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# The effects of malachite green on the composition of the blood of rainbow trout

by

Roger Robert Hlavek

A Dissertation Submitted to the
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# INTRODUCTION

Effective control of toxic substances and disease is often the major factor determining success of fish culture or management of wild fish populations. Even subtle physical changes in aquatic environments may result in stress leading to an impairment of animal health or deterioration of the quality of tissue for human consumption (National Research Council, 1973). Rapid and accurate detection of sublethal toxic effects and of impending disease is vital to timely control.

Fish blood is being increasingly studied for toxicological research, for environmental monitoring, and as a possible indicator of physiological or pathological change in fishery management and disease investigations (Mulcahy, 1975). The great value of a study of fish blood lies in its possibilities of revealing conditions within the body long before there is any outward manifestation of disease (McCay, 1928). Since only a thin epithelial membrane separates the blood of the fish from the water in which it swims, every unfavorable environmental change should be reflected in the circulatory system.

The basis for the present study stems from a report that malachite green, a common hatchery therapeutant used in fish culture for decades, may have a leukocytotoxic effect in young salmonids. Glagoleva and Malikova (1968) indicated a severe leukopenia in Baltic salmon (Salmo salar) averaging 5 gm in weight 1 to 2 days after exposure to concentrations of malachite green as low as 1.33 ppm for 20 to 60 min.

Malachite green (4-[p-(dimethyl amino)-α-phenyl-benzidine] -2,5 cyclohexadien-1-ylidene dimethyl ammonium chloride) is a chemically active,

colored organic compound belonging to one of the largest groups of synthetic dyes, the triphenyl methane derivatives. It is a diamino derivative of triphenyl methane and was first synthesized by Fischer in 1877 (Ioachim, 1963) and again, independently, in 1878 by Döbner (Werth, 1967). Malachite green was initially used in the chemical and dye industries, but biological and medical workers soon became interested in the compound also (Werth, 1967). Nelson (1974) has prepared a comprehensive review of the literature on the use of malachite green in fisheries.

The objectives of my research were 1) to determine if malachite green causes a reduction in white cell count in juvenile rainbow trout (Salmo gairdneri); 2) if so, to determine the duration of leukopenia; 3) to determine effective exposure time and dosage to induce this condition; and 4) to provide specific information on the safe therapeutic and prophylactic levels of malachite green to facilitate its registration with the Food and Drug Administration and Environmental Protection Agency in accordance with more stringent regulations (Lennon, 1967). Information required for its registration covering toxicity, residues, efficacy, metabolites, and counteractions is incomplete (Bills et al., 1977).

One of the more important aspects of the use of fish hematology in research and in practice is the determination of normal or nonpathological values, which must be known in order to recognize pathological states (Hickey, 1976). Normal blood values may differ considerably among laboratory, wild, and hatchery fish due to differing environmental conditions. Because fish are creatures of their environment and are influenced accordingly, a "normal" value for one group of fish in one water system may be indicative of an abnormal condition for another group of fish of the same

age and species in another water system (Klontz and Smith, 1968). Field et al. (1943) pointed out that a "normal" fish is difficult to define, especially fish used in experiments, because they have been removed from their natural habitat and diet and placed in an artificial confining environment. Snieszko (1960) noted that even in "normal" fish the composition of blood may show considerable variation, and that it is necessary to determine the amplitude of such "normal" variation before examination of the blood can be used for the detection of "abnormal" or pathological conditions. Blood composition is modified by many factors, such as handling, stress, inflammation, metabolism, age, maturity, sex, diet, strain, and pollution (Mulcahy, 1975). Water temperature and chemistry and the number of fish per unit water volume must also be considered (Klontz and Smith, 1968). The vagueness of the concept of "normal" led Klontz et al. (1964)[1] to believe at that time it was not possible to produce a table of normal values for fish blood which might be used with "even the remotest degree of validity" because of the variation within even a single species. Mulcahy (1975) contended that such studies can be useful provided normal blood parameters are established from a sufficiently large and representative number of control fish to reflect the normal variation under the conditions of the study. In the future, hematology will no doubt play a large part in the diagnosis of disease in fish, but unless more basic research is done to establish more normal hematological ranges, this future will always remain distant (McCarthy et al., 1973). Reichenbach-Klinke and Elkan (1965) stated that the usefulness of blood counts in the diagnosis of fish

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[1] G. W. Klontz et al., unpublished report, 13 pp., University of Idaho.

diseases is limited because of the lack of normal species values. Blaxhall and Daisley (1973) felt the leukocyte total and differential counts could occupy as important a place in fish studies as they do in human medicine, but at present there is not sufficient evidence to prove the usefulness of differential white cell counts in fish. Such evidence will accumulate if the test is increasingly used by interested workers.

Katz (1950) believed that the use of stained differential blood smears would be a valuable aid in the diagnosis of fish diseases and could be applied with little trouble to hatchery problems by the pathologist, especially if the characteristic blood picture is reported along with descriptions of the lesions and bacteriological findings. Belova (1966) felt blood smears could give evidence of the intensity of the processes in hematopoietic organs and thus reflect the reactions of the hematopoietic system to factors in the fish's environment.

In a general discussion of normal human clinical hematological values, Beeler (1968) suggested that if test results conform reasonably well to a Gaussian distribution, values lying within 2 standard deviations of the mean might be considered compatible with health, those between 2 and 3 standard deviations as possibly indicative of disease, and greater than 3 standard deviations as probably indicative of disease. Lehmann et al. (1976) have adopted a similar classification in the most thorough enumeration of rainbow trout clinical hematological values published to date. They considered 1 standard deviation as normal range, 2 standard deviations as tolerable or acceptable, and 3 or more standard deviations as a warning level.

Another factor which contributes to the lack of agreement on normal fish hematology is the degree of difficulty in

identifying the blood cells, especially the leukocytes. Unlike mammals, undifferentiated white blood cells are often found in the peripheral circulation of healthy fish (Yuki, 1957). Watson et al. (1956) noted that reports on the morphology of normal fish blood cells are not scarce but are often contradictory. Since 1878 when Ehrlich classified the granular leukocytes of mammals on the basis of their staining reactions, many attempts have been made to apply this classification to the whole vertebrate kingdom (Duthrie, 1939). Downey (1909) found that while leukocytes in fish have a tendency to follow Ehrlich's scheme, they do not do so completely. Anderson (1974) pointed out that rainbow trout have many blood cells which are visually similar to mammalian blood cells, so that direct comparisons are often made. This field of study should remain fairly subjective until direct biochemical evidence is available to show the exact identity of the cells. Bell (1976) cautioned that leukocyte names based on morphological similarity alone may make unwarranted implications about the origin and function of the cell. Differences are probably related to differences in the sites of hematopoeisis. The anterior kidney and spleen are the major sites in fish instead of the bone marrow as in mammals (Klontz and Smith, 1968).

Interestingly, Fey (1966) found that granulocytes of 25 kinds of lower vertebrates were homologous to those of mammals, based on conventional electron microscope and cytochemical methods. However, there is still no firm cytological or physiological basis available for establishing a uniform nomenclature for specific types of leukocytes in teleosts (Dolsky, 1971).

In summary, problems in the use of fish blood parameters in disease diagnosis include determination of normal values for each species under different conditions, the wide range in values among healthy fish, and difficulty in identifying types of blood cells. Despite these problems, it seems that fluctuations of the blood cell constituents are utilizable as biological indicators in the study of fish physiology (Yuki, 1957).

Changes in the composition of the blood have also been related to stress. Stress has been defined as "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force" (Selye, 1950). Brett (1958) considered stress as a "state produced by any environmental or other factor which extends the adaptive response of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are reduced." He distinguished between a discriminate stress, which applies at any one time to individuals singly within a population and not to a group or stock as a whole, and indiscriminate stress, which applies to every member and is not discrete in its action. Physical and chemical stresses are almost invariably indiscriminate. Stress, in the widest sense of this word, is a very important factor in the health of aquatic animals. Therefore, in aquaculture the aim of management should be to reduce stress as much as possible to improve the health of the cultured animals, to reduce loss of animals from diseases, and to avoid low production (National Research Council, 1973). Brett (1958) noted that the assessment of stress cannot involve simply the determination of any change induced in the internal state of the animal. Physiological homeostatis

means a continual return to a mean level, rather than a steady state. The range of normal metabolic levels must be established before deviations can be attributed to stress. Stress caused by biological, physical and chemical factors reflects itself in various changes in the absolute and relative abundance of blood cells. Wedemeyer (1970) discussed general effects of stress on fish and indicated a common condition is a decreased number of circulating lymphocytes. This condition frequently leads to leukopenia because lymphocytes constitute the most abundant white blood cells in fishes (Yokoyama, 1960). Heck (1955) noted that leukopenia in rabbits, and at times loss of lymphocytes, resulted from many drugs. Not all of the drugs contained the benzene ring, but most were ring compounds of one type or another. Bushby (1970) indicated that depression of leukopoiesis is the commonest toxic action of drugs on leukocytes, but a rise in circulating neutrophils may also occur. In the study of Glagoleva and Malikova (1968) on malachite green-induced leukopenia, differential counts of white blood cells approximated a characteristic vertebrate stress syndrome (Selye, 1950).

Information on blood parameters offers potential for the evaluation of physiological responses to certain forms of stress (Houston and De Wilde, 1968). However, differing opinions regarding the validity of this kind of approach have been expressed.

Wedemeyer and Wood (1974) felt that quantitating the stress response in fish in terms of cellular elements (leukopenia, leukocytosis, eosinophilia) is of limited usefulness. Lehmann et al. (1976) indicated that it is still not practical to use hematological values for reliable diagnosis of disease in rainbow trout. Some studies have shown total white

cell count to be a somewhat insensitive indicator. Mulcahy (1975) obtained similar leukocyte counts in both control and Ulcerative Dermal Necrosis infected salmon, and Reichenbach-Klinke (1966) found that white cell count in diseased or parasitized whitefish (Coregonus wartmanni), and trout exposed to ammonia or copper sulfate, showed no definite trend with concentration of the stressor. However, Belova (1965) felt that the leukocytes react rapidly to a change in external conditions and serve as a more reliable indicator of short-term changes and stress than hematocrits, erythrocyte counts, or any other hemotological parameter. McLeay and Gordon (1977) support this suggestion. Their findings showed that coho salmon (Oncorhynchus kisutch) exposed to bleached kraft mill effluent responded with a leukopenia within several hours.

Slicher et al. (1966) demonstrated that sheepshead minnows (Fundulus heteroclitus) that were not accustomed to handling had significantly lower white cell counts than specimens which were used to this routine disturbance 2 to 4 weeks before examination. Bouck and Ball (1966) pointed out that various methods are used to capture fish for blood studies, and although none of these methods are known to cause a physiological stress reaction, they were aware of no studies which showed this was not the case.

Several studies employing cold shock as a stressor have shown an initial leukopenia at 1 hr followed by leukocytosis at 2 hr in sheepshead minnows and sailfin mollys (Poecilia latipinna) (Slicher and Ball, 1962a, 1962b; Slicher et al., 1962). Bennett and Neville (1975) found a significant alteration of the leukocyte percentages in 2 hr among cold-shocked goldfish (Carassius auratus). Enomoto (1969a) observed

lymphopenia following cold shock in filefish (Rudarius ercodes).

Colgrove (1966) noted a profound shift in the leukocyte distribution of sockeye salmon (Oncorhynchus nerka) in spawning condition. The highest white cell counts were found in early pre-spawning fish, with a mean value of 23,800/mm$^3$. In general, he found that the proportion of neutrophils increased from sea to spawning, whereas the proportion of lymphocytes progressively decreased. The proportion of neutrophils was highest in spawned-out fish. In salmon at sea, segmented neutrophils comprised about 9% of the white cells, while these cells constituted approximately 47% of the leukocytes in the spawned-out fish; lymphocyte fractions declined from 86% of the white cells to 28%.

Tugarina and Ryzhova (1970) noted a reduction of lymphocytes from 90-96% to 77-85% and an increase in polymorphonuclear neutrophils in the leukocyte formula of the black Baikal grayling (Thymallus arcticus baikalnesis) during and after the spawning period. Robertson and Wexler (1960) noted a decrease in the number of lymphoid cells in the spleen of maturing salmon of the genus Oncorhynchus. At full sexual maturity, the lymphocytes showed marked depletion to complete disappearance. Naumov (1959) observed a similar situation in spawning Murmansk herring (Clupea harengus harengus). Lymphocytes declined from 87.5 to 63.3%, while neutrophils increased from 4.5 to 16%. Einszporn-Orecka (1973) found an increase in PMN in male tench (Tinca tinca) after they spawned and again during the winter months of November and January. Female tench exhibited a third peak of neutrophilia in April and May. A pronounced lymphopenia accompanied each increase in PMN.

Changes in the differential leukocyte count were used as an indicator of physiological change in fathead minnows (Pimephales promelas) and long-nose dace (Rhinichthyes cataractae) exposed to acid stress for 28 days (Dolsky, 1971). At a pH of 5.9, lymphocyte percentages declined from about 91 to 52%, while neutrophil percentage increased from 8.6 to 46.6%. Fathead minnows exposed for 20 hr in two acidic streams (pH 5.2 and 4.8) showed a reduction in lymphocytes from 93.4 to 58.8% and from 91.3 to 77.6%. Vaala (1971) found a lymphopenia and neutrophilia in brook trout (Salvelinus fontinalis) acutely exposed to low pH.

Quick and Henderson (1974) noted leukocytopenia and thrombocytopenia in ladyfish (Albula vulpes) and mullet (Mugil cephalus) exposed to the red tide dinoflagellate, Gymnodinium brevis. Klontz et al. (1966) noted a significant decline in leukocyte counts 64-80 hr after rainbow trout were injected with the causative agent of furunculosis. White cell counts dropped from 15,600 to less than 500 per $mm^3$ in infected fish.

Kawatsu (1975) fed brook trout a folic acid-deficient diet for 24 weeks, at the end of which time he noted that the number of neutrophils was higher, but the number of lymphocytes showed no remarkable tendency in their numerical changes. A small sample size may have obscured his results.

Smirnova (1965) noted that the number of leukocytes in the burbot (Lota lota) was reduced by fasting. On the 15th day, white cell number was one-third of that in the control fish. Leukocyte numbers subsequently increased and by the 145th day again reached the maximal values. Fasting increased the percentage and absolute numbers of phagocytes (probably

implying neutrophils). After feeding, the total numbers of leukocytes increased, owing to an increase in the number of lymphocytes (implying the stress of fasting had caused a lymphopenia). The absolute number of phagocytes was reduced. Smirnova attributed the increase in leukocyte number to the fact they are directly involved in the digestive processes of fish and in the transport of fat particles and the secretion of digestive enzymes. Johansson-Sjöbeck et al. (1975a) studied the effects of starvation in eels (Anguilla anguilla) through 164 days and detected a successive decrease in white blood cell count during the entire starvation period.

Robertson et al. (1963) found that hydrocortisone caused a marked decrease in the number of lymphocytes in the thymus of immature rainbow trout. A moderate to profound reduction in lymphocyte numbers was also found in the spleen.

DeLaney et al. (1976) found that the number of macrophages and small lymphocytes decreased markedly in lungfish (Protopterus aethiopicus) during estivation, while the number of thrombocytes generally increased. They felt that the initial neutrophilia during the first month of estivation may have been a stress-related phenomenon.

Waluga (1966) noted a decrease of 40,000 leukocytes/$mm^3$ in bream (Abramis brama) exposed to phenol when compared to control fish. He could not make differential counts because blood cells were too damaged by the phenol.

Smirnova (1968) established a connection between the leukocyte composition of the blood and degree of satiation in fed and starving bream. He observed an increase in the number of circulating granulocytes in several

situations, such as intensive feeding, temperature increase, increased activity, electrical stimulation, and exposure to 2-methyl-5 vinyl pyridine.

Casillas (1974) found that the effect of hooking stress on the quantity of circulating thrombocytes was very dramatic in two varieties of rainbow trout. In his study, surprisingly, the lymphocytes did not respond to short-term stress, but this situation may have been due to his method of counting lymphocytes from fixed blood smears.

Johansson-Sjöbeck et al. (1975b) fed polychlorinated biphenyl to rainbow trout (1000 mg/kg body weight) every second day for up to two weeks. They noted no changes in the total leukocyte count or in the percentage of different white cells between test and control fish. Similarly, Amend and Smith (1974) found no change in the differential or total leukocyte count in rainbow trout infected with infectious hematopoietic necrosis.

McLeay (1973a) found that ACTH injections caused a marked decrease in the number of circulating small lymphocytes in juvenile coho salmon, while the percentage of large lymphocytes and neutrophils increased. McLeay (1973b) also found that all dosages of cortisol and dexamethasone used caused a marked decrease in the number of small circulating lymphocytes. Thrombocyte counts were significantly decreased by dexamethasone but not by cortisol injections.

Saad et al. (1973) noted a general increase in total white cell counts of Tilapia zilli taken from polluted waters, but their data on differentials do not appear to indicate a significant stress syndrome. Linn (1963) exposed goldfish to radium and found that white cell count increased

linearly as the radium content of the water increased following 5-days exposure at 60 F. Values returned to normal by the 10th day. After fish were exposed for 10 days at 50 F, Linn noted high white cell counts in low radium treatment levels and lower counts at high treatment levels. There was a return to normal leukocyte level by the 15th day. He attributed the changes in white blood cell number to the granulocytes because they contributed to the increased counts and were also absent in the decreased count. Sheckmeister et al. (1962) found that 100-5000 r caused a sharp decrease in the number of various leukocytic elements in the peripheral blood of goldfish within 24 hr. The recovery schedule was not reported.

Shpolyanskaya (1953) recorded an increase in blood monocytes and polymorphonuclears in crucian carp (Carassius carassius) infected with Ligula. Yokoyama (1960) noted an increase in white cell count, especially PMN, in pathological specimens of yellow perch (Perca flavescens). However, Khan (1977) found no changes in the differential blood picture of Atlantic cod (Gadus morhua) infected with Trypanosoma mumanensis.

Enomoto (1969b) detected an increase in immature leukocytes in ayu (Plectoglossus altivelis), yellowtail (Serolia quinqueradiata), eel (Angilla japonica), and rainbow trout when these fish were diseased or had inflammations. He felt the immature leukocytes may be immature granulocytes (possibly indicating impending neutrophilia).

Belova (1965) found that transporting humpback (pink) salmon (Oncorhynchus gorbuscha) fry under crowded conditions in polyethylene bags led to a sharp increase of up to 16% in phagocytic elements (monocytes and polymorphonuclear leukocytes) which resulted in a considerable reduction to 24% in the percentage of lymphocytes.

Hines and Spira (1973) noted that the overall white blood cell count in mirror carp infected with Ichthyophirius multifiliis remained within the range found in healthy carp (Cyprinus carpio) under similar conditions, with a concurrent rise in neutrophil percentage early in the infection. Leukocyte changes were similar to those found in other diseases and also in certain stress conditions.

Lagler et al. (1962) reported that a Pseudomonas infection in carp caused a reduction in lymphocytes from 92.0% to 49.4% of the total, while polymorphonuclears increased from 2.3% to 12.6%. Belova (1966) noted a high percentage of PMN in young pink salmon reared in hatcheries. However, he considered this a sign of normal blood formation because pink salmon from natural conditions also had high percentages of PMN.

Pickford et al. (1971) found that the response 2 hr after the injection of ACTH into intact sheepshead minnows depended on the dose: leukopenia occurred at low doses, leukocytosis at high doses. The 2-hr response to cortisol (5 ug/g) depended on the condition of the fish: leukocytosis occurred in sexually mature fish, leukopenia in sexually regressed fish.

McLeay (1973c) exposed juvenile coho salmon to pulp mill effluent for 12 hr or 25 days. The number of circulating lymphocytes decreased markedly after a 12-hr exposure. However, following the prolonged exposure, the number of lymphocytes returned to normal, whereas the number of circulating neutrophils increased. In another experiment, McLeay (1975) again exposed juvenile coho salmon to pulpmill effluents and found that white cell counts decreased markedly from 12-96 hr afterward, with greatest decline occurring

24 hr after. Leukocyte levels returned to stock values within 2-4 days, following the initial decline. McLeay felt the leukocyte response provided a reasonably rapid and sensitive method for measuring stressful levels of pulpmill effluents to salmon. Decline in white blood cell counts is attributable to reduced numbers of circulating lymphocytes, which could result in decreased resistance of stressed fish to disease. Mounting evidence strongly suggests lymphocytes are the executive cells of the specific immune mechanisms in fish (Ellis, 1977).

Iwama et al. (1976) found that total leukocyte counts in coho salmon decreased after 24 hr exposure to dehydroabietic acid (DHAA), a major component of kraft pulpmill effluent. Hatai (1972) found an increase in the proportion of neutrophils and a decrease in lymphocytes after 1 day in eels (Anguilla japonica) injected with pathogenic strain Y-62 of Aeromonas liquifaciens.

In recent studies on the effects of malachite green on fish hematology, Bills and Hunn (1976) chronically exposed juvenile coho salmon to 0.1 mg/l for up to 28 days but induced only a transient leukopenia approximately 4 days post-treatment.

Grizzle (1977) noted that channel catfish (Ictaluras punctatus) chronically exposed to 0.1 ppm malachite green exhibited neutrophilia 1 and 3 days after treatment. Leukopenia was noted 21 days post-treatment, but no other significant differences were found after 28 days of exposure.

# METHODS AND MATERIALS

The rainbow trout was used as the test fish, because it offers an excellent reference standard in hematological work. The species has a worldwide distribution, is easily cultured, and control over variables such as size, age, diet, water temperature, water quality, and holding facilities can be maintained (Barnhart, 1969). Weinreb (1958) indicated that the circulatory system of the rainbow trout is sensitive to foreign stimuli and reflects the homeostasis of the fish. Mawdsley-Thomas (1971) pointed out that salmonids, particularly trout, are more susceptible to various toxic chemicals than are many of the coarse fish. Klontz et al. (1966) proposed that the rainbow trout should be the reference standard in fish hematology, because nearly any facility, particularly in the U.S. Fish and Wildlife Service, has access to these fish, even though another species is being studied.

Three different sets of experiments were conducted to evaluate effects of malachite green on rainbow trout blood. The first set concerned the sub-acute (28 day) effects of a 30-min static exposure. The second set concerned the changes in leukocyte composition for the first 24 h after static exposure to malachite green for 30 min. The final set of experiments was designed to evaluate the leukocyte response during the first 24 h after a 5-min static dip treatment in relatively high concentrations of malachite green.

In the first set of experiments, three individual trials were conducted on three different lots of fish provided by the Fish Control Laboratory, LaCrosse, Wisconsin. Before the first trial, which was conducted

in March, 1976, fish were held in a flow-through system consisting of two fiberglass tanks each divided into four individual compartments. Each compartment contained about 75 liters of water with a replacement time of about 90 min. Water temperature was maintained at 12 C with 8 hr of artificial light daily.

Ammonia ranged from 0.05 to 0.50 ppm with no trend during the course of the experiment and averaged 0.30 ppm, pH remained near 6.5, and dissolved oxygen 9-10 ppm. Water hardness was not monitored precisely. Previous measurements revealed the test and holding water in our laboratory contained 300-410 ppm $CaCO_3$.

Fish were impartially distributed to the 8 holding compartments by adding one to each compartment and repeating the process until each compartment contained 18-20 fish (Environmental Protection Agency, 1975). Fish averaged about 40 g each (S.E. 2.4) and were maintained on a diet of Purina Trout Chow with two feedings per day (Hunn et al., 1968). Excess food and egesta were siphoned out daily.

Concentrations of 0.00, 1.35, and 13.5 mg/l malachite green were used (Glagoleva and Malikova, 1968). Treatments were assigned randomly to the compartments so that each tank of 3 compartments had a control, low-, and high-concentration compartment. Fish were acclimated two weeks before testing.

The fish were statically exposed for 25 min to malachite green at 12 C $\pm$ 1 C in subdued light to reduce photosensitization (Van Duijn, 1973). Food was withheld the day before and day of the experiments. Fish were transferred from holding tanks to a test tank which contained 452 liters of solution. Fish density was 3 g/l. Fish in each test group were

simultaneously introduced and removed from the test solution. No fish perished during treatment, and all were returned to their original compartments.

Blood samples were taken 1, 4, 14, and 28 days after exposure to malachite green. Fish were captured in a small net with minimal stress (McLeay, 1975) and killed with a sharp blow to the head (Catton, 1951; Conroy, 1972). The caudal area was thoroughly blotted and the caudal peduncle then severed with a sharp blade (Blaxhall, 1972; Anderson, 1974). One fish was taken for sampling from each compartment in rotation to reduce the cumulative stress among fish in a single compartment. Ethylene diamine tetra-acetic acid (EDTA) was used as the anti-coagulant because of its preservation of blood components over a long time and its exceptionally effective anti-coagulant action (Blaxhall, 1972). A small amount of EDTA was sprinkled on a watch glass (Snieszko, 1960), the blood was allowed to drip onto the watch glass from the severed tail, and mixed with a gentle swirling action. Blood taken directly from the caudal peduncle without anti-coagulant was used for differential smears (McKnight, 1966). Blood collection was completed in about 30 seconds.

Rees-Ecker fluid (Klontz and Smith, 1968) was used as the diluent for blood counts because it can be stored in the refrigerator for months, is more stable than Shaw's two-part solution (Shaw, 1930), and does not involve mixing just prior to use as does Yokoyama's solution (Yokoyama, 1960). The blood was drawn up to the 0.5 mark in a red-cell diluting pipette and filled to the proper mark with recently filtered Rees-Ecker fluid. The pipette with blood and diluting fluid was shaken for 30-60

seconds, the first few drops were discarded, and the tip of the pipette touched to the edge of a Neubauer hemocytometer between the cover slip and chamber. Capillary action distributed the diluted cell suspension (Hesser, 1960).

