A Preliminary Investigation of the Effect of 2,4 - Dichlorophenoxyacetic Acid (Butoxy Ethanol Ester) on Juvenile Rainbow Trout

B.C. Pearce and J. McBride

Water Quality Unit
Habitat Protection Division
Resource Services Branch
Fisheries and Marine Service
1090 West Pender Street
Vancouver, British Columbia V6E 2P1

October, 1978

Fisheries & Marine Service Manuscript Report No. 1478

Fisheries and Marine Service
Manuscript Reports

These reports contain scientific and technical information that represents an important contribution to existing knowledge but which for some reason may not be appropriate for primary scientific (i.e. *Journal*) publication. They differ from Technical Reports in terms of subject scope and potential audience: Manuscript Reports deal primarily with national or regional problems and distribution is generally restricted to institutions or individuals located in particular regions of Canada. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Fisheries and Marine Service, namely, fisheries management, technology and development, ocean sciences and aquatic environments relevant to Canada.

Manuscript Reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report will be abstracted by *Aquatic Sciences and Fisheries Abstracts* and will be indexed annually in the Service's index to scientific and technical publications.

Numbers 1-900 in this series were issued as Manuscript Reports (Biological Series) of the Biological Board of Canada, and subsequent to 1937 when the name of the Board was changed by Act of Parliament, as Manuscript Reports (Biological Series) of the Fisheries Research Board of Canada. Numbers 901-1425 were issued as Manuscript Reports of the Fisheries Research Board of Canada. The series name was changed with report number 1426.

Details on the availability of Manuscript Reports in hard copy may be obtained from the issuing establishment indicated on the front cover.

Service des pêches et des sciences de la mer
Manuscrits

Ces rapports contiennent des renseignements scientifiques et techniques qui constituent une contribution importante aux connaissances actuelles mais qui, pour une raison ou pour une autre, ne semblent pas appropriés pour la publication dans un journal scientifique. Ils se distinguent des Rapports techniques par la portée du sujet et le lecteur visé; en effet, ils s'attachent principalement à des problèmes d'ordre national ou régional et la distribution en est généralement limitée aux organismes et aux personnes de régions particulières du Canada. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du Service des pêches et de la mer, notamment gestion des pêches; techniques et développement, sciences océaniques et environnements aquatiques, au Canada.

Les Manuscrits peuvent être considérés comme des publications complètes. Le titre exact paraît au haut du résumé de chaque rapport, qui sera publié dans la revue *Aquatic Sciences and Fisheries Abstracts* et qui figuera dans l'index annuel des publications scientifiques et techniques du Service.

Les numéros de 1 à 900 de cette série ont été publiés à titre de manuscrits (Série biologique) de l'Office de biologie du Canada, et après le changement de la désignation de cet organisme par décret du Parlement, en 1937, ont été classés en tant que manuscrits (Série biologique) de l'Office des recherches sur les pêcheries du Canada. Les numéros allant de 901 à 1425 ont été publiés à titre de manuscrits de l'Office des recherches sur les pêcheries du Canada. Le nom de la série a été changé à partir du rapport numéro 1426.

La page couverture porte le nom de l'établissement auteur où l'on peut se procurer les rapports sous couverture cartonnée.

• Cover design by Christine Rusk

Fisheries and Marine Service
Manuscript Report No. 1478
1978

A PRELIMINARY INVESTIGATION OF THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (butoxy ethanol ester) ON JUVENILE RAINBOW TROUT

B.C. Pearce
J. McBride*

Water Quality Unit
Habitat Protection Division
Resource Services Branch
Fisheries and Marine Service
Pacific Region

1090 West Pender Street
Vancouver, B.C.

*Research Scientist, Resource Services Branch, Fisheries and Marine Service, 4160 Marine Drive, West Vancouver, B.C. V7V 1N0

TABLE OF CONTENTS

ABSTRACT

PEARCE, B.C. AND J. McBRIDE, 1978. A preliminary investigation of the effect of 2,4-dichlorophenoxyacectic acid (butoxy ethanol ester) on juvenile rainbow trout. Fish. Mar. Ser. Manuscript Report No. 1478.

The effect of the butoxy ethanol ester of 2,4-dicholorphenoxyacetic acid (BEE 2,4-D) on juvenile rainbow trout (*Salmo gairdneri* Richardson) was examined in the field and in the laboratory using bioassay techniques.

High mortalities of caged rainbow trout were recorded in both treatment and control areas of the North Arm of Okanagan Lake, British Columbia, where BEE 2,4-D was used experimentally to control Eurasian water milfoil (*Myriophyllum spicatum* L.). The maximum concentration of 2,4-D recorded in the treatment area was 0.14 mg l^{-1}, 2 days after herbicide treatment. Excessive periphyton growths on the bioassay cages may have decreased ventilation of the cages and contributed to fish mortality.

In laboratory bioassays the 96 h LC_{50} of BEE, 2,4-D to juvenile rainbow trout was 3.05 mg l^{-1}.

