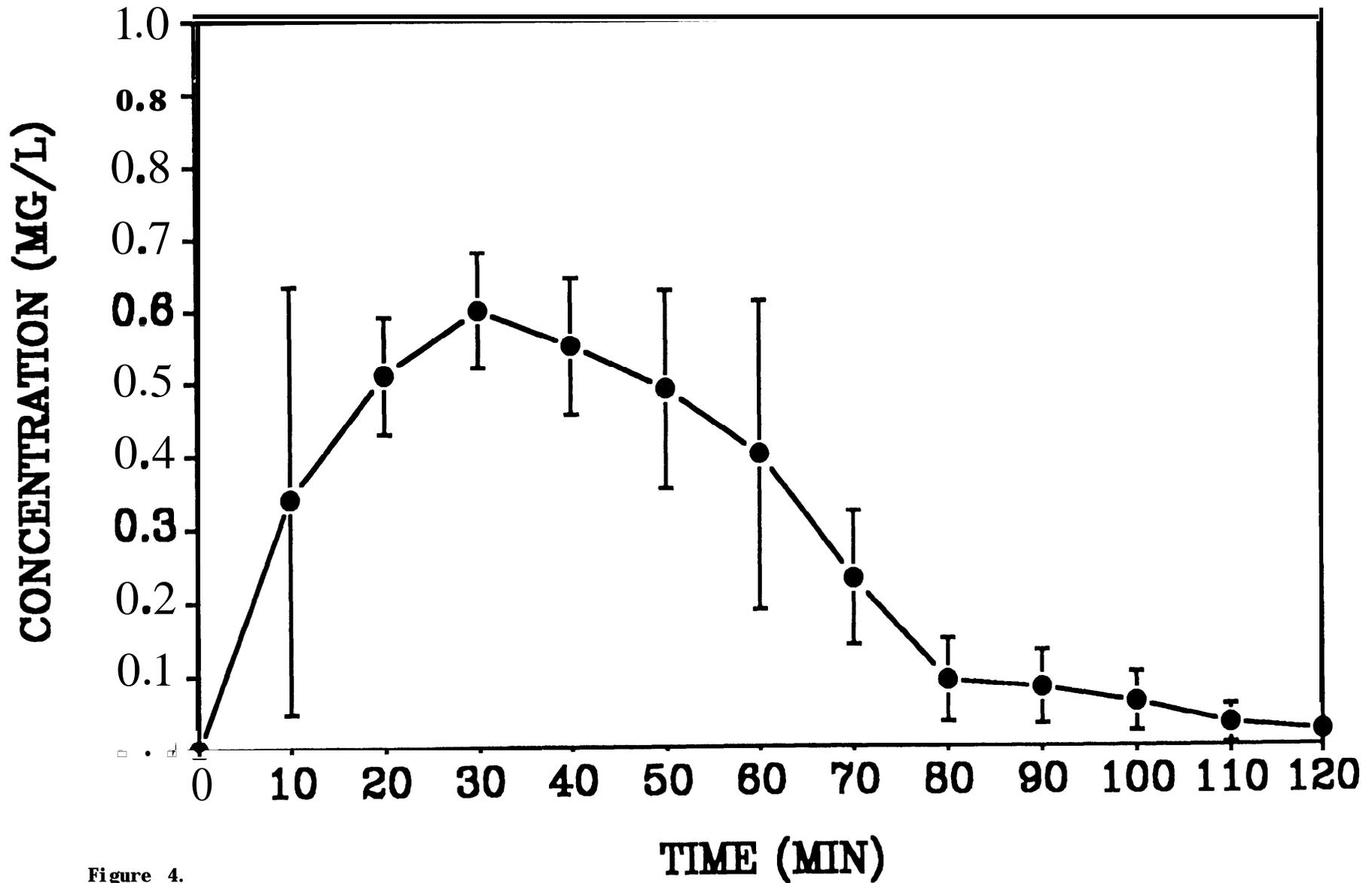


Figure 4.

