THE EFFECTS OF DINOSEB AND ENDOSULFAN FROM AGRICULTURAL DRAINAGE ON THE BIOTA IN THE NICOMEKL RIVER WATERSHED VOLUME I

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Environmental Conservation
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## EXECUTIVE SUMMARY

Environment Canada is conducting an ongoing study determining the effects of pesticides on aquatic biota in the Fraser River Estuary Management Program (FREMP) and adjacent areas. This report examines the effects of dinoseb and endosulfan on the aquatic biota of the Nicomekl River. Recommendations for future pesticide monitoring projects in the FREMP and adjacent areas are also provided.

This study integrated chemical analyses, laboratory bioassays, and field monitoring. Two study ditches (Burrows, Logging) and four river stations were selected after a preliminary survey. Concentrations of endosulfan and dinoseb, plus numerous other water quality parameters, were measured monthly from November, 1989 to July, 1990, and again in November, 1990 in the ditch and river water and sediments. A battery of laboratory tests assessing the effects of the ditch waters was conducted from November, 1989 to April, 1990. Artificial substrates were used throughout the study to assess effects on macroinvertebrates. An in situ test, using rainbow trout embryos/alevins, was conducted in November, 1990.

Concentrations of dinoseb and endosulfan in the ditches and river exceeded CCREM (1987) guidelines only in February - March, after heavy rains. Concentrations in the river stations only slightly exceeded guideline levels, but concentrations in the ditches reached levels. which were above those known to have effects in previous laboratory tests. Concentrations of many metals, especially aluminum, in the ditches and river routinely exceeded B.C. MOE (Ministry of Environment) Water Quality Criteria. The ditches had high nutrient ( $\mathrm{P}, \mathrm{N}$, organic C ) levels, and nutrient concentrations in the river tended to increase from upstream to downstream:

No negative effects were observed in laboratory bioassays despite the high concentrations of the target pesticides, at least in February - March, and of other contaminants. Plants (algae, duckweed) responded positively to the nutrient-rich ditch waters, showing increased growth and reproduction. Ceriodaphnia also produced more young in the ditch waters, which may have contained high numbers of bacteria, than in standard control water ( $20 \%$ Perrier water). The control waters for these bioassays are unsuitable for comparison with nutrient-rich waters. Therefore, we also compared the effects of the ditch waters with those of the receiving waters (taken from a station upstream of the ditch outleis). There were no significant differences, except for a slight increase in the total number of duckweed fronds produced in the ditch waters relative to the river water.

In general, algae and invertebrates are not sensitive to the target pesticides, and show an obvious response to nutrient enrichment. The most sensitive organisms are fish, usually at early life stages. Therefore, we also examined effects on growth, development and yolk conversion efficiency of rainbow trout alevins. The alevins performed better in ditch and river waters than in dechlorinated Vancouver tap water. Our subsequent studies have demonstrated that this is an effect of water hardness. There were no significant differences in alevin response between ditch and river waters, even in February - March when endosulfan and dinoseb concentrations were high. We attribute the absence of effects to: sorption or complexation associated with high organic carbon and iron levels, or pesticide loss during static-renewal bioassays. Similar interactions, such as between metals and hardness, may also have limited the effects of other contaminants; these influences are standard problems in this type of testing.

Rainbow trout alevins incubated in ditches in November, 1990, had lower survival, growth and yolk conversion efficiency than those incubated in the river. No differences were observed between ditch and river waters when the alevins from the field were reared for 10 d in laboratory water. Low dissolved oxygen was the most
probable cause of the poor performance in the ditches; dinoseb and endosulfan concentrations were less than detection limits at the time.

The macroinvertebrate community colonizing artificial substrates was dominated by Oligochaeta and Chironomidae. Lesser taxa included Ephemeroptera, Plecoptera, and several marine crustaceans. Total abundance was greater in spring and summer than in fall and winter, and abundance increased downstream. The abundance of Ephemeroptera and Plecoptera (EP taxa, both absolute and relative to Chironomidae) decreased downstream. These trends were interpreted as an indication of impact from physical and chemical stressors. A downstream increase in abundance (especially of the tolerant Oligochaeta and Chironomidae) is usually indicative of enrichment, and a downstream decrease in the abundance of the intolerant EP taxa is usually indicative of physical or chemical stress. There was no evidence that this downstream change in the macroinvertebrate communities was greatest when pesticide levels were highest (February - March). As integrators, the macroinvertebrates probably reflect the overall impacts on the Nicomekl River from multiple stresses.

The analyses and tests described could only detect chronic (greater than a few days) exposure to, and effects of the target pesticides. Acute effects may occur immediately after spraying, through aerial drift of the pesticides into ditches or the Nicomekl River. To address this issue, a chemical and toxicologiocal assessment of the ditch and river waters immediately after spraying was planned as part of the project. The elevated pesticide levels measured in February - March, 1990, suggested that the target pesticides were being used in 1989, although not neccessarily in the immediate study area. The spring and summer of 1990 provided the only opportunity to measure exposure and effects immediately after spraying, but the farmers in the immediate study area stated that they no longer used the target pesticides. Thus, it was not possible to conduct tests at the time of spraying, and the possibility that acute effects from the target or other pesticides occur immediately after spraying cannot be discounted. In retrospect, the tests should have been conducted during spraying, eyen if the pesticides used were not the target pesticides.

The results of this study led to the following conclusions and recommendations:

## 1. Dinoseb and Endosulfan

Use of the target pesticides, especially dinoseb, should continue to decline. The major concerns are the effects of accumulated endosulfan in sediments, and in the marine environment of Boundary Bay.

## 2. Status of the Nicomekl River

The Nicomekl River is subject to multiple stressors, and remediation will be difficult. The primary concerns are low summer oxygen levels, nutrients, metals, and channelization/drainage control. The effects of runoff from agricultural fields could be decreased by reducing, if possible, fertilizer and manure application and including education about environmentally sound farming practices with Agriculture Canada extension services.

## 3. Pesticide Monitoring in Other Areas

Monitoring studies should split effort equally between chemical analyses, laboratory bioassays and field monitoring. The focus should shift from chemical concentrations in ditches to biota in receiving rivers. Chemical QA/QC programs and interlaboratory studies should focus on estimating and reducing temporal and spatial variation in actual field concentrations, as well as in-laboratory and instrument error. Guidelines should be developed using a consistent and logical procedure (a regression approach is suggested), and compared with concentrations causing effects in the field. Effects of pesticides will be difficult to distinguish from those of other factors, as found in this study. This problem may be partially solved by determining NOECs (No Observed Effect Concentrations)
using receiving water samples spiked with the target pesticides, and by comparing effects in the field between times of high and low pesticide concentrations.

Acute effects from aerial drift can be assessed by measuring chemical concentrations and conducting in situ toxicity or other biological tests during and immediately after spraying. To some degree, the -Nicomekl River may be protected from acute effects during and after spraying because the pesticides may only enter the river directly from aerial drift. Ditches, especially those with flap, gates, are unlikely to be discharging during spring and summer when spraying occurs. In other areas, there may be an important additional load from either uncontrolled ditch or tributary discharge, or from direct run-off into rivers and streams.
4. Assessment of Integrated Pesticide Management (IPM)

Both the federal and provincial governments are committed to implementing IPM practices, and have begun to do so as of 1993. Implementation of IPM should reduce the amount of pesticide applied, the use of pesticides during times when effects on non-target organisms are greatest, and the use of the most toxic pesticides. The success of IPM practices in reducing effects on local biota could be assessed in monitoring programs by comparing areas where the practices are applied with areas where they are not applied, or by comparing the areas before and after implementation of IPM.

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### 1.0 INTRODUCTION

Following the introduction of DDT in 1942, an increasing number of synthetic organic chemicals have become available and widely used in pesticide formulations. Although the advantages of these products, both in preserving valuable crops from attack by pests and destroying vectors of communicable disease, attracted much attention initially, the environmental hazards arising from large-scale use of pesticides have caused increasing concern to legislators in Canada (MacKenzie et al., 1975). The Water Quality Branch, Inland Waters Directorate is in the process of developing and implementing an approach to determine the effects of selected pesticides on aquatic biota, and applying the approach in the Fraser River Estuary Management Program (FREMP) study area.

A multi-year project assessing the potential toxic effects of agricultural crop pesticides on aquatic biota within the Lower Fraser Valley, B.C., began in late 1988. Phase I (McLeay, 1989) developed a site-specific approach for predicting and monitoring the potential toxic effects on aquatic biota of pesticides entering a receivingwater body by surface water runoff from commercial farms. Based on this appraisal, dinoseb (herbicide) and endosulfan (insecticide) were selected for investigation of their presence and potential toxicity to aquatic life in the study area. Prospective study sites were visited and a decision made to confine the project to a portion of the Nicomekl River in Lower Mainland, B.C., and adjacent drainage/irrigation ditches (Figures 1 and 2).

Based on the foregoing, an approach for future site-specific toxicity assessments of the study area was presented. The present study implemented the recommended approach with revisions as described.

### 1.1. Problem Statement

In spite of agricultural success using pesticides, there are environmental concerns regarding the presence of these compounds in the aquatic environment. Run-off can transport pesticides through surface and groundwater from the point of application. In the study area, irrigation and/or drainage ditches discharge seasonal runoff from adjoining commercial farms directly to the Nicomekl River. In the summer (when the ditches are used for irrigation) pesticides in the ditches may be returned to fields. The two most important concerns regarding pesticides are short and long term effects. Pesticide drift or runoff shortly after application can cause direct (i.e., short-term) damage to fish or other organisms. The long-term persistence of pesticides in the aquatic environment can impact adversely on the productivity of fish and/or other aquatic organisms (e.g., algae, invertebrates) which ultimately support fish resources. Definitive information is not available regarding the effects of exposure to the target pesticides on native aquatic plants and animals.

For this reason, a seasonal study of concentrations and biological effects of dinoseb and endosulfan was undertaken to assess possible impacts on biota in the Nicomekl River and to form the basis for recommendations to mitigate any adverse effects. Since each ditch outlet can be considered a point source
of pollution, the ditch waters were treated as effluents, with their effects on the receiving waters of the Nicomekl River examined.