The hemacytometer was placed under a microscope and allowed to settle for a minute. Blood cells were then counted according to Hesser (1960). White blood cells and thrombocytes were counted together because small lymphocytes and immature thrombocytes could not be reliably distinguished in a fresh preparation (Klontz and Smith, 1968).

Differential smears were also prepared according to Hesser (1960), fixed in methyl alcohol 3-5 min, and stored for subsequent staining with a combination Leishman-Geimsa stain (Anderson, 1974).

The Leishman stain was prepared by dissolving 0.15 gm of stain in 100 ml of absolute methyl alcohol, heating in a water bath, and filtering when cool (Gurr, 1962).

Stock Geimsa stain was made by dissolving 1.0 gm of Geimsa powder in 66 ml of glycerin, heating in a 60 C oven for 1 hr, then adding 66 ml of absolute methyl alcohol. The working Geimsa solution was prepared by diluting 3.5 ml of stock solution with 50 ml of pH 6.0 phosphate buffer (Anderson, 1974).

The fixed blood smear was flooded with Leishman stain for 3 min and covered to reduce evaporation of the methanol base. An equal amount of Geimsa working solution was then added and the combination allowed to set for 6 min. Mixing was effected by gently blowing on the stain (Ashley and Smith, 1963). A green metallic sheen appeared on the surface when properly

done. The slides were then immersed in water to float off the excess stain, rinsed in running water, and allowed to dry 24 hr before mounting medium and cover slips were applied.

Microhematocrits were measured in heparinized tubes prepared according to Larsen and Snieszko (1961). Blood was centrifuged at 7000 RPM for 5 min and the hematocrit determined from a microhematocrit tube reader card. One person did all blood work to reduce variability as much as possible (Wedemeyer and Yasutake, 1977).

A second and third trial were conducted in August and November, respectively. Ammonia (as ammonia nitrogen) levels averaged 0.2 to 0.3 ppm (range 0.10-0.55) in the holding compartments during trial 2 and 0.15 to 0.2 ppm (range 0.05-0.40) during trial 3. The pH was 8.1. Temperature was maintained at 12 C, and oxygen concentrations were maintained at saturation with airstones. Holding compartments were again about 75 liters with a turnover rate of 75-80 min.

During trials 2 and 3, 23 specimens were statically treated in 35 liters of solution (5-6 gr of fish/l) at 12 C for 30 min in subdued light. Concentrations of 0.00, 1.35, 13.5, and 21.0 mg/l were used. Fish averaged 10 g (S.E. 0.40) in weight. Again, fish were introduced and removed simultaneously as a group from the test solution. Blood samples were again taken 1, 4, 14, and 28 days post-treatment. Use of wax-coated watch glasses to collect the fresh blood greatly reduced the need for an anticoagulant. Sampling on each day required 12-13 hr, so values are actually an average for the sampling day, not precise hourly values.

Results from corresponding levels of treatment were pooled because of the great inherent variability in leukocyte counts. The analysis of

variance among the concentrations on each sampling day was examined to determine differences in the total leukocyte count and relative and absolute counts for thrombocytes, lymphocytes, and polymorphonuclear neutrophils, the three most numerous components in the differential (McCarthy et al., 1973). No distinction was made between large and small lymphocytes (Watson et al., 1963) because the division between types is somewhat arbitrary (Ellis, 1977). Differential percentages were converted to absolute numbers because these figures are much more informative (Bushby, 1970). Groups with significant F values were then analyzed by the "t" test in which mean values of the test groups were individually compared with the controls.

A second set of experiments was conducted to investigate possible effects of malachite green on leukocytes during the first 24 hr after exposure. Three trials were conducted on two different lots of fish. Fish weighed about 8 g (S.E. 0.15). After acclimation to holding compartments at 12 C and 8 hr daylight, groups of 24 fish were randomly selected and statically exposed to 0.00, 1.35, or 13.5 mg/l malachite green, respectively, for 30 min. The 21.0 mg/l concentration was not used in these experiments because it produced high mortality (up to 44%) in the previous experiment. Three fish each were then randomly distributed among the 8 experimental compartments. Fish from each compartment were randomly selected at hourly intervals, so that the 24 treated fish were sampled in 8 hr, and each group of 3 from the same compartment served as a sample representing progressive hours after treatment. Each post-treatment day was broken into three 8-hr periods for each concentration tested to encompass the first 24-hr post-exposure. Randomization of the sequence insured

a more homogeneous distribution of fish among the concentrations so that size stratification of the original lot of fish in the holding tank would not be a factor. Hematological methods were the same as in the long-term study.

Linear regressions were computed for total white blood cell-thrombocyte count, relative and absolute thrombocyte, lymphocyte, and polymorphonuclear leukocytes, and hematocrit versus time to see if the slopes differed significantly from 0 during the first 24 hr after exposure. Separate linear regressions were computed for total leukocyte count and total lymphocyte count to see when the greatest effect occurred. McLeay and Gordon (1977) noted little change in leukocyte composition in the first few hours after they exposed fish to stressors, so I divided the first 24 hr into the following time periods: less than 3 hr, 3-8 hr, and 8-24 hr. Slopes were then determined for these segments. The latter two periods (3-24 hr) were also combined and fit to a curvilinear model.

Another method to determine the greatest period of stress was to compute the ratio between the pooled average lymphocyte count and the pooled average of the polymorphonuclear leukocyte count for each hour or day sampled. Michael (1949) has shown that this ratio is reduced by stress.

A final set of experiments was conducted in duplicate to determine the leukocytic effect of a brief dip exposure to strong concentrations of malachite green, a common hatchery practice used to control ectoparasites (Foster and Woodbury, 1936; Davis, 1953; Hoffman, 1970). I used concentrations of 0.00, 42.0, and 75.0 mg/1. Fish averaged 18 g (S.E. 0.75) in weight. Fish were acclimated as in the previous experiments, immersed in groups of 15 in the test solution for 5 min, removed as a group, and

returned to fresh water. Samples of 3 fish were taken immediately after exposure and at 2-, 4-, 6-, and 24-hr post-exposure. Again, the sequence was randomized. Data were analyzed by the Statistical Analysis System. The analysis of variance was used to detect any significant differences among groups at each sampling period. The "t" test was used to determine which test groups differed from the control.

# RESULTS

The three most prevalent leukocytes (thrombocyte, lymphocyte, and polymorphonuclear leukocyte) were categorized mainly by the schemes of Lehmann and Stürenberg (1976) and Finn and Nielson (1971).

The young or not fully mature thrombocyte is ovoid, with faint blue cytoplasm. The immature nucleus is kidney-shaped, usually with a noticeable indentation. As the cell matures, it elongates, and a fully mature thrombocyte commonly has two "spikes" of cytoplasm at either end of the nucleus. These spindle shaped cells may be from 11-17μ long and 2-8μ wide.

The stained lymphocyte looks much like its mammalian counterpart. It is rounded, 7-8μ in diameter, with sparse, deep blue cytoplasm, which frequently has irregular pseudopod-like blebs (Wedemeyer and Yasutake, 1977).

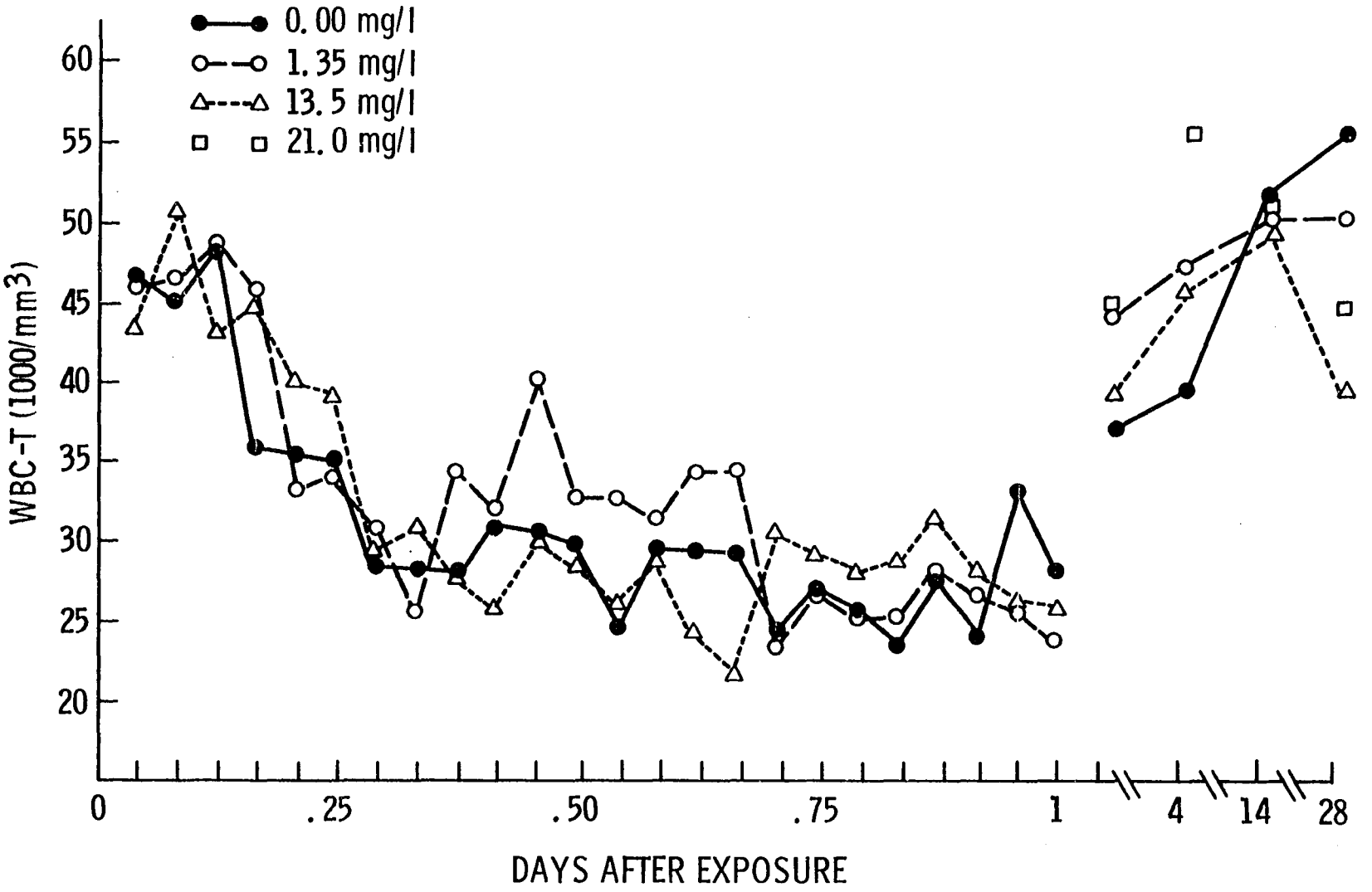
Polymorphonuclear leukocytes (PMN) may be up to 13μ in diameter. They have a light gray cytoplasm and a lobed nucleus, with 2-5 lobes. Most mature PMN's observed had 3 lobes.

The total white blood cell-thrombocyte count (WBC-T) decreased significantly ($P=.0001$) during the first 24 hr after 30 min exposure in controls and in 1.35 and 13.5 mg/l test groups, following an initial lag period of 3-4 hr (Figure 1). The response in WBC-T during the first 24 hr after exposure was not significantly different among control and test groups, probably due to handling. All could be described by the same curvilinear equation for values beyond the 3rd hr post-treatment:

$$WBC\text{-}T = 48.329 - 2.3628\ Hr + 0.0638\ Hr^2$$

where WBC-T is in terms of $1000/mm^3$ and Hr is hrs after treatment.

Figure 1. Changes in average total white blood cell-thrombocytes counts of rainbow trout statically exposed to malachite green for 30 min


0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
WBC-T (1000/mm3)
60
55
50
45
40
35
30
25
20
0
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

A significant increase (P=.01) occurred in the WBC-T count in fish exposed to 21.0 mg/l on the 4th post-treatment day as compared to the controls. Leukocyte totals were similar in magnitude in all groups by the 14th day after exposure, although a significant decrease (P=.01) occurred on the 28th day in fish exposed to 13.5 mg/l when compared to controls. This decline was probably a tank effect, because WBC-T counts in fish treated with 21.0 mg/l were not significantly different from control values, although the average number of leukocytes was lower. The standard error in all samples was large, comparable to those found by Lehmann et al. (1976) in rainbow trout and McLeay (1975) in coho salmon.

Regarding thrombocytes, the percentage of this cell type increased significantly (P = .0001) in all test groups and control within 24 hr of exposure, indicating a relative thrombocytosis (Figure 2). The control and 1.35 mg/l groups showed no significant increase in absolute numbers of thrombocytes during the first 24 hr, but I noted a significant increase (P=0.0001) in total thrombocyte number during this time period in the 13.5 mg/l group. An absolute as well as relative thrombocytosis was indicated (Figure 3). A significant increase (P=0.01) occurred in the absolute number of thrombocytes in fish exposed to 21.0 mg/l when compared to controls on the first day post-treatment. Fish in the 13.5 mg/l group had a higher average number of thrombocytes per $mm^3$ of blood than did fish from the control and 1.35 mg/l groups. Standard errors were larger in the groups exposed to the two higher concentrations of malachite green. After the first post-treatment day, thrombocytes exhibited no significant changes in relative percentage or absolute number on any subsequent day.

Figure 2. Changes in relative thrombocyte percentage in differential leukocyte counts of rainbow trout statically exposed to malachite green for 30 min

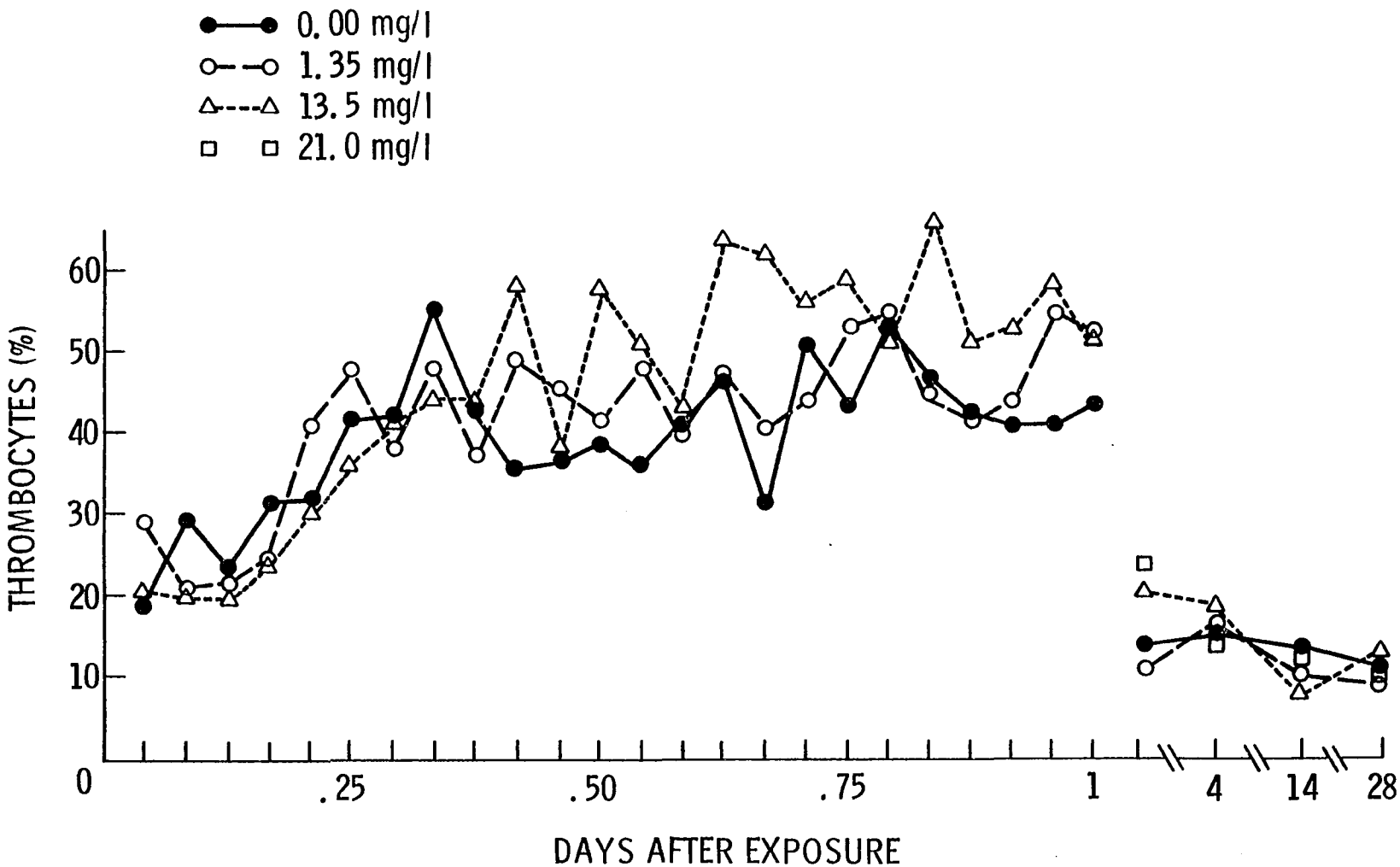
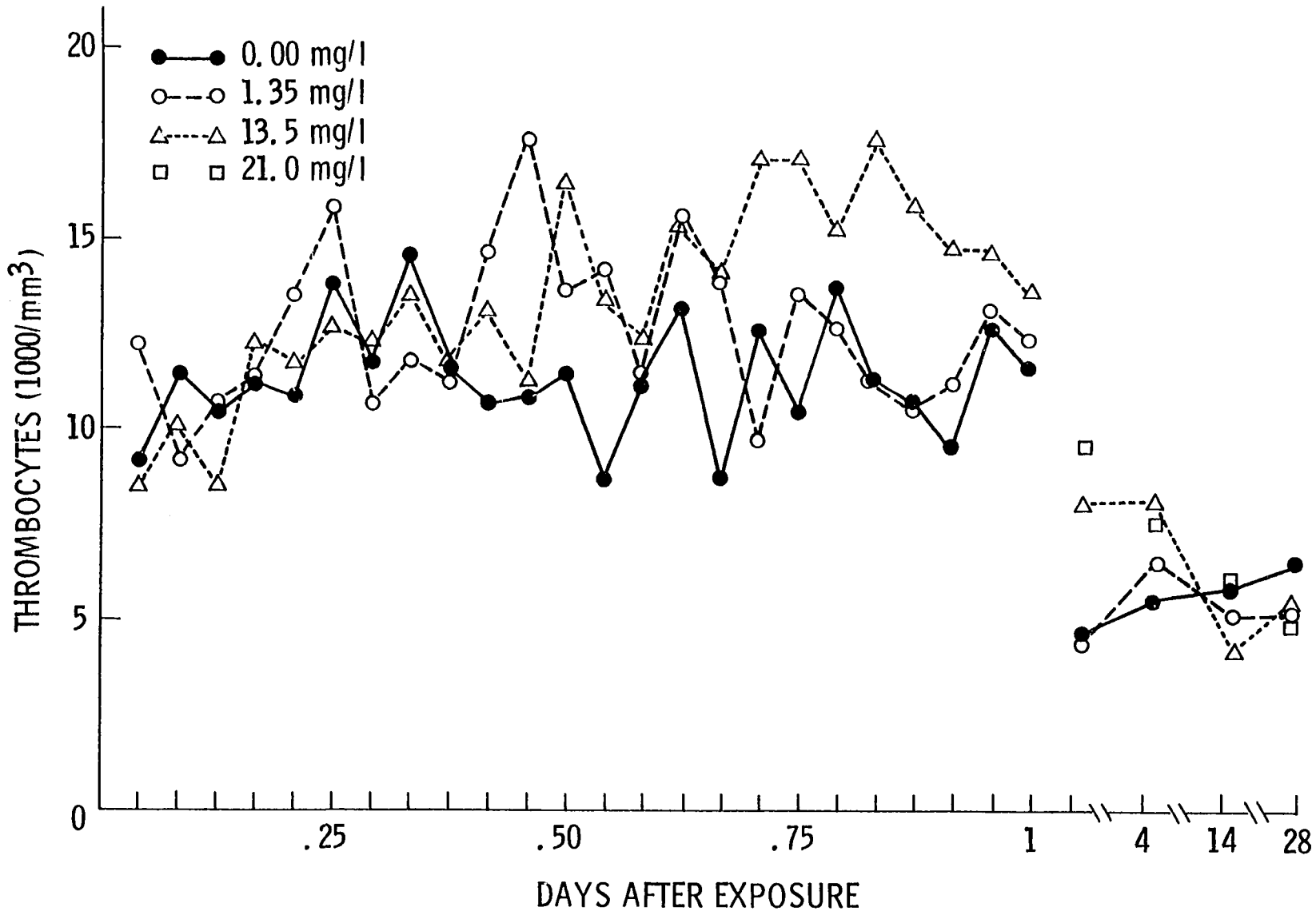

0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
THROMBOCYTES (%)
60
50
40
30
20
10
0
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

Figure 3. Changes in the absolute number of thrombocytes in the differential leukocyte counts of rainbow trout statically exposed to malachite green for 30 min


0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
THROMBOCYTES (1000/mm3)
20
15
10
5
0
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

In all test groups and control, both a relative and an absolute lymphopenia developed within the first 24 hr after exposure (Figures 4 and 5). All groups were not significantly different in total number of lymphocytes, and responses were described by the same curvilinear equation beyond the 3rd hour after exposure.

$$\text{Lymphocytes} = 37.543 - 2.795\ \text{Hr} + .00779\ \text{Hr}^2$$

where lymphocytes are in terms of $1000/mm^3$ and Hr is hrs after treatment.

A significant decrease (P=0.05) occurred in the percentage of lymphocytes in both the 13.5 and 21.0 mg/l groups when compared to control values on the first day after exposure. Fish exposed to 13.5 mg/l also had an overall decrease in lymphocyte percentage when compared to the control and 1.35 mg/l results. No other significant changes were found in this parameter on the 4th, 14th, or 28th days post-treatment.

The absolute number of lymphocytes showed no significant changes on the first day post-treatment. Values for fish from the 21.0 mg/l group, however, were significantly higher (P=0.05) on the 4th day after exposure. The number of lymphocytes in control fish and all three test groups was almost identical by the 14th day after exposure and remained at a similar level, except for a significant (P=0.05) decline in fish from the 13.5 mg/l group on day 28. This drop in lymphocytes parallels that of the total WBC-T count because the lymphocyte is the predominant leukocyte in the peripheral circulation of the rainbow trout.