# MALACHITE GREEN

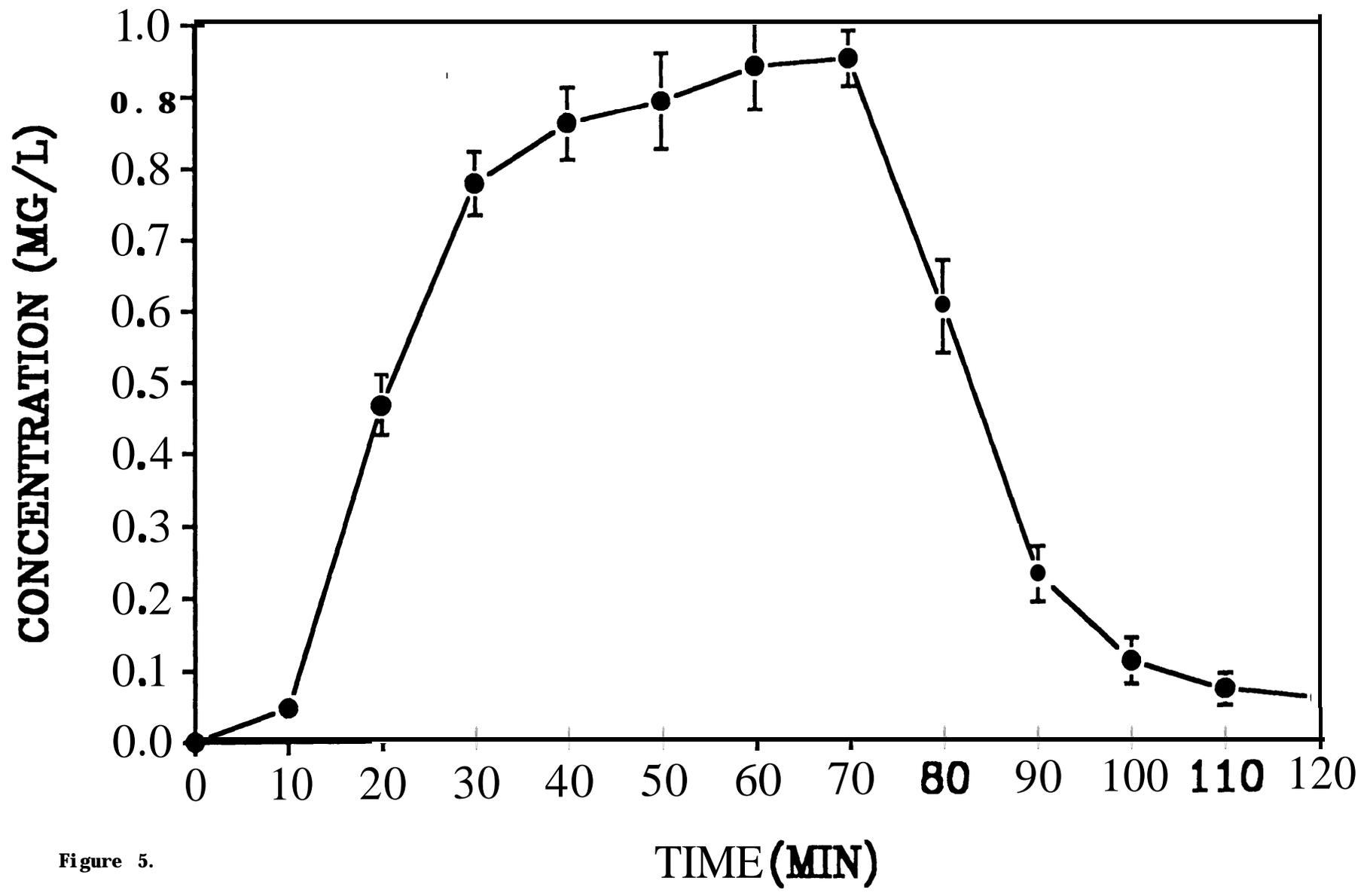


Figure 5.

**APPENDIX**

**Appendix 1. Procedure for analysis of malachite green in water.**

Malachite green can be detected directly in water at concentrations of 0.1 g/mL by colorimetry. Lower concentrations can be detected by passing a large volume of water through a C<sub>18</sub> column (Waters C<sub>18</sub>, Sep-Pak) and eluting malachite green from the column with AFA (ethyl alcohol-formalin-acetic acid:85-10-5).

A procedure effective for detection of 0.01 or more µg/g is as follows:

1. Condition a C<sub>18</sub> Sep-Pak by passing 2 mL of methanol through the cartridge followed by 2 mL of deionized or distilled water.
2. Pass 200 mL of the water being analyzed through the cartridge at approximately 40 mL/min. Discard the water.
3. Pass 5.0 mL of AFA through the column and collect it in a cuvette for colorimetric analysis.
4. Determine the absorbance (or % transmittance) of the resultant AFA on a Spec 20 or similar colorimeter at 615 nm
5. Compare the absorbance of the solution to the absorbance of an analytical standard of malachite green in AFA to determine the concentration of malachite green in the water.
6. A procedural blank should be run on water known to be free of malachite green.

