AN ABSTRACT OF THE THESIS OF

Melvin Dean Fuqua for the Master of Science

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Title: Effects of Paraquat on Various Stages of Rainbow Trout

Abstract approved: Gaylen Neufeld

The toxicity of the herbicide paraquat upon two larval stages of rainbow trout (Salmo gairdneri) was studied. The histopathological effects and tissue accumulation of ^{14}C-labeled paraquat were also investigated. Static bioassays were conducted for 24 and 48 hours at concentrations of 150, 205, 281, 385 and 528 ppm paraquat. Using four replicates, prolarval fish (12 days old) had LC_{50} values of 282, 313, 366 and 329 ppm paraquat. In another experiment with postlarval fish (53 days old), 50% died after 48 hour exposure to 528 ppm paraquat (no replicates). Results of these experiments indicate that toxicity is both time and age dependent.

Tissues from the skin, gills, intestine, kidney and liver of postlarval fish were examined after exposure to 724 ppm paraquat for 48 hours. Microscopic examination revealed no evidence of tissue degeneration, hypertrophy or lesions.

Whole-body autoradiographs were used to locate any sites of paraquat accumulation in the tissue. Prolarval fish (24 days old) were exposed to 20 ppm ^{14}C-paraquat dichloride (50 µCi) in 250 ml of water for 48 hours. Autoradiograms revealed extensive grain around the columnar epithelium of pyloric cecae. Extensive study of other organ systems failed to show any dense grain development which could be interpreted as paraquat localizations.

EFFECTS OF PARAQUAT ON VARIOUS STAGES OF RAINBOW TROUT

A Thesis
Submitted to
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Master of Science

by
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Approved for Major Department

Approved for Graduate Council

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INTRODUCTION

Widespread usage of agricultural pesticides poses a potential hazard to the biota of the aquatic ecosystem. These chemical contaminants released into the aquatic system by inaccurate application, accidental spillage or runoff, may have irreparable effects on the zooplankton, benthic fauna, and fish community.

Deteriorating water quality and the growing concern for wildlife and public health have produced research aimed at determining "safe levels" of pesticide concentrations. The majority of studies has been focused on the chlorinated hydrocarbon and organophosphorus insecticides. The herbicides and their respective toxicological properties have not been investigated as extensively.

Knowledge regarding the toxicological characteristics of paraquat upon the larval stages of fish is scarce. A thorough understanding of its larvicidal properties could prevent inadvertent fish kills and ecological imbalances.

Paraquat, a bipyridylium herbicide (1,1'-dimethyl=4,4'-bipyridylium ion) is a registered general contact herbicide for non-crop usage. It is used for post-emergence weed control, preharvest desiccation, and aquatic weed control (Calderbank and Yuen, 1965). Paraquat is effective in controlling aquatic macrophytes (Brooker and Edwards, 1973). The mechanism of action in plants is primarily inhibition of the photosynthetic processes.

According to the data of Earnest (1971) a mortality of 34% was observed among bluegill (Lepomis macrochirus) after a 48 hour exposure to 1.14 ppm of paraquat. Paraquat residue studies were also made for four fish species after a 1.14 ppm treatment. Twenty-four hours after exposure

the rainbow trout, channel catfish, green sunfish and bluegill whole body tissue analysis revealed concentrations of 1.86, 1.32, 0.58 and 1.22 ppm, respectively. Microscopic examination showed no abnormalities of the eye, skin or gills of any fish tested. However, the pancreas of most fish was afflicted with a granuloma. Fibrous tissue forming whorls infiltrated the adipose tissue of the pancreas.

Similar bioassays with paraquat were conducted by Fytizas (1980) on three marine organisms. The test organisms included one fish, Mugil cephalus, a decapod crustacean, Pagurus sp., and a gastropod mollusc, Murex brandaria. The fish exhibited an LC_{50} of 10 ppm within 1 hour. However, the LC_{50} values for the crustacean and gastropod were 36 and 24 hours for the same treatment concentration. Highest paraquat accumulations were found in the digestive tube and skin of the fish. Histopathological examination revealed damage to the epithelium of the secondary lamella in both Pagurus sp. and M. cephalus. Fibrotic tissue had formed at the bases of the distended gill filaments.

Diquat, a herbicide closely related to paraquat, also has been evaluated using static bioassay tests. Gilderhus (1967) found the 96-hour LC_{50} levels for diquat of five fish species to be as follows: bluegill, 35 ppm; goldfish, 35 ppm; northern pike, 16 ppm; rainbow trout, 11.2 ppm; and walleye, 2.1 ppm. Peak levels of diquat in the tissue were observed about 10 days after treatment. Complete disappearance from the tissue was noted approximately 6 to 12 weeks following the last exposure. Examination of excised tissue after a 3 ppm treatment did not indicate any morphological changes in the nervous tissue, digestive organs, testes, heart, or kidney which might have been caused by the chemical.

Brown trout (Salmo trutta) fingerlings were reported to have TL_m's

(total lethal mortality) of 32.6, 22.4, and 20.4 milligrams diquat cation per liter at 24, 48 and 96 hours, respectively (Simonin and Skea, 1977). A synergistic effect was expressed when diquat and cutrine were used in combination.

Comparable LC_{50} values were observed among adult Louisiana crayfish (Procambarus clarkii) as reported by Leung et al. (1980). A 57% mortality resulted after exposure to 35 ppm paraquat for a 48 hour period. Death rates increased consistently with time. For the same concentration a 100% mortality occurred at 96 hours. Juvenile crayfish appeared to be more susceptible than adults. One hundred percent mortality was noted after a 48 hour exposure to eight milligrams paraquat per liter.

Naqvi et al. (1980) conducted bioassays with two genera of freshwater copepods (Diaptomus sp. and Eucyclops sp.). In comparative tests using the two herbicides diquat and paraquat, he found that paraquat was seven times more toxic than diquat during a 24 hour exposure period. At 30 ppm, paraquat was 100% lethal for both taxa of animals in 24 hours.

Several authors have reported that paraquat is absorbed rapidly by soils after initial application. Earnest (1971) performed analyses of water, mud and vegetation after a 1.14 ppm application. Twenty-four hours following initial exposure the surface waters contained only 0.57 ppm, mud, 2.15 ppm, Chara, 11.0 ppm, and Spirogyra 190 ppm. Dramatic shifts were observed in the residue concentrations after four days. Surface water decreased to 0.01 ppm and mud samples decreased moderately to 0.77 ppm. The greatest changes were visible in the tissue samples from Chara and Spirogyra which yielded 840 and 1300 ppm respectively.