Concerning polymorphonuclear leukocytes or neutrophils, the fish exposed to either 1.35 or 13.5 mg/l developed neutrophilia during the first 24 hr after exposure (P=0.0001), but the control fish did not (Figure 6). Only the 13.5 mg/l group showed a significant absolute neutrophilia during

Figure 4. Changes in the relative lymphocyte percentage in the differential leukocyte counts of rainbow trout statically exposed to malachite green for 30 min

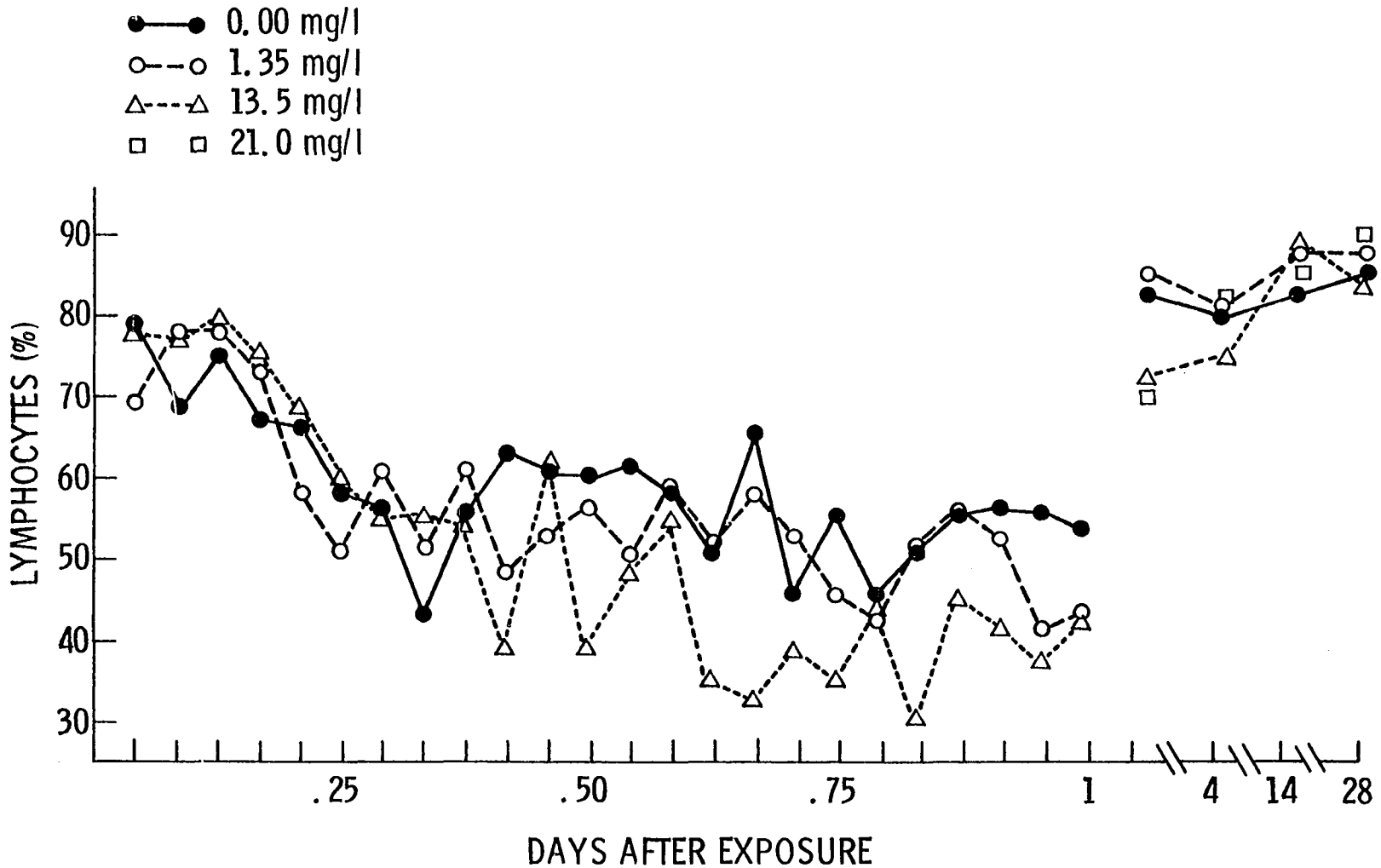

0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
LYMPHOCYTES (%)
90
80
70
60
50
40
30
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

Figure 5. Changes in the absolute number of lymphocytes in the differential leukocytes count of rainbow trout statically exposed to malachite green for 30 min

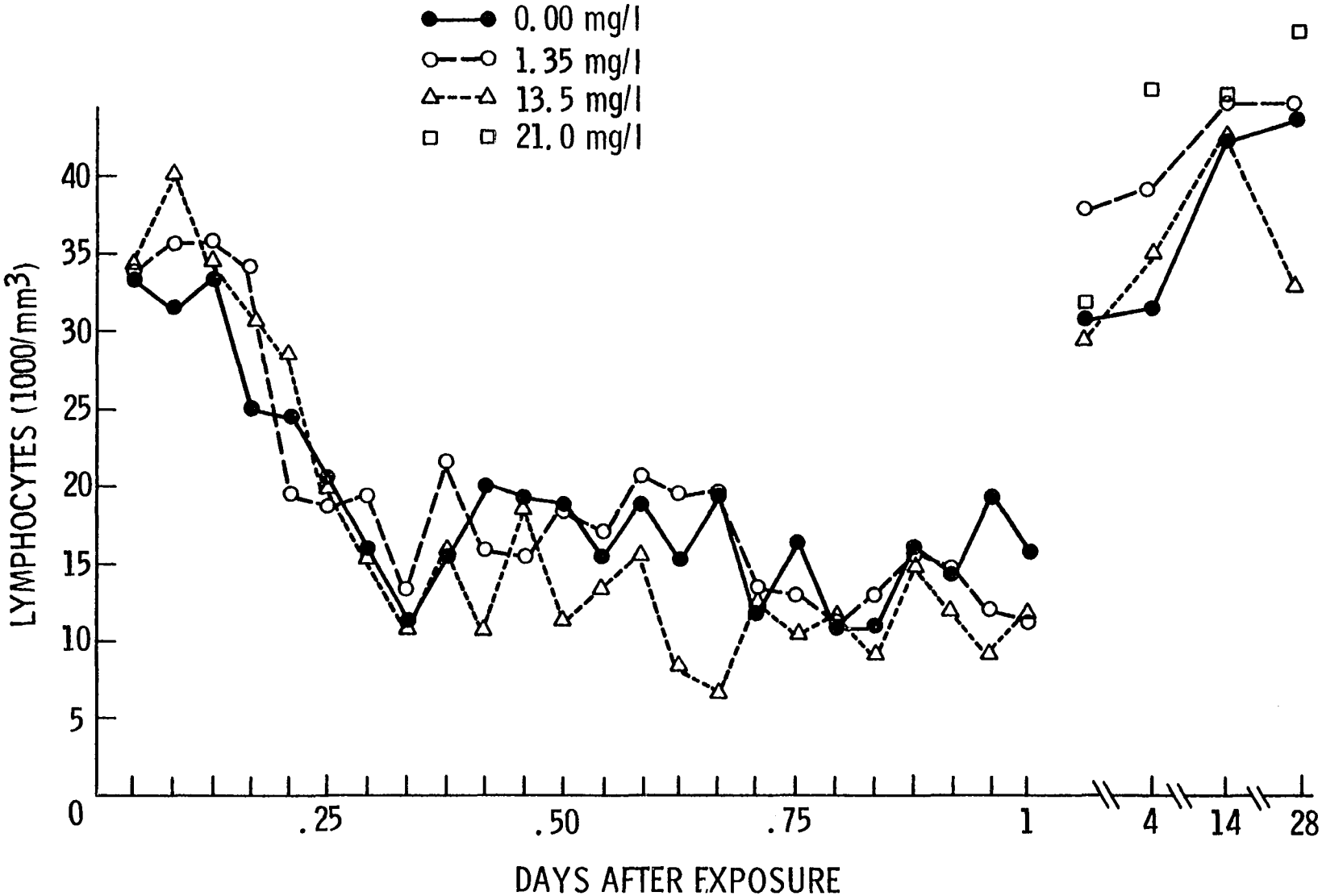
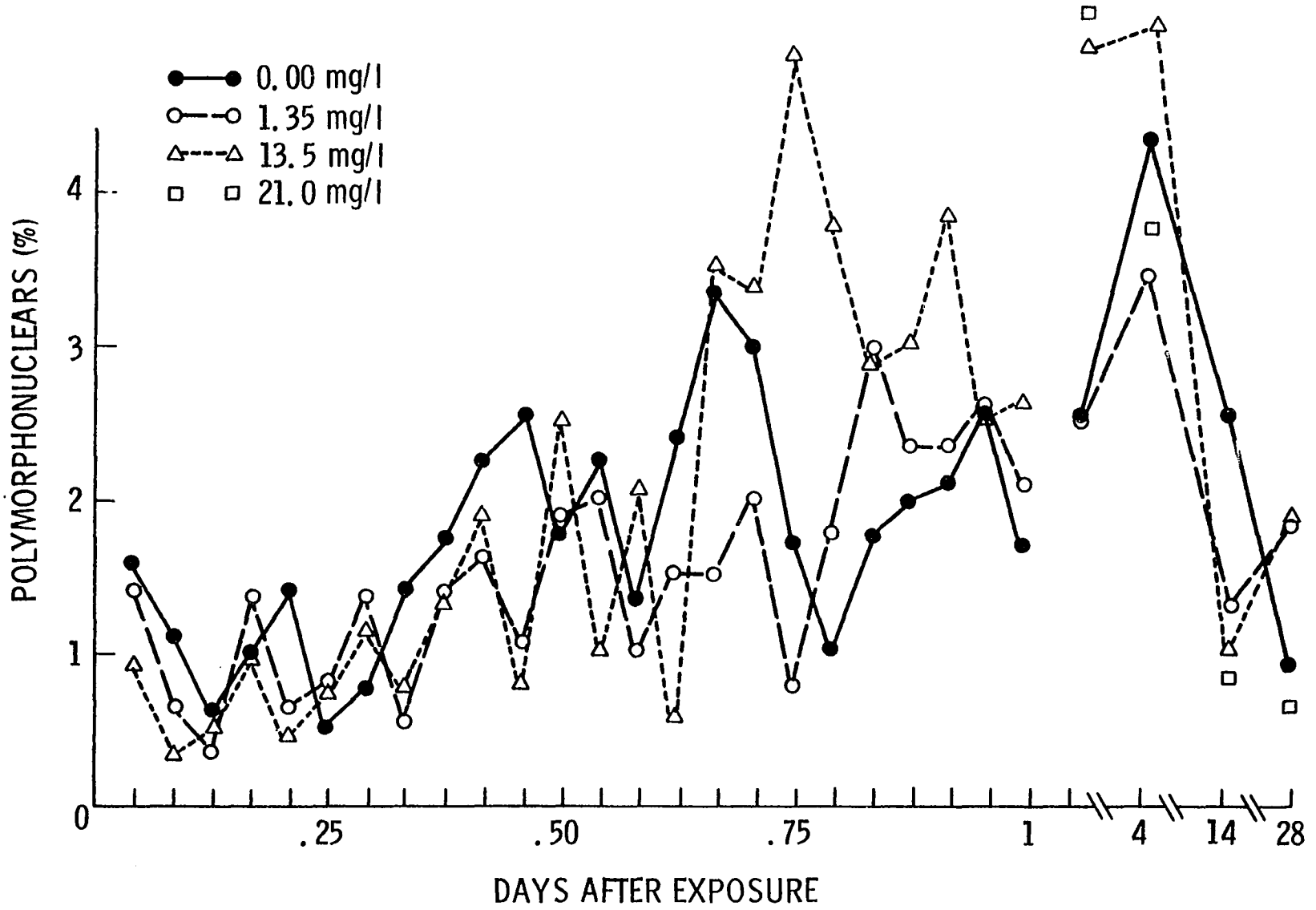

0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
LYMPHOCYTES (1000/mm3)
0
5
10
15
20
25
30
35
40
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

Figure 6. Changes in the relative percentage of polymorphonuclear leukocytes in the differential leukocyte count of rainbow trout statically exposed to malachite green for 30 min


0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
POLYMORPHONUCLEARS (%)
0
1
2
3
4
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

this time period (P=0.0001). The percentage of polymorphonuclear neutrophils revealed no significant changes on any of the post-treatment sampling days. The average percentage was higher, however, on the first day after exposure in fish from the 13.5 and 21.0 mg/l groups. Neutrophil percentage remained elevated through the 4th post-treatment day, then gradually declined.

The average absolute number of neutrophils was higher through the 4th post-treatment day in fish exposed to 13.5 to 21.0 mg/l but not significantly so (Figure 7). Values in fish in all test groups diminished by the 14th day after exposure and remained at low levels through the end of the experiment.

Overall slopes calculated for the relative and absolute numbers of thrombocytes, lymphocytes, and polymorphonuclear leukocytes to determine if they differed significantly from 0 are presented in Table 1.

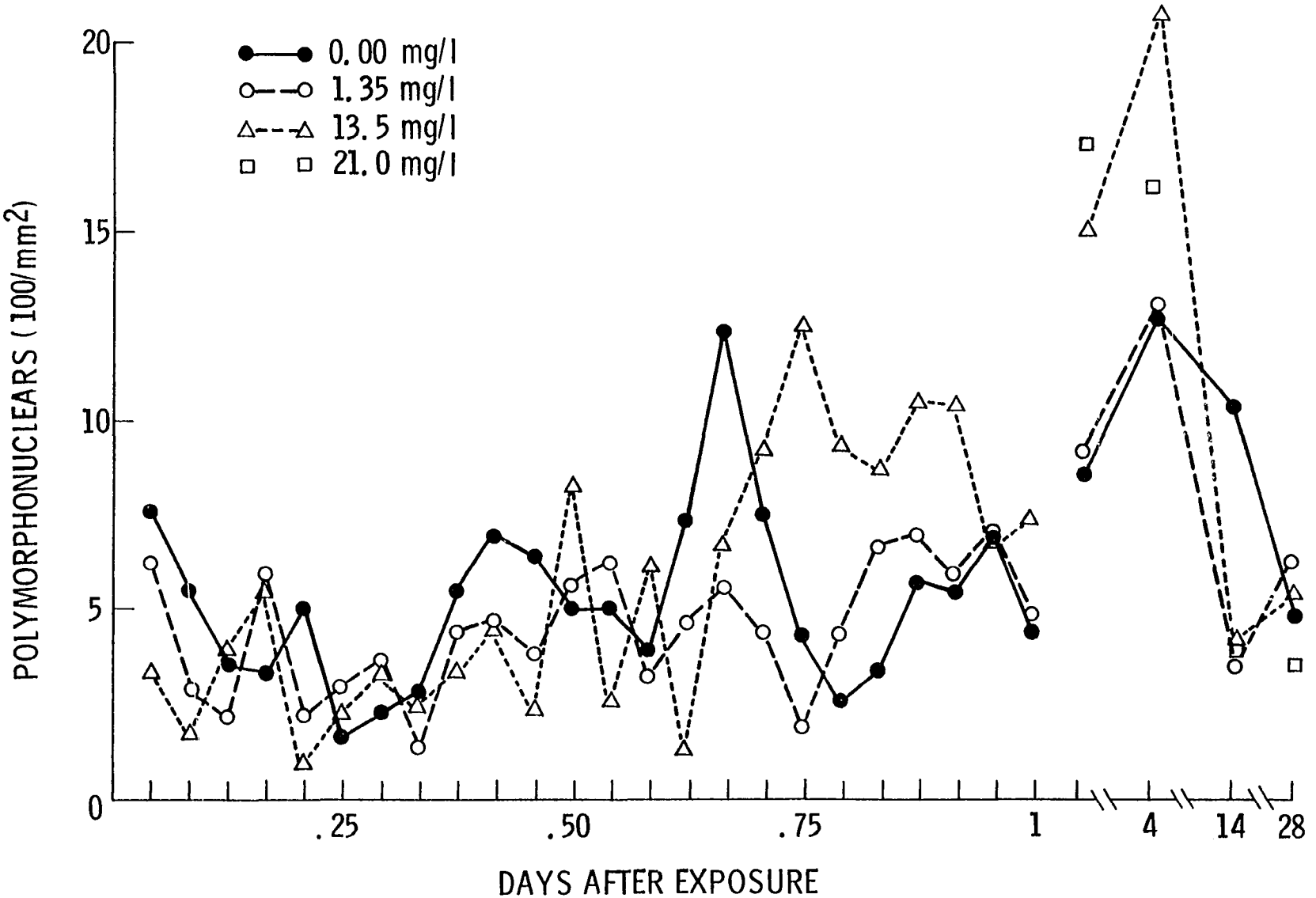
Table 1. Slopes (b values) of absolute and relative leukocyte numbers in rainbow trout vs. time for the first 24 hr after static exposure to malachite green for 30 min at 12 C

| Parameter | 0.00 mg/l | 1.35 mg/l | 13.5 mg/l |
|---|---|---|---|
| Total WBC-T count | -.84**** | -.86**** | -.75**** |
| Thrombocyte (%) | .85**** | 1.08**** | 1.56****[a] |
| Thrombocyte (#) | .04 | .04 | .24**** |
| Lymphocyte (%) | -.89**** | -1.16**** | -1.69**** |
| Lymphocyte (#) | -.81**** | -.92**** | -1.02**** |
| Neutrophil (%) | .03 | .07**** | .14**** |
| Neutrophil (#) | .00 | .01 | .03**** |
| Hematocrit | .05 | .04 | .02 |

[a] Underlined values significantly different from control value at P = 0.01.

**** Slope significantly different from 0 at P=0.0001.

Figure 7. Changes in the absolute number of polymorphonuclear leukocytes in the differential leukocyte count of rainbow trout statically exposed to malachite green for 30 min


0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
POLYMORPHONUCLEARS (100/mm²)
20
15
10
5
0
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

Slopes calculated for the separate time intervals showed no significant decline in total WBC-T or lymphocytes in test groups or controls up to the 3rd hour post-exposure, establishing the existence of a physiological lag. The greatest changes in the leukograms occurred between 4-8 hr after treatment, when all groups showed a leukopenic response, which tended to stabilize.

The lymphocyte/polymorphonuclear ratio tended to reiterate the stress pattern shown by the total WBC-T or lymphocyte counts (Figure 8). The ratio increased dramatically during the first few hours after exposure (especially 3-5 hr), then underwent a rapid decline, reaching its low point 18-24 hr post-treatment. The ratio remained depressed up to 4 days after exposure but approximated initial values by 28 days after treatment, after vascillation about 14 days after exposure.

Inasmuch as the slopes for leukocyte values from control fish showed a rapid decline similar to those from fish exposed to the chemical, I conducted t-tests to compare results of each tested group with those from control fish. Significant elevations (P=0.01) occurred in the relative and absolute thrombocyte counts, relative lymphocyte count, and absolute neutrophil count in fish exposed to 13.5 mg/l of malachite green. However, no significant differences occurred in fish exposed to 1.35 mg/l when compared with control fish.

Trends in the hemotocrit showed no significant changes during the first 24 hr after treatment, although the 13.5 mg/l group had a slightly higher average compared to the 1.35 mg/l and control groups (Table 2). Hematocrits were significantly higher 1 day after (P=0.0001) and 4 days after (P=0.0005) in the 21.0 mg/l group when compared to controls. No

Figure 8. Changes in the lymphocyte/polymorphonuclear ratio in the peripheral circulation of rainbow trout statically exposed to malachite green for 30 min

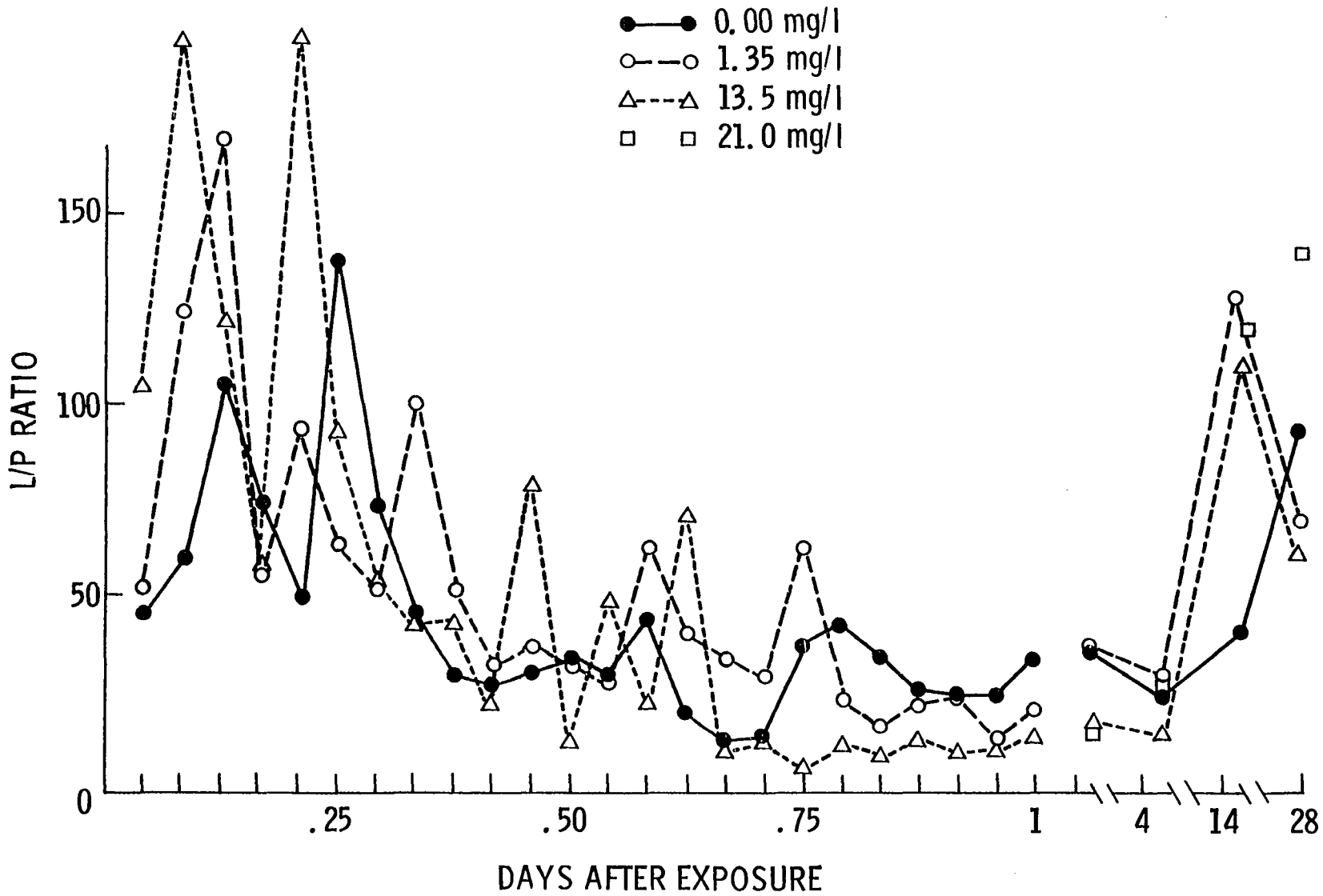

0.00 mg/l
1.35 mg/l
13.5 mg/l
21.0 mg/l
L/P RATIO
150
100
50
0
.25
.50
.75
1
4
14
28
DAYS AFTER EXPOSURE

Table 2. Average hematocrit changes over time in juvenile rainbow trout statically exposed to selected concentrations of malachite green for 30 min at 12 C. Number of fish examined in parentheses

| Time | 0.00 mg/l | 1.35 mg/l | 13.5 mg/l | 21.0 mg/l |
|---|---|---|---|---|
| 1 hr | 32.1 (8) | 30.3 (7) | 32.8 (11) | -- |
| 2 hr | 27.0 (8) | 30.9 (9) | 36.1 (9) | -- |
| 3 hr | 30.6 (8) | 29.7 (9) | 31.8 (6) | -- |
| 4 hr | 30.2 (6) | 29.4 (8) | 33.8 (11) | -- |
| 5 hr | 30.5 (8) | 28.6 (8) | 34.6 (11) | -- |
| 6 hr | 29.4 (8) | 30.3 (7) | 32.5 (10) | -- |
| 7 hr | 29.9 (8) | 30.4 (8) | 31.3 (11) | -- |
| 8 hr | 30.1 (7) | 29.7 (7) | 32.9 (12) | -- |
| 9 hr | 29.1 (8) | 32.3 (7) | 32.0 (9) | -- |
| 10 hr | 30.1 (7) | 31.0 (8) | 32.4 (8) | -- |
| 11 hr | 30.4 (7) | 30.3 (8) | 31.1 (9) | -- |
| 12 hr | 29.7 (7) | 30.8 (8) | 33.7 (8) | -- |
| 13 hr | 29.3 (8) | 29.3 (7) | 31.9 (8) | -- |
| 14 hr | 30.8 (8) | 31.8 (7) | 32.0 (6) | -- |
| 15 hr | 34.0 (4) | 31.3 (7) | 32.8 (6) | -- |
| 16 hr | 30.3 (6) | 31.7 (6) | 30.7 (7) | -- |
| 17 hr | 30.5 (8) | 30.0 (8) | 33.1 (11) | -- |
| 18 hr | 31.5 (6) | 30.0 (8) | 31.9 (11) | -- |
| 19 hr | 30.6 (8) | 29.9 (9) | 32.9 (9) | -- |
| 20 hr | 31.6 (8) | 30.9 (8) | 34.1 (10) | -- |
| 21 hr | 31.8 (8) | 31.2 (9) | 32.7 (7) | -- |
| 22 hr | 32.3 (7) | 29.0 (8) | 33.3 (9) | -- |
| 23 hr | 30.5 (6) | 32.4 (8) | 32.8 (10) | -- |
| 24 hr | 31.3 (6) | 30.9 (7) | 34.1 (9) | -- |
| 1 day | 22.0 (30) | 21.0 (29) | 24.0 (30) | 28.0 (20) |
| 4 days | 22.5 (30) | 23.6 (29) | 25.6 (30) | 28.1 (16) |
| 14 days | 27.4 (26) | 26.5 (29) | 26.7 (26) | 25.6 (15) |
| 28 days | 27.7 (21) | 27.7 (28) | 26.5 (22) | 28.2 (8) |

significant differences were noted on days 14 and 28.

Fish statically exposed for 5 min to 0.00, 42.0, or 75.0 mg/l malachite green showed hematological effects similar to those of fish exposed for 30 min (Table 3). The total WBC-T was not as low after 24 hr, on the average, but was depressed from original levels. Thrombocyte

Table 3. Relative (%/100 leukocytes) and absolute (number/$mm^3$) changes in blood characteristics over time in juvenile rainbow trout statically exposed to malachite green for 5 min at 12 C

| No. | Hr after exposure | WBC-T[a] | Thrombocytes % | Thrombocytes No. | Lymphocytes % | Lymphocytes No. | Polymorphs % | Polymorphs No. | HCT[b] | L/P[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.00 mg/l | | | | | |
| 6 | 0 | 50625 | 30.7 | 14820 | 68.8 | 35477 | 0.67 | 329 | 27.8 | 107.8 |
| 6 | 2 | 51208 | 31.7 | 15399 | 67.8 | 35570 | 0.50 | 240 | 30.4 | 148.2 |
| 6 | 4 | 48500 | 38.0 | 18300 | 61.8 | 30135 | 0.17 | 66 | 29.2 | 460.0 |
| 6 | 6 | 40792 | 34.3 | 13448 | 64.8 | 26941 | 0.83 | 404 | 26.6 | 66.7 |
| 6 | 24 | 34375 | 54.3 | 18743 | 42.8 | 14630 | 2.83 | 1011 | 31.3 | 14.5 |
| | | | | | 42.0 mg/l | | | | | |
| 6 | 0 | 49708 | 32.3 | 15794 | 67.3 | 33798 | 0.33 | 167 | 31.7 | 202.6 |
| 6 | 2 | 54042 | 24.0 | 11964 | 75.5 | 41867 | 0.50 | 211 | 32.3 | 198.1 |
| 6 | 4 | 44458 | 30.2 | 14208 | 69.2 | 29988 | 0.67 | 263 | 30.8 | 114.2 |
| 6 | 6 | 39875 | 32.3 | 11978 | 67.2 | 27744 | 0.50 | 153 | 28.7 | 181.0 |
| 5 | 24 | 31850 | 69.0 | 21552 | 29.2 | 9716 | 1.80 | 583 | 32.0 | 16.7 |
| | | | | | 75.0 mg/l | | | | | |
| 8 | 0 | 44844 | 25.1 | 10988 | 72.9 | 32953 | 2.00 | 903 | 29.0 | 36.5 |
| 9 | 2 | 61806 | 30.6 | 18246 | 69.1 | 43324 | 0.33 | 236 | 33.0 | 184.4 |
| 8 | 4 | 47219 | 33.5 | 15633 | 66.3 | 31486 | 0.25 | 99 | 30.7 | 317.1 |
| 9 | 6 | 35944 | 40.7 | 14655 | 59.3 | 21290 | 0.00 | 00 | 29.9 | -- |
| 16 | 24 | 36625 | 58.1 | 21284 | 40.1 | 14682 | 1.75 | 659 | 33.0 | 22.3 |

[a]White blood cells-thrombocytes/$mm^3$.