### 1.2 Objectives

The principal objective of this study was to examine the effects of endosulfan and dinoseb from agricultural runoff in drainage (irrigation) ditches on biota in the Nicomekl River watershed. Specific objectives were:

1. Interview federal and provincial agencies (and, if necessary, users responsible for the application of pesticides to crops in the Nicomekl River watershed) to determine where and when dinoseb and endosulfan are used as the predominant pesticides;
2. Select three agricultural drainage (irrigation) ditches for initial evaluation of toxicity and for measurement of dinoseb and endosulfan concentrations in both sediment and water,
3. Select one ditch for further study utilizing laboratory bioassays as well as in situ evaluation of effects of endosulfan and dinoseb on fish, invertebrates, and algae;
4. Recommend practical mitigative procedures (if warranted) to reduce the impact of toxicity from drainage ditches to the Nicomekl River.

Objective 3 was expanded so that two ditches, and their effects on the receiving waters of the Nicomekl River, were studied. Four stations on the river were established so that increases in contaminant concentrations and effects downstream of ditch outlets could be monitored. Objective 4 was expanded to include the provision of recommendations for monitoring pesticide effects in other areas. An interim report (Paine, 1990) provided the results of the study, covering the period from November, 1989 to March, 1990. The present report provides the complete study results, from November, 1989 to November, 1990. Raw data have been provided separately in an accompanying report (Volume II).

The study was also originally intended to examine exposure and effects from aerial drift immediately after spraying of the target pesticides. However, local farmers stated that they were not using the target pesticides in the spring and summer of 1990, even though there was evidence that the compounds had been used in 1989. Therefore, the studies during and after spraying could not be conducted as planned.

## 1.3 . Study Area

### 1.3.1 Nicomekl River Watershed

The Nicomekl River watershed is shown in Figure 1. The river is approximately 34 km long, originating east of Langley, and emptying into Boundary Bay. The river becomes progressively more turbid downstream, and the substrate shifts from gravel/rubble to a very fine silt or mud. The shift to the mud substrate occurs just before our study area (inset in Figure 1; Figure 2). Upstream of the study area, the river receives discharges from stormwater runoff, some domestic sewage, several industries, a landfill, and several feedlots (Swain and Holms, 1988a; Swain and Holms, 1988b). In the study area, the river receives runoff from vegetable croplands via drainage ditches. Swain and Holms (1988b) and McLeay (1989) identified the following parameters of concern: metals, phosphorous, ammonia, nitrite, faecal coliforms, and low dissolvéd oxygen. These authors concluded that the largest impact on the water quality of the Nicomekl River came from diffuse agricultural operations. These impacts were largely due to nutrient input, but the authors also expressed concern about the impacts of pesticides from agricultural drainage.

The Nicomekl and adjacent watersheds are used by salmonids, including Pacific salmon (Oncorhynchus species), cutthroat trout ( $O$. clarki), and steelhead ( $O$. mykiss) (Figure 1). Spawning is largely confined to the upper reaches or tributaries. All of these salmonids are anadromous, with the young migrating to sea a few weeks to several years after hatching. Escapement of coho salmon ( $O$. kisutch) in the Nicomekl River ranges from <100-7500, but is usually 1000-2000 (Hancock and Marshall, 1985). Spawning occurs through November and December, with a peak in late November. The embryós incubate over winter, and hatch as alevins in early spring. Some young migrate downstream as alevins immediately after emerging from the gravel; others migrate as juveniles a year later. Thus, the major fisheries concern in the study area is impacts on rearing and downstream migration of coho alevins or juveniles, which occurs in spring-summer (April to August). Anadromous cutthroat spawn in the early spring rather than fall, but the young would be migrating downstream through the study area at roughly the same time as coho.

### 1.3.2 Study Reach

The study reach is shown in detail in Figure 2. The river is slow, and $5-20 \mathrm{~m}$ wide in this area. Midstream depth varies considerably, reaching a maximum of 5 m during high water, and a minimum of $<1 \mathrm{~m}$ during the summer. The substrate is fine mud, except at the extreme upstream end. The only known salmonid spawning grounds in the study area are in what Swain and Holms (1988b) referred to as the "Old Logging Ditch" (Figure 1). This is not the same as the ditch McLeay (1989) and ourselves refer to as the "Logging Ditch" (Figure 2). Instead it is a smaller ditch entering the river opposite Erickson ditch. Apparently, cutthroat trout have been observed spawning in this ditch in the past (B. Clark, B.C. M.O.E., personal communication) although no , surveys have'been made recently. Many of the ditches in the area follow old temporary or permanent stream
beds, it is not surprising that trout would use the ditches as spawning areas. However, it is not expected that the embryos survive, as the ditch substrate is also mud.

There are numerous ditches in the study area; we have only shown the three we studied. Burrows and Logging ditches are the two largest, but large ditches also run parallel to north-south streets. There are irrigation as well as drainage pumps at the outlets of Burrows and Logging ditches. The pumphouses and ditch system are maintained by the Surrey Diking District, whose commissioners are local farmers. During periods of heavy rain, usually September to April or May, the drainage pumps move water from the ditches into the river. Flap valves at the end of outlet pipes prevent backflow from the river. During the summer, irrigation pumps move water from the river into the ditches to irrigate fields.

The major crops in the study area are root vegetables - potatoes, onions, and carrots. Insecticide application may be more or less continuous through the spring and summer. Herbicides are used in the spring prior to emergence of crop plants to control weeds. They are often applied again in August for pre-harvest top kill. Generally, insecticides are applied in the spring (April-May), with possible repeated applications through the spring and summer. Previous studies (e.g., McLeay, 1989; Moody, 1989; Wan, 1989) had identified dinoseb and endosulfan as pesticides used extensively, althoughi the local farmers indicated to us that they had recently discontinued dinoseb use, and did not plan to use endosulfan in 1990.

### 1.4 Properties and Effects of Dinoseb and Endosulfan

The following review of the two target pesticides is taken from CCREM (1987), McLeay (1989), MacDonald et al. (1990); these publications should be consulted for more details.

### 1.4.1 Dinoseb

Dinoseb (2-sec-butyl-4,6-dinitrophenol) has been registered for use since 1947. In 1990, use was restricted to raspberry, bean and pea crops (Agriculture Canada, 1990), and use will be restricted to beans and peas after the 1993 growing season. Registration is expected to be withdrawn eventually. The U.S. EPA suspended the registration of dinoseb products in 1986. The primary concerns of both Canadian and U.S. agencies was the risk of teratogenic effects, cataract formation, and effects on male reproduction (i.e., direct risk to users; indirect risk to people in agricultural areas).

The major use of dinoseb was as a top-kill herbicide for potatoes, although it has also been used as an insecticide and miticide. Dinoseb tends to be a transitory compound, as it is quickly removed from soils and does not persist from one season to the next. The compound may be present as the parent compound, various salts, or a phenol. Dinoseb acetate is also a herbicide. The solubility of dinoseb in water at neutral $\mathbf{p H}$ is 52 $\mathrm{mg} / \mathrm{L}$, which is high for a pesticide, and certainly higher than concentrations producing effects. The propensity
of dinoseb to leach into water from soils led Agriculture Canada to rate it high on their list of potential contaminants.

Dinoseb is lethal to salmonids at concentrations ranging from $32-1400 \mu \mathrm{~g} / \mathrm{L}$, with effects decreasing with increasing pH . Long-term or chronic mortality can occur at concentrations as low as $12 \mu \mathrm{~g} / \mathrm{L}$. The lowest concentration known to produce sublethal effects on fish is $\leq 0.5 \mu \mathrm{~g} / \mathrm{L}$ or $500 \mathrm{ng} / \mathrm{L}$ (Woodward 1976; this was the lowest concentration tested so the No Observed Effects Concentration or NOEC could not be determined). This concentration caused reduced growth in young lake trout (Salvelinus namaycush) which had been exposed continuously for 81 d from the eyed egg stage. Salmonids are the most sensitive fish, as dinoseb concentrations causing lethal or sublethal effects in other species are higher than those given above.

Invertebrates and plants are less sensitive to dinoseb than are fish, although effects on the earty life stages of invertebrates have not been examined extensively. LCsos for invertebrates range from 100 to $2800 \mu \mathrm{~g} / \mathrm{L}$; sublethal effects may occur at lower concentrations. Effects on algae (inhibition of photosynthesis) have been observed at concentrations $>500 \mu \mathrm{~g} / \mathrm{L}$. MacDonald et al. (1990) found only one study of dinoseb effects on aquatic plants (O'Brien and Prendeville, 1979). Dinoseb concentrations of $24 \mu \mathrm{~g} / \mathrm{L}$ affected membrane permeability, so that electrolytes leaked into the surrounding medium. The extent of leakage was not quantified, and its significance is obscure.

Bioaccumulation of dinoseb does not appear to be a major concern (MacDonald et al., 1990). Bioconcentration factors (BCFs) are $<100$, and the compound is rapidly eliminated. Thus, there is a low potential for significant accumulation from food and subsequent food chain biomagnification. MacDonald et al. (1990) concluded that direct lethal and sublethal effects, primarily on salmonid fishes, were the fundamental concern.

Based on their review, MacDonald et al. (1990) recommended $0.05 \mu \mathrm{~g} / \mathrm{L}(50 \mathrm{ng} / \mathrm{L})$ as an appropriate guideline for the protection of aquatic life. This was derived by dividing the lowest concentration affecting aquatic life, $500 \mathrm{ng} / \mathrm{L}$ from Woodward (1976); by a safety factor of 10.

### 1.4.2 Endosulfan

Endosulfan is an insecticide, primarily used to control aphids, mites, and insects on vegetable crops. There are 11 products containing endosulfan registered for use in Canada; Thiodan is probably the most common brand name. . Endosulfan is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide. Technical grade endosulfan consists of two isomers, $\alpha$ - and $\beta$-endosulfan, in the usual ratio of 7:3. Endosulfan sulfate is formed by oxidation of endosulfan under aerobic conditions. $\alpha$ endosulfan appears to decompose in soils much faster than does $\beta$-endosulfan; neither is very soluble in water (solubilities are 0.15 and $0.06 \mathrm{mg} / \mathrm{L}$ at neutral pH , two to three orders of magnitude lower than those for dinoseb). Endosulfan sulfate can persist in soils for several years. Thus, endosulfan does not enter aquatic
systems as quickly as dinoseb does, except through aerial drift immediately after spraying, but persists longer in soils and sediments.