As mentioned earlier, the larvacidal properties of paraquat upon fish species are relatively unknown. The following investigation was

undertaken to determine the lethal threshold of the herbicide paraquat upon the prolarval and postlarval stages of the rainbow trout (Salmo gairdneri). The rainbow trout is being used in many laboratories as a representative of the cool-water salmonid family (Sprague, 1973). This represents a trend to use standard species that may be representative of a broad spectrum of fish. The histopathological effects of paraquat were examined in a variety of tissues at subacute and acute exposure levels. An additional experiment utilized C^{14} labeled paraquat and autoradiographic techniques to detect localization of the chemical in fish tissue. Data from these experiments will aid in the evaluation of paraquat use and it's effect upon non-target species. Techniques used in this research may also be used as a foundation for further investigation into cellular attraction of toxicants. A knowledge of tissue accumulation may suggest cellular interactions with paraquat as the organism attempts to cope with this form of environmental stress. This information might indicate possible metabolic modifications necessary to decrease paraquat toxicity.

METHODS AND MATERIALS

The rainbow trout (Salmo gairdneri) used in this study were purchased from Troutdale Ranch located in Gravois Mills, Missouri. Approximately 1000 eyed-eggs were transported in a special container to maintain high moisture and cool temperatures. The eggs and subsequent life stages were maintained in a Sherer Model Cel 25-7 plant growth chamber. The temperature was set at 14°C constant. Lighting was supplied by two 100 watt cool-white fluorescent lamps and four 15 watt incandescent lamps to provide a balanced spectrum. The photoperiod was set at 8 hour light and 16 hour dark cycle.

A special hatching trough was constructed from nylon netting with a one millimeter mesh size. The trough was 46 cm long, 20 cm wide, and 14 cm in depth. It was constructed to facilitate the cleaning of the aquarium while not disturbing the eggs. The trough was suspended in a 75 liter aquarium and positioned inside the growth chamber. A second aquarium was used to age tap water. Constant aeration was supplied, and oxygen levels of 7.5-8.5 ppm were measured inside the trough; pH ranged from 6.5-6.8. Partial water changes, approximately 25% of total volume, were made every 48 hours.

The test compound was a herbicide formulation of paraquat known as ORTHO Paraquat CL. It contained 29.1% paraquat dichloride active ingredient (A.I.) and 70.9% inert materials. Hanson-Kimmel of Emporia, Kansas was the supplier.

Toxicity Test

Estimation of the LC_{50} (lethal concentration for 50 percent of the individuals) was by a method devised by Weil (1952). This method affords the investigator a statistical method for determination of median-effective

dose while utilizing a limited number of test animals. The general formula for the calculation of an LC_{50} may be reduced to:

$$\log m \cong \log D_a + d \cdot (f + 1)$$

The formula permits the use of 2,3,4,5,6 or 10 test animals per dosage, with four or more dosage levels being evaluated. Each successive dosage concentration must be increased by a constant value (R). The variable (d) in the equation is the logarithm of the geometric progression constant R. Log D_a represents the log of the lowest successive dosage levels used in the experiment. The value (f) is taken from a table designed specifically for this formula. The resulting LC_{50} is equal to (m).

All toxicity tests were conducted under controlled conditions in the growth chamber to prevent interaction of environmental factors. Static short-term bioassay tests were performed according to methods outlined by Sprague (1973). Forty-eight hour bioassays were preferred in order to limit the synergistic effects of the metabolites. Preliminary tests indicated that the 48 hour LC_{50} was between 187.5 and 375 ppm paraquat.

The first toxicity series was designed to determine the 48 hour LC_{50} for the prolarval stages. The embryos were approximately 12 days old (days after hatching), 20 mm in length and 0.12 g in weight. They were characterized by possessing a large yolk sac. Six animals were placed in each of several large glass culture dishes containing 250 ml of aquarium water. There were four replicates in each of the following treatment concentrations; control, 150, 205, 385 and 528 ppm paraquat (A.I). Mortality was recorded at 24 and 48 hours.

Similar bioassays were initiated with the postlarva. These fish were 60 days old. Average length and weight for this group of animals were 43 mm and 0.72 grams, respectively. No replications were made due

to a shortage of test animals. Paraquat concentrations for the bioassay were control, 150, 205, 281, 385, 528 and 724 ppm (A.I). Observations were made at 24 and 48 hours.

To obtain a mortality curve (mortality vs time for a specific treatment concentration) 80 postlarval trout were placed in an all glass aquarium holding seven liters of water. The treatment concentration used was 322 ppm (A.I) which was the calculated 48 hour LC_{50} value in the first toxicity test. Mortality was recorded at 24 and 48 hours.

Histopathological Experiment

For the histopathological investigation, advanced postlarval trout (72 days after hatching) were subjected to subacute and acute levels of paraquat concentrations. Five specimens were placed in each of four one-liter beakers. One beaker contained untreated water which was used as the control. The other three beakers contained paraquat in the following concentrations; 150, 385 and 722 ppm (A.I). Forty-eight hours later the surviving specimens were removed, injected intraperitoneally with fixative and then placed in Bouin's fixative overnight. Prior to embedding the picric acid was removed with several changes of saturated lithium carbonate and 70% ethyl alcohol. Following is the dehydration, clearing and infiltration series used in this investigation:

1.	95% ethyl alcohol	- 1 hour
2.	100% ethyl alcohol	- 2 hours
3.	100% ethyl alcohol	- 2 hours
4.	Ethyl alcohol-xylene (1:1)	- 2 hours
5.	Xylene	- overnight
6.	Xylene	- 1 hour
7.	Xylene-Paraplast (1:1)	- 2 hours
8.	Paraplast	- 2 hours
9.	Paraplast	- 2 hours
10.	Embed in Paraplast	

Paraplast was used as the embedding medium from which serial cross sections

seven micrometers in thickness were prepared. A standard staining series as described by Humason (1979) was utilized. Delafield hematoxylin and Eosin Y were the primary stains used with staining times of 30 seconds and three minutes, respectively. Tissues examined under the light microscope were from the eye, skin, gills, digestive tract, liver and kidneys.

Autoradiography

For the processing of autoradiograms, four 24-day trout embryos were exposed to ^{14}C-paraquat for 48 hours. The fish were placed in a beaker containing 250 ml of aquarium water containing 20 ppm paraquat dichloride and 50 µCi (methyl-^{14}C) paraquat chloride. The ^{14}C-paraquat chloride (S.A. = 425 µCi/mg) was purchased from Amersham Corporation. Analabs supplied the paraquat dichloride 99% (1,1'-Domethyl-4,4'-bipyridylium dihydrochloride). After exposure the fish were rinsed with uncontaminated water and fixed in Bouin's solution. The dehydration, clearing, infiltration and sectioning procedures were identical to the histopathological investigation.

The emulsion selected was Eastman Kodak NTB 2. The average grain size was 0.26 micrometers. Type NTB 2 is effective for recording low energy beta particles. Emulsion coating of the prepared slides was by the Dipping Method (Joftes, 1963). This method was preferred due to its ease and speed of preparation plus the intimate contact between emulsion and specimen obtained. In this method, tissue slides are dipped in a quantity of melted bulk emulsion, removed and the excess removed from the bottom of the slide. The emulsion coating was performed under dark room conditions. The slides were placed in plastic slide boxes with approximately 25 grams of bundled Drierite. The boxes were wrapped twice

with aluminum foil and stored for 10 days at 4°C for development.