[b]Hematocrit.

[c]Lymphocyte/polymorphonuclear ratio based on absolute counts.

percentages were comparable to those of the 30-min exposure.

Lymphocyte percentages and absolute numbers dropped more in the 42.0 and 75.0 mg/l groups than in controls. Values from the 75.0 mg/l group were nearly equal to corresponding values in the control group 24 hr after exposure.

No remarkable trends occurred in the polymorphonuclears, although the 75.0 mg/l group showed a significant increase (P=0.05) immediately after exposure. However, there were no other significant differences to suggest an immediate neutrophilia.

There was a significant increase (P=0.02) in the hematocrit immediately after exposure in the 42.0 mg/l group. This trend did not continue, and I noted no other significant changes in the hematocrit, although the averages were slightly higher in the 42.0 and 75.0 mg/l groups when compared to controls.

The lymphocyte/polymorphonuclear ratios declined markedly in both test group and controls and were similar by 24 hr after exposure. Values of the same magnitude were noted in the 30-min exposure in both test groups and control also 24 hr after treatment.

# DISCUSSION

## Thirty-Minute Exposure

### Total leukocyte counts

There is an increasing awareness that determining and establishing safe limits to exposure to effluents (and chemicals in general) in the aquatic environment will require measurements of various meaningful sublethal toxic responses in fish. Such responses must include sensitive stress indicators (McLeay and Howard, 1977). Casillas and Smith (1977) feel the use of hematological parameters as indicators of sublethal effects of stress can provide information on the physiological responses fish make to a changing environment because of the close association of the circulatory system with the external environment and every tissue.

It should be noted that the number and range of fluctuation of leukocytes in the blood of fish are very high compared to those in terrestrial vertebrates. Kalashnikova (1976) feels this quality affords fish a higher degree of protection because pathogens and toxicants are usually more concentrated in water than in air.

My results show that the leukocytes of rainbow trout exhibit certain measurable responses to exposure to strong concentrations of malachite green, both in total white blood cell-thrombocyte count and in the changes in absolute numbers of the individual cell types, particularly during the first 24 hr after treatment. Pickford *et al.* (1971) point out the need for defining the immediate effects of stressful stimuli.

The general pattern of the changes in total white blood cell-thrombocyte count that I found in the first 24 hr after exposure has been noted in

other species. McLeay and Gordon (1977) exposed coho salmon to bleached kraft mill effluent for 4 hr but noted no leukocyte response during the exposure period. My data showed an initial lag of about 4 hr before a leukocyte response occurred. McLeay and Gordon believed their findings imply that either a longer exposure to effluent is required before the toxic constituents elicit a stress response, or a longer period of observation after exposure (sampling beyond 4 hr) is necessary before the decrease in circulating thrombocytes and/or leukocytes is manifested. A number of the fish I exposed to 13.5 or 21.0 mg/l malachite green for 30 min died within a few hours after exposure. The initial lack of response in survivors was probably not due, therefore, to insufficient concentration of the chemical.

Dougherty and White (1944) injected 1 mg of pituitary adrenotrophic hormone into mice and caused a slight drop in white cell count after 1 hr, a progressive drop 3-9 hr post-injection and a gradual increase from 9-24 hr back to near normal levels. A similar blood picture was noted in rabbits. A 0.5-ml injection of aqueous adrenal cortical extract caused a leukopenia/lymphopenia 3 hr after injection in mice and rats. Nelson (1953) found that the 4-hr maximum fall observed in lymphocytes in humans injected with 17-hydroxycorticosteroids was seen to be a delayed response to elevations in blood steroids, which are usually maximal at least 3 hr before the maximum effect on the leukocytes. Bushby (1970) points out that depression of leukopoiesis is the commonest toxic action of drugs on leukocytes.

Gordon (1955) indicated that the total white cell count generally increases in animals that have a greater proportion of neutrophils to

lymphocytes (e.g., man) and decreases in forms such as the rabbit, mouse, and rat, in which the lymphocyte is the predominating circulating white blood cell. The rainbow trout falls into the latter category. Mazeaud et al. (1977) felt the white cell picture seems to be essentially the same in fish as in some higher vertebrates, because nonspecific stress induced lymphopenia.

The duration of alterations I noted in the total white blood cell-thrombocyte count of control fish and trout exposed to malachite green persisted at least through the 4th post-treatment day. After day 4 test and control groups were not significantly different. Glagoleva and Malikova (1968) noted the greatest degree of leukopenia within 48 hr of treatment with 13.3 mg/l malachite green and found a 95% reduction of white cells. A concentration of 1.33 mg/l reduced the leukocyte count by a factor of 5-8 times. The leukopenia was only about one-half as severe by the 6th day after exposure to 1.33 mg/l. McLeay (1975) found that coho salmon exposed to pulpmill effluent or zinc required 2-4 days for white blood cell levels to recover from an initial decline, a recovery schedule similar to that noted by Bills and Hunn (1976) for juvenile coho salmon chronically exposed to 0.1 mg/l malachite green. Bills and Hunn found that the total leukocyte count was generally lower by the 4th post-treatment day but not after 7 days when compared with controls. Iwama et al. (1976) observed that total leukocyte count in coho salmon decreased by the 24th hr after exposure to dehydroabeitic acid.

Mazeaud et al. (1977) showed that metabolic changes from transient neuroendocrine disturbances caused changes in blood parameters which lasted for several days. In other words, a brief stress can bring about

long-lasting disturbances, which are not limited to the 2-3 hr suggested by some earlier investigations (Michael, 1949).

Jones (1976) believed that a drop in white cell count may result from cell egress to tissues as an immediate response of the circulatory system to irritation. The blood leukocytes, however, whether they are destroyed in the circulation or because they leave it, constitute a shifting cell population and represent an equilibrium between the new formation of leukocytes to be discharged into the blood and the destruction or migration from the blood of cells already in it (Yoffey, 1955).

My data indicate that the stress produced in fish by immersion in malachite green for 30 min did not appear to have a lasting effect. All test groups had similar leukograms 14 days post-treatment, including fish treated with concentrations which are far above those used in control of fish disease for prolonged exposure. McKim et al. (1970) exposed brook trout to copper and found hematological changes persisted after 21 days but not after 337 days. They felt that the disappearance of initial blood changes after extended exposure suggests the transient nature of these early responses. McLeay (1973c) found no reduction in lymphocytes in coho salmon exposed to kraft mill effluent for 25 days. Vaala (1971) found normal leukocyte counts in brook trout after they were exposed to low pH (4.9) for 28 days. Similarly, McLeay and Brown (1974) noted no prolonged hematological changes in coho salmon exposed to bleached kraft mill effluent for 200 days, which again suggests the initial stress response is transitory. Data on fathead minnows exposed to graded severities of pH (5.0-7.4) for 7 months showed that chronic exposure had no effect on the leukocyte count

of fish exposed to any degree of acidity tested (Dolsky, 1971). Dolsky assumed that the fish had acclimated to these conditions.

Differential leukocyte counts

The differential white cell count in my experiments revealed changes in the absolute number of thrombocytes, lymphocytes, and polymorphonuclears. A significant increase in the absolute number of thrombocytes occurred within 24 hr of exposure only in the 13.5 mg/l group. Casillas (1974), using hooking stress in rainbow trout, found a three-fold increase in the number of thrombocytes 20-30 min after the stress period. Much of the increase was recorded 10 min after the applied stress. Values recovered to pre-stress levels in 180 min. Schreck et al. (1976) found an apparent doubling of the thrombocyte number in rainbow trout immediately after electrical shock, with a return to normal levels within 1 hr. Jones (1976) exposed bluegills (Lepomis macrochirus) to 3 stressors (turbidity, chloramine, and Flexibacter columnaris) and noted an increase in thrombocytes. She attributed this increase to the body's protective mechanism in a stressful situation. Pickford et al. (1971) noted a significant increase in thrombocyte number in Fundulus within 3 min after injecting fish with 0.025 µg/g of cortisol. This elevation lasted about 2-4 hr. Klontz and Smith (1968) indicated that thrombocytes are thought to be retained in the spleen and anterior kidney under moderate stress, whereas stronger stimuli elicit their release into the peripheral vascular system in great numbers. My study showed a significant increase in thrombocytes 1 day post-treatment in fish exposed to 21.0 mg/l. This situation indicates that malachite green in strong concentrations can cause an increase in the

number of circulating thrombocytes but is not as stressful as some other stimuli.

DeLaney et al. (1976) reported that thrombocytes generally increased in estivating lungfish. Hattingh and van Pletzen (1974) found an increase in thrombocyte count 2-3 days after mudfish (Labeo umbratus) were stressed. Weinreb (1958) found, however, that thrombocytopenia occurred in rainbow trout 72 hr after they were stressed or injected with ACTH or cortisone. McLeay (1973a) found that daily ACTH injections in coho salmon for 7 days caused a reduced thrombocyte count, as did dexamethasone (McLeay, 1973b). He felt the thrombocytopenic response might serve as an available supply of protein and other cellular constituents required during stress, as well as releasing clotting factors in preparation for repair of tissue damage. I found no evidence of malachite green-induced thrombocytopenia.

Changes in the differential are most vividly seen in the lymphocyte response. My results showed that the lymphocytes tend to reiterate the general pattern shown by the total white blood cell-thrombocyte count of fish exposed to all concentrations of malachite green tested as well as in controls. The initial lag of 3-4 hr is followed by a rapid decline 4-8 hr post-exposure. A period of relative stability ensues, and recovery tends to occur 1-4 days after exposure. Glagoleva and Malikova (1968) noted a drop of 10-12% in lymphocyte percentage in fish exposed to 1.33 or 13.3 mg/l malachite green 1 or 2 days after treatment. However, relative percentages may be deceiving, and these authors gave no absolute counts. They found that lymphocyte percentage approximated the control values 6 days post-exposure. Belova (1965) pointed out that a reduction in the number of lymphocytes is one of the indices of an unfavorable state in the fish.

Gordon (1955) observed that the pattern of peripheral lymphocyte behavior evoked by stress has an initial rise in the level of circulating lymphocytes (Phase I), followed by a characteristic lymphopenia (Phase II), after which there is a return to normal levels (Phase III). The timing of these phases may differ in different species.

Hatai (1972) found that injections of different strains and concentrations of Aeromonas liquifaciens into eels caused a drop in lymphocyte percentage 5-7 hr after inoculation. Pickford et al. (1971) found that a low dose of cortisol significantly reduced the percentage of circulating lymphocytes in sheepshead minnows 4 hr after fish were injected, following an initial lag.

Weinreb and Weinreb (1969) injected rainbow trout with thoroplast. Examination of blood samples over a 26-hr period showed that lymphopenia persisted for 6 hr. The reaction decreased after 12 hr, and the blood picture began to normalize.

Other investigators have found different time limits for the response of lymphocytes to stressors. Casillas (1974) noted that the lymphocytes of rainbow trout did not respond to short-term hooking stress. The lack of response, which he found unusual, may have been due to his small sample size, his indirect method of estimating lymphocyte numbers in terms of relative abundance compared to red blood cells, or a short (5 hr) observation period. My results indicate a lymphocyte response probably requires more than 5 hr to manifest itself.

Dolsky (1971) showed that acute (17-20 hr) and subacute (28 day) exposure of fathead minnows and longnose dace to acid stress resulted in a lymphopenia. McLeay (1973c) noted a decrease in lymphocytes in coho salmon

exposed to kraft pulp mill effluent for 12 hr. Hines and Spira (1973) noted that carp infected with Ichthyophthirius multifiliis showed 2 periods of reduced lymphocytes: 1-5 days after infection and 12-15 days after infection.

Regarding higher vertebrates, Dougherty and White (1944) found that adrenal corticosteroids in oil (0.1 mg), corticosterone (0.25 mg), or 17-hydroxycorticosterone acetate (0.025 mg) all produced lymphopenia in adrenalectomized mice within 3-6 hr. Aqueous adrenal cortical extracts (0.5 ml) induced lymphopenia in rats 3 hr after administration. In all cases, lymphocytes recovered in about 24 hr. Dougherty and Frank (1953), using epinephrine as a stressor in mice, noted lymphopenia in 30 min and reconstruction in 4 hr. Hills et al. (1948) noted that a single intramuscular injection of 25 mg of ACTH given at 8:00 AM resulted in a 40% decrease in lymphocytes 4 hr later in human subjects.

McLeay and Howard discussed the mechanism of lymphopenia. They felt that the depression of white blood cell-thrombocyte counts in salmonid fish resulted from increased secretion of corticosteroid stress hormones, which lyse circulating lymphocytes, resulting in leukopenia/lymphopenia. Dougherty and White (1944) showed that stress and corticosteroids caused lymphopenia in mammals. Gordon (1955) indicated it might be difficult to decide to what extent migration of lymphocytes to the tissues contributed to peripheral lymphopenia induced by cortical factors or stress. He felt lymphopenia developing in early stages of stress resulted from destruction of lymphocytes within lymphatic organs, resulting in a decreased delivery of the cells to the peripheral circulation.

McLeay (1973b) discussed the possible adaptive significance of lymphopenia and noted that lysis of lymphocytes provides a source of protein for gluconeogenesis. Additionally, lymphocyte destruction releases already-formed antibodies and provides nuclear and cytoplasmic materials for phagocytosis by reticuloendothelial cells and renewed differentiation (Dougherty, 1960).

Polymorphonuclears (PMN) are not a sensitive parameter for assessing stress in salmonids because of their low numbers per volume of blood. However, they become more numerous under the influence of severe stimuli. In my study, the PMN showed a gradual overall increase during the first 24-hr post-exposure in the controls and 1.35 mg/l groups. Fish treated with 13.5 or 21.0 mg/l malachite green had higher average numbers of polymorphonuclears 1 and 4 days post-treatment when compared to controls, a situation also found by Grizzle (1977) in catfish chronically exposed to 0.1 mg/l malachite green. PMN counts in my work were similar or essentially the same 14 and 28 days after exposure in all test groups and controls.

Glagoleva and Malikova (1968) noted that the percentage of PMN approximately doubled in salmon 24 hr after treating fish with 1.33 mg/l malachite green and tripled in fish 48 hr after treatment with 13.3 mg/l. Percentages were virtually identical by 6 days post-treatment. Hatai (1972) detected a progressive increase in the percentage of PMN, which peaked after 1-2 days in eels injected with strains of Aeromonas liquifaciens. Fathead minnows exposed to a pH of about 5 for 17-20 hours showed a 3-6 fold increase in PMN percentages (Dolsky, 1971). Hines and Spira (1973) showed that mirror carp infected with Ichthyophthirius exhibited an almost immediate change in leukocyte proportions, producing a reversal of

the ratio of neutrophils to lymphocytes between days 0 and 3. A five-fold increase in PMN occurred by day 6. Weinreb (1958) noted that thoroplast caused a neutrophilia which persisted for 6 hr in rainbow trout but decreased before the end of 1 day. Gardner and Yevich (1969) found an increase in eosinophils in Fundulus (the only granulocytic white cell in this species) in fish exposed to 50 ppm cadmium in 4 hr, with a progressive increase up to 24 hr. Pickford et al. (1971) also found a significant elevation in eosinophil percentage after 4 hr when they injected Fundulus with low doses of cortisol. Finn and Nielson (1971) injected complete Freund's adjuvant into rainbow trout and detected a slight neutrophilia which occurred from 12 hr to 2 days afterward, with a peak at 1 day. Yokoyama (1960) injected turpentine into yellow perch and produced a heterophilia (her term for neutrophilia) after 24 hr.

Hills et al. (1948) noted an increase of 102% in neutrophils in 4 hr in humans injected with 25 mg of ACTH. McLeay (1973a) found that ACTH increased the neutrophil percentage in coho salmon. Conversely, Amend and Smith (1974) reported that infectious hematopoietic necrosis caused an absolute reduction in neutrophils in rainbow trout. McLeay (1973c) found a significant elevation in neutrophils in coho salmon up to 25 days after continuously exposing fish to kraft pulpmill effluent. My results show that all test groups had similar PMN values by 28 days after treatment. The exposure period I used was only 30 min, but there may also be differences due to species. McLeay and Gordon (1977) indicated that leukocyte responses in rainbow trout were less consistent than in coho salmon exposed to effluents of the same concentration. Prolonged neutrophilia was not seen in coho salmon continuously exposed to bleached kraft mill effluent

for 200 days (McLeay and Brown, 1974). Similarly, chronic (7 month) exposure of fathead minnows to acid stress produced no lasting neutrophilia, although acute and subacute exposure did (Dolsky, 1971). Poels and Strik (1975), however, noted significant increases in granulocytes in rainbow trout exposed to polluted Rhine River water after 3 and 9 months but not after 6 months.

Ellis (1976) considered variations in neutrophils a response to natural challenge from factors in the fish's environment. The kidney of teleosts contains large numbers of neutrophils, which may, under appropriate stimulation, be released into the peripheral circulation. McLeay (1973a) noted that in teleost fish and mammals the number of circulating neutrophils is apparently unaffected by corticosteroids but elevated by stress and ACTH. McLeay and Brown (1974) noted that if the teleost neutrophil actively phagocytizes as does its mammalian counterpart, an elevated neutrophil count suggests some tissue damage or entrance of a foreign substance, as well as increased ACTH secretion. Gordon (1955) felt that the neutrophilia induced by stress and pituitary factors may involve, at least in part, extra-adrenal mechanisms and may be associated with nonspecific reactions to foreign proteins or other stressful stimuli.

The lymphocyte-to-polymorphonuclear or PMN ratio (L/P) makes the lymphocyte count an indirect function of the neutrophil count. Because both a lymphopenia and neutrophilia result from adrenocorticosteroid activity, appropriate combination of numerical values of both the lymphocyte and neutrophil counts into a single value enhances the sensitivity of the leukocyte count as an indicator of adrenocorticoid activity (Michael, 1949). This ratio is difficult to measure in individual fish, because

neutrophils are often not found on a blood smear. However, a pooled average for each cell type for each sampling period and concentration produces a ratio which does show the characteristic lymphopenia/neutrophilia of a stress response. This ratio, to my knowledge, has not been used before in fish hematology studies.

Using the average values for lymphocytes and neutrophils in over 2000 rainbow trout examined by Lehmann et al. (1976), a "normal" ratio is 46:1. My results show that the ratio is depressed mainly about 12-24 hr after exposure to malachite green and continues to remain depressed up to 4 days afterward. Higher dosages depressed the ratio more. To compare my results to those of Glagoleva and Malikova (1968), I calculated ratios on the basis of differential percentages and absolute counts (Table 4). It appears Glagoleva and Malikova (1968) found a dose-related response, although my data failed to show that 1.35 mg/l was any more stressful than the routine handling response exhibited by the controls.

I also applied this technique to data from McLeay (1973c), who showed effects of kraft mill effluent on blood cell counts in juvenile coho salmon (Table 5). Evidently the lymphocyte/polymorphonuclear leukocyte ratio can be used as a general indicator of stress in salmonid fish. Low ratios suggest the fish are under stress.

Hematocrits

An increased hematocrit can be brought about by increase in erythrocyte size, increase in red cell number, or a decrease in plasma volume (Schiffman and Fromm, 1959). Houston and DeWilde (1968) obtained coefficients of 0.6-0.3 for hematocrit vs. red blood cell counts. Because

Table 4. Lymphocyte/polymorphonuclear ratios based on differential leukocyte percentages for salmon and rainbow trout exposed to malachite green. Ratios for salmon derived from data of Glagoleva and Malikova (1968)

| Conc. (mg/l) | 1 day[a] | 1 day[b] | 2 days[b] | 4 days[a] | 6 days[b] |
|---|---|---|---|---|---|
| 0.00 | 32.00 (36.75)[c] | 16.98 | 15.62 | 18.30 (25.76) | 15.06 |
| 1.33 | 33.83 (39.00) | 8.16 | -- | 23.25 (30.36) | 13.64 |
| 13.3 | 14.91 (19.86) | 6.29 | 4.33 | 14.60 (16.53) | -- |
| 21.0 | 13.00 (19.03) | -- | -- | 21.70 (29.00) | -- |

[a]Based on my data.

[b]Based on data from Glagoleva and Malikova (1968).

[c]Ratios in parentheses based on pooled average absolute counts from my data.

Table 5. Lymphocyte/polymorphonuclear ratios in control and kraft pulp mill effluent-exposed juvenile coho salmon. Data from McLeay (1973c)[a]

| | 12-hr exposure | 25-day exposure |
|---|---|---|
| Control fish | 28.35 | 20.40 |
| Effluent-exposed fish | 10.18 | 5.64 |

[a]Based on differential leukocyte counts (no./3000 cells).

erythrocyte counts contain a fair degree of inherent error (± 20%) when carried out visually (Brown, 1973), greater reliance is usually placed on the hematocrit as an indicator of fish health.

Lower concentrations of malachite green (1.35 mg/l) in my studies had virtually no effect on hematocrit, although 13.5 mg/l caused a slight increase in average hematocrit, while 21.0 mg/l caused a significant elevation. Strik et al. (1975) noted a significant increase in hematocrit of rainbow trout exposed to chromium for 15-22 days. Casillas (1974) found that hooking stress caused an increase in hematocrit in rainbow trout within 10 min, but values returned to pre-stress levels within a few hours. Vars (1934) reported that the toxicity of ammonium salts raised the mean hematocrit of carp from 32 to 39.

Glagoleva and Malikova (1968) found a drop in salmon erythrocyte count 24 and 48 hr after exposing fish to 1.33 or 13.3 mg/l malachite green for 40 or 60 min, but this situation abated by the 6th day post-treatment. McLeay (1975) and McLeay and Howard (1977) noted that erythrocyte counts in juvenile coho salmon showed little response to kraft mill effluent or zinc. Klontz et al. (1966) detected no change in red cell count within 80 hr after rainbow trout became infected with furunculosis. O'Conner and Fromm (1975) found no lasting changes in the hematocrit of rainbow trout exposed to methyl mercury. This apparent lack of response possibly indicated a transient effect, because they did not take their initial sample until the 4th week post-exposure.

Malachite green has been shown to cause possible irritation to gills. Grizzle (1977) noted a slight erythrocytosis and increase in hematocrit in catfish chronically exposed to 0.1 mg/l malachite green. He felt these

changes may have been due to impairment of gas exchange because the lamellar epithelium of the gills thickened after treatment. Waluga (1971) found extensive necrobiotic changes in the respiratory epithelium of the gills of rainbow trout exposed to 0.2 mg/l malachite green for 10 days. Lanzing (1965) reported that sand whiting (Sillago ciliata) exhibited an increase in respiratory activity when transferred to a 1:160,000 malachite green solution. He obtained the same results when the chemical was slowly added over a 1-hr period, which indicated the hyperventilation was due to the chemical rather than to excitation of the fish.

### Five-Minute Exposure

Investigations on the hematological effects of a brief exposure of fish to a stressor have not been numerous. Slicher and Ball (1962a) immersed sheepshead minnows and sailfin mollys in ice water (0 C) for 2-3 min. A marked leukopenia was elicited after 1 hr, followed by leukocytosis at 2 hr. Bennett and Neville (1975) exposed goldfish raised at 22 C and 24 C to 2 C water for 3-4 min. These authors found significant lymphopenia and neutrophilia 1 and 2 hr after cessation of cold shock, but values recovered and stabilized by 4 hr post-treatment. My results showed only a significant elevation in polymorphonuclears within minutes after exposure to 75 mg/l malachite green for 5 min. This increase was probably not physiologically significant, because the number of PMN did not increase in samples taken 2, 4, 6, or 24 hr later. No marked lymphopenia was noted in either test group when compared with the control fish. However, in both controls and test groups, there was an average increase in the number of PMN and a noticeable decrease in lymphocyte number 24 hr after exposure.

It is difficult to compare effects of the 5-min exposure with those of the 30-min treatment because sampling schedules and concentrations were different. Handling probably caused some stress reaction, even in the controls. However, it appears that short exposure (5 min) to strong concentrations (42 or 75 mg/l) of malachite green are not significantly more stressful to rainbow trout than is routine mechanical transfer. The upper concentration (75 mg/l) is stronger than the 67 mg/l often used as a dip treatment for 15-30 seconds to control ectoparasites in fish (Wellborn, 1971).

# CONCLUSIONS

1. The total and differential white blood cell-thrombocyte counts of rainbow trout, despite their large inherent variability, can serve as an indicator of physiological stress.