Fish were periodically sampled from the field and laboratory experiments and examined histologically. The fish from the laboratory bioassay exhibited distinct degenerative changes in liver structure at concentrations of 0.37 to 1.48 mg l^{-1} 2,4-D. The pathology however, was observed only in the region adjacent to the gall bladder or the bile ducts where degenerate cells contained pyknotic nuclei and amorphous-like cytoplasm. Minor alterations (focal areas of edema and tubular degeneration) were also noted in the kidneys of the fish which were exposed to BEE 2,4-D in the field (max. 0.14 mg l^{-1} 2,4-D) but not in the kidneys of control fish or fish exposed to BEE 2,4-D in the laboratory experiments.

Further studies to assess the sublethal effects of 2,4-D on salmonids and aquatic systems are recommended before large scale use of 2,4-D for aquatic weed control is undertaken in British Columbia.

Key Words

2,4-D Rainbow Trout, Histopathology, Toxicity, Water Milfoil, *Myriophyllum spicatum*, Aquatic Weeds, Okanagan Lake.

RÉSUMÉ

PEARCE, B.C., and J. MCBRIDE, 1978. A preliminary investigation of the effect of 2,4-dichlorophenoxyacetic acid (butoxy ethanol ester) on juvenile rainbow trout. Fish. Mar. Ser. Manuscript Report No. 1478

Nous avons étudié les effets du BEE 2,4-D (produit de l'estérification de l'acide dichloro-2,4 phénoxyacétique avec le butoxy-éthanol) sur des Truites arc-en-ciel juvéniles (*Salmo gairdneri* Richardson) au moyen de techniques de bioanalyse pratiquées sur le terrain et en laboratoire.

Une forte mortalité des truites en cage a été enregistrée à la fois dans les zones de traitement et les zones témoins du bras nord du lac Okanagane, en Colombie-Britannique, où on a utilizé du BEE 2,4-D dans des expériences de répression du Myriophylle verticilé (*Myriophyllum spicatum* L.). La concentration maximale de 2,4-D, 2 jours après l'application, était de 0,14 mg l^{-1}. Une croissance excessive du périphyton sur les cages pourrait avoir réduit l'oxygénation et contribué à la mortalité des poissons.

En laboratoire, la CL_{50} après 96 h du BEE 2,4-D pour les Truites arc-en-ciel juvéniles était de 3,05 mg l^{-1}.

Des poissons ont été prélevés périodiquement sur le terrain et en laboratoire, et soumis à des examens histologiques. Les poissons provenant des bioanalyses en laboratoire montraient une dégénérescence visible de la structure du foie, à des concentrations de 2,4-D et de 0,37 à 1,48 mg l^{-1}. Des signes pathologiques n'ont toutefois été relevés que dans la région adjacente à la vésicule et aux canaux biliaires, où les cellules dégénérées contenaient des noyaux pycnotiques et du cytoplasme amorphe. On a aussi noté des changements mineurs (zones localisées d'oedèmes et de dégénérescence des tubules) dans les reins des poissons qui avaient été exposés au BEE 2,4-D sure le terrain (conc. max. de 2,4-D de 0,14 mg l^{-1}), mais pas dans ceux des poissons témoins ou des poissons exposés au BEE 2,4-D en laboratoire.

Nous recommandons que d'autres recherches soient entreprises pour évaluer les effets sublétaux du 2,4-D sur les Salmonidés et les écosystèmes aquatiques, avant qu'on adopte le 2,4-D pour la lutte à grande échelle contre les plantes aquatiques en Colombie-Brittanique.

Mots clés: 2,4-D, Truite arc-en-ciel, histophatologie, toxicité, Myriophylle, *Myriophyllum spicatum*, plantes aquatiques, lac Okanagane.

I. INTRODUCTION

Since 1972 the Water Investigations Branch (WIB) of the British Columbia Ministry of Environment has been involved in ecological and management studies of the Eurasian water milfoil (*Myriophyllum spicatum* L.), a plant which has spread rapidly throughout the Okanagan lakes. Most of the WIB studies have been directed towards evaluating the efficacy of various physical and chemical weed control techniques. The collection of basic information on the biology of water milfoil and the effect of control technologies on aquatic organisms of British Columbia has been limited.

On May 31, 1976 the WIB initiated an experiment in Okanagan Lake to assess the efficacy of BEE 2,4-D granules (AQUA-KLEEN^{R})[1] as an aquatic herbicide for the control of Eurasian water milfoil. The WIB monitored herbicide efficacy, water quality, and the impact of the herbicide treatment on invertebrates (Water Investigations Branch 1976). The WIB also carried out, under the direction of the British Columbia Fish and Wildlife Branch, *in situ* bioassays with juvenile rainbow trout.

The Fisheries and Marine Service carried out laboratory bioassays to determine the concentrations of 2,4-D that were lethal to juvenile rainbow trout. Those juvenile rainbow trout that survived in the laboratory and *in situ* bioassays were examined histologically.