The effects of endosulfan have been tested on a broad range of organisms. The mean for the most sensitive species, rainbow trout juveniles, is $0.34 \mu \mathrm{~g} / \mathrm{L}(340 \mathrm{ng} / \mathrm{L})$. Acute effects on invertebrates occur at concentrations ranging from 2.3 to $740 \mu \mathrm{~g} / \mathrm{L}$; algae are generally not affected by concentrations $<1000 \mu \mathrm{~g} / \mathrm{L}$. The concentrations given refer to technical grade formulations. The toxicity of $\alpha$-endosulfan is $16-33$ times that of $\beta$-endosulfan, and the toxicities of the formulations are intermediate. The lowest LC50 values have been recorded in flow-through tests, as considerable loss from solution may occur in static tests. Toxicity does not appear to be affected by pH , but increases with temperature.

Chronic effects on mortality of fathead minnows (Pimephales promelas) occur at concentrations of $280 \mathrm{ng} / \mathrm{L}$ Concentrations as low as $50 \mathrm{ng} / \mathrm{L}$ can produce biological effects such as physiological or histopathological alterations. Invertebrates are less sensitive, with sublethal effects occurring at concentrations an order of magnitude higher than those affecting fish. Bioaccumulation appears to be a minor concern as BCFs range from $<10$ to 1000 , and endosulfan is rapidly eliminated, at least by fish.

The current CCREM (1987) guideline for protection of aquatic life is $20 \mathrm{ng} / \mathrm{L}$ endosulfan. The guideline was derived by applying an application factor of 0.05 to the average LC50 ( $340 \mathrm{ng} / \mathrm{L}$ ) of the most sensitive species, rainbow trout. U.S. EPA (1987) water quality criteria for freshwater biota are $220 \mathrm{ng} / \mathrm{L}$ for acute effects and $56 \mathrm{ng} / \mathrm{L}$ for chronic effects. The EPA criteria are simply the lowest concentrations that have been observed to produce acute or chronic effects. Note the difference between the derivation of Canadian guidelines for endosulfan and dinoseb. If the endosulfan guideline were derived in the same way as that for dinoseb, it would be the lowest concentration causing chronic effects ( $56 \mathrm{ng} / \mathrm{L}$ ) divided by a safety factor of 10 , or $5 \mathrm{ng} / \mathrm{L}$. If the guideline for dinoseb were calculated in the same way as that for endosulfan, it would be $40 \mu \mathrm{~g} / \mathrm{L}$ (approximate average LC50 at low to neutral pH for juvenike lake trout) multiplied by an application factor of 0.05 , or 2 $\mu \mathrm{g} / \mathrm{L}$ ( $2000 \mathrm{ng} / \mathrm{L}$, or 4 times the concentration known to have chronic effects). These discrepancies are noted not as a criticism of the derivation of the guidelines, but to guide the comparison of guidelines with observed concentrations of the target compounds. Exceedance of the guideline for endosulfan is likely to have greater consequences than exceedance of the guideline for dinoseb.

## 2.0 <br> METHODS AND MATERIALS

2.1 : Study Design

The study was initially intended to consist of three phases: a preliminary survey; an extensive round of toxicity testing; and subsequent seasonal monitoring. For the preliminary phase, water and sediment samples were collected in November, 1989, from Erickson, Burrows, and Logging ditches, and Stations 1-4 in the Nicomekl River (see Figure 2 for locations). These samples were analyzied for pesticides, and used for several screening bioassays. After the preliminary' phase, the second and third phases were conducted more or less simultaneously, from December, 1989, through to July, 1990, and resumed again in November, 1990. These second and third phases consisted of chemical analyses of water and sediment samples, laboratory bioassays, and field biomonitoring (in situ rainbow trout alevin bioassay, artificial substrates).

Testing and monitoring during the second and third phases were restricted to Burrows and Logging ditches, and the four river stations; Erickson ditch was under construction for much of the study period...The basic approach was to determine the effects of the ditch waters, in laboratory bioassays or through biomonitoring at the river stations, and relate these effects to concentrations of the target pesticides and other chemicals present in the ditches or river.

### 2.2 Water and Sediment Samples

### 2.2.1 : Routine Sampling and Analyses

Water and sediment samples were collected from the river by boat, and from the ditches from pumphouse platforms. Water samples from the Nicomekl River and the ditches were collected by holding a 4 L dark glass bottle 5 cm below the water surface and allowing it to fill. The bottles were stored overnight at $4^{\circ} \mathrm{C}$ in the dark, then delivered to the analysts. Samples for laboratory bioassays were usually collected at the same time. Sediment samples were collected with a Petite Ponar grab. These samples were composites of at least three subsamples taken along a transect across the river or ditch. Individual subsamples within a transect were transferred from the Ponar to a stainless steel dish. After all subsamples had been taken, the contents of the dish were thoroughly mixed, and a 500 mL composite spooned into a brown glass jar. These composite samples were also stored overnight at $4^{\circ} \mathrm{C}$ and then delivered to the analyst.

Analyses of pesticides was performed by Zenon Environmental of Burnaby, B.C. The focus was on dinoseb and endosulfan, but other pesticides were noted and their concentrations recorded. The protocol for these analyses is summarized in Figures 3 and 4. Because of problems with the Florisil columns, B-endosulfan and endosulfan sulfate concentrations were not measured prior to February, 1990. The results of a QAQC program evaluating the methodology are provided in Appendix I. Chromatographs have been provided separately to the Scientific Authority, Mr. Fred Mah of Environment Canada.

Sediment and water samples were also collected for analysis of physical parameters (suspended solids, turbidity, colour) and inorganic compounds (ions, nutrients, metals) by Environment Canada laboratories. These samples were collected at the same time as samples for pesticide analyses. Basic water quality data such as dissolved oxygen and conductivity were usually recorded in the field, and were routinely recorded as part of laboratory bioassays.

Water for the bioassays was collected in a stainless steel bucket, and stored in 57 L glass jugs in the dark at $4^{\circ} \mathrm{C}$. The stored test water was usually replaced with a fresh sample within a week, and sometimes more frequently. Bioassays were usually initiated with water that had been collected the previous day. Concentrations of the target pesticides were measured in at least one, and often two or more, samples of each test water. The results from all three components could easily be integrated because samples for pesticide analysis, inorganics analysis, and bioassay testing were often taken simultaneously, and from the same sites.

One minor difference between samples used for chemical analyses and those used for bioassays should be noted. All chemical analyses were conducted on samples collected at River Station 1, but bioassays were conducted using water collected from a site further upstream, at the 184th Street bridge ("Upstream"; see Figure 2). The Upstream samples were used as an on-site control; and as a diluent. Since large volumes were often required on a frequent basis, it was much more convenient to collect samples from the bridge, rather than from Station 1, as a boat was not required. However, it was not practical to conduct field bioassays near the bridge, because of the increased probability of vandalism. Station 1 was more suitable for field bioassays, as it was not visible from the bridge.

### 2.2.2 Oil Hydrocarbons

On June 7, 1990, an oil spill from an industry upstream of the study area occurred on the Nicomekl River. An oil slick was visible in the study area by the morning of June 8. Several water and sediment samples were collected during June and July for measurement of oil hydrocarbons. Water samples were collected from the Upstream site, and the two ditches, on June 8. Water samples were also collected July 3 from the ditches, and from all four river sites. Oil and grease concentrations in these samples were measured in accordance with Standard Methods (APHA, 1985) by ASL Analytical Service Laboratories of Vancouver, B.C. Oil and grease concentrations were also measured in sediment samples from the ditches, and from Stations 1 and 4 on July 2. These analyses were conducted by Zenon; the samples were originally collected for pesticide analysis, but the analysts suggested that the oil would interfere with the analysis.

## 2.3

Laboratory Bioassays

Protocols for laboratory bioassays are provided below. The bioassays can conveniently be divided into screening tests (rainbow trout pass/fail, residual oxygen bioassay) designed to provide a rapid assessment of
lethal effects, and sublethal tests (rainbow trout alevin, Ceriodaphnia; Selenastrum, and duckweed) measuring effects on growth and reproduction.

### 2.3.1 <br> Screening Bioassays

### 2.3.1.1 Rainbow Trout Pass/Fail

This bioassay assesses the acute lethal effects of undiluted test waters on rainbow trout júveniles. . Replicates were 40 L aquaria and each contained 20 L of test water and 10 trout juveniles. Mortality was monitored daily, and dead trout removed. For regulatory purposes, an effluent passes if mortality is $<50 \%$ over 4 d . Control mortality should not exceed $10 \%$. Following McLeay (1989), mortality $>10 \%$ in the test waters was considered an indication of lethal effects. The procedure for the test is described in detail in Environment Canada (1980).

The test was used to examine the effects of waters in the Erickson, Burrows, and Logging ditches during the preliminary survey in November, 1989. Two replicates were used for each of the ditch waters and the laboratory control (dechlorinated city tap water).

### 2.3.1.2 Residual Oxygen Bioassay

The residual oxygen bioassay measures the effect of a toxicant on the ability of a fish to withstand oxygen depletion. The test is actually a challenge test, rather than an acute lethal bioassay. However, it is useful as a rapid means of assessing the relative effects of different toxicants. The test procedure is described in detail in Vigers and Maynard (1977). Each replicate was a 300 mL BOD bottle, filled completely with test water plus three rainbow trout juveniles, then sealed. Test waters were aerated beforehand to achieve $100 \%$ oxygen saturation. The bottles were then monitored until the trout died from lack of oxygen (usually 4-8 h). The time to death was recorded, and the oxygen remaining in the bottles measured. Toxicants usually decrease the ability of the fish to withstand low oxygen levels, leading to a higher oxygen content in the bottles at the time of $100 \%$ mortality. Effects on time to death are more variable, as the toxicant may actually increase time to death by reducing metabolic activity and oxygen uptake rate.

Residual oxygen bioassays were conducted during November, 1989, on water and sediment elutriates from Erickson, Burrows, and Logging ditches. Elutriates were prepared by placing 200 g of sediment in 4 L of laboratory water in a 4 L jar. The sealed jar was then placed on a belt-driven mixer which rotated the jar for 24 h . The fluid (=elutriate) was then decanted from the jar and placed in test bottles. There were two BOD bottles containing fish for each water and elutriate sample, plus a third bottle without fish. The fishless bottles provided an indication of the BOD and COD of the samples. Controls (laboratory water) were also included.