2. The major changes caused by a 30-min static exposure to 0.0 to 13.5 mg/l malachite green occur within 24 hr. After an initial lag period of 3-4 hr, the total leukocyte count declines rapidly by about 50%, then reaches a relatively stable level.

3. The differential white blood cell picture shows that the leukopenia results mainly from a decrease in lymphocytes, which are probably lysed by stress-evoked corticosteroids. The relative percentage of thrombocytes increases within 24 hr after exposure. The absolute number, however, did so only after exposure to 13.5 mg/l or greater. The polymorphonuclear leukocytes show a general increase during the first 24 hr and appear to reach a maximum count 1-4 days after exposure, with the higher average numbers occurring in fish treated with 13.5 or 21.0 mg/l.

4. Recovery probably occurs by the 14th day after treatment, as indicated by the similarity of leukograms from all test groups. No changes were noted on the 28th day after treatment, showing that the changes in blood composition are of a transient nature.

5. The lymphocyte/polymorphonuclear ratio can be used as a general indicator of stress in salmonids. The ratio is noticeably depressed within 24 hr after exposure to malachite green but recovers approximately parallel to the total white blood cell-thrombocyte count.

6. The hematocrit was higher, on the average, in fish statically exposed to higher concentrations of malachite green for 25-30 minutes, particularly 21.0 mg/l. However, this condition was only transient and not detectable by the 14th post-treatment day.

7. Fish exposed to short-term 5 min static exposure to 42 or 75 mg/l malachite green also showed some changes in leukocytes. The onset of leukopenia was not as rapid or its manifestation quite as severe when compared to the longer exposures. Total leukocyte counts were lower after 24 hr; a marked lymphopenia and mild neutrophilia occurred. The hematocrits were again higher, on the average, in the fish exposed to malachite green compared to those for controls.

8. Changes similar to those in the exposed fish in both the 30-min and 5-min static treatments also occurred in the controls. These changes were probably caused by routine handling when transferring the fish to the test solutions.

9. My results indicate that leukocytic changes caused by exposure to malachite green are not chronic and are not caused by a specific leukocytotoxic effect of this compound. According to the literature, similar results (leukopenia/lymphopenia and neutrophilia) have been elicited by a wide variety of stressors and even by natural physiological cycles.

10. I submit that my results were produced by a nonspecific vertebrate stress syndrome. Concentrations of malachite green similar to those used in routine therapeutic and prophylactic hatchery treatments did not appear to affect fish any more than did routine handling.

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# APPENDIX A. GLOSSARY OF TERMS

Eosinophil - a granulocytic leukocyte which contains acidic granules in its cytoplasm, giving an overall red appearance in the stained preparation. Seldom if ever found in the peripheral circulation in rainbow trout.

Eosinophilia - a condition where the number of eosinophils is increased.

Granulocytes - leukocytes which possess cytoplasmic granules.

Hematocrit - the proportion of whole blood which is comprised of blood cells, mainly erythrocytes. Also known as packed cell volume.

Leukocyte - a white blood cell.

Leukocytosis - an increased number of circulating white blood cells.

Leukocytotoxic - the selective destruction of leukocytes.

Leukogram - a clinical overview of the entire blood cell picture, both in absolute numbers and relative percentages.

Leukopenia - a decrease in the number of circulating white blood cells.

Lymphocytes - small circular white blood cells in which the sparse cytoplasm stains as a blue halo, outlining a large, round purple nucleus.

Lymphopenia - a decrease in the number of circulating lymphocytes.

Macrophages - phagocytic cells, having a vacuolated cytoplasm and twisted nucleus. Sparse in the peripheral circulation.

Neutrophilia - an increased number of circulating neutrophils or polymorphonuclear leukocytes.

Polymorphonuclear leukocyte - a leukocyte with a lobed nucleus and grey cytoplasm without granules. Also called neutrophils or heterophils.

Thrombocyte - an ovoid cell about the size of a red blood cell nucleus. The cytoplasm, if visible, appears as feint spikes at opposite ends of the cell. Thought to function much as a platelet does in the clotting of blood.

Thrombocytopenia - a decrease in the number of circulating thrombocytes.

Thrombocytosis - an increase in the number of circulating thrombocytes.

# APPENDIX B.

## LEUKOGRAMS OF FISH EXPOSED TO MALACHITE GREEN FOR 25-30 MIN (EXAMINED 1, 4, 14, AND 28 DAYS)

Table B-1. Leukograms of trout examined 1 day after static exposure to 0.00 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 162 | 39.23 | 18500 | 1110 | 16650 | 740 |
| 147 | 28.98 | 43000 | 1290 | 40850 | 860 |
| 132 | 21.71 | 13250 | 133 | 12720 | 133 |
| 151 | 34.25 | 29500 | -- | -- | -- |
| 155 | 31.78 | 31750 | 4763 | 25718 | 1270 |
| 164 | 39.11 | 28000 | 2520 | 25480 | 0 |
| 135 | 25.18 | 21500 | 1290 | 16770 | 3440 |
| 149 | 33.03 | 35750 | 5005 | 30388 | 358 |
| 130 | 18.30 | 20500 | 4920 | 15375 | 205 |
| 148 | 29.72 | 23750 | 6880 | 16150 | 713 |
| 99 | 12.33 | 44000 | 11880 | 31240 | 880 |
| 77 | 5.04 | 67500 | 3375 | 62775 | 675 |
| 112 | 12.10 | 50250 | 6533 | 43215 | 503 |
| 97 | 9.03 | 45500 | 5460 | 40040 | 0 |
| 69 | 3.22 | 40000 | 3600 | 35200 | 1200 |
| 79 | 5.42 | 26566 | 1590 | 24645 | 265 |
| 109 | 13.43 | 36750 | 7350 | 28665 | 735 |
| 100 | 9.44 | 51750 | 4658 | 46658 | 1035 |
| 88 | 6.66 | 48750 | 2438 | 45338 | 975 |
| 80 | 5.40 | 58500 | 1170 | 54990 | 1176 |
| 105 | 9.80 | 32500 | 7800 | 24050 | 650 |
| 115 | 13.36 | 39500 | 5530 | 33970 | 0 |
| 98 | 8.95 | 53250 | 5858 | 47393 | 0 |
| 102 | 8.93 | 26500 | 8215 | 16695 | 1325 |
| 110 | 12.41 | 40500 | 9720 | 29970 | 810 |
| 85 | 6.06 | 17500 | 6650 | 10150 | 700 |
| 91 | 6.46 | 32500 | 5850 | 25350 | 1300 |
| 84 | 5.23 | 51250 | 1025 | 45613 | 4100 |
| 114 | 13.67 | 37750 | 10570 | 26803 | 378 |
| 93 | 7.22 | 44750 | 2685 | 41618 | 448 |

[a]Total white blood cell-thrombocytes per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

[b]Number per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

Table B-2. Leukograms of trout examined 1 day after static exposure to 1.35 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 175 | 48.30 | 28000 | 840 | 27160 | 0 |
| 174 | 50.74 | 30750 | 3998 | 26753 | 0 |
| 160 | 40.41 | 16750 | 1843 | 13903 | 1005 |
| 175 | 55.30 | 44500 | 3115 | 39605 | 1780 |
| 176 | 45.40 | 29500 | 2950 | 23010 | 3540 |
| 170 | 49.63 | 36250 | 0 | 32263 | 3988 |
| 180 | 52.88 | 32000 | 4160 | 25600 | 1920 |
| 137 | 24.51 | 20750 | 4358 | 16185 | 208 |
| 155 | 33.44 | 15000 | 3300 | 11700 | 0 |
| 78 | 4.50 | 62000 | 1860 | 57660 | 2480 |
| 87 | 6.69 | 45750 | 9150 | 35228 | 1373 |
| 89 | 7.00 | 85500 | 11115 | 74385 | 0 |
| 102 | 10.43 | 61250 | 7963 | 52675 | 613 |
| 93 | 7.42 | 52750 | 0 | 50640 | 2110 |
| 89 | 7.30 | 84500 | 0 | 82810 | 1690 |
| 94 | 8.54 | 36000 | 360 | 32040 | 3600 |
| 77 | 4.94 | 59250 | 2370 | 56880 | 0 |
| 85 | 5.85 | 69750 | 3488 | 64170 | 2093 |
| 124 | 17.80 | 55000 | 3850 | 50600 | 550 |
| 96 | 7.16 | 54250 | 2713 | 51538 | 0 |
| 115 | 14.21 | 37250 | 6705 | 30540 | 0 |
| 96 | 8.30 | 40000 | 8000 | 32000 | 0 |
| 120 | 16.78 | 37500 | 1500 | 36000 | 0 |
| 96 | 8.29 | 44500 | 13795 | 30260 | 0 |
| 108 | 12.22 | 18500 | 2590 | 15355 | 555 |
| 92 | 8.00 | 47750 | 1910 | 45840 | 0 |
| 78 | 4.13 | 48750 | 9750 | 38513 | 488 |
| 93 | 6.86 | 38750 | 8525 | 29450 | 775 |
| 110 | 12.16 | 48000 | 8640 | 39360 | 0 |

Table B-3. Leukograms of trout examined 1 day after static exposure to 13.5 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 162 | 35.87 | 18250 | 0 | 13505 | 4745 |
| 179 | 46.55 | 15500 | 4960 | 9455 | 1085 |
| 159 | 37.19 | 23500 | 235 | 22560 | 705 |
| 147 | 24.25 | 17000 | 5100 | 16200 | 1700 |
| 171 | 42.14 | 21500 | 2795 | 13760 | 4945 |
| 178 | 44.91 | 36500 | 3285 | 32120 | 1095 |
| 148 | 28.57 | 46000 | 3220 | 38180 | 4600 |
| 137 | 20.68 | 13250 | 5963 | 6890 | 398 |
| 125 | 19.52 | 27750 | 18870 | 8325 | 555 |
| 156 | 29.64 | 26250 | 9450 | 16013 | 788 |
| 72 | 4.53 | 41250 | 1650 | 38775 | 825 |
| 89 | 7.48 | 62500 | 6250 | 55625 | 625 |
| 80 | 4.95 | 52250 | 4703 | 45980 | 1568 |
| 78 | 4.48 | 37750 | 755 | 33598 | 3398 |
| 90 | 7.27 | 58750 | 6463 | 51113 | 1175 |
| 90 | 6.70 | 47000 | 3760 | 43240 | 0 |
| 110 | 11.16 | 61500 | 7380 | 53505 | 615 |
| 88 | 6.89 | 54750 | 13688 | 39968 | 1095 |
| 96 | 9.12 | 59000 | 8260 | 50740 | 0 |
| 68 | 2.75 | 56250 | 6750 | 45563 | 3375 |
| 78 | 4.64 | 43500 | 6225 | 36540 | 435 |
| 76 | 4.48 | 24750 | 8168 | 13118 | 2970 |
| 97 | 9.01 | 30500 | 9760 | 19825 | 915 |
| 90 | 6.81 | 48750 | 15113 | 31688 | 1950 |
| 95 | 8.61 | 20000 | 6400 | 13400 | 0 |
| 114 | 11.16 | 42000 | 17220 | 24360 | 420 |
| 87 | 6.00 | 30500 | 2440 | 27755 | 305 |
| 75 | 4.15 | 73750 | 17700 | 54575 | 1475 |
| 105 | 10.08 | 44250 | 8408 | 34515 | 1328 |
| 97 | 8.23 | 51750 | 37260 | 11903 | 2070 |

Table B-4. Leukograms of trout examined 1 day after static exposure to 21.0 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 114 | 13.34 | 68250 | 5460 | 62108 | 683 |
| 93 | 8.07 | 46500 | 5580 | 40920 | 0 |
| 114 | 10.86 | 86000 | 14620 | 71380 | 0 |
| 116 | 10.69 | 33500 | 14740 | 14405 | 4355 |
| 75 | 3.85 | 64750 | 4533 | 54390 | 5828 |
| 76 | 4.04 | 36000 | 11880 | 20880 | 3240 |
| 104 | 10.16 | 73500 | 8085 | 63210 | 2205 |
| 89 | 7.45 | 54250 | 5968 | 48283 | 0 |
| 79 | 5.17 | 34500 | 4140 | 28635 | 1725 |
| 100 | 9.90 | 28250 | 12148 | 14690 | 1413 |
| 116 | 13.60 | 30500 | 11590 | 17995 | 915 |
| 100 | 9.79 | 38000 | 6460 | 28880 | 2280 |
| 103 | 10.27 | 26250 | 6300 | 18375 | 1575 |
| 88 | 5.92 | 17750 | 3550 | 7810 | 6213 |
| 92 | 6.84 | 21000 | 14280 | 5460 | 1260 |
| 118 | 14.63 | 29500 | 4720 | 24190 | 590 |
| 88 | 5.61 | 36750 | 0 | 36015 | 735 |
| 103 | 9.90 | 49000 | 29400 | 18620 | 980 |
| 102 | 9.16 | 40250 | 4025 | 35823 | 403 |
| 118 | 13.66 | 64000 | 21120 | 42880 | 0 |

Table B-5. Leukograms of trout examined 4 days after static exposure to 0.00 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 160 | 34.01 | 32500 | 4875 | 19825 | 7800 |
| 156 | 39.78 | 24750 | 990 | 23513 | 248 |
| 132 | 17.99 | 21750 | 4785 | 15660 | 1305 |
| 159 | 39.00 | 32750 | 0 | 31113 | 1638 |
| 124 | 16.60 | 25000 | 12000 | 8750 | 4000 |
| 141 | 24.75 | 12750 | 383 | 9945 | 2295 |
| 155 | 37.28 | 15250 | 2135 | 10828 | 2288 |
| 140 | 23.69 | 27250 | 5450 | 20710 | 818 |
| 166 | 42.31 | 42000 | 9660 | 28560 | 3780 |
| 184 | 63.46 | 24250 | 2910 | 19400 | 1940 |
| 102 | 9.53 | 63250 | 6325 | 55660 | 633 |
| 87 | 6.74 | 39750 | 5168 | 34185 | 398 |
| 79 | 5.00 | 54250 | 6570 | 47198 | 543 |
| 78 | 5.21 | 53750 | 1075 | 52675 | 0 |
| 85 | 6.92 | 89750 | 8078 | 81673 | 0 |
| 115 | 17.69 | 49250 | 5910 | 41370 | 1970 |
| 86 | 7.60 | 64500 | 645 | 63855 | 0 |
| 82 | 6.04 | 32250 | 2580 | 29670 | 0 |
| 83 | 5.66 | 57250 | 2863 | 53815 | 0 |
| 75 | 4.46 | 44750 | 1790 | 42513 | 448 |
| 85 | 6.83 | 34250 | 9590 | 23975 | 685 |
| 117 | 14.28 | 22750 | 2048 | 20475 | 228 |
| 110 | 13.41 | 40000 | 18400 | 20800 | 800 |
| 99 | 8.92 | 36500 | 8030 | 27375 | 730 |
| 92 | 7.71 | 13000 | 5330 | 7540 | 130 |
| 103 | 9.15 | 59250 | 4148 | 52733 | 2370 |
| 84 | 5.93 | 30250 | 3630 | 26620 | 0 |
| 95 | 7.25 | 54250 | 8680 | 43400 | 2170 |
| 90 | 7.36 | 44000 | 6160 | 37840 | 0 |
| 110 | 12.58 | 48000 | 15360 | 31680 | 960 |

Table B-6. Leukograms of trout examined 4 days after static exposure to 1.35 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 138 | 22.02 | 30000 | 12900 | 8700 | 8400 |
| 176 | 54.17 | 39500 | 3160 | 30415 | 5925 |
| 138 | 28.11 | 30500 | 9760 | 17690 | 3050 |
| 149 | 35.20 | 40750 | 1223 | 37490 | 1630 |
| 157 | 40.62 | 31250 | 4063 | 24688 | 2500 |
| 165 | 47.28 | 65500 | 2620 | 58950 | 3275 |
| 169 | 47.60 | 33000 | 3630 | 25410 | 3960 |
| 141 | 31.14 | 39000 | 4680 | 33930 | 390 |
| 141 | 29.18 | 23000 | 8050 | 14030 | 920 |
| 93 | 9.00 | 71750 | 0 | 69598 | 2153 |
| 93 | 8.16 | 41500 | 12035 | 29050 | 415 |
| 99 | 9.67 | 57000 | 9120 | 47880 | 0 |
| 100 | 9.84 | 67250 | 2690 | 63888 | 0 |
| 115 | 11.72 | 60250 | 3615 | 56635 | 0 |
| 101 | 10.58 | 58250 | 1748 | 56503 | 0 |
| 120 | 19.97 | 39500 | 7110 | 31995 | 395 |
| 104 | 10.20 | 42250 | 8028 | 33800 | 423 |
| 89 | 7.09 | 50000 | 5500 | 43500 | 1000 |
| 90 | 7.52 | 66250 | 5963 | 59625 | 663 |
| 102 | 10.51 | 53000 | 10070 | 42400 | 530 |
| 122 | 16.77 | 54250 | 3798 | 49368 | 1085 |
| 89 | 7.59 | 43000 | 2150 | 40850 | 0 |
| 102 | 9.07 | 38500 | 14245 | 23870 | 385 |
| 97 | 8.46 | 50250 | 11558 | 38693 | 0 |
| 120 | 16.31 | 53500 | 4280 | 49220 | 0 |
| 90 | 6.94 | 48000 | 8160 | 39840 | 0 |
| 114 | 14.08 | 49750 | 16915 | 32835 | 0 |
| 109 | 13.54 | 40750 | 4890 | 35860 | 0 |
| 92 | 7.26 | 50500 | 5555 | 44440 | 505 |

Table B-7. Leukograms of trout examined 4 days after static exposure to 13.5 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 167 | 35.55 | 50000 | 19500 | 14500 | 16000 |
| 150 | 32.28 | 35500 | 2130 | 30175 | 3195 |
| 130 | 20.74 | 22000 | 14300 | 6600 | 1100 |
| 159 | 43.79 | 45000 | 4500 | 36450 | 4050 |
| 164 | 36.62 | 25500 | 8415 | 12240 | 4845 |
| 169 | 45.75 | 19500 | 0 | 19305 | 195 |
| 158 | 36.64 | 53000 | 17490 | 21730 | 13780 |
| 170 | 49.68 | 33500 | 7705 | 24455 | 1340 |
| 172 | 47.87 | 32750 | 2293 | 27838 | 2620 |
| 153 | 26.00 | 38750 | 2325 | 36425 | 0 |
| 102 | 11.19 | 74000 | 12580 | 60680 | 0 |
| 100 | 11.17 | 92750 | 1855 | 90895 | 0 |
| 89 | 7.91 | 49250 | 1970 | 46295 | 985 |
| 99 | 10.20 | 60500 | 8470 | 52030 | 0 |
| 108 | 13.03 | 53000 | 1060 | 51940 | 0 |
| 87 | 6.28 | 70000 | 14700 | 53200 | 2100 |
| 111 | 13.79 | 58250 | 4078 | 54173 | 0 |
| 111 | 13.67 | 58750 | 10575 | 48175 | 0 |
| 96 | 9.29 | 47500 | 2375 | 44175 | 950 |
| 96 | 8.55 | 27250 | 4088 | 22618 | 545 |
| 123 | 15.73 | 52500 | 12600 | 38850 | 1050 |
| 105 | 11.91 | 43750 | 12250 | 30188 | 1313 |
| 102 | 13.50 | 52000 | 14040 | 37960 | 0 |
| 122 | 16.79 | 40500 | 12150 | 22680 | 5670 |
| 77 | 4.46 | 45250 | 15385 | 27885 | 0 |
| 94 | 7.60 | 32500 | 12675 | 16250 | 3250 |
| 79 | 4.86 | 32750 | 1310 | 30458 | 983 |
| 92 | 6.41 | 56500 | 4520 | 51415 | 565 |
| 111 | 12.16 | 46250 | 6475 | 39775 | 0 |
| 98 | 8.25 | 33250 | 9975 | 22943 | 333 |

Table B-8. Leukograms of trout examined 4 days after static exposure to 21.0 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 64 | 2.27 | 53000 | 6360 | 46110 | 530 |
| 96 | 9.25 | 42250 | 10563 | 16055 | 14788 |
| 82 | 5.64 | 76000 | 9880 | 66120 | 0 |
| 96 | 8.18 | 51250 | 10763 | 38438 | 2050 |
| 115 | 16.28 | 58250 | 2330 | 54173 | 1748 |
| 89 | 6.69 | 29500 | 11770 | 24485 | 2065 |
| 110 | 15.73 | 92500 | 1850 | 89725 | 925 |
| 79 | 5.33 | 33750 | 3713 | 28350 | 1688 |
| 106 | 11.85 | 78500 | 3140 | 75360 | 0 |
| 113 | 12.76 | 57000 | 4560 | 49020 | 570 |
| 100 | 8.89 | 42250 | 9718 | 32533 | 0 |
| 115 | 13.50 | 50000 | 2000 | 48000 | 0 |
| 88 | 6.29 | 59250 | 5925 | 53325 | 0 |
| 110 | 11.71 | 53000 | 23320 | 28090 | 1590 |
| 91 | 7.87 | 60750 | 9720 | 51030 | 0 |
| 112 | 12.81 | 67000 | 15410 | 51590 | 0 |

Table B-9. Leukograms of trout examined 14 days after static exposure to 0.00 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 185 | 54.74 | 43750 | 5250 | 37625 | 438 |
| 173 | 43.90 | 26250 | 14175 | 11813 | 263 |
| 225 | 91.97 | 20500 | 3280 | 17720 | 0 |
| 125 | 15.05 | 24250 | 4365 | 19885 | 0 |
| 130 | 19.67 | 15500 | 3875 | 10850 | 775 |
| 225 | 104.45 | 37000 | 12580 | 7700 | 16650 |
| 95 | 8.70 | 44000 | 1320 | 42240 | 440 |
| 99 | 10.32 | 73500 | 3675 | 67620 | 2205 |
| 100 | 9.34 | 67500 | 0 | 67500 | 0 |
| 96 | 8.73 | 84250 | 6740 | 76668 | 843 |
| 99 | 9.72 | 80500 | 7245 | 73255 | 0 |
| 90 | 7.43 | 62000 | 4340 | 55180 | 1860 |
| 94 | 9.18 | 77750 | 0 | 77750 | 0 |
| 88 | 7.52 | 68250 | 2730 | 65520 | 0 |
| 98 | 9.88 | 52000 | 6240 | 45760 | 0 |
| 109 | 12.32 | 77500 | 3875 | 72850 | 775 |
| 98 | 9.34 | 52000 | 9360 | 42640 | 0 |
| 126 | 17.71 | 62250 | 19920 | 42330 | 0 |
| 126 | 16.67 | 44500 | 1780 | 42720 | 0 |
| 111 | 11.76 | 63250 | 6325 | 53763 | 3163 |
| 108 | 12.99 | 47500 | 4275 | 43225 | 0 |
| 112 | 14.44 | 63750 | 8925 | 54825 | 0 |
| 90 | 7.20 | 35000 | 2100 | 32900 | 0 |
| 109 | 12.76 | 31000 | 5580 | 25420 | 0 |
| 89 | 6.46 | 49500 | 4950 | 44550 | 0 |
| 89 | 6.85 | 51250 | 11788 | 38950 | 513 |

Table B-10. Leukograms of trout examined 14 days after static exposure to 1.35 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 172 | 45.39 | 24750 | 4950 | 19553 | 248 |
| 150 | 33.29 | 35750 | 715 | 34678 | 358 |
| 155 | 33.97 | 16500 | 1155 | 14685 | 660 |
| 157 | 35.50 | 41750 | 7098 | 33400 | 1253 |
| 135 | 19.95 | 8750 | 788 | 7525 | 350 |
| 137 | 22.86 | 45000 | 450 | 42300 | 2250 |
| 187 | 60.52 | 20500 | 2665 | 17220 | 615 |
| 160 | 41.21 | 21500 | 860 | 19780 | 860 |
| 130 | 17.96 | 24250 | 1698 | 20370 | 2183 |
| 102 | 11.68 | 72000 | 3600 | 68400 | 0 |
| 87 | 7.05 | 55000 | 550 | 54450 | 0 |
| 83 | 6.25 | 61000 | 610 | 60390 | 0 |
| 84 | 6.55 | 96000 | 8040 | 87360 | 0 |
| 117 | 16.41 | 64250 | 4498 | 59753 | 0 |
| 89 | 7.25 | 55000 | 22200 | 52800 | 0 |
| 126 | 20.18 | 73250 | 5128 | 68123 | 0 |
| 96 | 8.78 | 51000 | 3570 | 47430 | 0 |
| 115 | 15.92 | 66250 | 1325 | 64925 | 0 |
| 90 | 7.29 | 69500 | 695 | 68805 | 0 |
| 97 | 8.65 | 38750 | 15113 | 23637 | 0 |
| 115 | 14.89 | 69500 | 4865 | 64635 | 0 |
| 121 | 16.63 | 62250 | 14318 | 47933 | 0 |
| 117 | 15.55 | 75250 | 15050 | 59448 | 753 |
| 123 | 17.21 | 55000 | 10450 | 44550 | 0 |
| 90 | 7.31 | 30000 | 8100 | 21300 | 600 |
| 107 | 11.40 | 74000 | 0 | 74000 | 0 |
| 113 | 14.15 | 59250 | 14220 | 45030 | 0 |
| 83 | 6.42 | 50250 | 8543 | 41708 | 0 |
| 103 | 10.45 | 50500 | 3030 | 47470 | 0 |