This report documents the results of the laboratory and field bioassays and the results of the histological examination of the juvenile rainbow trout that survived in these experiments.

II. SITE DESCRIPTION AND HERBICIDE APPLICATION *In situ* EXPERIMENT

Three plots, each approximately 0.6 hectares in size, were established in dense beds of water milfoil in the littoral area of the northwest corner of the North Arm of Okanagan Lake, between Deep and Irish Creeks (Figure 1). The plots were arranged parallel to the shoreline approximately 50 m apart and 100 m off shore. Water depths within

1. AQUA-KLEEN^{R} - butoxy ethanol ester 2,4-D, 20% by weight 2,4-D acid equivalent - Amchem Products, Inc. Ambler, Pa. Clinton, Iowa, St. Joseph, Mo., Fermont, California.

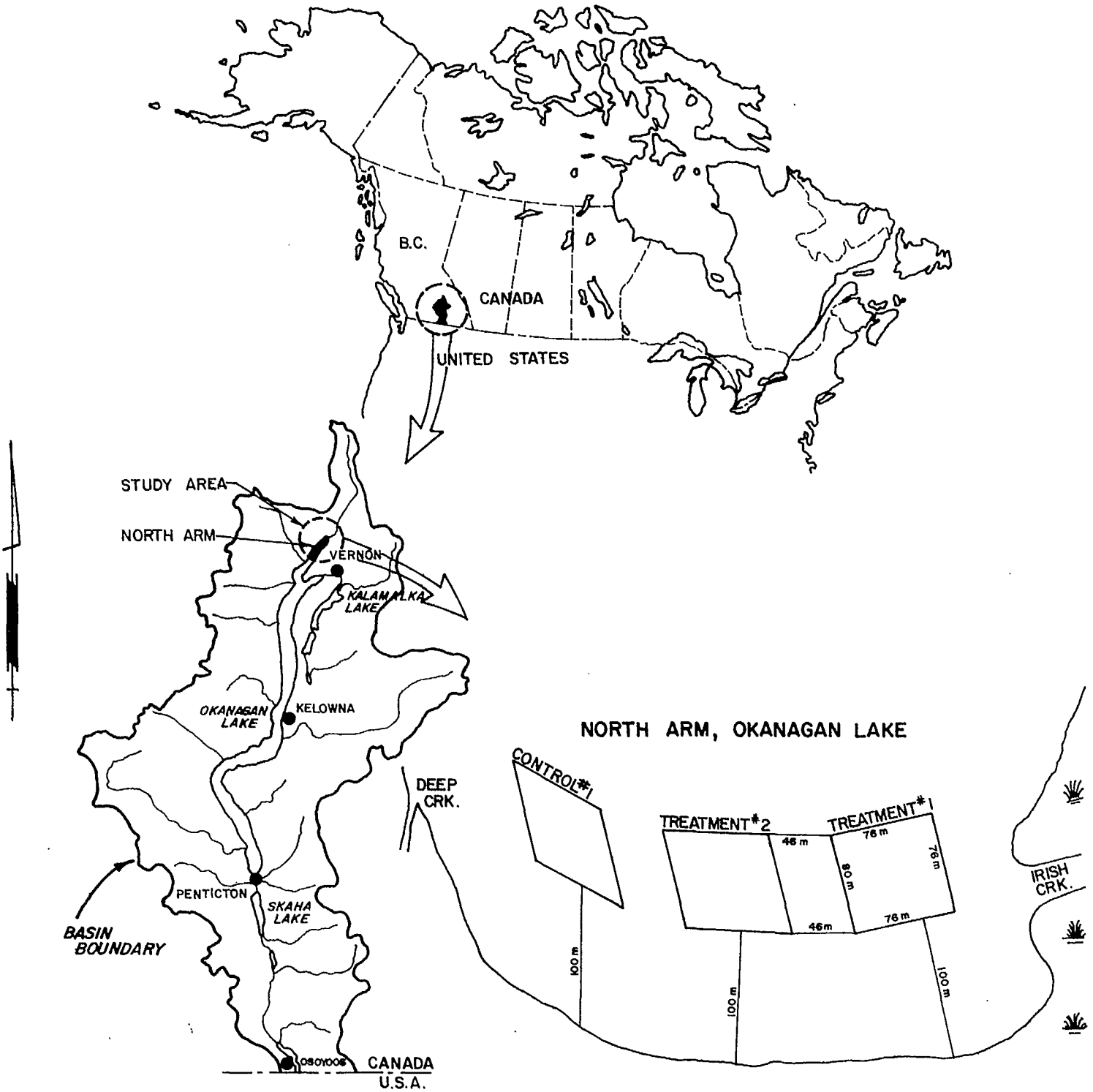


FIGURE 1. OKANAGAN BASIN, BRITISH COLUMBIA AND 2,4-D STUDY AREA AT NORTH ARM OF OKANAGAN LAKE.