Bogoroch (1972) outlined the particular method of processing autoradiograms followed in this investigation. The tissue slides were developed in Kodak D-19 developer for three minutes, rinsed in distilled water for 15 seconds and fixed in Kodak Acid Fixer for six minutes. To prevent separation of the specimen and slide during development all processing was performed at 4°C.

Poststaining procedures and necessary criteria presented by Bogoroch (1972) were utilized. Mayer's hematoxylin and Eosin Y were used with staining times of two minutes and three minutes, respectively. The same staining series as in the histopathological experiment was followed.

RESULTS

Toxicity Tests

A preliminary bioassay was performed to establish the upper and lower ranges of toxicity. Results of the 24 and 48 hour preliminary bioassay are presented in Table 1. No mortality was observed in the control or 188 ppm treatment groups in any of the 24 or 48 hour replicates. Mortality was first seen in the 375 ppm treatment groups of both exposure periods. In the 24 hour experiment three of the 24 prolarva did not survive. A sharp contrast can be seen in the 48 hour bioassay in which only two of the 24 embryos survived. Since the exposure period was doubled, this response, although abrupt, was not unexpected. The mortality response appears to be time-dependent as shown by the increased mortality at 375 ppm in the 48 hour bioassay. No embryos survived the 750 or 1500 treatment levels in the 48 hour bioassay.

Data from the preliminary toxicity tests were used to design a more precise bioassay. One hundred and fifty parts per million of paraquat was selected as the initial concentration. A geometric interval ratio of 1.37 gave the desired treatment concentrations of 205, 282, 385 and 528 ppm. Table 2 summarizes the results of the 24 and 48 hour toxicity experiments. Comparison of survival at the 281 ppm treatment level showed no mortality in the 24 hour bioassay; at 48 hours, 25% of the embryos died. Total lethal mortality (TLM) was not encountered in the 24 hour test (Table 2). In contrast TLM in the 48 hour bioassay was reached at 528 ppm. Only 25% of the larva in the 48 hour test survived the 385 treatment concentration.

Having met all the requirements necessary for determination of the

Table 1. Mortality of prolarval rainbow trout after 24 and 48 hours exposure to paraquat. The numbers represent the dead from an original number of six.

Treatment Concentrations (ppm)	24 hour				48 hour			
	Replicates				Replicates			
	I	II	III	IV	I	II	III	IV
Control	0	0	0	0	0	0	0	0
187.5	0	0	0	0	0	0	0	0
375	1	0	2	0	6	5	5	6
750	5	5	6	6	6	6	6	6
1500	6	6	6	6	6	6	6	6

Table 2. Mortality of prolarval rainbow trout after 24 and 48 hours exposure to paraquat. The numbers represent the dead from an original number of six.

Treatment Concentration (ppm)	24 hour				48 hour			
	Replicates				Replicates			
	I	II	III	IV	I	II	III	IV
Control	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0
205	0	0	0	0	0	0	0	0
281	0	0	0	0	3	1	0	2
385	3	0	2	2	6	6	4	4
528	5	6	4	5	6	6	6	6

median effective dose (Weil, 1952), LC_{50} values were calculated for each replicate. Respective 48 hour LC_{50} values for replicates I through IV (Table 2) were 282, 313, 366 and 329 ppm. The calculated 95% confidence limits for the four replicates were 264-299, 299-326, 349-383 and 305-353 ppm respectively. The mean of the four replicates was calculated as 323 ppm.

Using the calculated mean of 323 ppm, an experiment was designed to allow the construction of a time-dependent toxicity curve. The experiment, using post-larval fish, was performed for 48 hours with observations on mortality being made every two hours. Embryos used in the experiment were 53 days old. Unexpectedly no mortality was observed throughout the exposure period. This indicated that an unknown factor had influenced the results. A review of the experimental design indicated that age of the test organisms was the only apparent variable. Larvae in the first bioassay were approximately 12 days old, while those in the subsequent experiment were 53 days old. This abrupt shift with age in the toxicity threshold necessitated the determination of approximate LC_{50} levels for the postlarval fish.

A shortage of research animals did not permit replication of the postlarval bioassay. Results from the postlarval toxicity experiment are presented in Table 3. The upper limit of paraquat concentration was increased to 724 ppm due to the increased tolerance observed in the previous experiment. Paraquat concentrations were much higher than those values reported with other fish species. There was no larval mortality during the 24 hour exposure period even at the 724 treatment concentration. After 48 hours three of the five embryos survived exposure to 724 ppm paraquat. Although the experiment could not be replicated it can be used

Table 3. Mortality of post-larval rainbow trout after 24 and 48 hour exposure to paraquat. The numbers represent the dead from an original number of five.

Treatment Concentration (ppm)	24 hour	48 hour
Control	0	0
150	0	0
205	0	0
281	0	0
385	0	2
528	0	3
724	0	2

as a reliable indicator of age-dependent paraquat tolerance. The LC_{50} value for the postlarvae could conservatively be said to exceed 528 ppm and, possibly, surpass the 724 treatment concentration.

Histopathological Experiment

Examination of tissues from the prepared slides revealed no changes in morphology or signs of tissue degeneration after exposure to paraquat concentrations of 724 ppm. Tissues examined were from the eye, skin, gill, digestive tract, kidney and liver. The slides were viewed with a light microscope and representative photomicrographs were made from each tissue under investigation. Identification of cellular components was aided referring to the observations of Leeson and Leeson (1979), Reith and Ross (1977), and Andrew and Hickman (1974).

Representative sections of skin from the control and treatment larvae are shown in Figures 1A and 1B. Several layers of epithelial cells (EC) with underlying basal membrane (BM) are clearly seen. Striated muscle (SM) from the hypaxial muscle group is immediately beneath the epidermis. Interspersed throughout the epidermal cells are numerous secretory cells (SC). These are mucus-secreting cells that may aid in protection from foreign intervention. There were no eruptions in the epidermis or alterations in the number of secretory cells which could be attributed to high paraquat concentrations.

Gill tissue from the control and treatment embryos are shown in Figures 2A and 2B. The long thin filaments are secondary lamella (SL) separated by numerous water spaces (WS). Squamous epithelium (Ep), one cell layer thick, covers the lamellae of each filament. This is respiratory epithelium through which the diffusion of gases occurs. Capillaries carry erythrocytes (Er) the entire length of the lamellae. Supporting

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Fig. 1A. Skin from control. (x400.)