If more definitive results are required, the AFA eluted from the C<sub>18</sub> Sep-Pak can be analyzed by HPLC. The HPLC system to be used is a Waters Nova Pak C<sub>18</sub> column (15 cm x 4 mm) with a mobile phase of 80% acetonitrile and 20% 0.05 M toluene sulfonic acid at 1.0 mL/min. A UV-visible detector set at 615 nm should give good response for 1 ng of malachite green oxalate with a retention volume of about 4 mL.

**Appendix 2. Concentrations of malachite green in water treated at 1.0 mg/L during filtration through a minicolumn simulation unit at a flow of 500 gpm**

<b>Sample ID No.</b>	<b>Simulated days</b>	<b>Simulated gallons (x 1,000)</b>	<b>Concentration (mg/L)</b>
26	109	78,176	0.00
31	126	91,000	0.00
36	144	103,824	0.00
39	155	111,519	0.01
40	159	114,084	0.01
41	162	116,648	0.02
47	183	132,037	0.02
48	187	124,602	0.03
50	194	139,731	0.03
51	198	142,296	0.02
52	201	144,697	0.02
53	215	155,120	0.10
54	219	157,685	0.11
55	223	160,250	0.09
56	226	160,815	0.10
60	255	183,333	0.16
65	272	196,157	0.20
75	308	221,805	0.16
80	337	242,323	0.17
85	354	255,147	0.17
90	372	267,971	0.17
94	386	278,230	0.22
95	390	280,795	0.22
100	408	293,619	0.27
104	422	303,878	0.25
110	443	319,267	0.34
115	461	332,091	0.37
118	472	339,785	0.38
120	479	344,915	0.32
125	497	357,739	0.89
130	515	370,563	0.67
132	522	375,693	0.67

**Appendix 3. Concentrations of malachite green in water treated at 1.0 mg/L during filtration through a minicolumn simulation unit at a flow of 1,000 gpm**

<b>Sample ID No.</b>	<b>Simulated days</b>	<b>Simulated gallons (x 1,000)</b>	<b>Concentration (mg/L)</b>
21	48	68,591	0.00
22	50	72,003	0.00
23	53	75,415	0.04
24	55	78,827	0.06
25	57	82,239	0.08
26	60	85,650	0.09
27	62	89,062	0.11
28	64	92,474	0.19
29	67	95,886	0.23
30	69	99,297	0.29
32	74	106,121	0.31
35	81	116,356	0.36
37	86	123,180	0.40
39	91	130,003	0.43
40	93	133,415	0.50
42	98	140,239	0.36
44	102	147,063	0.35
46	107	153,886	0.31
47	110	157,298	0.36
49	114	164,121	0.37
52	121	174,357	0.50
55	129	184,592	0.52
60	140	201,651	0.50
66	155	222,122	0.52

**Appendix 4. Concentrations (ng/L) of malachite green in holding pond containing about 1,000 adult Pacific Salmon during and after a 1-hour application with a flow rate of 500 gpm Data for Figure 4.**

Time (min)	Trial number			Mean	+ SD
	1	2	3		
0	<0.01	<0.01	<0.01	<0.01	0
10	0.54	<0.01 <sup>a</sup>	0.48	0.34	0.293
20	0.57	0.42	0.57	0.51	0.081
30	0.52	0.68	0.60	0.60	0.080
40	0.45	0.64	0.55	0.55	0.095
50	0.34	0.61	0.51	0.49	0.137
60	0.16	0.56	0.48	0.40	0.212
70	0.13	0.30	0.27	0.23	0.091
80	0.03	0.14	0.10	0.09	0.057
90	0.03	0.13	0.08	0.08	0.050
100	0.02	0.06	0.10	0.06	0.040
110	0.01	0.02	0.06	0.03	0.026
120	<0.01 <sup>a</sup>	0.01	0.04	0.02	0.019

<sup>a</sup><0.01 = 0.005 for calculation of mean  $\pm$  SD.

Appendix 5. Concentrations (mg/L) of malachite green in a salmon egg incubation unit during and after a 1-hour application of 1.0 mg/L with a flow rate of 50 gpm. Data for Figure 5.