S.P.

the plots ranged from 0.9 to 2.5 m.

On May 31, 1976 granular BEE 2,4-D was applied from a boat to two of the plots using a mechanical spreader. The manufacturer's recommended application rate for control of water milfoil (22.4 kg acid equivalent (a.e.) hectare^{-1}) was used. The third plot, which was not treated, was used as a control (Figure 1).

III. METHODS

1. LABORATORY EXPERIMENTS WITH JUVENILE RAINBOW TROUT

a) Acute Toxicity Studies

Bioassays were carried out in self-contained, temperature controlled rooms using groups of nine juvenile rainbow trout; mean fork length 6.1 cm (range 5.1 to 6.8 cm), mean weight 2.5 g (range 1.5 to 3.5 g).

Concentrations of 5.6, 4.2, 3.2, 2.4, and 1.8 mg a.e. l^{-1} BEE 2,4-D were made up by mixing AQUA-KLEEN^R in glass aquariums, with 30 l of dechlorinated Vancouver City tap water (pH 6.3, hardness 3.9 mg l^{-1} as $CaCO_3$). A 30 l aquarium that had no herbicide added to it was used as a control. The mean weight of fish per unit volume was 0.8 g l^{-1}. The water temperature was maintained at 13 ± 1°C in the controlled environment rooms and dissolved oxygen was maintained near saturation with compressed air.

b) Histological Studies

A histological examination was carried out on groups of 10 juvenile rainbow trout, mean fork length 5.2 cm (range 4.3 to 5.8 cm) mean weight 1.5 g (range 0.8 to 2.4 g), which were exposed to BEE 2,4-D. The histological examination was limited to the liver, kidney, testes, and alimentary tract.

Concentrations of 5.0, 1.48, 0.74, and 0.37 mg a.e. l^{-1} BEE 2,4-D were made up by mixing AQUA-KLEEN^R in glass aquariums, with 30 l of dechlorinated Vancouver City tap water (pH 6.4, hardness 3.9 mg l^{-1} as $CaCO_3$). An aquarium that had no herbicide added to it was used as a control. The mean weight of fish per unit volume was 0.5 g l^{-1}. The water temperature was maintained at 13 ± 1°C and dissolved oxygen was maintained near saturation with compressed air.

Five fish were removed from each concentration, with

the exception of the fish within the 5.0 mg l^{-1} concentration, after 8 days exposure to BEE 2,4-D and preserved in Bouin-Hollande-Sublimate fixative. The remaining five fish were removed and preserved in the same manner after a total 14 days exposure.

The fish exposed to 5.0 mg l^{-1} BEE 2,4-D died within 24 hours and were not examined histologically.

2. *In situ* EXPERIMENTS WITH JUVENILE RAINBOW TROUT

Three small bioassay cages (10 x 10 x 40 cm) and one large bioassay cage (45 x 45 x 60 cm) were placed in each treatment and control plot five days prior to the herbicide treatment. The bioassay cages were constructed of high impact plexiglass. Holes 0.5 cm diameter, were spaced, at intervals of 1 cm, uniformly in the sides of each cage to provide ventilation. The cages were supported in the center of each plot at a depth of approximately one metre with wooden stakes embedded in the lake bottom.

Juvenile rainbow trout were obtained from the Fish and Wildlife Branch hatchery at Summerland, British Columbia. Thirty rainbow trout were placed in each of the larger cages and 20 rainbow trout were placed in each of the smaller cages four days prior to the herbicide application.

The fish from the large bioassay cages were to be used to replace any fish that died in the small bioassay cages during the acclimating period prior to the herbicide application. A number of fish from these cages were ultimately used in the histological evaluation because of deaths in the small bioassay cages.

a) Acute Toxicity Studies

The fish in one of the three small bioassay cages in each plot were used to indicate the acute toxicity of BEE 2,4-D. The fish were observed, and the number of deaths recorded, immediately before herbicide treatment and 3, 7, 11 and 16 days after treatment.

b) 2,4-D Residue Studies

The WIB sampled the water column of the treatment and control plots immediately prior to the herbicide treatment and at 7 hours, and 2, 3, 8, 10, and 17 days after the herbicide treatment. The water samples were analyzed for 2,4-D residues by the British Columbia Ministry of Agriculture Pesticide Laboratory in Vancouver using a gas chromatography

method (see Water Investigations Branch 1976 for details of sampling methods).

c) Histological Studies

The fish from two of the three small bioassay cages and the large bioassay cage in each plot were subsampled and examined 3, 11, and 16 days after the herbicide treatment.

Due to severe mortalities in the treatment and control cages and a storm that resulted in the destruction of one set of treatment cages, the following sampling regime was used for the histological evaluation.

Days following herbicide application	Number of fish sampled from each plot		
	Treatment I	Treatment II	Control
3	12	12	12
11	0	0*	10
16	11	0*	15

*cages destroyed by a storm that occurred between day 3 and 11.