KEY

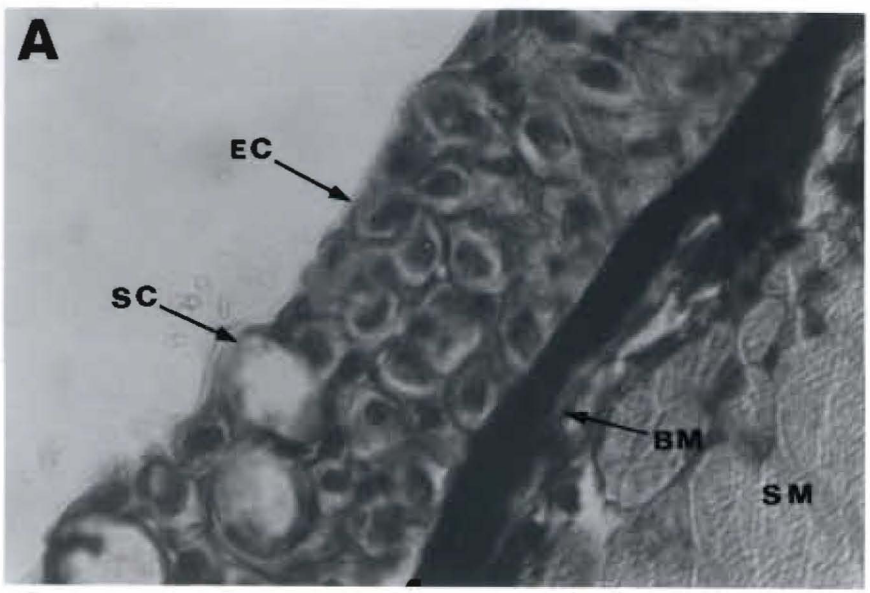
BM, basal membrane

EC, epithelial cells

SC, secretory cells

SM, striated muscle

Fig. 1B. Skin from experimental. (x400.)


A
EC
SC
BM
SM

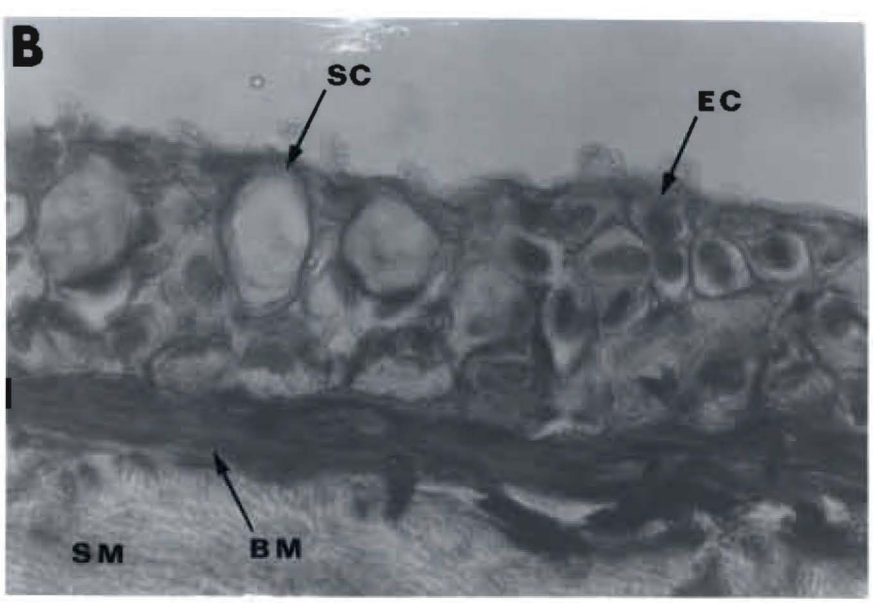

B
SC
EC
SM
BM

Fig. 2A. Gill lamellae from control. (x1000.)

KEY

Ep, epithelium

Er, erythrocytes

GC, granule cell

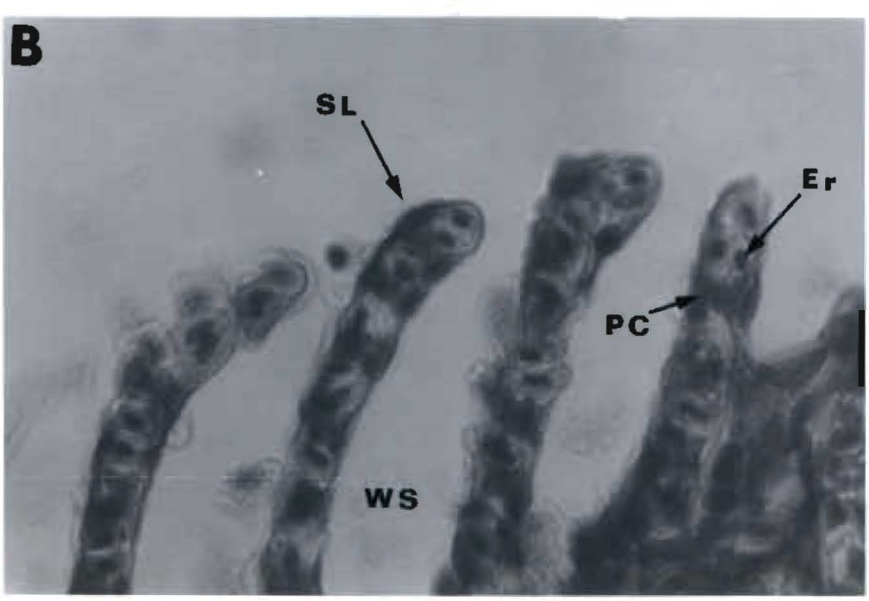
PC, pillar cell

SL, secondary lamellae

WS, water space

Fig. 2B. Gill lamellae from experimental. (x1000.)


A
SL
GC
PC
Ep
WS


B
SL
Er
PC
WS

structures called pillar cells (PC) and large granule cells (GC) are also components of each gill filament. Degeneration of the gill tissue or fibrosis of lamellae was not observed in the larvae exposed to a paraquat level of 724 ppm.

Andrew and Hickman (1974) described the trout intestine as a rather uncomplicated tube that passes forward as the duodenum and then curves backward and passes to the anus. Two slightly different sections of small intestine are represented in Figures 3A and 3B. Numerous intestinal villi are seen in the control (Fig. 3A) encircling the lumen (Lu). Major elements of the mucosa seen in these photographs are the columnar epithelium (CE) and many goblet cells (GC). The absorbing cells are interspersed with the mucus-secreting goblet cells. Beneath the mucosal villi lies the submucosa (Sb), followed by a layer of circular muscle (CM). The same cell types and structures although of different size can be seen in the intestinal section of the paraquat exposed fish (Fig. 3B). There were no signs of tissue destruction which might have been caused by acute exposure to paraquat.

Typical sections from the larval kidney are illustrated from the control (Fig. 4A) and experimental (Fig. 4B) animals. The kidney is a long organ originating anterior and dorsal to the air bladder and terminating above the cloaca. The kidney is bounded by the air bladder membrane (AB) on one side and the striated muscles (SM) of the epaxial muscle group on the other. A large medial blood vessel (BV) runs through most of its length, and erythrocytes (Er) can be seen filling this structure in Figure 4A. Lateral to the blood vessel are numerous mesonephric tubules (MT). Pigment cells occur normally in the kidney, and hemopoietic tissue is abundant. Figure 4A contains a glomerulus (Gl) surrounded by

Fig. 3A. Intestine from control. (x100.)