Tests (LCSOS) with reference toxicants indicated that trout alevins were more sensitive than juveniles. Therefore, alevins (8 per bottle) were used in subsequent residual oxygen bioassays. The first such test was conducted in December, 1989, using undiluted water from Burrows and Logging ditches, the Upstream site, and laboratory water. There were three replicates (one fishless) for each of these test waters. Unfortunately, the test results were not valid because the control fish died within hours at a high oxygen level ( $8 \mathrm{mg} /$ ). We have no explanation for this; chlorine analysis indicated that the laboratory water was being effectively dechlorinated. The next test was conducted March, 1990, using test waters from the same sites, but with four replicates (one fishless) of each.

### 2.3.2 Sublethal Effects Bioassays

### 2.3.2.1 Experimental Design and Statistical Analysis

In order to compare the results from the various tests for sublethal effects, the same experimental design and analysis was used for all tests. This approach differed from the approach generally required by regulatory agencies (e.g., U.S. EPA), but is statistically more sound, and directly addressed hypotheses relevant to our study objectives. Any minor deviations from the standard approach are noted in the individual test protocols.

The same set of treatments was used for the rainbow trout alevin, Ceriodaphnia, Selenastrum, and duckweed bioassays. These treatments were:

- laboratory control (varies with test; see protocols)
- water from Upstream site
- $10,30,100 \%$ Burrows ditch water
- $10,30,100 \%$ Logging ditch water.

In all tests initiated in 1989, lab control water was used to dilute the ditch waters, because the chemical and toxic properties of the Upstream water were not known. Subsequently, the Upstream water was used as the diluent to more closely simulate the dilution of ditch waters in the Nicomekl River.

Analyses of variance (ANOVAs) or covariance (ANCOVA) and orthogonal contrasts were used to analyze all data. Most agencies (e.g, U.S. EPA) use multiple comparisons (e.g., Dunnett's Test) to compare individual treatment means with control means. There were other comparisons which were of interest to us and these comparisons can be made using contrasts. The variation among treatments in any ANOVA can be divided into several independent comparisons or contrasts, following procedures described in Sokal and Rohlf (1980).

Each contrast can be considered a mini-ANOVA, testing a specific hypothesis. Contrasts are discussed in more detail in Appendix II. The following contrasts were used:

- laboratory control versus all other treatments - does the control mean differ from the grand mean for all river and ditch waters combined?
- Upstream (receiving) water versus ditch waters - does the Upstream mean differ from the grand mean for all ditch waters combined?
- between ditches - do the grand means for the two ditches differ?

The first contrast simply tests the differences between laboratory and natural (river, ditch) waters. It indicated more about the appropriateness of the various required control waters than it did about any effects likely to occur in the study area. The second contrast was the key test of whether the ditch waters would have a negative impact on the biota in the river, because the Upstream water was the experimental and natural diluent. The third contrast was useful for testing whether any differences in the concentrations of chemicals between ditches led to differences in effects. There are other contrasts that could have been used. For example, it would be possible to test the significance of differences in concentrations of the ditch waters, or to test for a log-linear or quadratic dose-response relationship. However, these other contrasts were of little interest given the results observed.

### 2.3.2.2 Rainbow Trout Alevin Bloassay

This bioassay measures the lethal and sublethal effects of toxicants on rainbow trout embryos and alevins. We are still refining the procedures and analyses, and attempting to adapt the test to field conditions. The test is based on similar studies conducted by Hodson and Blunt $(1981,1986)$ on rainbow trout. Tests were conducted December, 1989 - January, 1990; February - March, 1990; and November, 1990.

Eyed embryos were obtained from a local hatchery, and transported to the EVS laboratory. They were transferred to a 40 L aquarium containing aerated dechlorinated tap water, and acclimated in darkness to $15 \pm 3^{\circ} \mathrm{C}$ for 48 h . The embryos were then transferred to the test containers, 2 L glass beakers with 1 L of test solution in each. Twenty embryos were placed in each beaker, with three beakers (replicates) per test solution. The embryos were held in $\mathbf{9 0} \mathrm{mm}$ diameter glass petri dishes attached with silicone sealant to aylon mesh dip nets. These incubation baskets could easily be removed from the beakers when solutions were changed (three times weekly). Test containers were gently aerated, and held in darkness-at $15^{\circ} \mathrm{C}$.

Dead alevins and embryos were counted and removed daily. Embryos were considered dead when they did not move, and when the eggs were opaque. Alevins were considered dead when they did not move, lacked a
heart beat, and were rigid and opaque. Dissolved oxygen, pH , and temperature of the test solutions were monitored three times weekly, just before solutions were changed.

Tests were conducted until most alevins reached the swim-up stage (approximately 20 d from the start of the experiment). The objective was to terminate the test before the alevins used all their yolk, but to allow sufficient time for any differences in mortality and development to manifest themselves. At the end of the experiment, total (body plus yolk) and body (yolk removed) wet weight of surviving alevins were measured. All surviving alevins within a replicate were pooled before weighing, then the mean weights calculated by dividing by the number weighed.

Acute lethality tests using the reference toxicant sodium dodecyl sulfate (SDS) were also conducted on alevins, $\mathbf{2 0 ~ d}$ after hatch. These tests followed the same protocol as the longer tests, except that they lasted for only 96 h and solutions were not changed. One replicate was used for each of seven concentrations ( $0,3.2,5.6$, $10.0,18.0,32.0$, and 56.0 ppm SDS); two of these tests were conducted. Alevin mortality was monitored daily. 96 h LC50s were calculated for comparison with 96 h LC50s for juveniles. Tests on juveniles are conducted routinely because SDS is one of our standard reference toxicants; thus, there was no need to conduct any specifically for this project.

The response variables measured in this bioassay included embryo and alevin mortality, developmental rate, and yolk conversion efficiency. These are all simple yet biologically important measures calculated from the daily counts of dead individuals plus the weights of the alevins.

## Embryo and Alevin Mortality

Embryo mortality was the percentage of embryos that failed to hatch. Alevin mortality was the number of individuals dying after hatching, expressed as a percentage of the individuals that hatched successfully. Both variables required an arcsin square root transformation prior to analyses.

## Developmental Rate

Yolk is converted to body tissue as an embryo or alevin develops. Therefore, body weight relative to yolk weight can be used as a measure of developmental rate. Rigorous statistical analysis of developmental rate is difficult, because yolk conversion efficiency can be affected by toxicants, altering the body weight:yolk weight relationship (see below). However, if yolk weight is significantly lower, and body weight significantly greater, in one treatment relative to controls, it seems reasonable to conclude that the treatment has increased developmental rate.

## Yolk Conversion Efficiency (YCE)

Yolk conversion efficiency is the efficiency with which yolk is converted to alevin body tissue (Hodson and Blunt, 1986), and can be calculated directly by dividing the change in body weight over some interval by the change in yolk weight. Alevins with a greater efficiency will produce more grams of body tissue for each gram of yolk used. Yolk conversion efficiency should not be confused with yolk conversion rate (the same as developmental rate, as defined above). For example, one group of alevins may be converting yolk to body tissue at a faster rate than another, but might have a lower efficiency, producing less body tissue per gram of yolk used.

Statistical analysis of conversion efficiency involves comparing body weights at a fixed yolk weight using analysis of covariance (ANCOVA). We could not calculate YCE directly, as the initial yolk and body weights can be hard to obtain from embryos (the yolks are harder to remove, and the embryos have to be excised from egg envelopes). There should be a negative relationship between body and yolk weight. A sample of several individuals taken at some time should produce such a relationship, provided that some variation in developmental rate exists. That relationship should be similar to the relationship that exists over time for an individual alevin, and the slope of the line should theoretically be equal to the average YCE (there are several statistical reasons why the slope is almost invariably less than YCE calculated directly, although the difference rarely affects conclusions). Alevins that are efficient at converting yolk to body tissue will lie above the line; alevins that are inefficient will lie below the line. An ANCOVA simply tests whether the position of alevins with respect to the overall line differs among treatments.

### 2.3.2.3 Ceriodaphnia 7-day Life Cycle Bioassay

This test measures the effects of toxicants on mortality and reproduction of Ceriodaphnia dubia. Each replicate consisted of a single female placed in 15 mL of test solution in a 50 mL vial. These females were all $<\mathbf{2 4} \mathrm{h}$ old and had not yet produced their first brood of young. Occasionally one of the individuals turned out to be a male, although a healthy population generally produce only females (via parthenogenesis). Males were, of course, not included in the results. Ten replicates were used per treatment. The control water consisted of $\mathbf{2 0 \%}$ Perrier water in deionized water, which ensures a standard control in every laboratory regardless of local water chemistry. In all Ceriodaphnia tests, test waters were filtered through a $50 \mu \mathrm{~m}$ filter to remove any potential predators. Test waters were changed daily, and any young produced were removed and counted. Previously, U.S. EPA protocols called for a 7-day test, which allowed for the production of three broods. New protocols aliow the test to be extended for a few extra days until the controls have produced three broods (U.S. EPA, 1989).

The data analyzed were mortality and the total number of young produced per female. If mortality does not differ significantly among treatments, dead females (usually with 0 young produced) are normally included in the analysis of the number of young produced. We have reservations about this, but followed the established
protocol in order to comply with external standards. Test results are considered valid if $\geq 60 \%$ of the control females produce 15 young each, and if control mortality does not exceed $10 \%$.

Two tests were initiated during 1989. In the first test, no control female produced 15 young, and few experimentals did. In the second test, only five (or $50 \%$ ) control females produced $\geq 15$ young, although many experimentals did. We suspected that our laboratory stock culture was losing its vigour after many generations of asexual reproduction and consequent accumulation of deleterious mutations, and replaced it. A third test was, therefore, conducted during February, 1990, using the new culture. Even then, the test had to be extended to 10 days for $60 \%$ of the control females to produce 15 young, although most experimentals produced double that amơnt.

Transformations of the data were not necessary for the first two tests.' In the third test, there were one or more females producing few young in most treatments. These outliers skewed distributions and led to heterogeneous variances. The data were, therefore, rank-normalized, which is equivalent to conducting a nonparametric test, but enables contrasts to be calculated.