3. HISTOLOGICAL TECHNIQUES

Prior to the fish being placed in Bouin-Hollande-Sublimate fixative a longitudinal incision was made in their ventral surface, between the pectoral fins and the anus, to ensure rapid penetration of the fixative solution into the fish tissues. The portion of the fish posterior to the anal fin was discarded.

Following a minimum of 24 hours in the Bouin-Hollande-Sublimate fixative, representative samples of the liver, kidney, stomach, gill and gonad were excised, embedded in Paraplast (mp 56°C) and sectioned at 5 µm. Sections were stained with Mayer's hematoxylin and eosin as well as Masson's trichrome method (Culling, 1957).

IV. RESULTS

1. ACUTE TOXICITY STUDIES

a) Laboratory Bioassays

Results of the laboratory bioassays are presented in Table 1. All fish subjected to 5.6 and 4.2 mg l^{-1} BEE 2,4-D died within the first 24 hours. In concentrations of 3.2 and

2.4 mg l^{-1} BEE 2,4-D, 4 and 2 fish respectively died within 96 hours. There were no deaths in 1.8 mg l^{-1} BEE 2,4-D or in the control. The 96 h LC_{50} of BEE 2,4-D to juvenile rainbow trout was 3.05 mg l^{-1}.

Table 1. Mortality of juvenile rainbow trout subjected to various concentrations of BEE 2,4-D in laboratory bioassays.

Concentration mg l^{-1}	Test Volume(l)	No.of Fish	Initial pH	Final pH	Mortality at Time (hrs) 24	48	72	96
5.6	30	9	6.4	6.6	9	-	-	-
4.2	30	9	6.4	6.4	9	-	-	-
3.2	30	9	6.4	6.7	0	4	4	4
2.4	30	9	6.4	6.6	0	2	2	2
1.8	30	9	6.4	6.3	0	0	0	0
Control	30	9	6.4	6.6	0	0	0	0

96 hour LC_{50} = 3.05 mg l^{-1}

b) *In situ* Bioassays

The bioassay cages placed in one treatment plot in Okanagan Lake were destroyed by a storm immediately following the herbicide treatment. Consequently, results of the *in situ* investigation are available for the control and one treatment plot only.

i) Fish Mortality

Fish deaths recorded in the *in situ* bioassay are presented in Table 2. All the fish (20) that were held within the bioassay cage that was placed in the treatment plot were dead 11 days after the herbicide treatment. In contrast, in the control plot 50 percent (10) of the fish in the bioassay cage were dead at 11 days. After 11 days no further deaths were recorded in the control cage.

Table 2. Percent mortality of juvenile rainbow trout held in control and treatment plots in Okanagan Lake.

	No. of Fish	% Mortality at Time Before Treatment	Day 3	Day 7	Day 11	Day 16
Treatment	20	0	5	10	100	--
Control	20	0	0	45	50	50

ii) 2,4-D Residues

Prior to the herbicide treatment 2,4-D was not detected in samples collected from either the treatment or control plots.

After the herbicide treatment, 2,4-D was not detected in the water samples collected from the control plot. In the treatment plots mean 2,4-D levels ranged from 0.003 to 0.005 mg l^{-1} within the first three days after the herbicide treatment. The maximum concentrations of 2,4-D measured in samples collected from the treatment plots were 0.06 mg l^{-1} at 7 hours, 0.14 mg l^{-1} at 2 days and 0.025 mg l^{-1} at 3 days after treatment. There was no 2,4-D detected in samples collected 8, 10 or 17 days after the herbicide treatment (Water Investigations Branch 1976).

Herbicide granules were observed on top of, and inside the treatment cages immediately following treatment; however, the granules were absent by the second day after treatment.

2. HISTOLOGICAL STUDIES

a) Laboratory Bioassays

The only organ to show a distinct degenerative change in the fish from the laboratory bioassays was the liver.

i) Incidence of Liver Pathology

The incidence of liver damage at each 2,4-D concentration is presented in Table 3.

Table 3. Incidence of degenerative changes in the liver of rainbow trout exposed to 2,4-D.

	Days of Exposure			
	8		16	
Concentration	No. of fish with liver pathology	No. of fish examined	No. of fish with liver pathology	No. of fish examined
Control	0	5	1	5
0.37 mg l^{-1}	2	5	4	5
0.74 mg l^{-1}	2	5	5	5
1.48 mg l^{-1}	4	5	5	5
5.0 mg l^{-1}	-	0*	-	0

*all fish exposed to 5.0 mg l^{-1} BEE 2,4-D died within 24 hours and were not examined.

ii) Description of Liver Pathology

With one exception, the liver cells of the control fish contained a well defined oval or circular nucleus containing a single prominent nucleolus. The cytoplasm consisted of a fine, evenly distributed granular mass often containing a few small vacuoles. The latter sac-like structures in all probablility represented lipid deposits extracted during processing. A number of small haemorrhagic lesions were noted in the liver of one control fish sampled after 16 days; however, the degenerative areas did not appear to be restricted or related to a particular area or structure.