Key

CE, columnar epithelium

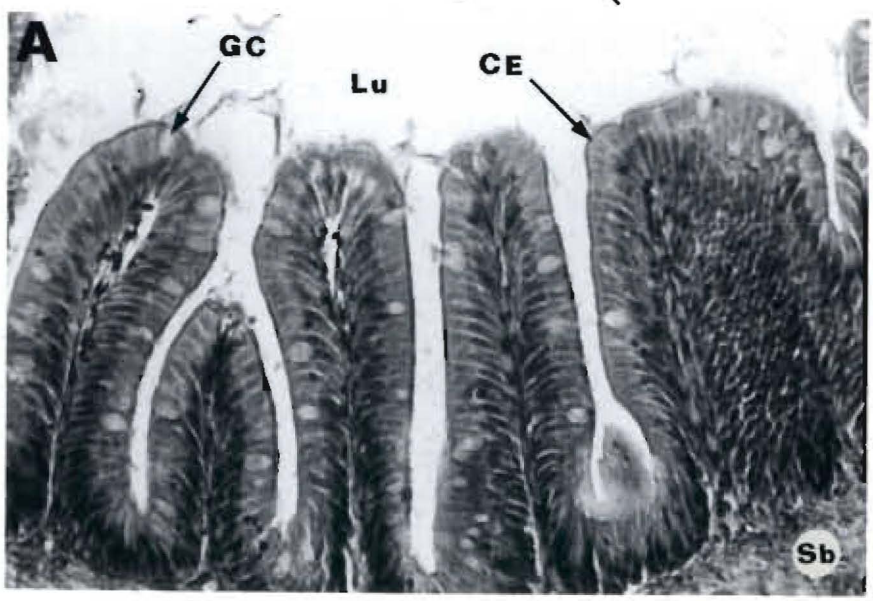
CM, circular muscle

GC, goblet cell

Lu, lumen

Sb, submucosa

Fig. 3B. Intestine from experimental. (x100.)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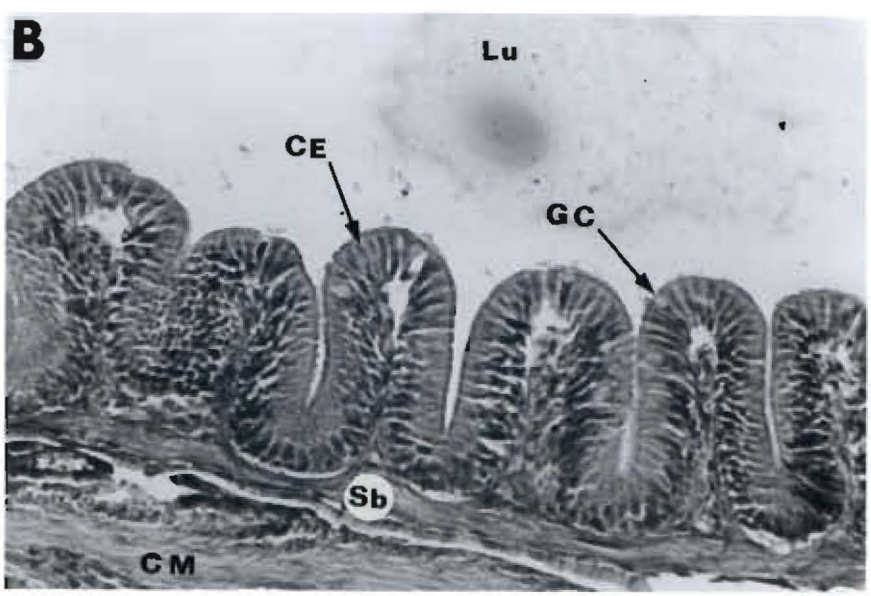

B
Lu
CE
GC
Sb
CM

Fig. 4A. Kidney from control. (x100.)

KEY

AB, air bladder membrane

BV, blood vessel

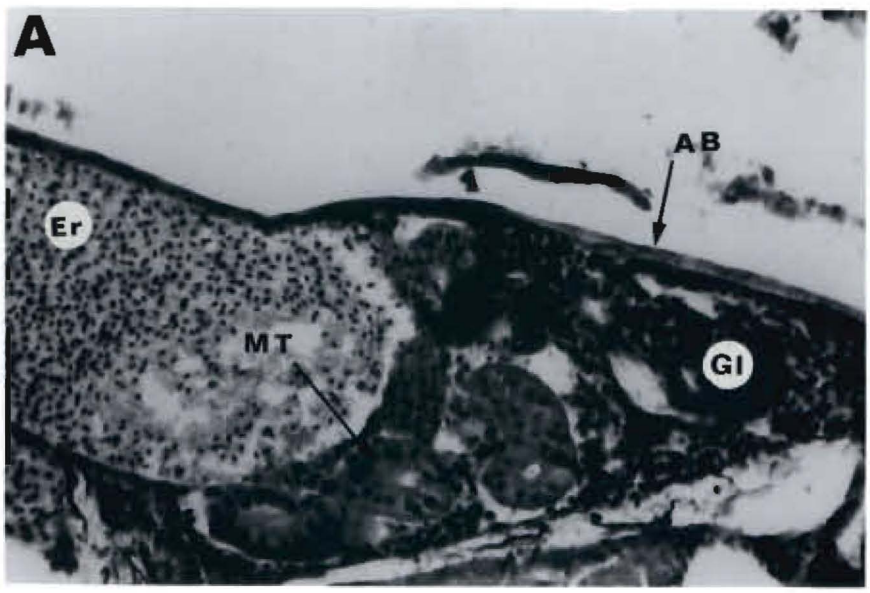
Er, erythrocytes

Gl, glomeruli

MT, mesonephric tubules

SM, striated muscle

Fig. 4B. Kidney from experimental. (x100.)


A
AB
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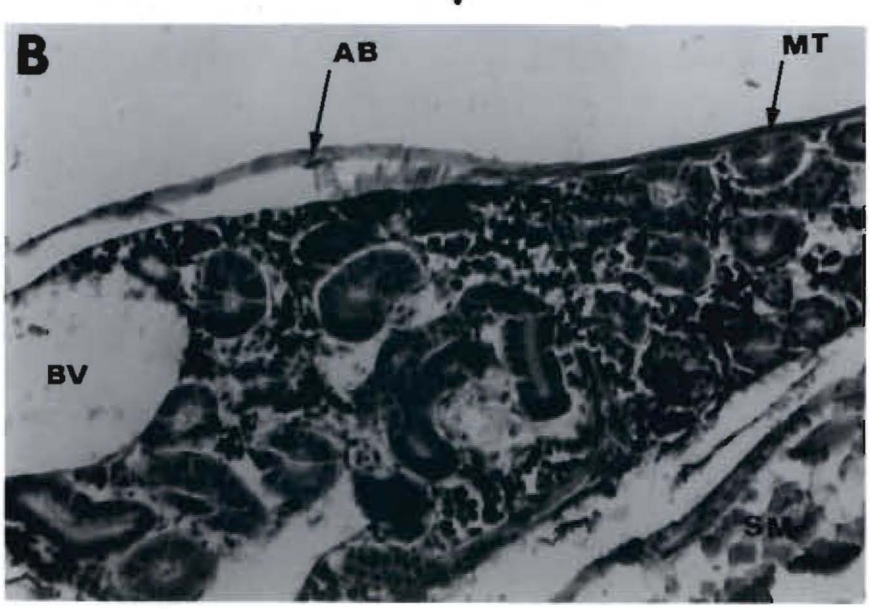
MT
GI


B
AB
MT
BV
SM

the parietal layer of Bowmen's capsule. No observable differences were detected between the tissue of the control and experimental animals.