### 2.3.2.4 Selenastrum 4-d Bioassay

This test measures the effects of treatment waters on population growth of the freshwater alga Selenastrum capricomutum. Procedures are described in detail in U.S. EPA (1989). Flasks containing the test waters were inoculated with a standard density algal culture, and nutrients added. The addition of nutrients supposedly ensured that any inhibitory effects of a test water were not due to nutrient deficiency. The flasks were incubated at $24^{\circ} \mathrm{C}$ for 4 days. After 4 d , the densities of algal cells in each flask was estimated by counting the number of cells in a fixed area on a gridded slide. Two counts were made for each flask; if they were not within $10 \%$ of each other further counts were made. The densities were then expressed as number of cells per unit volume (our approach here) or as a percentage increase (=stimulation) or decrease (=inhibition) relative to controls.

Our test was conducted during December 1989 during Phase 2. All test water was filtered through a $1 \mu \mathrm{~m}$ filter to remove other algae and herbivores. Three replicates (flasks) were used for each treatment. Algal densities were log-transformed prior to analysis.

### 2.3.2.5 Duckweed Bloassay

Various versions of this test exist, using a number of different Lemna species. The ASTM is currently attempting to standardize the procedures, and define appropriate guidelines for controls. All protocols are similar in that duckweed fronds are cultured in flasks containing test waters for $4-14 \mathrm{~d}$. The number of fronds
(each plant consists of several fronds), and usually their weight, is then determined. As in the Selenastrum bioassay, nutrients are added to each flask.

Duckweed were collected in April from a highway ditch in Richmond, B.C. The area near the ditch was not developed for agricultural activities, and presumably lacked high levels of contaminants including pesticides. The plants were cultured for approximately one week in laboratory water supplemented with a commercial nutrient solution. Ten plants ( $32-40$ fronds) were then placed in each test container ( $\mathbf{2 0 0} \mathbf{m L}$ flask). Three test containers were used for each test water. One $m \mathrm{~L}$ of the commercial nutrient solution was added to each container to provide an excess of nutrients; 100 mL of test water were used per container. Water quality measurements were made on selected containers throughout the bioassay.

The test ran for 7 days under constant light, with temperature ranging from $21-23^{\circ} \mathrm{C}$. At the end of the experiment, the fronds were counted, dried overnight at $60^{\circ} \mathrm{C}$ and weighed. The response variables were therefore dry weight, and the number of fronds: Fronds were classified visually as green or pale (a potential indicator of chlorophyll deficiency). The initial dry weight was also measured, so that the biomass increase over the 7-d test period could be calculated.

### 2.4 Field 'Bloassays and Monitoring

2.4.1 Artificial Substrates

### 2.4.1.1 Procedures,

Artificial substrates were used to examine effects on macroinvertebrates. Normally, to determine whether the benthic community in the Nicomekl River changed downstream as more ditch water entered, samples of benthic invertebrates would be collected at each site with a grab or other sampling device. However, any changes observed might be due to changes in substrate which were not related to water quality. If a standard substrate is placed in the stream at each station, and left for $6-8$ weeks to be colonized, problems due to heterogeneity of substrate are removed. Normally, these substrates would be placed near the river bottom, but in the Nicomekl, the substrates would have sunk into the predominantly muddy natural substrate. Therefore, they were suspended below the surface but well above the substrate.

Similar artificial substrates have been successfully used to document changes in macroinvertebrate communities in the Peace River, B.C., associated with an improvement in wastewater treatment (Gibbons, 1991). Because the substrates were suspended above the natural substrate, they were probably colonized by drift organisms rather than by the resident benthos. In a comparison of natural and artificial substrates, Munkittrick et al. (1990) noted that the communities from artificial substrates were more a function of water quality than sediment quality; the reverse was true for communities from natural substrates.

Artificial substrates were placed in the Nicomekl River for 4-8 weeks on four occasions:

- December 14, 1989 - January 29, 1990 (47 d);
- February 23 - April 12, 1990 (49 d);
- April 26 - July 4, 1990 (70 d);
- November 2-27, 1990 ( 26 d ).

On each occasion, six substrates were placed at each of the four river stations. The original plan was to leave the substrates in the river for the same amount of time on each of the four occasions. However, they were left in place for an additional 3 weeks in June, 1990, to assess the effects of the oil spill which occurred June 7. They were removed early in November, 1990, because of the danger that the high discharge in the river would wash them away.

Rocks for the artificial substrates were obtained from a local quarry. The rocks were sorted by size by the quarry operators, and were approximately 5 cm diameter. The rocks were placed in wire cylindrical barbecue baskets approximately 30 cm long and 15 cm diameter. These baskets were then clipped to floats, which were in turn tied to anchors. The floats kept the baskets suspended 1 m below the surface; the anchors prevented the baskets from being washed downstream. After some floats and substrates were lost during DecemberJanuary, an additional weight was added midway along the anchor rope. The extra weight sat on the bottom during low water, maintaining the basket in midstream, but lifted off the bottom during high water with the anchor still holding the basket in place. Approximately one substrate per site was lost (detached from float, drifted to bank or downstream) during each 4-8 week monitoring period, except in November, 1990. All substrates at Station 1 were lost in November, as vandals apparently detached the baskets from the floats. (The floats were recovered at the 176th Street bridge, upstream of other stations; the equipment belonging to the Erickson ditch construction crew was also vandalized at the same time.)

The baskets were removed by lifting them out of the water and unclipping them from the floats. A net was held under the baskets during lifting to catch any organisms washed out of the rocks. The baskets were placed in tubs of water and taken to shore. There the baskets were opened and the rocks thoroughly scrubbed while to dislodge any invertebrates. The contents of the tubs were washed through a $250 \mu \mathrm{~m}$ sieve, and the invertebrates retained by the sieve were preserved in $5 \%$ formalin. The invertebrates collected were returned to the laboratory, transferred to $\mathbf{7 0 \%}$ ethyl alcohol, and later sorted, identified and counted. In some cases, samples were split because of the large number of organisms present. Insects were identified to genus, and species if possible, except for Chironomidae, which were identified only to family. The level of identification differed for non-insect taxa, but large crustaceans and gastropods were identified to genus or species whenever possible. Identification was provided by Mr. Bob Wisseman of Corvallis, Oregon, an expert on Pacific Northwest invertebrates.

### 2.4.1.2 Statistical Analysis

For the purposes of analyses, the invertebrate taxa were lumped into 10 broad groups (cf. Results). Differences in abundance and community composition among seasons and stations were the primary focus of analyses. Changes from upstream to downstream (downstream trends) were the site differences of interest. The expectation was that any positive or negative impact of discharges from the ditches would result in a downstream trend in abundance or composition. Contrasts were used to test for the existence of these downstream trends (cf. Appendix II), and various other relevant hypotheses (cf. Results). The standard analyses were ANOVAs, with station and season as factors. Each analysis was conducted twice -with all stations included and November data excluded; and with Stations 1 excluded and November data included. The two approaches were necessary because there were no data from Station 1 in November.

The first step was to conduct multivariate ANOVAs (MANOVAs), with abundances of the 10 taxonomic groups (transformed to $\log (x+1\})$ as variables. These MANOVAs tested for overall differences among seasons or sites, and for any other differences specified by contrasts. Vectors ( $=$ discriminant functions or canonical vectors), or linear combinations of the variables, were created which maximized the differences among sites or seasons. The relationship between the original variables and these vectors, or new variables, is usually expressed by giving the correlation between them. Thus, if abundance of a particular taxon is highly correlated with a vector, then that taxon contributes substantially to the differences among groups. The signs of the correlations are also important. Suppose that abundances of two taxa are highly correlated (but with opposite signs) with the vector that best describes downstream changes. This means that one taxon increases, while the other decreases, in a downstream direction. In ecological terms, this would be replacement of one taxon by another.

If only a few taxa are considered, the information obtained from a MANOVA can easily be obtained from simple inspection and comparison of means for individual variables. However, if there are more than three or four variables, patterns can be more easily determined by MANOVA. In our analyses we used MANOVA to identify these patterns, and suggest some appropriate simple indices to analyze further. Plafkin et al. (1989) provides a good review of indices used for assessing impacts on macroinvertebrate communities. Many of these indices are considerably more complex than vectors from MANOVAs. Commonly used indices are actually similar to MANOVA vectors in that they are combinations of the original variables. For any specific study, the MANOVA vector provides a better description of the differences among stations or seasons. However, vectors will differ from study to study, and can be difficult to relate to environmental impacts. Indices are the same from study to study, and the relationship between changes in index values and environmental impacts are better known. Thus, identifying appropriate indices by MANOVA and then analyzing the indices combined the best of both approaches.

### 2.4.2 Rainbow Trout Alevin in situ Bioassay

This test measures the same responses as does the laboratory test. Embryos or alevins were incubated in the river and ditches in November, 1990, in containers known colloquially as "egg sandwiches". Our containers differed slightly from those traditionally used (e.g., Mohr et al., 1990; we would recommend the traditional design for future research), as our sandwiches were made of the gridded plastic crating used to cover fluorescent lights (Figure 5). In the laboratory, embryos ( 20 per sandwich) were placed in the central compartments formed by the grid. The gridded plates making up the sandwich were then bolted together, and the sandwiches transported in cool water to the river on October 31, 1990. The sandwiches were suspended in small ( $25 \times 15 \times 20 \mathrm{~cm}$ ) mesh cages (two sandwiches per cage) and firmly attached by cord to the sides of the cages. The objective was to maintain the sandwiches in a fixed position in the water, without subjecting them to physical damage from drifting objects, fish, birds and mammals. Three cages were used at each of the four river stations and in each ditch. Cages at the river stations were clipped to floats used to suspend artificial substrates; cages in the ditches were secured to pumphouse intake grates, away from the central part of the intake current.

The sandwiches were left in place for 4 weeks; they were checked weekly, and dead embryos removed if possible. The sandwiches from Station 1 were lost when the floats and artificial substrates were vandalized; one pair of sandwiches was also lost from Station 3. By November 27, the embryos had hatched, but the temperature was decreasing. The subsequent growth and survival of the alevins was likely to be minimal. The surviving alevins in one sandwich from each cage were immediately counted and preserved. The survivors from the remaining sandwiches were returned to the laboratory and reared for 9 d in EVS laboratory water. By that time, they had consumed most of their yolks, and the bioassay ended. Procedures and containers for the laboratory rearing were the same as those used for the laboratory bioassay. All alevins were later dissected and weighed.