Table B-11. Leukograms of trout examined 14 days after static exposure to 13.5 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 192 | 67.72 | 42750 | 1283 | 40613 | 855 |
| 160 | 35.20 | 29250 | 1463 | 26910 | 878 |
| 151 | 30.90 | 40000 | 1600 | 38400 | 0 |
| 184 | 57.98 | 26000 | 1560 | 23400 | 1040 |
| 148 | 29.03 | 29750 | 2380 | 26775 | 595 |
| 167 | 38.23 | 28500 | 5415 | 19950 | 3135 |
| 116 | 17.48 | 47500 | 1425 | 46075 | 0 |
| 108 | 13.09 | 93000 | 4650 | 87420 | 930 |
| 94 | 8.93 | 54250 | 4883 | 49368 | 0 |
| 120 | 17.12 | 81250 | 4063 | 77188 | 0 |
| 83 | 5.69 | 60750 | 0 | 60750 | 0 |
| 90 | 7.24 | 33000 | 5940 | 27060 | 0 |
| 104 | 10.97 | 48750 | 13650 | 35100 | 0 |
| 118 | 18.27 | 63250 | 3795 | 58823 | 633 |
| 113 | 13.84 | 65750 | 658 | 63778 | 1315 |
| 89 | 8.12 | 37000 | 4440 | 32560 | 0 |
| 93 | 7.87 | 54750 | 6570 | 48180 | 0 |
| 102 | 10.19 | 38000 | 1520 | 36480 | 0 |
| 103 | 10.16 | 65750 | 9205 | 56545 | 0 |
| 109 | 13.02 | 65250 | 7178 | 58073 | 0 |
| 112 | 11.56 | 59750 | 598 | 59152 | 0 |
| 95 | 8.48 | 33250 | 6983 | 26268 | 0 |
| 110 | 14.00 | 61250 | 3063 | 57575 | 613 |
| 97 | 9.61 | 44500 | 5785 | 38270 | 445 |
| 94 | 8.13 | 54750 | 547 | 54203 | 0 |
| 90 | 7.21 | 35000 | 5600 | 29400 | 0 |

Table B-12. Leukograms of trout examined 14 days after static exposure to 21.0 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 104 | 10.97 | 56500 | 1695 | 54805 | 0 |
| 105 | 12.66 | 54750 | 1643 | 52560 | 548 |
| 81 | 5.48 | 48250 | 3860 | 43908 | 483 |
| 115 | 10.55 | 50250 | 1005 | 48240 | 1005 |
| 95 | 8.60 | 49756 | 0 | 49750 | 0 |
| 91 | 7.30 | 46500 | 3255 | 42780 | 0 |
| 115 | 11.84 | 67500 | 2700 | 64125 | 675 |
| 120 | 15.26 | 50500 | 505 | 49490 | 505 |
| 112 | 14.41 | 98500 | 10835 | 87665 | 0 |
| 95 | 6.66 | 32250 | 15480 | 16125 | 645 |
| 105 | 10.92 | 47000 | 2820 | 43240 | 940 |
| 108 | 12.16 | 46500 | 15810 | 29760 | 930 |
| 105 | 11.10 | 54000 | 7020 | 46980 | 0 |
| 122 | 16.40 | 43250 | 11678 | 31572 | 0 |
| 119 | 17.41 | 43750 | 11375 | 32375 | 0 |

Table B-13. Leukograms of trout examined 28 days after static exposure to 0.00 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 215 | 156.40 | 38750 | 5425 | 32550 | 775 |
| 99 | 9.93 | 52000 | 520 | 50440 | 520 |
| 106 | 12.72 | 46250 | 463 | 45325 | 463 |
| 126 | 21.52 | 45250 | 12670 | 32580 | 0 |
| 118 | 17.80 | 71750 | 15785 | 55965 | 0 |
| 77 | 4.89 | 67000 | 2680 | 63650 | 670 |
| 124 | 20.30 | 75750 | 1515 | 73478 | 758 |
| 123 | 19.79 | 56250 | 2813 | 52313 | 563 |
| 115 | 16.33 | 60750 | 5468 | 54068 | 608 |
| 114 | 14.70 | 58500 | 4095 | 53820 | 585 |
| 127 | 21.70 | 60000 | 7200 | 51600 | 1200 |
| 110 | 13.76 | 56500 | 15255 | 40680 | 565 |
| 111 | 13.93 | 62250 | 3113 | 59137 | 0 |
| 95 | 8.28 | 74250 | 18562 | 55688 | 0 |
| 116 | 14.19 | 49000 | 7840 | 41160 | 0 |
| 113 | 12.43 | 71000 | 6390 | 63190 | 1420 |
| 128 | 17.97 | 63500 | 6350 | 55880 | 1270 |
| 135 | 23.26 | 39250 | 7065 | 31793 | 0 |
| 114 | 14.31 | 36750 | 5145 | 30870 | 735 |
| 108 | 12.29 | 41750 | 10437 | 31313 | 0 |
| 100 | 9.03 | 41500 | 830 | 40670 | 0 |

Table B-14. Leukograms of trout examined 28 days after static exposure to 1.35 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 192 | 60.08 | 29500 | 7670 | 21535 | 295 |
| 198 | 68.03 | 23500 | 3525 | 18800 | 1175 |
| 200 | 78.46 | 36750 | 6983 | 27563 | 2205 |
| 123 | 15.59 | 24500 | 1225 | 22785 | 245 |
| 168 | 40.90 | 17500 | 525 | 14000 | 2625 |
| 158 | 33.78 | 30500 | 3050 | 25315 | 1525 |
| 228 | 124.64 | 26250 | 1050 | 24675 | 525 |
| 198 | 78.31 | 48000 | 1440 | 45600 | 960 |
| 110 | 14.70 | 54250 | 14648 | 39603 | 0 |
| 91 | 7.12 | 93750 | 10313 | 83438 | 0 |
| 122 | 18.81 | 55000 | 550 | 53900 | 550 |
| 115 | 15.77 | 74250 | 8168 | 65340 | 743 |
| 110 | 12.01 | 51250 | 5125 | 45613 | 513 |
| 133 | 22.38 | 56750 | 7378 | 48805 | 568 |
| 90 | 7.72 | 47000 | 3290 | 43710 | 0 |
| 108 | 12.53 | 83750 | 10050 | 72025 | 1675 |
| 103 | 12.32 | 86500 | 9515 | 76120 | 865 |
| 91 | 6.97 | 49250 | 3448 | 45310 | 493 |
| 91 | 5.57 | 64000 | 3200 | 60800 | 0 |
| 105 | 10.37 | 47750 | 2865 | 43453 | 955 |
| 114 | 13.70 | 66250 | 0 | 66250 | 0 |
| 99 | 9.65 | 64500 | 16770 | 47085 | 645 |
| 121 | 16.19 | 64000 | 10880 | 53120 | 0 |
| 102 | 10.59 | 65750 | 657 | 65093 | 0 |
| 109 | 11.78 | 39250 | 1570 | 37287 | 393 |
| 118 | 15.02 | 41750 | 835 | 40498 | 418 |
| 123 | 18.13 | 42250 | 1268 | 40983 | 0 |
| 86 | 6.77 | 50250 | 6030 | 43717 | 503 |

Table B-15. Leukograms of trout examined 28 days after static exposure to 13.5 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 162 | 40.36 | 16500 | 3465 | 11880 | 990 |
| 215 | 77.50 | 39750 | 6360 | 29018 | 3975 |
| 228 | 102.48 | 11000 | 660 | 9900 | 440 |
| 185 | 56.05 | 36250 | 2175 | 31900 | 2175 |
| 170 | 44.59 | 42250 | 2535 | 38870 | 845 |
| 94 | 8.78 | 38250 | 6503 | 32185 | 0 |
| 128 | 24.88 | 72000 | 10080 | 61920 | 0 |
| 137 | 24.61 | 55000 | 10450 | 44550 | 0 |
| 100 | 8.51 | 34000 | 1700 | 29580 | 1700 |
| 105 | 11.42 | 72750 | 8730 | 64020 | 0 |
| 103 | 11.84 | 39750 | 11527 | 27825 | 0 |
| 105 | 12.32 | 19500 | 9165 | 8970 | 1365 |
| 101 | 9.48 | 54250 | 4883 | 49367 | 0 |
| 128 | 18.21 | 45750 | 3202 | 42548 | 0 |
| 125 | 18.39 | 42000 | 5880 | 36120 | 0 |
| 130 | 21.25 | 44500 | 4005 | 40495 | 0 |
| 96 | 8.49 | 35000 | 350 | 34300 | 350 |
| 95 | 8.29 | 20000 | 400 | 19600 | 0 |
| 122 | 16.22 | 39750 | 11925 | 27825 | 0 |
| 132 | 20.65 | 34500 | 1035 | 33465 | 0 |
| 127 | 19.92 | 41250 | 3713 | 37537 | 0 |
| 115 | 13.37 | 23500 | 5405 | 18095 | 0 |

Table B-16. Leukograms of trout examined 28 days after static exposure to 21.0 mg/l malachite green for 25-30 min at 12 C

| L (mm) | Wt (g) | WBC-T[a] | Thrombocytes[b] | Lymphocytes[b] | Polymorphs[b] |
|---|---|---|---|---|---|
| 117 | 17.25 | 68500 | 4795 | 62335 | 1370 |
| 111 | 11.76 | 51500 | 515 | 50985 | 0 |
| 84 | 6.18 | 63000 | 1260 | 61740 | 0 |
| 120 | 17.38 | 50500 | 13130 | 37370 | 0 |
| 102 | 11.41 | 66000 | 0 | 66000 | 0 |
| 119 | 16.78 | 44500 | 445 | 43165 | 890 |
| 95 | 7.52 | 54000 | 15660 | 37800 | 540 |
| 130 | 23.24 | 48250 | 4343 | 43907 | 0 |

# APPENDIX C.

## LEUKOGRAMS OF FISH EXPOSED TO MALACHITE GREEN FOR 30 MIN (EXAMINED HOURLY DURING FIRST 24 HR)

Table C-1. Leukograms of trout examined hourly during the first 24 hr after static exposure to 0.00 mg/l malachite green for 30 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 91 | 7.67 | 24000 | 4320 | 16080 | 3600 |
| 0 | 105 | 12.53 | 30500 | 10675 | 18910 | 915 |
| 0 | 117 | 15.97 | 50750 | 13195 | 36540 | 1015 |
| 0 | 117 | 16.13 | 50250 | 4523 | 45727 | 0 |
| 0 | 105 | 11.19 | 58750 | 4700 | 51113 | 2937 |
| 0 | 140 | 26.99 | 66750 | 1335 | 65415 | 0 |
| 0 | 148 | 31.09 | 73500 | 13965 | 5880 | 735 |
| 1 | 95 | 8.00 | 43500 | 3480 | 39150 | 435 |
| 1 | 98 | 9.25 | 46750 | 16362 | 27583 | 2805 |
| 1 | 73 | 4.08 | 27750 | 6938 | 20535 | 288 |
| 1 | 63 | 2.56 | 43750 | 4813 | 38500 | 438 |
| 1 | 85 | 5.86 | 36000 | 7920 | 27360 | 720 |
| 1 | 93 | 9.33 | 74750 | 18688 | 55315 | 748 |
| 1 | 90 | 8.33 | 38000 | 5700 | 32300 | 0 |
| 1 | 76 | 5.68 | 65750 | 9205 | 55888 | 658 |
| 2 | 90 | 6.52 | 76250 | 3813 | 71675 | 763 |
| 2 | 85 | 5.52 | 41750 | 17535 | 24215 | 0 |
| 2 | 87 | 6.34 | 25500 | 10710 | 14535 | 255 |
| 2 | 82 | 5.57 | 63500 | 6985 | 55245 | 1270 |
| 2 | 72 | 4.14 | 41500 | 9130 | 31540 | 830 |
| 2 | 100 | 11.20 | 36250 | 8700 | 27550 | 0 |
| 2 | 99 | 11.55 | 35250 | 15158 | 20093 | 0 |
| 2 | 82 | 5.38 | 41000 | 19270 | 20500 | 1230 |
| 3 | 85 | 5.05 | 88000 | 10560 | 76560 | 880 |
| 3 | 96 | 7.66 | 49750 | 7463 | 42288 | 0 |
| 3 | 87 | 7.90 | 30250 | 9983 | 19965 | 303 |
| 3 | 83 | 6.22 | 39000 | 8580 | 30420 | 0 |
| 3 | 69 | 3.45 | 41500 | 10790 | 30295 | 415 |
| 3 | 123 | 19.00 | 45250 | 13123 | 32128 | 0 |
| 3 | 87 | 6.64 | 34750 | 10773 | 23478 | 0 |
| 3 | 108 | 9.73 | 58750 | 12338 | 45238 | 1175 |

[a]Total white blood cell-thrombocytes per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

[b]Number per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

Table C-1. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 4 | 91 | 7.16 | 40500 | 8910 | 31590 | 0 |
| 4 | 87 | 6.26 | 58250 | 16893 | 40775 | 583 |
| 4 | 75 | 4.29 | 21250 | 7013 | 13813 | 425 |
| 4 | 75 | 4.01 | 39500 | 12245 | 27255 | 0 |
| 4 | 67 | 2.99 | 23750 | 8075 | 15438 | 238 |
| 4 | 72 | 3.82 | 42000 | 10080 | 31920 | 0 |
| 4 | 89 | 8.16 | 32250 | 12578 | 19028 | 645 |
| 4 | 75 | 4.60 | 34250 | 13015 | 20550 | 685 |
| 5 | 95 | 7.65 | 44750 | 2238 | 41170 | 1343 |
| 5 | 90 | 7.11 | 53750 | 13438 | 39775 | 538 |
| 5 | 76 | 4.56 | 27000 | 14310 | 12150 | 540 |
| 5 | 69 | 3.12 | 33750 | 8438 | 25313 | 0 |
| 5 | 67 | 3.21 | 30500 | 17080 | 13115 | 305 |
| 5 | 95 | 9.17 | 22750 | 5915 | 16608 | 228 |
| 5 | 78 | 5.36 | 34500 | 8625 | 24840 | 1035 |
| 5 | 104 | 11.15 | 44000 | 17600 | 26400 | 0 |
| 6 | 93 | 7.25 | 48250 | 9650 | 38600 | 0 |
| 6 | 82 | 4.96 | 31000 | 18290 | 11780 | 930 |
| 6 | 91 | 7.08 | 28000 | 8960 | 19040 | 0 |
| 6 | 74 | 4.24 | 28000 | 10640 | 17080 | 280 |
| 6 | 75 | 4.32 | 29000 | 17400 | 11600 | 0 |
| 6 | 108 | 12.46 | 35750 | 13968 | 22523 | 0 |
| 6 | 104 | 11.49 | 49500 | 15345 | 34155 | 0 |
| 6 | 96 | 10.46 | 32500 | 17550 | 14950 | 0 |
| 7 | 99 | 8.88 | 39500 | 8295 | 30415 | 790 |
| 7 | 84 | 4.86 | 25250 | 17170 | 7575 | 505 |
| 7 | 83 | 6.31 | 27500 | 13750 | 13200 | 275 |
| 7 | 75 | 4.35 | 29000 | 8700 | 20010 | 0 |
| 7 | 70 | 4.04 | 19000 | 7980 | 10830 | 190 |
| 7 | 114 | 14.67 | 25500 | 9945 | 15555 | 0 |
| 7 | 106 | 13.08 | 34750 | 19460 | 15290 | 0 |
| 7 | 106 | 12.02 | 29000 | 8700 | 20300 | 0 |
| 8 | 90 | 6.38 | 43000 | 26230 | 16770 | 0 |
| 8 | 91 | 6.01 | 48500 | 15520 | 32980 | 0 |
| 8 | 82 | 5.79 | 21750 | 12398 | 9353 | 0 |
| 8 | 57 | 2.11 | 19250 | 11165 | 7508 | 578 |
| 8 | 86 | 6.81 | 20000 | 9600 | 9600 | 800 |
| 8 | 98 | 10.26 | 27500 | 16775 | 10725 | 0 |

Table C-1. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 8 | 70 | 3.83 | 25000 | 12750 | 12000 | 250 |
| 8 | 93 | 8.74 | 19000 | 13680 | 4750 | 570 |
| 9 | 97 | 8.63 | 31500 | 10080 | 21105 | 315 |
| 9 | 105 | 10.19 | 34250 | 13015 | 19523 | 1713 |
| 9 | 100 | 9.91 | 29250 | 7898 | 20475 | 1463 |
| 9 | 91 | 7.81 | 23500 | 11280 | 11985 | 235 |
| 9 | 79 | 5.52 | 21750 | 9353 | 12398 | 0 |
| 9 | 102 | 10.79 | 35000 | 15050 | 19950 | 0 |
| 9 | 93 | 8.36 | 26000 | 11960 | 14040 | 0 |
| 9 | 69 | 3.58 | 20750 | 13488 | 6848 | 415 |
| 10 | 94 | 7.61 | 34250 | 11988 | 21235 | 1028 |
| 10 | 104 | 9.59 | 31250 | 5000 | 25938 | 313 |
| 10 | 96 | 8.84 | 39250 | 10598 | 29045 | 393 |
| 10 | 77 | 5.36 | 28500 | 6555 | 21090 | 855 |
| 10 | 103 | 9.47 | 28250 | 13560 | 13560 | 1130 |
| 10 | 90 | 7.94 | 30750 | 17220 | 12915 | 615 |
| 10 | 90 | 8.11 | 41750 | 10020 | 30895 | 835 |
| 10 | 98 | 10.96 | 20000 | 10400 | 9200 | 400 |
| 11 | 99 | 8.69 | 39250 | 9028 | 29830 | 393 |
| 11 | 100 | 8.81 | 31750 | 3175 | 27940 | 318 |
| 11 | 86 | 6.53 | 16500 | 7095 | 8085 | 1320 |
| 11 | 90 | 7.51 | 39250 | 17270 | 21588 | 393 |
| 11 | 92 | 9.80 | 32500 | 10075 | 22425 | 0 |
| 11 | 98 | 9.80 | 27750 | 14985 | 10823 | 1943 |
| 11 | 93 | 8.56 | 29250 | 14040 | 15210 | 0 |
| 12 | 95 | 7.85 | 28250 | 7063 | 20623 | 565 |
| 12 | 97 | 7.74 | 27750 | 9990 | 16373 | 1388 |
| 12 | 62 | 2.55 | 25000 | 8750 | 16250 | 0 |
| 12 | 98 | 10.19 | 52000 | 21321 | 30160 | 520 |
| 12 | 60 | 2.10 | 30000 | 9000 | 20700 | 300 |
| 12 | 78 | 4.91 | 24500 | 13475 | 10780 | 245 |
| 12 | 97 | 8.87 | 27500 | 14300 | 13200 | 0 |
| 12 | 89 | 6.57 | 24250 | 6790 | 16490 | 970 |
| 13 | 104 | 10.93 | 33000 | 4620 | 27720 | 330 |
| 13 | 98 | 8.58 | 33750 | 3038 | 30713 | 0 |
| 13 | 90 | 7.69 | 22750 | 8873 | 13423 | 455 |
| 13 | 101 | 10.26 | 24250 | 10913 | 12368 | 970 |
| 13 | 86 | 6.64 | 12500 | 250 | 11750 | 500 |

Table C-1. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 13 | 101 | 10.61 | 21500 | 11825 | 9460 | 215 |
| 13 | 97 | 10.23 | 23500 | 15510 | 7050 | 705 |
| 13 | 99 | 10.62 | 26250 | 14700 | 10763 | 788 |
| 14 | 95 | 7.76 | 39250 | 11775 | 27475 | 0 |
| 14 | 100 | 8.99 | 38250 | 9945 | 26775 | 1530 |
| 14 | 97 | 9.30 | 21000 | 15540 | 5250 | 210 |
| 14 | 81 | 6.40 | 50750 | 11673 | 39078 | 0 |
| 14 | 88 | 6.22 | 24250 | 13095 | 10185 | 728 |
| 14 | 90 | 7.18 | 18250 | 11498 | 6570 | 183 |
| 14 | 95 | 8.78 | 21500 | 860 | 20640 | 0 |
| 14 | 83 | 6.69 | 26250 | 13650 | 12075 | 525 |
| 15 | 95 | 7.47 | 38000 | 9500 | 26980 | 1520 |
| 15 | 93 | 7.07 | 26750 | 17120 | 9095 | 535 |
| 15 | 64 | 2.54 | 46500 | 17205 | 29295 | 0 |
| 15 | 80 | 4.62 | 21000 | 14700 | 6300 | 0 |
| 15 | 69 | 3.62 | 16250 | 4225 | 9805 | 0 |
| 15 | 75 | 4.62 | 23500 | 11280 | 11985 | 235 |
| 15 | 82 | 5.56 | 35000 | 14700 | 18900 | 1400 |
| 15 | 88 | 7.20 | 26750 | 16585 | 8025 | 2140 |
| 16 | 95 | 8.56 | 16000 | 3360 | 12320 | 320 |
| 16 | 112 | 12.17 | 24000 | 4560 | 19440 | 0 |
| 16 | 85 | 6.68 | 30750 | 7380 | 22448 | 923 |
| 16 | 61 | 2.00 | 30000 | 13200 | 14400 | 2400 |
| 16 | 69 | 3.15 | 21000 | 5040 | 15540 | 420 |
| 16 | 83 | 5.53 | 23750 | 10688 | 12825 | 238 |
| 16 | 90 | 8.35 | 26250 | 14175 | 11550 | 525 |
| 16 | 77 | 5.00 | 60750 | 11543 | 43740 | 5468 |
| 17 | 91 | 6.90 | 22250 | 8677 | 13128 | 445 |
| 17 | 94 | 7.33 | 19750 | -- | -- | -- |
| 17 | 81 | 5.63 | 24000 | 12480 | 10320 | 1200 |
| 17 | 86 | 5.63 | 15750 | 11183 | 3938 | 630 |
| 17 | 88 | 7.07 | 28000 | 13160 | 14840 | 0 |
| 17 | 78 | 10.01 | 25000 | 15000 | 9500 | 500 |
| 17 | 82 | 5.06 | 31000 | 15810 | 14260 | 930 |
| 17 | 95 | 9.63 | 32250 | 12578 | 18060 | 1613 |
| 18 | 99 | 8.85 | 23250 | 8370 | 14880 | 0 |
| 18 | 86 | 6.69 | 24750 | 14850 | 9405 | 495 |
| 18 | 98 | 8.66 | 42750 | 8978 | 33773 | 0 |

Table C-1. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 18 | 98 | 9.00 | 9250 | 6105 | 2683 | 463 |
| 18 | 103 | 10.99 | 39000 | 10530 | 26520 | 1950 |
| 18 | 67 | 2.93 | 23500 | 10340 | 13160 | 0 |
| 18 | 86 | 6.57 | 28000 | 13160 | 14840 | 0 |
| 19 | 89 | 7.03 | 22250 | 2225 | 20025 | 0 |
| 19 | 101 | 9.41 | 26250 | 15750 | 10500 | 0 |
| 19 | 89 | 7.46 | 19750 | 10270 | 9085 | 395 |
| 19 | 102 | 9.64 | 27250 | 12808 | 14443 | 0 |
| 19 | 89 | 6.84 | 21000 | 14070 | 6720 | 210 |
| 19 | 82 | 5.64 | 26500 | 16165 | 10335 | 0 |
| 19 | 71 | 3.87 | 29500 | 15635 | 12980 | 1180 |
| 19 | 92 | 7.48 | 31500 | 23940 | 7245 | 315 |
| 20 | 100 | 9.09 | 26750 | 5350 | 20865 | 535 |
| 20 | 93 | 7.44 | 22000 | 8140 | 13200 | 660 |
| 20 | 74 | 3.90 | 24500 | 15435 | 8820 | 245 |
| 20 | 73 | 3.97 | 14250 | 11115 | 2993 | 143 |
| 20 | 75 | 4.95 | 7750 | 1085 | 6355 | 310 |
| 20 | 84 | 6.50 | 31500 | 15120 | 16380 | 0 |
| 20 | 98 | 9.10 | 31000 | 15810 | 14880 | 310 |
| 20 | 99 | 9.52 | 26750 | 17655 | 8560 | 535 |
| 21 | 92 | 7.66 | 45750 | 14183 | 30195 | 1373 |
| 21 | 95 | 7.49 | 24250 | 6063 | 18187 | 0 |
| 21 | 86 | 6.01 | 30500 | 15250 | 14335 | 915 |
| 21 | 84 | 6.17 | 17250 | 15698 | 1553 | 0 |
| 21 | 83 | 6.00 | 26250 | 16800 | 8925 | 525 |
| 21 | 62 | 2.65 | 15250 | 2440 | 12505 | 305 |
| 21 | 73 | 4.15 | 40750 | 6928 | 33415 | 408 |
| 21 | 81 | 6.36 | 20500 | 9430 | 10045 | 1025 |
| 22 | 95 | 7.69 | 29000 | 12760 | 13920 | 2320 |
| 22 | 88 | 5.84 | 21250 | 7650 | 12750 | 850 |
| 22 | 93 | 8.18 | 17250 | 13283 | 3795 | 173 |
| 22 | 92 | 7.13 | 25250 | 9343 | 15908 | 0 |
| 22 | 58 | 1.86 | 18000 | 4140 | 13500 | 360 |
| 22 | 105 | 10.48 | 24750 | 8663 | 15345 | 495 |
| 22 | 81 | 4.87 | 20750 | 10375 | 10375 | 0 |
| 22 | 75 | 4.78 | 39500 | 9875 | 28440 | 0 |
| 23 | 96 | 8.49 | 30250 | 11495 | 16940 | 1210 |
| 23 | 82 | 5.60 | 30000 | 10800 | 18900 | 300 |