The liver (Fig. 5A and 5B) has been described as a homogeneous mass of cells forming the hepatic parenchyma (Pa). Sinusoids (Si) form a great anastomosing network. Hepatic veins (HV) are also numerous. Figures 5A and 5B show striated muscle (SM) and stomach (St). As with the other tissues under investigation there was no evidence of degeneration, surface eruptions, fibrotic accumulations, hypertrophy or significant disproportion of cellular components.

Autoradiography Experiment

All tissues were examined under the light microscope for silver grain development indicating paraquat localization. Background grain development was found in all slides. It was, however, sparse and well dispersed. Care had to be taken not to confuse grain development with the presence of numerous chromatophores (Ch) in the dermis (Fig. 6). They are large, branched, pigment cells containing dark brown melanin granules (MG). Humason (1979) describes melanin pigment as brown, gray, or almost black granules.

Thorough examination of tissue sections revealed extensive grain development about the epithelium of the pyloric cecae. The cecae are tubular pouches which open into the duodenum. Figure 7 illustrates the relative position and structure of the cecum. In this photograph two pyloric cecae (PC) are lying next to the muscular stomach (St) and contained within a matrix of connective tissue (CT). The epidermis (Ep) is seen overlying these organs. Higher magnification of the two cecae enhanced the visibility of silver halide grains. Figures 8 and 9 are photomicrographs from portions of these two cecae. The silver grains

Fig. 5A. Liver from control. (x100.)

KEY

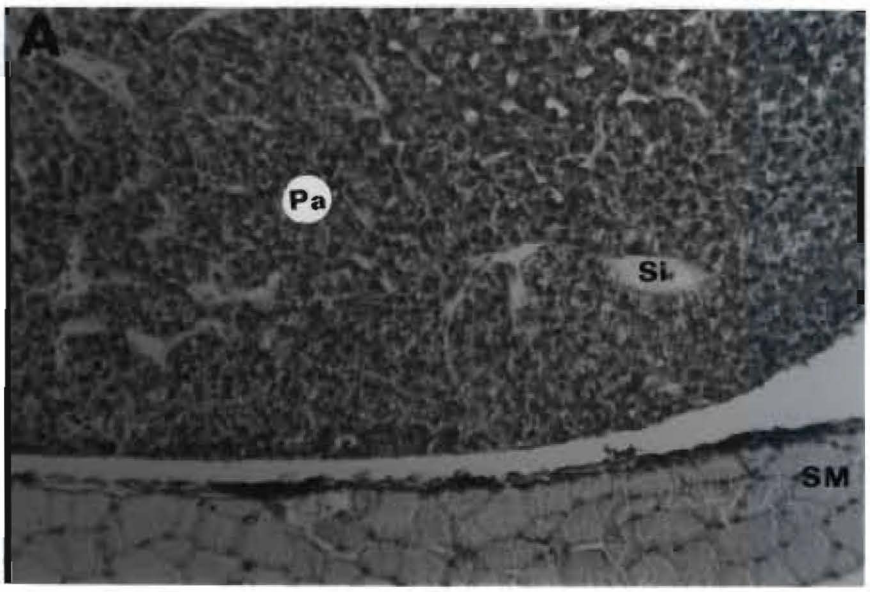
HV, hepatic vein

Si, sinusoids

SM, striated muscle

Pa, parenchyma cells

Fig. 5B. Liver from experimental. (x100.)


A
Pa
Si
SM

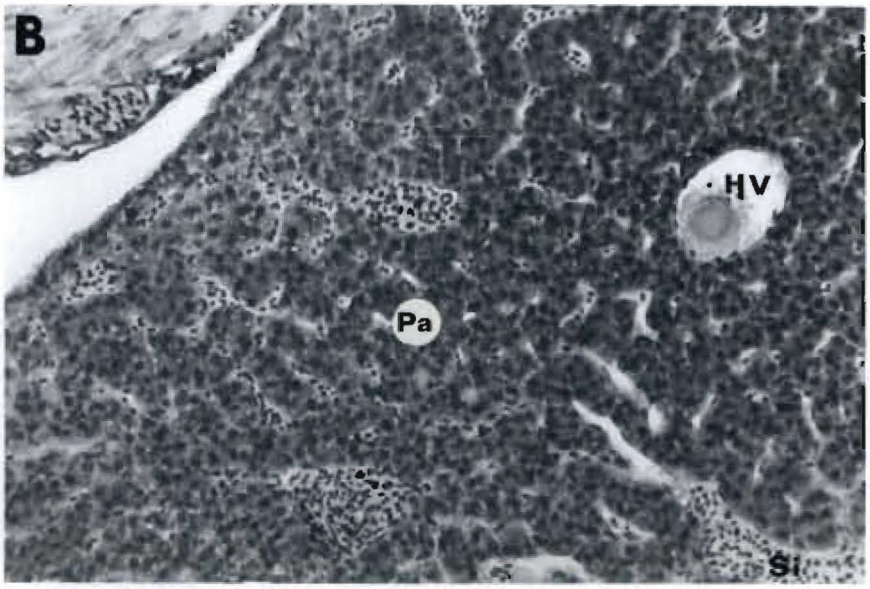

B
HV
Pa
Si

Fig. 6. Chromatophores from epidermis. (x1000.)