The variables analyzed for both the field and laboratory components were \% mortality, yolk and body weight, and yolk conversion efficiency (YCE). ANOVAs or ANCOVAs were used to compare sites (stations plus ditches). Contrasts were used to compare ditches with river stations, and to determine if response variables increased or decreased from upstream to downstream. The pairing of sandwiches allowed us to calculate YCE directly for laboratory reared alevins. Remember that alevins from one sandwich of each pair were preserved just prior to the start of the laboratory rearing. We assumed that the weights of these alevins approximated the initial weight of alevins from the remaining sandwich in the pair, and then calculated changes in yolk and body weight over the $10-\mathrm{d}$ period.

### 3.0 RESULTS

3.1 Background

### 3.1.1 Climate

Temperature and precipitation patterns for Surrey Municipal Hall in Newton (the nearest weather monitoring station) are provided in Figure 6. The temperature records reflect the north temperate climate. The precipitation records show that precipitation was greatest from October through March, and very low during. July and August. Even in winter, most of the precipitation is rain rather than snow.

### 3.1.2 Hydrology

Figure 7 provides discharge in the Nicomekl River, measured at a gauging station at 203rd Street $\mathbf{( ~} \mathbf{~} \mathbf{3 k m}$ upstream of the study area). Discharge was greatest from October to April, reflecting rainfall patterns; there is no spring/summer contribution from mountain snow melt as there is in other B.C. streams. Daily average flows at this site have historically ranged from 0.125 to $35.4 \mathrm{~m}^{3} / \mathrm{s}$; the ten year seven day average low flow (7Q10) is $0.13 \mathrm{~m}^{3} / \mathrm{s}$ (Swain and Holms, 1988b). Water temperatures during sampling trips are given in Table 1. The river temperatures followed changes in air temperature, but the extremes were not as great. The ditches were colder than the river in winter, and warmer in summer.

The pumps at the Burrows and Logging ditch pumphouses have a maximum discharge of 0.75 and $0.95 \mathrm{~m}^{3} / \mathrm{s}$, respectively ( E . Johnson, Surrey Municipality, personal communication). Theoretically, the maximum discharge could be double these values, since two pumps, set to go on at different water levels, are employed at each pumphouse. However, both pumps are rarely operating for extended periods (if they did there would be no flood control), and the pumps are less efficient at high tide when the water level in the river rises. Thus, with a discharge of $=1 \mathrm{~m}^{3} / \mathrm{s}$ from each ditch, dilutions factors should be $\approx 1: 10$ at times of high discharge in the river ( $10 \mathrm{~m}^{3} / \mathrm{s}$ ). Dilution factors at lower river discharges would be lower than this during the infrequent periods when the pumps were operating, and would be higher when ditch discharge was passive or zero. There are several other large ditches in the study area discharging to the river. Some of these are equipped with pumps with a capacity similar to that of the study ditch pumps. As a result, the dilution provided by the river at Logging ditch would be greater than at Burrows ditch. The extra dilution would consist of ditch water, providing little benefit in terms of reducing contaminant concentrations. Instead, contaminant concentrations would increase downstream, assuming that the ditches were the source of contaminants.

### 3.1.3 Pesticide Use in the Study Area

The farmers in the area generally applied herbicides in the spring (April) prior to emergence of crop plants, and then again in August as top-kill. Insecticides were applied at various times through the spring and summer. None of the three farmers in the immediate area of the study ditches would admit to using dinoseb when questioned in 1989, and all were aware of the potential hazards to their health. The results of our chemical analyses (see below) suggest that dinoseb was being used in the area in 1989, although not necessarily by the farmers questioned. The farmers also indicated that endosulfan use was decreasing in the area; Mr. Ward Strong, a local pesticide consultant, suggested that there were concerns that endosulfan was becoming less effective because the target organisms were developing resistance.

The farmers indicated that herbicides used were glyphosate (applied prior to ploughing), chlorpropham (used as a top-killer), and paraquat. Chlorpyrifos was used by one farmer for insect control. The Municipality of Surrey rarely uses pesticides for mosquito control, but may use Aquashade (a combination of dyes which reduces light penetration) to control algal growth. The Municipality indicated copper sulfate had not been used for algal control for years, and may never have been used. In general, users in the area were aware of concerns about various pesticides from the perspective of effects on human health, and had been switching to less toxic alternatives over the years.

## $3.2 \quad$ Water and Sediment Quality

Federal (CCREM) and provincial (B.C. MOE) water quality guidelines or criteria for freshwater aquatic biota are given in Table 2. Our discussion below, and Figures 8-18, are largely restricted to basic water quality parameters, nutrients, and metals which exceeded the criteria. B.C. MOE criteria, rather than CCREM guidelines, are shown in the Figures only because they are available for more parameters (in many cases the two are the same). Swain and Holms (1988a,b) provide some objectives specifically for the Nicomekl River but these have been superseded by the B.C. MOE criteria. Freshwater sediment criteria are not available. The complete chemical data are provided in Volume II.

### 3.2.1 Basic Water Quality

Basic water quality data are given in Table 1 and Figures 8-10. Dissolved oxygen levels in the ditches were usually less than in the river, but rarely below $8 \mathrm{mg} /$, except in November, 1990 (Table 1). On some sampling dates, there was a slight downstream decline in oxygen levels, presumably the result of the addition of ditch waters with lower oxygen levels. The two study ditches probably did not contribute to this trend. The pumphouses pump the ditch water into the river with such force that considerable turbulence occurs. Further oxygenation occurred when water levels were low in the river because the exit pipes from the ditches were then

[^0]above water. Water pumped from the Logging ditch resulted in higher oxygen levels in the river (e.g., Station 4) on February 12.

The river water was neutral to slightly alkaline, and hard (Figure 8). pH in the ditches was similar to that in the river, but hardness was elevated on most sampling dates, especially in Burrows ditch (Figure 8). The water was usually softer at Site 1 than at the other three river stations, suggesting that discharge from the ditches elevated hardness in the river. Note that when the ditches were not discharging in July, hardness was similar at all river stations. Except in July, conductivity was greater in the ditches than in the river and increased in a downstream direction (Figure 9). Concentrations of individual ions followed the same trends. We have shown sodium levels in Figure 9 to demonstrate that the ditches, and not tidal action and salt water intrusion, were responsible for the downstream increases in ion concentrations.

The water in the ditches, especially Burrows ditch, was highly coloured relative to the river water (Figure 10). Colour in the river increased from upstream to downstream, especially below Burrows ditch (from Station 2 to 3). Even in July, when the water in the ditches was pumped from the river, colour was greater in the ditches. Suspended solids (non-filterable residue) levels in the ditches were not markedly elevated over levels in the river (Figure 10; turbidity values showed the same pattern). There was some tendency for suspended solids and turbidity levels to increase downstream. However, the major source of the solids was probably not the study ditches, but other ditches that drained directly into the river. The pumps in the study ditches draw water from 1 m above the substrate, and would not be expected to disturb and discharge sediment particles.

### 3.2.2 Nutrients

The concentrations of nutrients in the river and ditches are shown in Figure 11. Levels of nitrogen, phosphorous, and organic carbon were elevated in the ditches, and tended to increase in the river from upstream to downstream. Levels of these nutrients in the sediments were also higher in the ditches than in the river. Probably because of the high nutrient levels, the ditches can become overgrown with algae and duckweed in the summer, our bioassay results (Section 3.3) certainly were consistent with the nutrient data.

### 3.2.3 <br> Metals

Levels of aluminum, cadmium (Figure 12), copper (Figure 13), iron (Figure 14), lead (Figure 15), manganese (Figure 16), mercury (Figure 17), and zinc (Figure 18) in the river or ditch waters exceeded provincial water quality criteria on one or more occasions. Aluminum concentrations always exceeded the criterion for acute effects. Arsenic, barium, beryllium, cobalt, chromium, lithium, molybdenum, nickel, selenium, strontium, and vanadium levels never exceeded the provincial criteria.

Concentrations of the metals followed several different patterns, not all of which indicated that the ditches were important sources. Zinc and manganese levels were generally higher in the ditches than in the river, and levels in the river increased downstream. This suggests that these metals originated from the ditches, perhaps as trace elements in fertilizer. Most of the metal levels were lower at Station 1 than at other river stations, but not necessarily higher in the ditches. Contribution from other sources in the study area (e.g., roadside ditches) is suggested. Aluminum levels are a good example of this type of pattern (Figure 12). However, even in July, when run-off from both agricultural and roadside ditches is minimal, aluminum levels exceeded the criterion for acute effects. There are upstream and natural metal sources on the Nicomekl (Swain and Holms, 1988a,b) which might explain elevated levels of metals at Station 1, and the absence of higher levels in the ditches and any downstream increase (mercury is a good example of a metal occurring at elevated levels with no apparent increase in the study area). Finally, metal levels in the ditch sediments tended to be as high as, or higher than, those in the river sediments (Figure 13-18), reflecting either a higher binding capacity or a source near the ditches.

### 3.2.4 Target Pesticides

Concentrations of dinoseb and endosulfan in water and sediment from the ditches and river stations are given in Tables 3 and 4. Raw data, including concentrations of some other pesticides, are provided in Volume II.

The detection limits for dinoseb and endosulfan matched the federal (CCREM) guidelines given in Section 1.4, so any detectable quantities would exceed those guidelines. Exceedance was restricted to February and early March, 1990, when concentrations of both pesticides in the ditches were very high. Unfortunately, samples from Station 1 and 3 from February were destroyed (broken bottles). The concentrations of dinoseb, and $\beta$-endosulfan and endosulfan sulfate, increased from Station 2 to 4 , presumably reflecting the input from the ditches. The increases were consistent with a dilution factor of $\mathbf{1 : 1 0}$ for both ditches. Concentrations of $\alpha$-endosulfan did not increase between Station 2 and 4. The predicted increase, based on 1:10 dilution, would be small ( $20 \mathrm{ng} / \mathrm{L}$ ), and difficult to detect. The concentrations of all compounds in February were elevated at Station 2, suggesting input from upstream sources. Other ditches (e.g., Erickson) are more likely sources, but sources upstream of the study area cannot be ruled out because no data were available for Station 1.

The high concentrations of the target compounds in February were unexpected - February was not a particularly rainy month with respect to daily average or monthly total (Figure 6). However, the rainfall in the week preceding the February 12 sampling date was much heavier than before all other sampling dates except November 14, 1989 (Table 5). The major contributor was the 47.6 mm of rain on February 9, making that the rainiest day of the study period (some November, 1990, days may exceed this value, when the data become available). Thus, there may have been considerable runoff from nearby fields at this time, but one wonders why the same high concentrations did not occur in November, 1989 (or 1990).