Table C-1. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 23 | 65 | 2.85 | 32000 | 21760 | 9920 | 320 |
| 23 | 61 | 2.20 | 24750 | 15840 | 8168 | 743 |
| 23 | 83 | 6.32 | 34500 | 16905 | 17250 | 345 |
| 23 | 62 | 2.61 | 24500 | 5880 | 16170 | 1960 |
| 23 | 83 | 6.06 | 54500 | 6540 | 47960 | 0 |
| 24 | 105 | 12.12 | 24250 | 11883 | 12368 | 0 |
| 24 | 103 | 9.60 | 29250 | 15210 | 12578 | 1463 |
| 24 | 73 | 3.39 | 13750 | 5913 | 7563 | 275 |
| 24 | 83 | 5.94 | 25500 | 13005 | 12240 | 255 |
| 24 | 63 | 2.63 | 25750 | 11588 | 13133 | 1030 |
| 24 | 69 | 3.92 | 38250 | 15300 | 22950 | 0 |
| 24 | 77 | 4.94 | 38000 | 10260 | 27740 | 0 |

Table C-2. Leukograms of trout examined hourly during the first 24 hr after static exposure to 1.35 mg/l malachite green for 30 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 137 | 22.19 | 44250 | 15930 | 27435 | 885 |
| 0 | 144 | 26.74 | 44750 | 447 | 44303 | 0 |
| 0 | 129 | 18.97 | 47250 | 10395 | 36855 | 0 |
| 0 | 127 | 18.20 | 55250 | 1105 | 51935 | 2210 |
| 0 | 144 | 25.33 | 55250 | 24310 | 30940 | 0 |
| 0 | 78 | 3.71 | 25500 | 3060 | 22440 | 0 |
| 0 | 84 | 4.79 | 32250 | 5483 | 26445 | 323 |
| 1 | 101 | 9.39 | 40750 | 11003 | 28525 | 1223 |
| 1 | 96 | 8.05 | 42000 | 17220 | 24360 | 420 |
| 1 | 110 | 11.92 | 40750 | 15078 | 24450 | 1223 |
| 1 | 86 | 6.06 | 29000 | 11600 | 17110 | 290 |
| 1 | 82 | 5.73 | 30250 | 12100 | 18150 | 0 |
| 1 | 77 | 4.24 | 48750 | 4875 | 43388 | 488 |
| 1 | 96 | 9.12 | 74000 | 12580 | 60680 | 740 |
| 1 | 112 | 14.06 | 50250 | 8543 | 41708 | 0 |
| 1 | 91 | 7.45 | 56000 | 17360 | 37520 | 1120 |
| 2 | 104 | 10.54 | 51000 | 13770 | 36720 | 510 |
| 2 | 94 | 7.92 | 43000 | 1720 | 40850 | 430 |
| 2 | 101 | 9.64 | 37750 | 7550 | 30200 | 0 |
| 2 | 96 | 8.56 | 34750 | 13205 | 21545 | 0 |
| 2 | 69 | 3.47 | 25750 | 7210 | 18283 | 258 |
| 2 | 85 | 6.14 | 41000 | 5330 | 35670 | 410 |
| 2 | 95 | 8.05 | 55500 | 15540 | 39960 | 0 |
| 2 | 108 | 13.15 | 51500 | 8755 | 41715 | 1030 |
| 2 | 79 | 5.50 | 82250 | 8225 | 74025 | 0 |
| 3 | 97 | 8.87 | 42250 | 11408 | 30843 | 0 |
| 3 | 100 | 9.78 | 38750 | 5038 | 33713 | 0 |
| 3 | 90 | 7.01 | 35750 | 9295 | 26098 | 358 |
| 3 | 92 | 7.50 | 50750 | 7105 | 43645 | 0 |
| 3 | 82 | 5.84 | 21000 | 630 | 20370 | 0 |
| 3 | 82 | 5.54 | 63000 | 25200 | 37800 | 0 |
| 3 | 116 | 16.27 | 79000 | 8690 | 68730 | 1580 |
| 3 | 84 | 5.91 | 52250 | 12018 | 40599 | 0 |
| 3 | 87 | 7.47 | 49500 | 17325 | 32175 | 0 |
| 4 | 110 | 11.88 | 52250 | 13063 | 37620 | 1568 |
| 4 | 95 | 7.74 | 62250 | 16185 | 45443 | 623 |
| 4 | 97 | 6.26 | 36000 | 12960 | 21600 | 1440 |

Table C-2. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 4 | 96 | 8.52 | 64750 | 7123 | 56980 | 648 |
| 4 | 66 | 2.80 | 37000 | 7770 | 28120 | 1110 |
| 4 | 62 | 2.49 | 30250 | 3933 | 26318 | 0 |
| 4 | 104 | 11.80 | 34500 | 10350 | 24150 | 0 |
| 4 | 102 | 12.57 | 55250 | 14918 | 40333 | 0 |
| 4 | 96 | 10.50 | 43250 | 15138 | 28113 | 0 |
| 5 | 95 | 7.39 | 37250 | 16763 | 19743 | 745 |
| 5 | 110 | 13.07 | 71000 | 28400 | 42600 | 0 |
| 5 | 100 | 9.45 | 27500 | 13750 | 13750 | 0 |
| 5 | 77 | 4.81 | 24000 | 10560 | 12960 | 480 |
| 5 | 70 | 3.65 | 24250 | 5093 | 18915 | 243 |
| 5 | 82 | 5.79 | 32500 | 12025 | 20475 | 0 |
| 5 | 80 | 5.32 | 24250 | 8730 | 15520 | 0 |
| 5 | 105 | 12.42 | 21250 | 11050 | 10200 | 0 |
| 5 | 84 | 6.57 | 36250 | 15588 | 20300 | 363 |
| 6 | 81 | 5.44 | 51500 | 16480 | 33990 | 1030 |
| 6 | 101 | 9.84 | 34000 | 14960 | 19040 | 0 |
| 6 | 97 | 9.00 | 30000 | 15600 | 13800 | 600 |
| 6 | 81 | 5.82 | 24500 | 16660 | 7595 | 245 |
| 6 | 57 | 1.70 | 32500 | 20150 | 12025 | 325 |
| 6 | 85 | 6.27 | 27750 | 14430 | 13320 | 0 |
| 6 | 104 | 11.54 | 37250 | 17880 | 18998 | 373 |
| 6 | 66 | 2.70 | 39500 | 11455 | 28045 | 0 |
| 6 | 83 | 7.35 | 32000 | 15680 | 16640 | 0 |
| 7 | 75 | 4.54 | 36750 | 15068 | 20213 | 1470 |
| 7 | 101 | 9.51 | 30750 | 11070 | 19680 | 0 |
| 7 | 105 | 10.47 | 29000 | 10730 | 17690 | 580 |
| 7 | 95 | 8.36 | 27250 | 7085 | 19893 | 273 |
| 7 | 84 | 5.76 | 8250 | 5363 | 2640 | 248 |
| 7 | 81 | 5.24 | 40750 | 13040 | 27710 | 0 |
| 7 | 84 | 7.27 | 43500 | 14355 | 28710 | 435 |
| 7 | 97 | 9.76 | 29250 | 12578 | 16380 | 293 |
| 7 | 112 | 13.54 | 29750 | 6843 | 22908 | 0 |
| 8 | 95 | 8.00 | 25750 | 13390 | 12103 | 258 |
| 8 | 98 | 9.25 | 40000 | 12800 | 27200 | 0 |
| 8 | 97 | 8.40 | 20000 | 12000 | 8000 | 0 |
| 8 | 68 | 3.39 | 16500 | 4950 | 11550 | 165 |
| 8 | 60 | 2.04 | 19500 | 8385 | 11115 | 0 |
| 8 | 76 | 4.11 | 24250 | 13823 | 9700 | 728 |

Table C-2. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 8 | 89 | 7.28 | 23250 | 13950 | 9300 | 0 |
| 8 | 71 | 3.66 | 23000 | 9430 | 13570 | 0 |
| 8 | 99 | 10.55 | 34000 | 18700 | 15300 | 0 |
| 9 | 95 | 7.75 | 38750 | 12788 | 25575 | 388 |
| 9 | 92 | 6.40 | 52250 | 1045 | 51205 | 0 |
| 9 | 95 | 9.15 | 35000 | 10850 | 23800 | 350 |
| 9 | 86 | 7.03 | 29250 | 11993 | 17258 | 0 |
| 9 | 89 | 8.43 | 35000 | 5950 | 28350 | 700 |
| 9 | 89 | 7.43 | 31750 | 19368 | 12065 | 0 |
| 9 | 63 | 2.73 | 28250 | 11300 | 15255 | 1695 |
| 9 | 96 | 9.69 | 21500 | 15695 | 5590 | 215 |
| 10 | 77 | 4.67 | 36250 | 14138 | 20663 | 0 |
| 10 | 91 | 6.46 | 32500 | 14300 | 16250 | 1950 |
| 10 | 96 | 9.62 | 28000 | 11200 | 16520 | 280 |
| 10 | 92 | 9.32 | 40000 | 10800 | 29200 | 0 |
| 10 | 77 | 4.99 | 42750 | 15818 | 26078 | 855 |
| 10 | 86 | 7.02 | 14750 | 9440 | 4868 | 443 |
| 10 | 97 | 9.43 | 35750 | 25025 | 10725 | 0 |
| 10 | 87 | 7.16 | 24000 | 17280 | 6480 | 240 |
| 11 | 93 | 7.24 | 53750 | 16663 | 37088 | 0 |
| 11 | 78 | 4.69 | 40750 | 24858 | 15893 | 0 |
| 11 | 94 | 8.77 | 33500 | 18425 | 13735 | 1340 |
| 11 | 85 | 6.26 | 43750 | 21000 | 22313 | 438 |
| 11 | 88 | 7.61 | 49000 | 15190 | 33320 | 0 |
| 11 | 102 | 11.50 | 34250 | 17125 | 16440 | 685 |
| 11 | 87 | 6.82 | 29250 | 10688 | 12870 | 293 |
| 11 | 94 | 9.66 | 36000 | 11880 | 23760 | 360 |
| 12 | 94 | 8.11 | 51750 | 16560 | 34155 | 1035 |
| 12 | 90 | 5.99 | 38250 | 11475 | 26775 | 0 |
| 12 | 88 | 8.12 | 24250 | 9700 | 13338 | 1213 |
| 12 | 72 | 3.66 | 25500 | 4080 | 20910 | 510 |
| 12 | 75 | 4.75 | 28500 | 10260 | 17385 | 855 |
| 12 | 98 | 9.81 | 16250 | 9913 | 6175 | 163 |
| 12 | 99 | 10.49 | 55500 | 34965 | 19980 | 555 |
| 12 | 98 | 10.17 | 21500 | 12255 | 9030 | 215 |
| 13 | 87 | 6.24 | 42750 | 14108 | 28215 | 428 |
| 13 | 85 | 5.53 | 39250 | 16093 | 22765 | 393 |
| 13 | 76 | 4.58 | 41000 | 12300 | 27880 | 820 |

Table C-2. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 13 | 70 | 3.81 | 34000 | 8500 | 24820 | 680 |
| 13 | 85 | 6.27 | 36000 | 15480 | 20160 | 360 |
| 13 | 81 | 5.22 | 22000 | 15180 | 6820 | 0 |
| 13 | 110 | 13.82 | 21750 | 17618 | 3698 | 435 |
| 13 | 77 | 4.60 | 24500 | 14700 | 8085 | 1715 |
| 14 | 87 | 5.65 | 40750 | 8558 | 31785 | 0 |
| 14 | 93 | 7.80 | 41250 | 20213 | 20625 | 413 |
| 14 | 97 | 9.75 | 47250 | 9923 | 36383 | 945 |
| 14 | 103 | 11.50 | 44000 | 7920 | 35200 | 440 |
| 14 | 85 | 6.47 | 30000 | 13200 | 16500 | 300 |
| 14 | 107 | 12.45 | 29500 | 11210 | 17995 | 295 |
| 14 | 89 | 7.40 | 7750 | 4263 | 3333 | 155 |
| 14 | 94 | 9.20 | 20750 | 15148 | 5603 | 0 |
| 15 | 86 | 5.64 | 38500 | 14245 | 23100 | 1155 |
| 15 | 89 | 6.00 | 71750 | 21525 | 50225 | 0 |
| 15 | 109 | 14.11 | 28750 | 14663 | 13800 | 288 |
| 15 | 85 | 6.32 | 30750 | 11070 | 18450 | 1230 |
| 15 | 84 | 6.79 | 35000 | 15750 | 19250 | 0 |
| 15 | 95 | 8.21 | 20500 | 12505 | 7585 | 410 |
| 15 | 74 | 4.55 | 27250 | 13898 | 12808 | 545 |
| 15 | 99 | 9.55 | 19250 | 12513 | 6738 | 0 |
| 16 | 85 | 5.94 | 40500 | 20655 | 19035 | 810 |
| 16 | 88 | 6.58 | 38000 | 15200 | 22420 | 380 |
| 16 | 87 | 7.12 | 26750 | 12038 | 14178 | 535 |
| 16 | 100 | 10.74 | 34250 | 8220 | 25688 | 343 |
| 16 | 61 | 2.39 | 40250 | 16100 | 22138 | 2013 |
| 16 | 95 | 7.98 | 31000 | 11470 | 19530 | 0 |
| 16 | 65 | 3.13 | 26000 | 8580 | 17160 | 260 |
| 16 | 103 | 9.66 | 36750 | 19478 | 17273 | 0 |
| 17 | 103 | 9.84 | 31250 | 10313 | 20938 | 0 |
| 17 | 88 | 5.61 | 23000 | 1380 | 20240 | 1380 |
| 17 | 85 | 6.29 | 30500 | 10370 | 19825 | 305 |
| 17 | 72 | 3.54 | 24500 | 7105 | 17395 | 0 |
| 17 | 63 | 2.02 | 18250 | 11315 | 6388 | 548 |
| 17 | 65 | 2.46 | 14500 | 8265 | 5945 | 290 |
| 17 | 95 | 9.58 | 26250 | 11550 | 14175 | 525 |
| 17 | 96 | 8.92 | 23250 | 13253 | 9300 | 698 |
| 17 | 83 | 6.00 | 19250 | 15015 | 4043 | 193 |

Table C-2. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 18 | 94 | 6.99 | 21250 | 14025 | 7013 | 213 |
| 18 | 98 | 8.40 | 33750 | 22613 | 11138 | 0 |
| 18 | 97 | 8.35 | 32500 | 21775 | 10075 | 325 |
| 18 | 64 | 1.92 | 33250 | 1663 | 31588 | 0 |
| 18 | 75 | 3.55 | 21750 | 13920 | 7830 | 0 |
| 18 | 81 | 5.06 | 30750 | 10763 | 19080 | 308 |
| 18 | 93 | 8.22 | 17250 | 11558 | 5520 | 173 |
| 18 | 103 | 11.48 | 22000 | 14740 | 6820 | 440 |
| 18 | 93 | 8.34 | 30250 | 12705 | 17243 | 303 |
| 19 | 98 | 7.95 | 19750 | 12048 | 7505 | 198 |
| 19 | 105 | 9.93 | 29500 | 21535 | 6785 | 885 |
| 19 | 94 | 7.68 | 26000 | 9360 | 16640 | 0 |
| 19 | 65 | 2.21 | 18750 | 12375 | 5625 | 750 |
| 19 | 65 | 2.46 | 33500 | 13400 | 19765 | 335 |
| 19 | 55 | 1.24 | 15500 | 10385 | 4805 | 310 |
| 19 | 94 | 8.58 | 26750 | 11503 | 15248 | 0 |
| 19 | 95 | 9.68 | 28500 | 16815 | 10260 | 1425 |
| 19 | 89 | 7.82 | 30250 | 14520 | 15730 | 0 |
| 20 | 102 | 10.23 | 27500 | 14025 | 13475 | 0 |
| 20 | 98 | 8.45 | 25500 | 1275 | 24225 | 0 |
| 20 | 90 | 6.58 | 21750 | 10658 | 10440 | 653 |
| 20 | 66 | 2.59 | 18750 | 6750 | 10500 | 1500 |
| 20 | 67 | 2.45 | 19750 | 10863 | 8295 | 593 |
| 20 | 59 | 2.00 | 25750 | 9528 | 15708 | 515 |
| 20 | 98 | 8.82 | 37750 | 19253 | 18498 | 0 |
| 20 | 94 | 8.16 | 23250 | 17438 | 5348 | 465 |
| 20 | 65 | 2.84 | 25750 | 11845 | 11588 | 2318 |
| 21 | 90 | 7.42 | 25750 | 12103 | 13648 | 0 |
| 21 | 101 | 9.47 | 30250 | 12705 | 14218 | 3328 |
| 21 | 105 | 10.49 | 26000 | 13780 | 11700 | 520 |
| 21 | 77 | 4.14 | 15250 | 6863 | 8388 | 0 |
| 21 | 83 | 5.08 | 20250 | 10125 | 9720 | 405 |
| 21 | 90 | 6.37 | 41750 | 6263 | 35070 | 418 |
| 21 | 81 | 5.70 | 28000 | 13720 | 14280 | 0 |
| 21 | 86 | 6.24 | 25500 | 16065 | 8925 | 510 |
| 21 | 93 | 7.84 | 36500 | 5475 | 29930 | 1095 |
| 22 | 102 | 9.75 | 19750 | 12443 | 7308 | 0 |
| 22 | 106 | 11.24 | 25500 | 15810 | 9435 | 255 |
| 22 | 107 | 10.81 | 26000 | 3640 | 21580 | 780 |

Table C-2. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 22 | 83 | 5.11 | 31750 | 6668 | 25083 | 0 |
| 22 | 68 | 3.18 | 34250 | 9590 | 25660 | 0 |
| 22 | 73 | 3.97 | 12750 | 7140 | 4845 | 765 |
| 22 | 66 | 2.77 | 27250 | 16350 | 10900 | 0 |
| 22 | 88 | 7.27 | 31500 | 11655 | 17325 | 2520 |
| 22 | 78 | 5.19 | 29750 | 16660 | 12198 | 893 |
| 23 | 100 | 8.76 | 19750 | 14220 | 5530 | 0 |
| 23 | 96 | 7.95 | 25250 | 13383 | 11615 | 253 |
| 23 | 95 | 7.64 | 22750 | 17973 | 4550 | 223 |
| 23 | 76 | 3.94 | 20250 | 12555 | 7493 | 203 |
| 23 | 61 | 1.81 | 41250 | 3713 | 37538 | 0 |
| 23 | 75 | 3.86 | 14750 | 7965 | 6638 | 148 |
| 23 | 95 | 9.37 | 25000 | 12750 | 10500 | 1750 |
| 23 | 90 | 7.35 | 37250 | 19370 | 16018 | 1863 |
| 23 | 65 | 3.13 | 24000 | 15840 | 6240 | 1920 |
| 24 | 95 | 8.07 | 22500 | 10125 | 12150 | 0 |
| 24 | 92 | 7.22 | 28750 | 17538 | 10925 | 288 |
| 24 | 104 | 9.84 | 25000 | 3000 | 21250 | 750 |
| 24 | 60 | 1.72 | 20000 | 9800 | 9000 | 800 |
| 24 | 72 | 3.47 | 18250 | 14965 | 2738 | 730 |
| 24 | 57 | 1.47 | 19500 | 10335 | 9165 | 0 |
| 24 | 69 | 3.19 | 24500 | 17150 | 6615 | 735 |
| 24 | 78 | 4.41 | 31250 | 10000 | 20938 | 625 |
| 24 | 93 | 8.67 | 25750 | 19055 | 6180 | 515 |