KEY

Ch, chromatophores

CT, connective tissue

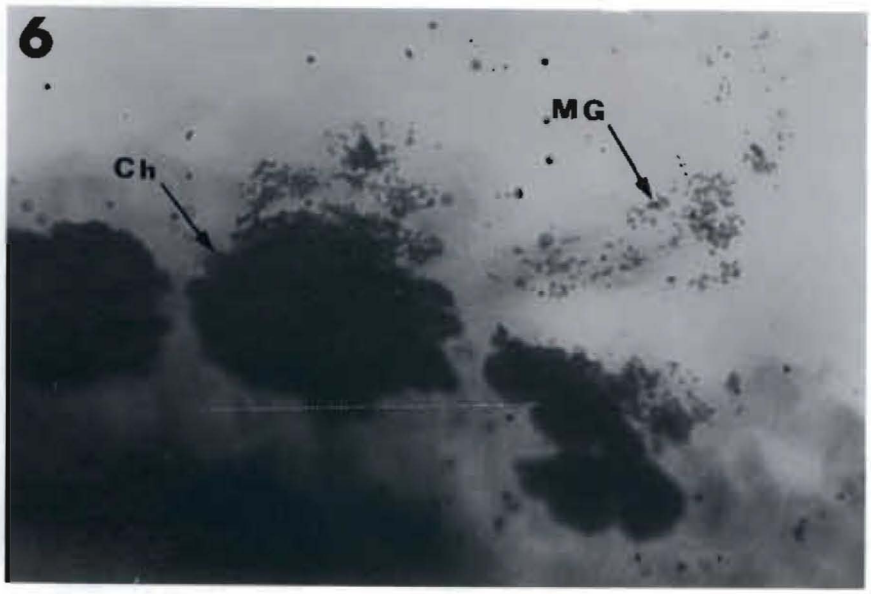
Ep, epidermis

MG, melanin granules

PC, pyloric cecae

St, stomach

Fig. 7. Pyloric cecum. (x100.)


6
MG
Ch

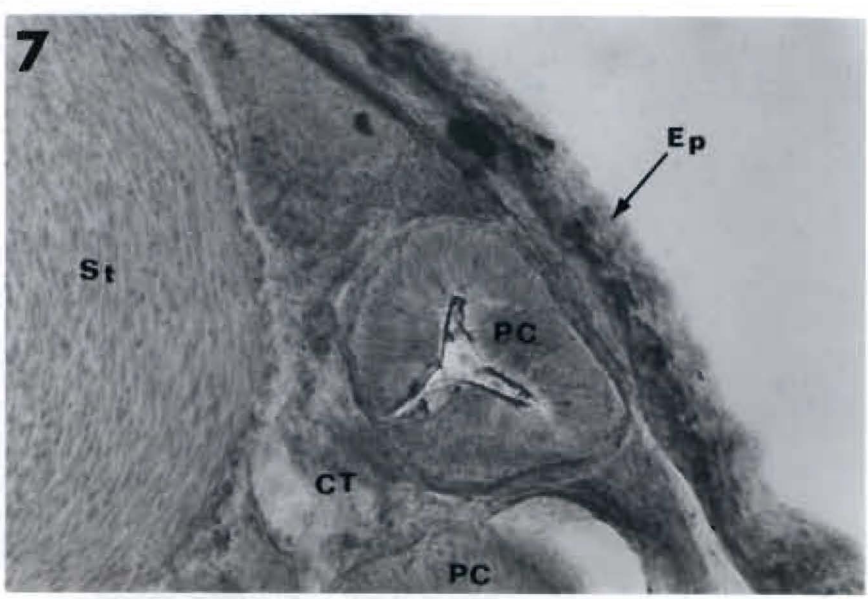

7
Ep
St
PC
CT
PC

Fig. 8. Pyloric cecae. (x1000.)

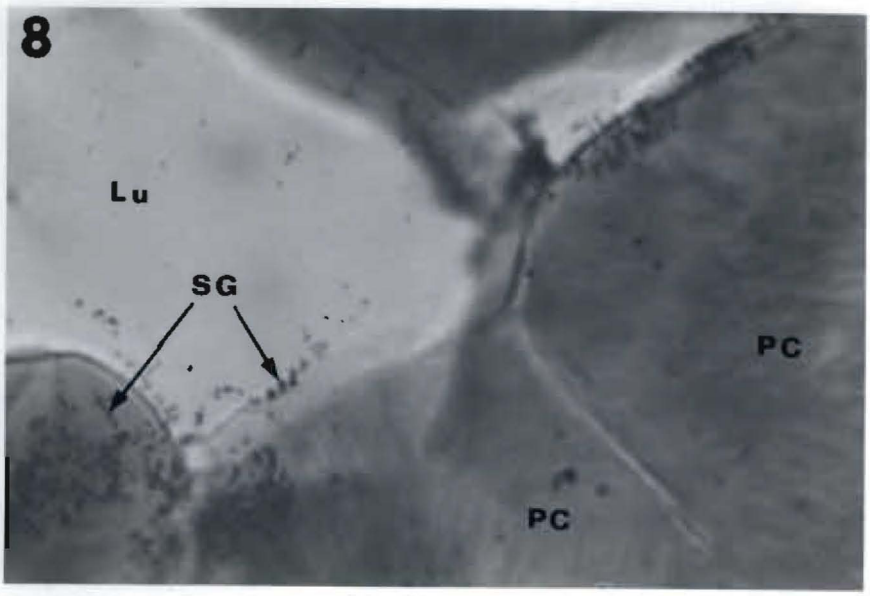
KEY

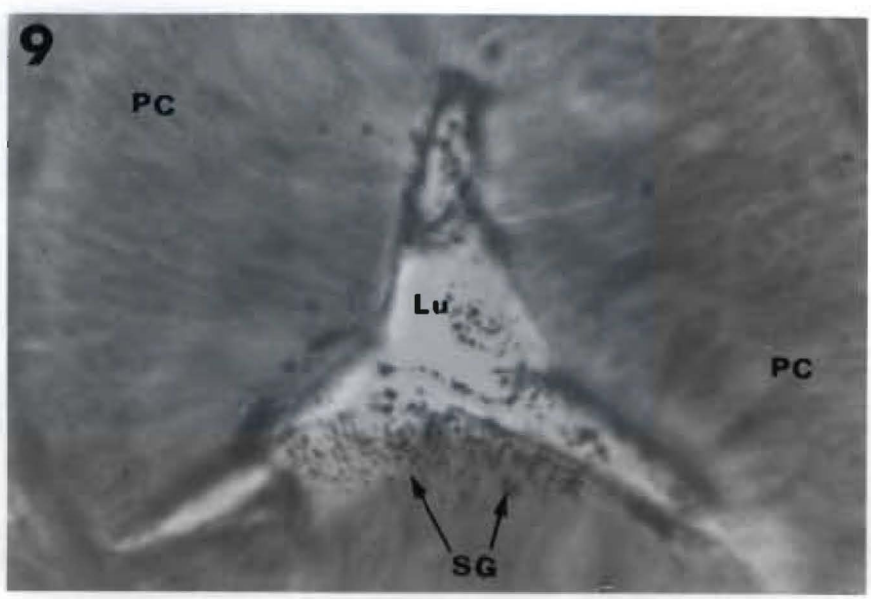
Lu, lumen

PC, pyloric cecae

SG, silver grains

Fig. 9. Pyloric cecae. (x1000.)


8
Lu
SG
PC
PC


9
PC
Lu
PC
SG

(SG) are visible on the columnar epithelium of the cecum and adjacent to the lumen (Lu).

Extensive study of other organ systems failed to show any dense grain development which could be interpreted as paraquat localizations.