The livers of the fish exposed to 2,4-D that exhibited histological change showed a very specific pathology in that the degenerative changes were noted only in the areas in proximity to the gall bladder or bordering the bile ducts. In contrast to the structure of the healthy tissue (Figures 2 and 3), the degenerative cells (Figures 2 and 4) contained pyknotic nuclei and amorphous-like cytoplasm. In a few cases, the hypertrophy of the degenerating cells appeared to have obliterated the sinusoids (Figure 4). Although these alterations were somewhat more well defined in the fish exposed to the maximum dose of 2,4-D the difference in response between the groups exposed to the minimum and maximum dosage was not striking.

Figure 2. Liver of a juvenile rainbow trout exposed to 0.74 mg l^{-1} BEE 2,4-D (butoxy ethanol ester) for a period of 8 days. The parenchyma cells located on the left side of the photo are normal in structure. The cells on the right side have pyknotic nuclei and amorphous-like cytoplasm. H and E x100.

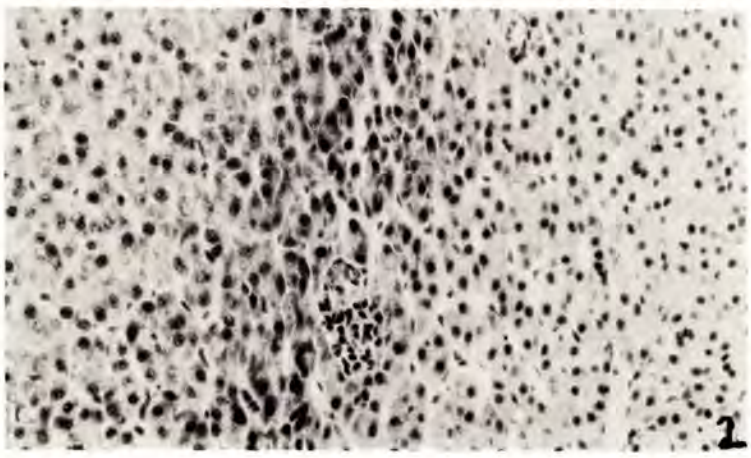


Figure 3. Liver of a juvenile rainbow trout exposed to 0.74 mg l^{-1} BEE 2,4-D (butoxy ethanol ester) for a period of 8 days. A higher magnification of the normal cells shown in Figure 2. Sinusoids containing erythrocytes separate the liver columns, 3 cells thick. Nucleoli are readily indentifiable. H and E x160.

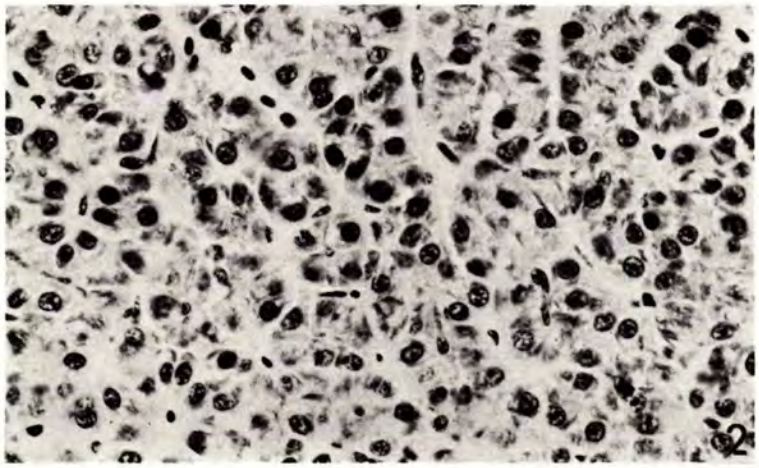
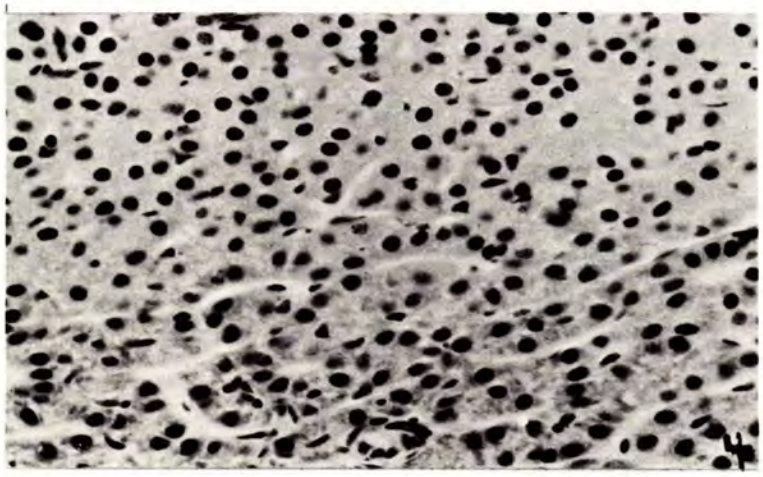