Table C-3. Leukograms of trout examined hourly during the first 24 hr after static exposure to 13.5 mg/l malachite green for 30 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 126 | 19.50 | 53750 | 10212 | 43000 | 538 |
| 0 | 135 | 22.00 | 53000 | 15900 | 34450 | 2650 |
| 0 | 87 | 5.92 | 28000 | 19040 | 8960 | 0 |
| 0 | 120 | 15.78 | 36500 | 5840 | 29200 | 1460 |
| 0 | 133 | 22.27 | 33250 | 14297 | 18288 | 665 |
| 0 | 132 | 21.45 | 37500 | 19500 | 18000 | 0 |
| 0 | 126 | 18.06 | 24750 | 17077 | 7425 | 248 |
| 1 | 108 | 11.41 | 48250 | 12063 | 36188 | 483 |
| 1 | 92 | 6.69 | 51500 | 9270 | 42230 | 0 |
| 1 | 103 | 10.96 | 52500 | 13125 | 38850 | 525 |
| 1 | 98 | 9.05 | 45250 | 3620 | 41630 | 0 |
| 1 | 107 | 12.45 | 59250 | 15998 | 43253 | 0 |
| 1 | 82 | 6.19 | 34750 | 2780 | 31970 | 0 |
| 1 | 89 | 7.44 | 32000 | 1920 | 27200 | 2240 |
| 1 | 87 | 6.79 | 42500 | 7650 | 34850 | 0 |
| 1 | 106 | 12.12 | 24250 | 12853 | 11155 | 243 |
| 1 | 103 | 10.76 | 46000 | 11960 | 34040 | 0 |
| 1 | 103 | 11.80 | 42750 | 2993 | 39758 | 0 |
| 2 | 118 | 15.86 | 51250 | 13325 | 37925 | 0 |
| 2 | 95 | 7.40 | 52750 | 3693 | 49058 | 0 |
| 2 | 111 | 13.00 | 82500 | 10725 | 70950 | 825 |
| 2 | 88 | 5.88 | 51250 | 1538 | 49713 | 0 |
| 2 | 110 | 12.50 | 71000 | 14200 | 56800 | 0 |
| 2 | 81 | 6.11 | 28000 | 9800 | 17640 | 280 |
| 2 | 89 | 7.19 | 25750 | 6438 | 19313 | 0 |
| 2 | 71 | 3.85 | 37500 | 4875 | 32250 | 375 |
| 2 | 98 | 10.31 | 62250 | 20543 | 41708 | 34 |
| 2 | 80 | 4.98 | 55500 | 11655 | 43845 | 0 |
| 2 | 98 | 9.64 | 54000 | 15120 | 38340 | 540 |
| 3 | 111 | 13.39 | 61750 | 1853 | 58663 | 1235 |
| 3 | 94 | 7.33 | 53250 | 3195 | 50055 | 0 |
| 3 | 106 | 11.63 | 45000 | 8100 | 36000 | 900 |
| 3 | 99 | 8.99 | 42250 | 8028 | 34223 | 0 |
| 3 | 62 | 2.42 | 26500 | 530 | 25970 | 0 |
| 3 | 58 | 2.29 | 24250 | 5335 | 18915 | 0 |
| 3 | 92 | 8.11 | 58500 | 21645 | 36855 | 0 |
| 3 | 91 | 8.29 | 39250 | 13738 | 25513 | 0 |
| 3 | 102 | 11.00 | 40750 | 13448 | 26895 | 408 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 4 | 105 | 10.44 | 71750 | 32288 | 38745 | 1435 |
| 4 | 100 | 9.15 | 53250 | 4793 | 48458 | 0 |
| 4 | 103 | 11.16 | 52250 | 17765 | 33963 | 523 |
| 4 | 107 | 11.59 | 50250 | 18090 | 30653 | 1508 |
| 4 | 93 | 7.75 | 52750 | 10550 | 41145 | 1055 |
| 4 | 85 | 6.57 | 17750 | 178 | 17573 | 0 |
| 4 | 62 | 2.00 | 23250 | 0 | 23250 | 0 |
| 4 | 72 | 4.48 | 41500 | 4565 | 36520 | 415 |
| 4 | 100 | 9.83 | 44750 | 15215 | 29535 | 0 |
| 4 | 76 | 4.65 | 47750 | 18145 | 28650 | 955 |
| 4 | 83 | 5.56 | 33500 | 12730 | 20770 | 0 |
| 5 | 90 | 6.89 | 52000 | 14560 | 37440 | 0 |
| 5 | 95 | 7.80 | 58250 | 18058 | 40193 | 0 |
| 5 | 118 | 16.12 | 42250 | 15633 | 26618 | 0 |
| 5 | 100 | 9.04 | 54250 | 7053 | 47198 | 0 |
| 5 | 99 | 9.67 | 42250 | 9295 | 32955 | 0 |
| 5 | 80 | 5.02 | 24000 | 6000 | 17760 | 240 |
| 5 | 92 | 8.23 | 24750 | 2723 | 22028 | 0 |
| 5 | 78 | 4.68 | 14500 | 6815 | 7250 | 435 |
| 5 | 111 | 13.61 | 41500 | 19505 | 21995 | 0 |
| 5 | 92 | 7.77 | 33500 | 13735 | 19430 | 335 |
| 5 | 108 | 12.05 | 53500 | 16585 | 36915 | 0 |
| 6 | 85 | 6.12 | 39750 | 15503 | 23850 | 0 |
| 6 | 96 | 7.99 | 43000 | 9890 | 32680 | 430 |
| 6 | 100 | 9.14 | 31500 | 14175 | 17010 | 0 |
| 6 | 89 | 6.73 | 39000 | 10140 | 27300 | 1560 |
| 6 | 103 | 11.24 | 38000 | 12540 | 25460 | 0 |
| 6 | 75 | 4.29 | 21750 | 9788 | 11745 | 0 |
| 6 | 50 | 1.20 | 27000 | 3510 | 23490 | 0 |
| 6 | 93 | 8.07 | 28750 | 6900 | 21850 | 0 |
| 6 | 110 | 13.93 | 36000 | 20520 | 15120 | 360 |
| 6 | 103 | 10.90 | 32500 | 21775 | 10725 | 0 |
| 6 | 108 | 12.65 | 30250 | 15428 | 14823 | 0 |
| 7 | 108 | 13.34 | 44500 | 11570 | 32485 | 0 |
| 7 | 85 | 5.17 | 31500 | 6930 | 24255 | 315 |
| 7 | 117 | 14.14 | 38250 | 17595 | 20273 | 0 |
| 7 | 101 | 8.56 | 29750 | 11785 | 26775 | 1190 |
| 7 | 108 | 12.07 | 28000 | 23800 | 4200 | 0 |
| 7 | 69 | 3.50 | 20500 | 10455 | 9225 | 615 |
| 7 | 80 | 4.83 | 21750 | 12615 | 8483 | 653 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 7 | 58 | 1.90 | 28250 | 10453 | 17515 | 283 |
| 7 | 100 | 10.23 | 13750 | 6050 | 7700 | 0 |
| 7 | 119 | 15.36 | 40500 | 19035 | 21465 | 0 |
| 7 | 101 | 10.88 | 26250 | 13125 | 12600 | 525 |
| 8 | 92 | 6.81 | 30750 | 12915 | 17835 | 0 |
| 8 | 90 | 6.06 | 46000 | 16100 | 28520 | 1380 |
| 8 | 107 | 11.68 | 28000 | 9240 | 18760 | 0 |
| 8 | 99 | 8.68 | 35250 | 5288 | 29610 | 353 |
| 8 | 107 | 10.90 | 27000 | 7560 | 19440 | 0 |
| 8 | 89 | 6.50 | 25000 | 18750 | 6000 | 250 |
| 8 | 58 | 1.71 | 28250 | 18928 | 9323 | 0 |
| 8 | 81 | 5.03 | 20750 | 6018 | 14525 | 208 |
| 8 | 98 | 9.24 | 30750 | 10763 | 19065 | 923 |
| 8 | 91 | 7.56 | 38500 | 24255 | 14245 | 0 |
| 8 | 90 | 7.33 | 34000 | 14960 | 19040 | 0 |
| 8 | 108 | 13.42 | 28000 | 17360 | 10640 | 0 |
| 9 | 91 | 6.57 | 30750 | 18758 | 11378 | 615 |
| 9 | 105 | 11.10 | 38750 | 12013 | 25963 | 775 |
| 9 | 95 | 7.93 | 45750 | 17843 | 27908 | 0 |
| 9 | 63 | 2.43 | 17250 | 12420 | 4658 | 173 |
| 9 | 92 | 6.88 | 26500 | 16165 | 10335 | 0 |
| 9 | 77 | 4.74 | 17750 | 3905 | 13490 | 178 |
| 9 | 78 | 4.47 | 30500 | 7625 | 22875 | 0 |
| 9 | 85 | 5.85 | 21500 | 7955 | 13330 | 215 |
| 9 | 82 | 6.15 | 19750 | 9480 | 9283 | 988 |
| 10 | 97 | 7.73 | 27250 | 13080 | 13898 | 273 |
| 10 | 99 | 8.17 | 20500 | 10455 | 8405 | 1640 |
| 10 | 113 | 13.63 | 29750 | 11900 | 17255 | 595 |
| 10 | 87 | 7.00 | 30000 | 16200 | 13800 | 0 |
| 10 | 86 | 6.02 | 23250 | 15345 | 7905 | 0 |
| 10 | 100 | 9.83 | 21500 | 13115 | 8170 | 215 |
| 10 | 84 | 5.98 | 31000 | 17980 | 12710 | 310 |
| 10 | 68 | 3.30 | 25250 | 18180 | 6060 | 1010 |
| 10 | 79 | 4.99 | 24000 | 18240 | 5760 | 0 |
| 11 | 100 | 8.59 | 33500 | 16080 | 17420 | 0 |
| 11 | 100 | 8.20 | 26000 | 5400 | 20020 | 260 |
| 11 | 98 | 8.99 | 34250 | 3083 | 30483 | 685 |
| 11 | 89 | 6.17 | 18750 | 9188 | 9375 | 188 |
| 11 | 84 | 5.60 | 28250 | 14408 | 13843 | 0 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 11 | 72 | 4.08 | 32000 | 8320 | 23360 | 320 |
| 11 | 95 | 8.28 | 42500 | 13600 | 28900 | 0 |
| 11 | 94 | 9.24 | 32000 | 21440 | 9920 | 640 |
| 11 | 80 | 5.17 | 23750 | 9025 | 14725 | 0 |
| 12 | 112 | 12.93 | 28000 | 8960 | 18760 | 280 |
| 12 | 81 | 5.08 | 20750 | 12865 | 7055 | 830 |
| 12 | 72 | 3.81 | 34500 | 15870 | 17940 | 345 |
| 12 | 70 | 3.22 | 23500 | 19740 | 3760 | 0 |
| 12 | 102 | 10.09 | 22500 | 11700 | 10350 | 450 |
| 12 | 79 | 5.34 | 32250 | 26123 | 5805 | 323 |
| 12 | 95 | 8.75 | 27000 | 14310 | 11880 | 810 |
| 12 | 66 | 3.16 | 38750 | 18213 | 16275 | 4263 |
| 12 | 77 | 4.63 | 31500 | 19845 | 11655 | 0 |
| 13 | 105 | 9.94 | 24250 | 13580 | 10185 | 485 |
| 13 | 102 | 10.19 | 38250 | 13770 | 24480 | 0 |
| 13 | 117 | 11.83 | 26750 | 5618 | 20330 | 803 |
| 13 | 65 | 2.63 | 25000 | 8000 | 17000 | 0 |
| 13 | 96 | 8.56 | 20000 | 11400 | 8800 | 0 |
| 13 | 81 | 5.08 | 22500 | 16650 | 5850 | 0 |
| 13 | 82 | 5.41 | 31000 | 22630 | 8060 | 310 |
| 13 | 74 | 4.52 | 24500 | 13965 | 10045 | 490 |
| 14 | 102 | 9.94 | 26750 | 6153 | 20063 | 535 |
| 14 | 86 | 5.71 | 33500 | 14405 | 17755 | 1340 |
| 14 | 100 | 8.99 | 21250 | 14450 | 5950 | 850 |
| 14 | 68 | 2.50 | 41750 | 10438 | 30478 | 835 |
| 14 | 81 | 5.53 | 30000 | 12600 | 16800 | 600 |
| 14 | 87 | 6.35 | 35250 | 25028 | 9870 | 353 |
| 14 | 60 | 2.06 | 26750 | 14713 | 12038 | 0 |
| 14 | 68 | 3.34 | 14250 | 2708 | 11400 | 143 |
| 14 | 69 | 4.04 | 25000 | 10000 | 14250 | 750 |
| 15 | 102 | 9.84 | 26500 | 18815 | 7685 | 0 |
| 15 | 105 | 10.19 | 22750 | 17063 | 5688 | 0 |
| 15 | 95 | 7.62 | 36500 | 21535 | 14965 | 0 |
| 15 | 68 | 2.94 | 23500 | 14335 | 8695 | 470 |
| 15 | 80 | 5.32 | 19000 | 11400 | 7410 | 190 |
| 15 | 78 | 4.78 | 21500 | 15695 | 5375 | 430 |
| 15 | 67 | 3.00 | 21250 | 18488 | 3613 | 0 |
| 15 | 68 | 3.29 | 23500 | 13865 | 9635 | 0 |
| 15 | 65 | 3.03 | 26750 | 9898 | 16318 | 0 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 16 | 92 | 7.44 | 29500 | 20940 | 7375 | 1180 |
| 16 | 102 | 9.33 | 23750 | 5938 | 17813 | 0 |
| 16 | 95 | 7.93 | 27500 | 17325 | 9075 | 1100 |
| 16 | 85 | 5.99 | 11750 | 9165 | 1998 | 588 |
| 16 | 66 | 2.99 | 22500 | 14400 | 6975 | 1125 |
| 16 | 58 | 2.11 | 7250 | 4857 | 2175 | 218 |
| 16 | 69 | 3.22 | 35000 | 27650 | 7350 | 0 |
| 16 | 86 | 6.15 | 14750 | 8850 | 4720 | 1180 |
| 16 | 67 | 3.01 | 26250 | 17063 | 8400 | 788 |
| 17 | 110 | 11.53 | 35500 | 22720 | 12425 | 355 |
| 17 | 100 | 9.12 | 27500 | 7975 | 19250 | 275 |
| 17 | 115 | 10.27 | 37500 | 24750 | 12375 | 375 |
| 17 | 96 | 6.95 | 37750 | 23028 | 11325 | 3020 |
| 17 | 115 | 10.35 | 33750 | 13163 | 20250 | 338 |
| 17 | 65 | 2.36 | 16750 | 10385 | 4188 | 2178 |
| 17 | 84 | 5.59 | 36250 | 9425 | 26825 | 0 |
| 17 | 82 | 5.05 | 38250 | 19508 | 17213 | 1530 |
| 17 | 112 | 13.37 | 24750 | 20295 | 3960 | 495 |
| 17 | 102 | 12.22 | 25250 | 22725 | 2020 | 505 |
| 17 | 77 | 5.18 | 28000 | 15120 | 11760 | 1120 |
| 18 | 102 | 9.92 | 28750 | 18688 | 9488 | 575 |
| 18 | 85 | 5.15 | 24750 | 13860 | 9900 | 990 |
| 18 | 104 | 10.53 | 38000 | 21280 | 15960 | 760 |
| 18 | 92 | 6.41 | 33500 | 20100 | 13065 | 335 |
| 18 | 104 | 10.74 | 30500 | 21960 | 7930 | 610 |
| 18 | 91 | 7.21 | 25000 | 16000 | 7000 | 2000 |
| 18 | 80 | 5.14 | 22250 | 9345 | 12460 | 445 |
| 18 | 97 | 7.27 | 22250 | 12015 | 4673 | 5563 |
| 18 | 104 | 12.03 | 35000 | 17850 | 16100 | 1050 |
| 18 | 100 | 11.23 | 36750 | 20580 | 15435 | 735 |
| 18 | 104 | 12.10 | 22500 | 16875 | 4950 | 675 |
| 19 | 102 | 9.92 | 29250 | 8775 | 18720 | 1755 |
| 19 | 89 | 6.36 | 42000 | 28980 | 11340 | 1680 |
| 19 | 98 | 8.57 | 30500 | 18605 | 10980 | 915 |
| 19 | 61 | 1.76 | 11250 | 1238 | 8888 | 1125 |
| 19 | 70 | 3.32 | 32750 | 13755 | 18668 | 328 |
| 19 | 88 | 6.96 | 27500 | 8525 | 18700 | 275 |
| 19 | 85 | 6.85 | 25250 | 13635 | 10100 | 1515 |
| 19 | 99 | 9.79 | 27250 | 25070 | 1908 | 273 |
| 19 | 101 | 9.84 | 24250 | 17945 | 5820 | 485 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 20 | 100 | 9.76 | 28500 | 21660 | 6270 | 570 |
| 20 | 98 | 8.77 | 37750 | 18498 | 18498 | 755 |
| 20 | 94 | 7.79 | 19000 | 16530 | 2280 | 190 |
| 20 | 89 | 5.98 | 51250 | 25625 | 24088 | 1538 |
| 20 | 95 | 7.54 | 33500 | 15075 | 17085 | 1340 |
| 20 | 83 | 5.84 | 12500 | 9875 | 2500 | 125 |
| 20 | 77 | 4.30 | 29750 | 20825 | 8628 | 298 |
| 20 | 75 | 4.21 | 19250 | 12705 | 6545 | 0 |
| 20 | 68 | 3.22 | 21500 | 15265 | 5160 | 1075 |
| 20 | 100 | 11.29 | 29750 | 25585 | 2975 | 1190 |
| 20 | 92 | 8.71 | 26750 | 14445 | 9898 | 2408 |
| 21 | 112 | 14.37 | 44500 | 17355 | 27145 | 0 |
| 21 | 95 | 6.63 | 32000 | 10880 | 20480 | 640 |
| 21 | 101 | 9.83 | 32000 | 13760 | 16640 | 1600 |
| 21 | 106 | 10.65 | 40250 | 24955 | 10465 | 4830 |
| 21 | 85 | 6.48 | 26250 | 18375 | 7875 | 0 |
| 21 | 67 | 2.88 | 26500 | 12720 | 11660 | 2120 |
| 21 | 67 | 2.58 | 23250 | 10230 | 13020 | 0 |
| 21 | 107 | 13.28 | 26500 | 19875 | 6625 | 0 |
| 21 | 80 | 6.01 | 42500 | 10200 | 31875 | 425 |
| 21 | 100 | 9.81 | 28000 | 21560 | 5600 | 840 |
| 22 | 101 | 12.62 | 23000 | 16330 | 5520 | 1150 |
| 22 | 95 | 8.08 | 41750 | 26303 | 14613 | 835 |
| 22 | 114 | 10.19 | 24000 | 15600 | 4560 | 3840 |
| 22 | 82 | 4.67 | 40750 | 9373 | 29340 | 1630 |
| 22 | 72 | 3.75 | 23500 | 11280 | 12220 | 0 |
| 22 | 92 | 7.14 | 20500 | 10250 | 10045 | 205 |
| 22 | 58 | 1.88 | 13500 | 1485 | 11880 | 135 |
| 22 | 113 | 12.92 | 35750 | 17160 | 17160 | 1430 |
| 22 | 89 | 7.25 | 31000 | 26660 | 4030 | 310 |
| 22 | 74 | 4.60 | 19250 | 15400 | 2695 | 1155 |
| 22 | 82 | 5.00 | 35250 | 14100 | 20445 | 705 |
| 23 | 115 | 10.99 | 29500 | 23305 | 5015 | 1180 |
| 23 | 102 | 8.17 | 35500 | 11715 | 23430 | 355 |
| 23 | 99 | 9.87 | 25750 | 19828 | 4635 | 1288 |
| 23 | 88 | 6.04 | 35500 | 25205 | 9585 | 710 |
| 23 | 101 | 10.50 | 28250 | 12713 | 14690 | 848 |
| 23 | 79 | 4.18 | 18000 | 10440 | 7200 | 540 |
| 23 | 75 | 4.20 | 11500 | 7935 | 3335 | 230 |
| 23 | 87 | 6.86 | 16750 | 10218 | 5863 | 670 |

Table C-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 23 | 80 | 5.04 | 22250 | 9345 | 12683 | 223 |
| 23 | 78 | 4.67 | 24250 | 11640 | 12125 | 485 |
| 23 | 96 | 9.89 | 31250 | 20938 | 9375 | 938 |
| 24 | 116 | 12.60 | 28250 | 15255 | 12713 | 283 |
| 24 | 95 | 8.45 | 45500 | 21385 | 22750 | 1365 |
| 24 | 105 | 10.53 | 29000 | 19720 | 8990 | 290 |
| 24 | 96 | 8.47 | 26000 | 13260 | 12220 | 520 |
| 24 | 95 | 8.83 | 22500 | 13725 | 7650 | 1125 |
| 24 | 81 | 5.38 | 26750 | 13108 | 13108 | 535 |
| 24 | 57 | 1.66 | 10250 | 3690 | 6150 | 410 |
| 24 | 64 | 2.45 | 10750 | 6128 | 4623 | 108 |
| 24 | 79 | 5.16 | 19000 | 11970 | 7030 | 0 |
| 24 | 71 | 3.83 | 48000 | 15840 | 29760 | 2400 |
| 24 | 94 | 7.99 | 22750 | 13423 | 7963 | 1138 |

# APPENDIX D.

## LEUKOGRAMS OF FISH EXPOSED TO MALACHITE GREEN FOR 5 MIN (EXAMINED 0, 2, 4, 6, AND 24 HR AFTER)

Table D-1. Leukograms of trout examined during the first 24 hr after static exposure to 0.00 mg/l malachite green for 5 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 118 | 15.49 | 44500 | 21360 | 22695 | 445 |
| 0 | 120 | 15.56 | 60750 | 1823 | 58928 | 0 |
| 0 | 135 | 25.42 | 38750 | 13563 | 25188 | 0 |
| 0 | 131 | 21.97 | 54250 | 17360 | 36348 | 543 |
| 0 | 114 | 17.58 | 56250 | 22500 | 33750 | 0 |
| 0 | 111 | 13.64 | 49250 | 12313 | 35953 | 985 |
| 2 | 131 | 22.94 | 49750 | 22388 | 26368 | 995 |
| 2 | 101 | 10.90 | 58500 | 13455 | 45045 | 0 |
| 2 | 119 | 17.62 | 70250 | 10538 | 59713 | 0 |
| 2 | 111 | 12.94 | 40000 | 13600 | 26400 | 0 |
| 2 | 149 | 32.46 | 44500 | 19580 | 24475 | 445 |
| 2 | 111 | 14.34 | 44250 | 12833 | 31418 | 0 |
| 4 | 121 | 16.77 | 53750 | 18275 | 35475 | 0 |
| 4 | 90 | 7.92 | 39250 | 18448 | 20410 | 393 |
| 4 | 140 | 26.14 | 41250 | 12788 | 28463 | 0 |
| 4 | 111 | 13.22 | 54250 | 15190 | 39060 | 0 |
| 4 | 122 | 18.78 | 54250 | 23870 | 30380 | 0 |
| 4 | 133 | 22.94 | 48250 | 21230 | 27020 | 0 |
| 6 | 108 | 12.17 | 38500 | 18865 | 19635 | 0 |
| 6 | 102 | 9.49 | 31250 | 11250 | 20000 | 0 |
| 6 | 128 | 22.26 | 55250 | 11603 | 42543 | 1105 |
| 6 | 135 | 23.99 | 36000 | 4680 | 31320 | 0 |
| 6 | 120 | 18.36 | 48000 | 12480 | 34560 | 960 |
| 6 | 136 | 27.28 | 35750 | 21808 | 13585 | 358 |
| 24 | 129 | 20.92 | 33250 | 23275 | 9643 | 33 |
| 24 | 96 | 8.50 | 41000 | 20090 | 16810 | 4100 |
| 24 | 148 | 30.85 | 41250 | 30113 | 11138 | 0 |
| 24 | 128 | 20.41 | 27250 | 19893 | 5723 | 1635 |
| 24 | 146 | 30.98 | 30000 | 12000 | 18000 | 0 |

[a]Total white blood cell-thrombocytes per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

[b]Number per $mm^3$. This footnote also applies to the rest of the tables in this appendix.

Table D-2. Leukograms of trout examined during the first 24 hr after static exposure to 42.1 mg/l malachite green for 5 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 102 | 10.52 | 44500 | 20025 | 24475 | 0 |
| 0 | 85 | 6.59 | 44500 | 9790 | 34710 | 0 |
| 0 | 125 | 22.06 | 35250 | 11985 | 23265 | 0 |
| 0 | 122 | 18.10 | 49750 | 17910 | 31343 | 498 |
| 0 | 122 | 17.00 | 50250 | 15075 | 34673 | 503 |
| 0 | 118 | 17.79 | 74000 | 19980 | 54020 | 0 |
| 2 | 153 | 38.22 | 71000 | 9230 | 61770 | 0 |
| 2 | 131 | 24.78 | 42250 | 18590 | 22393 | 1268 |
| 2 | 90 | 7.36 | 58250 | 8738 | 49513 | 0 |
| 2 | 133 | 23.79 | 57250 | 9160 | 48090 | 0 |
| 2 | 106 | 11.55 | 41000 | 13530 | 27470 | 0 |
| 2 | 123 | 17.78 | 54500 | 12535 | 41965 | 0 |
| 4 | 120 | 18.89 | 38250 | 12623 | 24480 | 1148 |
| 4 | 153 | 36.52 | 39000 | 10920 | 28080 | 0 |
| 4 | 125 | 21.60 | 34000 | 1700 | 32300 | 0 |
| 4 | 100 | 10.97 | 64250 | 25700 | 38550 | 0 |
| 4 | 126 | 20.26 | 42750 | 15390 | 26933 | 428 |
| 4 | 103 | 10.51 | 48500 | 18915 | 29585 | 0 |
| 6 | 118 | 17.08 | 53500 | 17655 | 35845 | 0 |
| 6 | 136 | 23.00 | 26000 | 15080 | 10920 | 0 |
| 6 | 113 | 14.68 | 29500 | 3245 | 25665 | 590 |
| 6 | 112 | 15.47 | 46250 | 18030 | 28213 | 0 |
| 6 | 129 | 21.83 | 51000 | 1020 | 49980 | 0 |
| 6 | 107 | 12.74 | 33000 | 16830 | 15840 | 330 |
| 24 | 143 | 29.62 | 27250 | 20438 | 6540 | 273 |
| 24 | 131 | 21.84 | 34000 | 28220 | 5440 | 340 |
| 24 | 107 | 12.30 | 34000 | 22780 | 10200 | 1020 |
| 24 | 120 | 15.06 | 24000 | 17520 | 6000 | 480 |
| 24 | 106 | 11.84 | 40000 | 18800 | 20400 | 800 |

Table D-3. Leukograms of trout examined during the first 24 hr after static exposure to 75.0 mg/l malachite green for 5 min at 12 C

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 0 | 127 | 21.12 | 29750 | 9223 | 19338 | 1190 |
| 0 | 112 | 12.43 | 32000 | 7040 | 24640 | 320 |
| 0 | 90 | 7.49 | 42250 | 11830 | 29998 | 423 |
| 0 | 112 | 15.12 | 44250 | 13718 | 30090 | 443 |
| 0 | 132 | 24.38 | 54000 | 11880 | 40500 | 1620 |
| 0 | 101 | 9.32 | 61000 | 11590 | 48190 | 1220 |
| 0 | 175 | 50.87 | 50250 | 9045 | 39195 | 2010 |
| 0 | 141 | 28.17 | 45250 | 13575 | 31675 | 0 |
| 2 | 107 | 12.65 | 42000 | 15540 | 26460 | 0 |
| 2 | 112 | 13.76 | 37250 | 13783 | 23468 | 0 |
| 2 | 123 | 17.11 | 56500 | 14690 | 41245 | 565 |
| 2 | 111 | 13.81 | 70750 | 24763 | 45988 | 0 |
| 2 | 90 | 7.99 | 58250 | 27960 | 30290 | 0 |
| 2 | 108 | 14.77 | 77750 | 24880 | 51315 | 1555 |
| 2 | 121 | 17.00 | 70250 | 23885 | 46365 | 0 |
| 2 | 151 | 34.16 | 70750 | 7075 | 63675 | 0 |
| 2 | 118 | 17.53 | 72750 | 11640 | 61110 | 0 |
| 4 | 90 | 6.77 | 46250 | 18500 | 27750 | 0 |
| 4 | 122 | 17.03 | 46500 | 11160 | 35340 | 0 |
| 4 | 117 | 14.29 | 49500 | 20295 | 29205 | 0 |
| 4 | 139 | 26.17 | 35500 | 13845 | 21300 | 355 |
| 4 | 118 | 15.22 | 51500 | 21115 | 30385 | 0 |
| 4 | 109 | 13.26 | 42750 | 9405 | 33345 | 0 |
| 4 | 118 | 17.62 | 61750 | 13585 | 48165 | 0 |
| 4 | 105 | 11.21 | 44000 | 17160 | 26400 | 440 |
| 6 | 99 | 9.25 | 45750 | 20588 | 25163 | 0 |
| 6 | 132 | 21.64 | 24000 | 6240 | 17760 | 0 |
| 6 | 93 | 8.22 | 30000 | 15600 | 14400 | 0 |
| 6 | 127 | 22.54 | 38000 | 16720 | 21280 | 0 |
| 6 | 131 | 23.19 | 30250 | 12403 | 17848 | 0 |
| 6 | 133 | 23.18 | 28250 | 13278 | 14973 | 0 |
| 6 | 127 | 21.16 | 41250 | 16088 | 25163 | 0 |
| 6 | 113 | 13.51 | 42500 | 14450 | 28050 | 0 |
| 6 | 120 | 17.13 | 43500 | 16530 | 26970 | 0 |
| 24 | 127 | 18.17 | 28000 | 18200 | 9800 | 0 |
| 24 | 115 | 13.84 | 34000 | 11560 | 21420 | 1020 |
| 24 | 92 | 7.90 | 44500 | 33820 | 9345 | 1335 |
| 24 | 126 | 19.72 | 36250 | 14863 | 21388 | 0 |

Table D-3. (continued)

| Hr | L (mm) | Wt (g) | WBC-T[a] | Thrombo-cytes[b] | Lympho-cytes[b] | Poly-morphs[b] |
|---|---|---|---|---|---|---|
| 24 | 131 | 21.77 | 31000 | 7440 | 23560 | 0 |
| 24 | 131 | 25.35 | 36750 | 21315 | 15435 | 0 |
| 24 | 93 | 7.52 | 40000 | 16000 | 22800 | 1200 |
| 24 | 89 | 6.52 | 33500 | 23785 | 9045 | 670 |
| 24 | 110 | 13.50 | 47250 | 34493 | 11340 | 1418 |
| 24 | 158 | 36.85 | 26500 | 19080 | 6890 | 530 |
| 24 | 97 | 8.43 | 41000 | 24190 | 16810 | 0 |
| 24 | 123 | 19.28 | 46000 | 23920 | 20700 | 1380 |
| 24 | 105 | 11.59 | 34250 | 28428 | 4795 | 1028 |
| 24 | 147 | 31.09 | 45000 | 23400 | 21600 | 0 |
| 24 | 116 | 17.72 | 28500 | 19950 | 8265 | 285 |
| 24 | 130 | 24.95 | 33500 | 20100 | 11725 | 1675 |