DISCUSSION

The experiments with paraquat described here did not reveal any evidence of tissue damage in rainbow trout larva following exposure to the herbicide. Numerous studies by other investigators have indicated specific tissue degeneration in many mammals resulting from paraquat exposure. Considered to be one of the safer herbicides available, paraquat has been responsible for over 400 human deaths following accidental ingestion. Death in man and most experimental animals is due primarily to progressive interstitial fibrosis and epithelial proliferation in lung tissue (Kimbrough, 1974). Transient effects have also been observed in the kidney, liver and heart. Electron microscopy examination of "paraquat lung" revealed degeneration of alveolar lining cells, absence of microvilli, exposure of the basal lamina, and fibroblast-like cells and macrophages at injury sites (Toner et al., 1970). Effects on other organs include hepatic centrilobular necrosis, renal tubular necrosis and myocarditis (Bullivant, 1966). Conversion of paraquat to a free radical and eventual conversion to a long-lived dihydroderivative are believed responsible for the pulmonary damage. This dihydroderivative is thought to cause transformation of normal alveolar epithelial cells to fibroblasts (Haley, 1979). In a literature review of paraquat toxicity, Haley (1979) provides the following comparative mammalian LD_{50}'s: rat, 100 mg/kg; rabbit, 150 mg/kg; sheep, 50-75 mg/kg; monkey, 50 mg/kg; and human, 15 gm/ml (fatal dose). There is no antidote for paraquat poisoning, however several methods are suggested to decrease the body burden. Hemodialysis, forced diuresis, mineral absorbents and prednisone (1000 mg/day) have been successful.

Results from this research suggest that small amounts of paraquat or its metabolites may be localized in some pyloric cecae of the rainbow trout. A computer assisted literature search did not reveal any prior research utilizing ^{14}C-paraquat whole body autoradiograms for determining herbicide localization in any of the fishes. In a study using mice, autoradiographs indicated that paraquat is rapidly distributed throughout the body, with the exception of brain and spinal cord tissue (Litchfield et al., 1973). Chemical localization was highest in the liver and cartilage. Bioaccumulation was not observed after feeding paraquat in concentrations of 50, 120 and 250 ppm for eight weeks. Ilett et al (1974) found that rats and rabbits receiving intravenous injections of ^{14}C-paraquat localized the herbicide at higher concentrations in the lung. Considering the effect of the chemical on the pulmonary system this would be the expected site of deposition. Quantitative studies with rainbow trout showed whole body levels of 0.5-0.6 ppm paraquat after a one ppm 16 day exposure (Haley, 1979). The herbicide was distributed throughout the gills, skin, gut and other organs.

Fytizas (1980) found lesions in the gills of M. cephalus, Pagurus sp. and M. brandaris after initial paraquat exposure. Due to the toxin's characteristic attack upon the respiratory system he suggested that gills are affected in a similar fashion. In aquatic organisms, lesions are more likely caused by exposure to concentrated ammonium compounds. No such lesions were observed in the current study.

Oxygen and paraquat act synergistically, increasing the toxicity of paraquat upon the pulmonary system (Haley, 1979). Formation of the hydroperoxide increases damage to the lung tissue. Kimbrough (1974)

reported that concentrations of paraquat lethal to aerobically grown E. coli have no effect on E. coli grown anerobically. For these reasons oxygen therapy is not recommended for the treatment of paraquat poisoning.

Daily niacin therapy in rats poisoned with paraquat have shown a delayed and reduced mortality (Brown et al., 1981). Paraquat was given intraperitoneally in doses of 30 mg per kilogram of body weight on two occasions. Initial deaths occurred 30 hours after injection and 50 percent of the animals died after 60 hours. The group of animals receiving niacin injections of 500 mg per kilogram of body weight every 24 hours for five days, began to die 36 hours later and experienced the 50 percent mortality rate at 120 hours. Animals given the niacin treatment also showed smaller weight losses. The mechanism for protection is unknown, but recent experiments with E. coli suggest that niacin circumvents the poisoning of quinolinate phosphoribosyltransferase. This enzyme is required for the synthesis of nicotinamide adenine dinucleotide (NAD). No evidence of tissue abnormalities or degeneration which might have been responsible for larval mortality were found in this study. Death, therefore, may have been caused by inhibition of specific enzymes essential for metabolism.

Considerable variation exists between the LC_{50} values in this study and those of other researchers among teleosts. The degree of these differences may be partially attributed to several factors. With the exception of the marine fish Mugil cephalus (Fytizas, 1980), most of the research has been conducted with warm water fish species. Being a cold water species, the rainbow trout would have a corresponding lower metabolic rate. This might have been sufficient to at least partially reduce or delay the effects of paraquat toxicity.

Eggs for this research project were obtained from a commercial fish hatchery. Selective breeding for increased viability and possibly increased environmental tolerance might also increase the lethal threshold for paraquat.

Study ponds were used by Earnest (1971) to determine toxicity levels. The pond had a surface area of 0.44 acre and an average depth of 4.2 feet. Vegetation consisted of Chara sp. and Spirogyra sp. Paraquat is an effective herbicide in small quantities and in his study surface dissolved oxygen reached a low of 2.5 ppm two days after treatment. He attributes the decrease to decaying aquatic vegetation. Thus the low quantities believed to cause fish mortality may have been secondary to the lowered dissolved oxygen caused by plant decomposition. The present study was conducted in glass vessels with dissolved oxygen levels above 7.0 ppm.

In summary this study found that paraquat concentrations necessary for adequate macrophyte control were apparently safe for rainbow trout larvae. These tests were conducted under laboratory conditions which are not typical of field sites. This study was also concerned with immature fish, and the effects on adult fishes cannot be determined from this research. It is necessary therefore to exercise caution because of possible interaction of other environmental factors.

SUMMARY

The toxicity of the herbicide paraquat upon two larval stages of rainbow trout (Salmo gairdneri) was studied. The histopathological effects and tissue accumulation of ^{14}C-labeled paraquat were also investigated. Static bioassays were conducted for 24 and 48 hours at concentrations of 150, 205, 281, 385 and 528 ppm paraquat. Using four replicates, prolarval fish (12 days old) had LC_{50} values of 282, 313, 366 and 329 ppm paraquat. In another experiment with postlarval fish (53 days old), 50% died after 48 hour exposure to 528 ppm paraquat (no replicates). Results of these experiments indicate that toxicity is both time and age dependent.

Tissues from the skin, gills, intestine, kidney and liver of postlarval fish were examined after exposure to 724 ppm paraquat for 48 hours. Microscopic examination revealed no evidence of tissue degeneration, hypertrophy or lesions.

Whole-body autoradiographs were used to locate any sites of paraquat accumulation in the tissue. Prolarval fish (24 days old) were exposed to 20 ppm ^{14}C-paraquat dichloride (50 μCi) in 250 ml of water for 48 hours. Autoradiograms revealed extensive grain around the columnar epithelium of pyloric cecae. Extensive study of other organ systems failed to show any dense grain development which could be interpreted as paraquat localizations.

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