Figure 4. Liver of a juvenile rainbow trout exposed to 0.74 mg l^{-1} BEE 2,4-D (butoxy ethanol ester) for a period of 8 days. A higher magnification of the abnormal cells located on the right side of Figure 2. Note pyknotic nuclei and absence of nucleoli. H and E x160.



b) *In situ* Bioassays

The tissues of the treatment and control fish that were sampled 3 days after the herbicide treatment and the tissues of the control fish that were sampled 11 days after the herbicide treatment all appeared to be normal in structure (no treatment fish were sampled 11 days after the herbicide treatment). After 16 days, minor alterations were noted in all of the kidneys and in a few of the livers of fish sampled from the treatment plot only. The livers and kidneys of control fish sampled 16 days after treatment appeared normal.

The kidney pathology consisted of focal areas of edema and tubular degeneration. The pathology of the liver consisted of a minor loss in cytoplasmic granulation. The liver pathology was not well defined.

V. DISCUSSION

1. ACUTE TOXICITY STUDIES

a) Laboratory Bioassays

The specific formulation of 2,4-D and the use of solvents and emulsifiers has been found to markedly affect the toxicity of 2,4-D to fish (McKee and Wolf, 1963; Meehan *et al.*, 1974). However, the 96 h LC_{50} value reported in this study (3.05 mg l^{-1} BEE 2,4-D) generally agrees with LC_{50} values reported by other investigators working with various formulations of 2,4-D. For example, Meehan *et al.* (1974) reported that the "no acute effect level" (the highest concentration tested that produced no mortality of test fish in 96 hours) varied from <1 mg l^{-1} for the butyl ester of 2,4-D to 50 mg l^{-1} for the pure acid.

Meehan *et al.* (1974) tested several different formulations of 2,4-D over a wide range of concentrations (1,5,10, 50 mg l^{-1}). They found that the butyl (n-butyl, isobutyl) ester and propylene glycol butyl ether ester were more toxic than the isooctyl ester or pure 2,4-D acid. In their study different species of salmonids, of similar "size", generally reacted similarly to the same concentration and formulation of 2,4-D; however, younger fish (fry) appeared to be more sensitive than older fish.

Concentrations of BEE 2,4-D less than 1 mg l^{-1} have been found to kill juvenile (alevin and swim-up fry) sockeye salmon (*Oncorhynchus nerka*) (J. Servizi, International Pacific Salmon Fisheries Commission, Cultus Lake, British Columbia, pers. comm.); however the establishment of the exact

concentration of BEE 2,4-D in static bioassays is complicated by the rapid conversion of 2,4-D ester to 2,4-D acid.

b) *In situ* Bioassays

Results of the *in situ* bioassays are somewhat inconclusive because of the mortality which occurred in fish in both control and treatment cages. Excessive periphyton growths which developed on the treatment and control bioassay cages may have prevented adequate ventilation of the cages resulting in depressed dissolved oxygen levels inside the cages. Low dissolved oxygen levels inside the cages may have been a contributing factor to fish mortality. Consequently the fish mortality in the cages within the treatment plot may not have been entirely due to 2,4-D toxicity.

The minimum dissolved oxygen value recorded in the water column of the treatment plots during the bioassay was 9.4 mg l^{-1} (Water Investigations Branch 1976). This concentration is considered adequate for the survival of rainbow trout (Davis, 1975).

The Tennessee Valley Authority (TVA) has used BEE 2,4-D for control of water milfoil in reservoirs. Smith and Isom (1967) monitored the impact of 112 kg (a.e.) hectare^{-1} BEE 2,4-D applied to Watts Bar reservoir for milfoil control. They used the abundance of the burrowing mayfly of the genus *Hexagenia* (a principal component of the benthic fauna in TVA reservoirs) as an index of the toxic effects of 2,4-D on benthos. The authors did not find a significant change in the abundance of *Hexagenia* between one pretreatment and three post-treatment sampling periods (1, 10, and 12 months after treatment).

Smith and Isom also assessed the impact of 2,4-D on fish. They reported a significant difference ($p \leq 0.05$) in the percent mortality of caged "native fish" that were held in treatment areas, when compared before and after herbicide treatment. Also, the mortality of fish held in treatment cages was significantly higher after 72 and 96 hours than the mortality of fish held in control cages. The authors, however, did not attribute the differences in mortality to 2,4-D alone since the maximum concentration of 2,4-D recorded in the water column (0.37 mg l^{-1}) was well below the "median tolerance limit" for the fish tested.

Smith and Isom concluded that there were no "adverse effects (of the herbicide treatments) on aquatic fauna or water quality" of Watts Bar reservoir.