Dinoseb was occasionally present in the ditch sediments, but never in the river sediments, at $>5 \mathrm{ng} / \mathrm{g}$ (Table 4). This is consistent with the high solubility of the compound. $\alpha$-endosulfan was also present at low levels only in the ditch sediments, and at trace or nondetectable levels in the river sediments. $\beta$-endosulfan and endosulfan sulfate were occasionally detected at low levels in the river sediments, and were almost always detected in the ditch sediments. Levels of the compounds were higher in February and March than later in the study period, probably reflecting adsorption from the water during February.

### 3.2.5 Oil Hydrocarbons

Oil and grease concentrations in water on June 8 were $5.7 \mathrm{mg} / \mathrm{L}$ at the Upstream site, $<1 \mathrm{mg} / \mathrm{L}$ in Burrows ditch, and $2.3 \mathrm{mg} / \mathrm{L}$ in Logging ditch. By July 3 , oil and grease concentrations in water were $<1 \mathrm{mg} / \mathrm{L}$ in both ditches and at all four river stations. At that time the ditch sediments had elevated levels of hydrocarbons ( 1140 and $1440 \mu \mathrm{~g} / \mathrm{g}$ for Burrows and Logging, respectively); the river sediments had lower levels (168 and $336 \mu \mathrm{~g} / \mathrm{g}$ for Stations 1 and 4, respectively). The most reasonable hypothesis is that hydrocarbons entered the ditch water, and eventually the sediment, when irrigation water was pumped from the river. Flushing rates for sediment hydrocarbons would subsequently be greater in the river, and the sorptive capacity of the less organic sediments would be lower. As a result, higher hydrocarbon levels would persist longer in the ditch sediments. If this hypothesis is true, the river actually discharged contaminants into the ditches.

### 3.3 Laboratory Bioassays

Bioassay results are provided below. Dates and water quality data, as well as dinoseb and endosulfan concentrations for one or more samples, are given for each test water.
3.3.1 Acute (Lethal) Effects Tests
3.3.1.1 Rainbow Trout Pass/Fail

Test Date: $\quad$ November 15-18, 1989
Samples Collected: November 14, 1989
Temperature ( ${ }^{\circ}$ ): $\quad 14-15$
DO ( $\mathrm{mg} / \mathrm{L}$ ): 8.6-9.8

| Site | pH | Dinoseb <br> $(\mathrm{ng} / \mathrm{L})$ | $\alpha$-endosulfan <br> $(\mathrm{ng} / \mathrm{L})$ |
| :--- | :---: | :---: | :---: |
| Control | $6.1-7.2$ | $<50$ | $<20$ |
| Burrows | $6.6-7.2$ | $27^{*}$ | $9^{*}$ |
| Logging | $6.6-7.1$ | $36^{*}$ | $6^{*}$ |
| Erickson | $6.6-7.2$ | $23^{*}$ | $9^{*}$ |

NOTE: Values given as < $x$ were below stated detection limits $(x)$, with no evidence of trace amounts (above background or blank); values marked "*" are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present

No mortalities were observed in any test waters in the pass/fail test.

### 3.3.1.2 Residual Oxygen Bioassays

Test Date: $\quad$ November 18, 1989
Samples Collected: $\quad$ November 14 (water), 16 (sediment), 1989
Temperature ( ${ }^{\circ} \mathrm{C}$ ): $\quad \approx 15$ (not measured)

| Site | Type | Oxygen <br> Demand <br> ( $\mathrm{mg} / \mathrm{L}$ ) | pH | Dinoseb $(n g / L)$ | $\alpha$-endosulfan <br> ( $\mathrm{ng} / \mathrm{L}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Control | Water | 2.2 | 6.4 | . <50 | $<20$ |
| Burrows | Water | 1.2 | 6.7 | 27* | 9* |
| - | Elutriate | 1.4 | 7.1 | 9* | $<20$ |
| Logging | Water | 0.6 | 6.8 | 36* | $6^{*}$ |
| $\because$ | Elutriate | 1.2 | 6.9 | $<50$ | 3* |
| Erickson | Water | 1.2 | 6.6 | 23* | 9* |
| - | Elutriate | 1.8 | 6.8 | $<50$ | 7* |

NOTE: Values given as $<x$ were below stated detection limits $(x)$, with no evidence of trace amounts (above background or blank); values marked "*" are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):

March 8, 1990
March 1, 1990
$=15$ (not measured)

| Site | Oxygen <br> Demand <br> ( $\mathrm{mg} / \mathrm{L}$ ) | pH | Dinoseb <br> ( $\mathrm{ng} / \mathrm{L}$ ) | $\begin{gathered} \alpha- \\ \text { (ng/L) } \end{gathered}$ | $\begin{gathered} \text { ndosulfa } \\ \beta- \\ (\mathrm{ng} / \mathrm{L}) \end{gathered}$ | Sulfate <br> (ng/L) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Control | 0.4 | 5.6 |  |  |  |  |
| Upstream | 0.6 | 7.5 |  |  |  |  |
| Burrows | 3.9 | 7.2 | 100 | 42 | 340 | 780 |
| Logging | 4.0 | 7.2 | 110 | 23 | 18* | $18^{*}$ |

NOTE: Values marked ${ }^{n * *}$ are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present

Results of the residual oxygen bioassays are given in Table 6. Trout in control laboratory water invariably expired at higher oxygen concentrations than did trout in test waters. Statistical analyses using contrasts showed that almost all of the variation among test waters was due to the difference between controls and others. This difference was significant in the November test $(P=0.02)$ and nearly so'in the March test ( $P$ $=0.07$ ). No other contrasts were significant, and in November there was no tendency for water and elutriate samples from the same ditch to differ. In November, concentrations of dinoseb and $\alpha$-endosulfan were lower in the elutriates than in the waters, and lower in Erickson ditch water than in other ditch waters. All concentrations were at trace levels and therefore must be regarded as negligible and similar.

### 3.3.2 <br> Sublethal Effects Bioassays

### 3.3.2.1 Rainbow Trout Alevin Bioassay

Test Date: • December 12, 1989 - January 2, 1990
Samples Collected: December 11, 14, 27, 1989
Temperature ( ${ }^{\circ} \mathrm{C}$ ): $\quad 14-15$
DO (mg/L): 9.2-10.0
Diluent: Control

| Site | pH | Dinoseb <br> $(\mathrm{ng} / \mathrm{L})$ | $\alpha$-endosulfan <br> $(\mathrm{ng} / \mathrm{L})$ |
| :---: | :---: | :---: | :---: |
| Control | $6.1-7.4$ |  |  |
| Upstream | $6.4-7.6$ | NM | NM |
|  |  | $<50$ | $<20$ |
|  |  | NM | NM |
| Burrows | $6.7-7.4$ | $<50$ | $<20$ |
|  |  | $<50$ | $<20$ |
|  |  | $<50$ | $<20$ |
| Logging | $6.2-7.4$ | $.25^{*}$ | $<20$ |
|  |  | $39^{*}$ | $<20$ |
|  |  | $<50$ | $<20$ |

$\mathrm{NM}=$ not measured ${ }^{\text {' }}$

NOTE: Values given as $<x$ were below stated detection limits $(x)$, with no evidence of trace amounts (above background or blank); values marked "*" are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present

1

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO (mg/L):
Diluent:

February 16 - March 7, 1990
February 12, March 1; 1990
13-17
8.8-10.8

Upstream

| Site | pH | Dinoseb <br> ( $\mathrm{ng} / \mathrm{L}$ ) | ( $\mathrm{ng} / \mathrm{L}$ ) | Endosulfan $\beta$ ( $\mathrm{ng} / \mathrm{L}$ ) | Sulfate ( $\mathrm{ng} / \mathrm{L}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Control | 5.6-6.5 |  |  |  |  |
| Upstream | 6.8-7.6 |  |  |  |  |
| Burrows | 6.4-7.6 | $\begin{gathered} 1600 \\ 100 \end{gathered}$ | $\begin{gathered} 170 \\ 42 \end{gathered}$ | $\begin{array}{r} 280 \\ \times \quad 340 \end{array}$ | $\begin{aligned} & 800 \\ & 780 \end{aligned}$ |
| Logging | 6.3-7.5 | 490 110 | 40 23 | $\begin{aligned} & 230 \\ & 18^{*} \end{aligned}$ | $\begin{aligned} & 680 \\ & 18^{*} \end{aligned}$ |

NOTE: Values marked ${ }^{* * *}$ are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present ,

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO (mg/L):
Diluent:

November 5-22, 1990
November 2, 1990
15-16
9.3-10.2

Upstream

| Site | pH | Dinoseb $(\mathrm{ng} / \mathrm{L})$ | $\alpha-$ <br> ( $\mathrm{ng} / \mathrm{L}$ ) | Endosulfan $\beta-$ (ng/L) | Sulfate <br> (ng/L) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Control | $\checkmark$ 6.6-7.7 |  |  |  |  |
| Upstream | 6.6-7.6 | $<50$ | - 20 | $<20$ | <20 |
| Burrows | 6.1-7.4 | $<50$ | $<20$ | $<20$. | <20 |
| Logging | 6.2-7.1 | <50 | <20 | $<20$ | $<20$ |

Embryo and alevin mortalities did not differ among test waters in any of the tests conducted, and were generally lower than $10 \%$, except in December-January (Table 7).

In all tests, alevins from laboratory controls had smaller bodies, and usually larger yolks, than did alevins from the ditch or river waters (Figures 19-21). The control alevins, therefore, developed more slowly. Yolk conversion efficiency (YCE) may also have been lower for the laboratory controls, but they were so far removed from the body-yolk weight relationship for the other alevins that comparisons would be unwise (Figures 19, 20). The retarded development of the laboratory controls was almost certainly a function of water hardness, as we have largely eliminated the problem in other studies by increasing hardness (Hamilton and Nix, 1991). The laboratory controls were not included in any subsequent statistical analyses, as their inclusion would cause problems in ANCOVAs. Differences between the controls and the other test waters are not relevant to this project.