Time (min)	Trial number			Mean	SD
	1	2	3		
0	0.00	0.00	0.00	0.00	0
10	0.07	0.03	0.03	0.04	0.023
20	0.48	0.49	0.42	0.46	0.038
30	0.82	0.76 <sup>a</sup>	0.73	0.77	0.046
40	0.91	0.84	0.81	0.85	0.051
50	0.96	0.86	0.83	0.88	0.068
60	1.00	0.92 <sup>a</sup>	0.88	0.93	0.061
70	0.99	0.92	0.92	0.94	0.040
80	0.67	0.52	0.59	0.59	0.075
90	0.27	0.19 <sup>a</sup>	0.20	0.22	0.044
100	0.14	0.09	0.08	0.10	0.032
110	0.08	0.06	0.04	0.06	0.020
120	0.06	0.04	0.04	0.05	0.012

<sup>a</sup>Concentrations checked by HPLC analysis at La Crosse National Fisheries Research Center.

**Appendix 6. Concentrations 9ng/L) of malachite green as determined by two analytical methods in treated and filtered Carson NFH spring water (pH = 6.8, total hardness = 16.3).**

Sample date and time	Analytical method			
	Colorimetric		HPLC <sup>a</sup>	
	Infl uent	Effluent	Influent	Effluent
08/08/88				
0800	12.17	<0.01	13.02	<0.1
<b>1300</b>	<b>10.07</b>	<b>&lt;0.01</b>	<b>12.19</b>	--
08/15/88				
0800	<b>9.23</b>	<b>&lt;0.01</b>	10.30	<0.1
1300	<b>9.23</b>	<b>&lt;0.01</b>	10.60	<0.1
<b>09/02/88</b>				
0800	9.12	0.01	10.99	<0.1
1300	9.23	0.02	11.17	<0.1
09/27/88 <sup>b</sup>				
1045	8.70	0.03	8.08	0.018
09/28/88 <sup>c</sup>				
0845	8.50	0.03	7.51	0.017

<sup>a</sup>High Performance Liquid Chromatography analysis at the National Fisheries Research Center, La Crosse, Wisconsin.

<sup>b</sup>Flow-through single chamber at about 1,000 gpm

<sup>c</sup>Flow-through single chamber at about 1,250 gpm

**Appendix 7. Concentrations (mg/L) of malachite green as determined by two analytical methods in treated and filtered Abernathy Salmon Culture Technology Center creek water (pH = 7.7, total hardness = 16.2 mg/L).**

Sample date and time	Analytical method			
	Colorimetric		HPLC <sup>a</sup>	
	Influent	Effluent	Influent	Effluent
<b>08/12/88</b>				
<b>0900</b>	9.59	0.07	11.05	<0.1
<b>1300</b>	<b>9.10</b>	0.06	11.77	<0.1
<b>08/26/88</b>				
0900	9.23	0.08	10.37	<0.1
1300	10.04	0.07	10.46	<0.1
<b>09/02/88</b>				
0900	8.78	0.07	10.18	<0.1
<b>1300</b>	9.05	0.08	10.74	<0.1
<b>10/07/88<sup>b</sup></b>				
<b>0900</b>	0.86	0.02	0.770	0.018
<b>0930</b>	0.98	0.03	0.862	0.025
<b>1000</b>	0.36	0.02	0.240	0.011
<b>10/14/88<sup>b</sup></b>				
0800	0.84	0.02	0.810	0.027
0830	1.01	0.04	1.020	0.044
0900	0.20	0.02	0.162	0.024
<b>10/21/88<sup>b</sup></b>				
0800	0.80	0.02	0.770	0.018
0830	0.97	0.04	1.050	0.031
0900	1.00	0.04	1.130	0.033
<b>11/15/88<sup>c</sup></b>				
1357	8.72	0.04	8.863	0.035
1358	8.34	0.04	8.964	0.039
<b>11/16/88<sup>d</sup></b>				
<b>1308</b>	12.41	0.14	12.26	0.139
<b>1309</b>	11.84	0.14	12.01	0.146
<b>11/28/88<sup>e</sup></b>				
<b>0914</b>	11.27	0.04	10.10	0.025
<b>0916</b>	11.46	0.03	9.94	0.026

<sup>a</sup>High Performance Liquid Chromatography analysis at the National Fisheries Research Center, La Crosse, Wisconsin.

<sup>b</sup>Abernathy well water (pH = 7.8, total hardness = 88.41 mg/L).

<sup>c</sup>Flow-through single chamber at about 100 gpm

<sup>d</sup>Flow-through single chamber at about 150 gpm

<sup>e</sup>Series flow through both chambers at about 150 gpm