2. HISTOLOGICAL STUDIES

a) Liver Pathology

i) Laboratory Bioassays

In fish from the laboratory bioassays, the damage to liver cells only in the regions adjacent to either the gall bladder or the bile ducts suggests that the liver pathology may be due to secondary effects of 2,4-D on the gall bladder or its contents. Hendricks *et al.* (1976) showed that pre- or post-mortem exposure of liver cells to bile caused peripheral liver necrosis in rainbow trout. The authors advised that extreme care should be taken in the collection of trout livers for histological examination in order to avoid spillage of bile onto the liver and resultant tissue damage. However, in the present study only the livers of rainbow trout subjected to 2,4-D exhibited pathology associated with the gall bladder, suggesting that the sampling technique was not responsible for the observed liver pathology.

ii) *In situ* Bioassays

The minor loss of cytoplasmic granulation in the livers of fish from the *in situ* bioassay, although not well defined, may nevertheless represent the initial stages of a developing pathological condition. Cope *et al.* (1970) observed hepatic lesions in bluegill (*Lepomis macrochirus*) that were exposed to 2,4-D (propylene glycol butyl ether ester). They exposed bluegills, for 196 days, in ponds that received single applications of 2,4-D at concentrations of 10, 5, 1, 0.5 and 0.1 mg l^{-1} (a.i.), subsampling the fish periodically for histological and biochemical analysis. In addition to the liver pathology which, unlike this investigation, involved the whole organ, the authors documented a marked depletion of liver glycogen, globular deposits in the blood vessels and stasis and engorgement of the circulatory system of the brain.

The pathological changes documented by Cope *et al.* were dependent upon both the duration of exposure and the concentration of 2,4-D. The changes were maximum after 14 days exposure and were present at concentrations as low as 0.1 mg l^{-1}. The single exposure to 2,4-D, while resulting in severe pathological changes, was followed by eventual complete recovery. After 84 days only an occasional fish that had been exposed to 10 mg l^{-1} of 2,4-D appeared to have a slightly increased capillary network within the brain; at subsequent sampling periods no abnormalities were observed.

b) Kidney Pathology

The pathology associated with the kidney is unclear. Lesions were noted in the kidneys of treated fish from the *in situ* bioassays but not in the kidneys of fish from the laboratory bioassays. It is possible that the low dissolved oxygen levels within the treatment cages may have contributed to the kidney pathology.

VI. CONCLUSIONS

Juvenile salmonids may be adversely affected following exposure to BEE 2,4-D, during aquatic weed control programs.

Pathological changes were observed in the kidneys of all rainbow trout and in the livers of a few rainbow trout that were held in cages within areas treated with 22.4 kg ha^{-1} a.e. BEE 2,4-D for Eurasian water milfoil control. Juvenile rainbow trout exposed to BEE 2,4-D in the laboratory, at concentrations which generally occur as a result of aquatic weed control programs (0.37 to 1.43 mg l^{-1}), had distinct pathological changes in their livers but not in their kidneys.

The histological changes documented in this study may represent a developing pathological condition and further studies should be carried out in order to assess the effect of repeated exposures of fish to 2,4-D, before large scale use of 2,4-D for aquatic weed control is undertaken in British Columbia.

VII. ACKNOWLEDGEMENTS

The authors wish to thank the many individuals who have contributed to this study, in particular the staff of the Fisheries and Environment Bioassay Laboratory in North Vancouver, W.R. Bennett, Resource Services Branch, West Vancouver, and the staff of the British Columbia Water Investigations Branch and Fish and Wildlife Branch.

Dr. I.K. Birtwell and M.D. Nassichuk reviewed the original drafts.

VIII. REFERENCES

Cope, O.B., E.M. Wood and G.H. Wallen, 1970. Some chronic effects of 2,4-D on the bluegill (*Lepomis macrochirus*). Trans. Am. Fish Soc. 99(1): 1-12.

Culling, C.A.F., 1957. Handbook of histopathological techniques. Butterworth and Company Ltd., London, England. 446 p.

Davis, J.C., 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32: 2295-2332.

Hendricks, J.D., L.J. Hunter and J.H. Wales, 1976. Postmortem bile damage to rainbow trout (*Salmo gairdneri*) livers. J. Fish. Res. Board Can. 33: 2613-2616.

McKee, J.E. and H.W. Wolf, 1963. Water quality criteria. Resour. Agric. Calif. State Water Quality Control Board, Publ. 3-A, 2nd ed., 548 pp.

Meehan, W.R., L.A. Norris, and H.S. Sears, 1974. Toxicity of various formulations of 2,4-D to salmonids in southeast Alaska. J. Fish. Res. Board Can. 31: 430-485.

Smith, G.E. and B.G. Isom, 1967. Investigation of effects of large-scale applications of 2,4-D on aquatic fauna and water quality. Pest. Mont. J.: 1(3): 16-21.

Water Investigations Branch, 1976. A field experiment with granular 2,4-D for control of Eurasian water-milfoil, 1976. 105 pp.