Differences in yolk and body weight among test waters occurred only in February-March (Table 8). Concentrations of the target pesticides were elevated in samples taken then, and were higher in Burrows than in Logging ditch. Thus, if the pesticides had any effect, test waters would be ranked Upstream > Logging > Burrows for any characteristic reflecting good condition or health. Instead, yolk and body weight did not differ between the ditches and Upstream, but did differ between the ditches (Table 8). The Burrows alevins developed slightly faster, and the Logging alevins developed slightly slower, than the Upstream alevins (Figure 20). This is best seen in the bottom graph. The Burrows alevins lie to the left (faster development), the Logging alevins to the right (slower development), and the Upstream alevins almost exactly in the middle. The observed pattern is entirely inconsistent with any hypothesis suggesting that the target pesticides present at the time had an effect. Note also that there were no differences in body weight relative to yolk weight (YCE; Table 8).

The regressions of body on yolk weight provided poor estimates of the actual yolk conversion efficiency. Slopes ranged from positive values (not significantly different from 0 , in November, 1990) to -1 to - 1.5 ( $\mathbf{m g}$

$$
!
$$ body/mg yolk) in the other tests. In our interim report (Paine, 1990), we stated that -1 to -1.5 was probably a reasonable estimate of the true conversion efficiency, as it matched that'measured directly by Hodson and Blunt (1981). The results of the alevin in situ bioassay, and the results of tests conducted for other clients (Hamilton and Nix, 1991), suggest that the actual YCE is -2 to -2.5. The slopes from this study underestimated the true YCE because there was not a large difference in yolk and body weights between treatments (excluding laboratory controls). Thus the range of data, and strength of correlations, was restricted. Again, we emphasize that this does not affect our conclusions about yolk conversion efficiency, as the in situ bioassay results demonstrate.

96-h LC50s for the reference toxicant SDS were 7 and 18 ppm for alevins $\mathbf{2 0} \mathrm{d}$ after hatch (measured in March, 1990). These values were lower than LC50s for juveniles obtained from the same source, which ranged from $25-40 \mathrm{ppm}$ ( 10 tests). over the first six months of the present study. Therefore, alevins appear more
sensitive than juveniles to contaminant effects. More extensive testing is required to determine if the difference is statistically and biologically significant.

In our interim report (Paine, 1990), we suggested several changes to the protocol for the rainbow trout alevin test, which have been implemented for other clients. Many of these changes have been incorporated in Environment Canada protocols for salmonid early life stage tests (Environment Canada, 1992). Those protocols emphasize short ( $7-\mathrm{d}$ ) tests on embryos rather than alevins. We did not change our protocols during this study, in order to maintain comparability among tests conducted at different times. However, our suggestions are still valid, and have proven successful. The test can be initiated with newly hatched alevins, rather than embryos, which removes any confounding effects of differences in time to hatch. If initial yolk and body weight are measured on a sample when the test starts, changes in yolk and body weight can be calculated, and YCE directly calculated. A 7-d test is sufficient for alevins to use $>50 \%$ of their yolk and, thus, for contaminants to affect the conversion of yolk to body tissue. A hard-water control ( $50-75 \mathrm{mg} / \mathrm{L} \mathrm{Ca}$ ) is demonstrably superior to softer City of Vancouver water ( $<15 \mathrm{mg} / \mathrm{L}$ Ca), and is more similar to the hardness in larger B.C. streams and rivers (such as the Columbia or the Nicomekl). With the recommended changes, the test takes less time than the Ceriodaphnia bioassay, and is comparable in cost. The major restriction on the use of the modified alevin bioassay would be that alevins are unavailable year-round.

### 3.3.2.2

Ceriodaphnia 7-day Life Cycle Bioassay

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO (mg/L):
Diluent:

December 12-19, 1989
December 11, 14; 1989
21-24
7.9-9.0

Control (20\% Perrier water)

| Site | pH | Dinoseb <br> (ng/L) | $\alpha$-endosulfan <br> $(\mathrm{ng} / \mathrm{L})$ |
| :--- | :---: | :---: | :---: |
| Control | $7.3-7.5$ |  |  |
| Upstream | $7.4-7.8$ | NM <br> $<50$ | NM <br> $<20$ |
| Burrows | $7.4-7.8$ | $<50$ <br> $<50$ | $<20$ <br> $<20$ |
| Logging | $7.2-7.5$ | $25^{*}$ <br> $39^{*}$ | $<20$ |

NOTE: Values given as $<x$ were below stated detection limits $(x)$, with no evidence of trace amounts (above background or blank); values marked ${ }^{* * *}$ are below stated detection limits, but indicate that apparent trace amounts of the target pesticides were present

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO ( $\mathrm{mg} / \mathrm{L}$ ):
Diluent:

December 28, 1989 - January 4, 1990
December 27, 1989
20-22
8.1-9.1

Control (20\% Perrier water)

| Site | pH | Dinoseb <br> $(\mathrm{ng} / \mathrm{L})$ | $\alpha$-endosulfan <br> $(\mathrm{ng} / \mathrm{L})$ |
| :--- | :---: | :---: | :---: |
| Control | $6.7-7.1$ |  |  |
| Upstream | $7.6-7.9$ |  |  |
| Burrows | $7.5-7.7$ | $<50$ | $<20$ |
| Logging | $7.5-7.7$ | $<50$ | $<20$ |

Test Date:
Samples Collected:
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO ( $\mathrm{mg} / \mathrm{L}$ ):
Diluent:

February 13-23, 1990
February 12; 1990
21-23
7.8-9.5

Upstream

| Site | $\because \mathbf{p H}$ | Dinoseb <br> (ngL) | $\begin{gathered} \alpha- \\ (\mathrm{ng} / L) \\ \hline \end{gathered}$ | $\begin{gathered} \text { Endosulfan } \\ \beta- \\ (\mathrm{ng} / \mathrm{L}) \\ \hline \end{gathered}$ | Sulfate $(\mathrm{ng} / \mathrm{L})$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Control | 7.1-7.9 |  | , |  |  |
| Upstream | 7.1-7.7 |  |  |  |  |
| Burrows | 7.0-7.3 | 1600 | 170 | 280 | 800 |
| Logging | 7.0-7.5 | 490 | 40 | 230 | 680 |

Resulis of tests conducted during December to February are shown in Figure 22; results of statistical analysis are provided in Table 9. In two cases (out of 24), 2 females (of 10 ) in a treatment died; in the remainder, 1 (three cases) or 0 females died. :Thus, there were no effects on mortality.

In the first December test, the number of young produced per female was similar in all test waters (Figure 22) and no contrasts were significant (Table 9). In the second December test the only significant trend was an increase in the number of young produced with increasing concentration of ditch water (Figure 22). Such a trend would be expected if the daphnids were eating the bacteria in the ditch waters; these bacteria would be less abundant in diluted ditch water and absent in controls. However, if this were true, the production of young even in the dilute ditch waters would be greater than that in the controls, which was not the case. The presence of bacteria in the test waters might explain the results of the February test. Reproduction was significantly lower in the controls than in any of the ditch or river waters, although we still expected an increase in reproduction with an increase in concentration. Reproduction was also significantly higher in Logging ditch waters than in Burrows ditch waters.

### 3.3.2.3 Selenastrum 4-day Bioassay

| Test Date: | December $15-19,1989$ |
| :--- | :--- |
| Samples Collected: | December 14, 1989 |
| Temperature (C): | $21-24$ |
| Diluent: | Control (prepared water) |


| Source | Dinoseb <br> $(\mathrm{ng} / \mathrm{L})$ | $\alpha$-endosulfan <br> $(\mathrm{ng} / \mathrm{L})$ |
| :--- | :---: | :---: |
| Control |  | $*$ |
| Upstream | $<50$ | $<20$ |
| Burrows | $<50$ | $1<20$ |
| Logging | $39^{*}$ | $<20$ |

NOTE: Values given as $<x$ were below stated detection limits $(x)$, with no evidence of trace amounts (above background or blank); values marked "*" are below stated detection' limits, but indicate that apparent trace amounts of the target pesticides were present

Algal numbers were significantly lower in the control water than in any of the other test waters (Figure 23, Table 9). The most obvious explanation is that nutrients are limiting in the controls, so that the addition of nutrients from the river or ditch waters promoted growth. Table 10 compares nutrient levels in the test waters and in the culture medium. The controls and culture medium are the same. The test waters contained the concentrations listed plus those listed for the culture medium (a fixed volume of a concentrated nutrient solution is added to each test flask, then the flask is topped off with deionized water and/or test water to
provide the required dilution). If phosphorous was the limiting nutrient, Figure 23 indicates that there was a large increase in growth between $0.19 \mathrm{mg} /$ (Control) and $0.29 \mathrm{mg} /$ (Upstream). Beyond that concentration only modest increases occurred. The best growth occurred in $10 \%$ Logging ditch water ( $0.32 \mathrm{mg} /$ ). Growth in $100 \%$ Burrows ( $3.0 \mathrm{mg} / \mathrm{L}$ ) and Logging ditch ( $1.52 \mathrm{mg} /$ ) waters was less than in Upstream water. Nitrogen may have been limiting in the ditch waters as $\mathrm{N}: \mathrm{P}$ was less than the $14: 1$ considered indicative of phosphorous limitation (Wetzel, 1983). Growth declined with increasing concentration of both ditch waters, suggesting that the positive effects of nutrient addition were less than the negative,effects of some other compounds. Ions, metals or decreased light penetration were the most probable causes of these negative effects. The target pesticides were present only at trace or non-detectable levels.

### 3.3.2.4 Duckweed Bioassay

Test Date:
April 27 - May 4, 1990
Samples Collected:
April 26, 1990
Temperature ( ${ }^{\circ} \mathrm{C}$ ):
DO (mgh):
20.5-23.0

Diluent:
7.8-11.4

Upstream

| Site | pH | Dinoseb <br> ( $\mathrm{ng} / \mathrm{L}$ ) | $\begin{gathered} \alpha- \\ (\mathrm{ng} / \mathrm{L}) \end{gathered}$ | $\begin{gathered} \text { Endosulfan } \\ \beta- \\ (\mathrm{ng} / \mathrm{L}) \end{gathered}$ | Sulfate ( $\mathrm{ng} / \mathrm{L}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Control | 7.2-7.6 |  |  |  |  |
| Upstream | 7.4-8.4 | <50 | $<20$ | <20 | 20 |
| Burrows | 7.0-8.9 | <50 | $<20$ | <20 | <20 |
| Logging | 7.2-8.1 | <50 | $<20$ | $<20$ | $<20$ |

Test results are shown in Figure 24, and Table 9. The laboratory controls produced fewer fronds, and less frond biomass, than the other test waters. The replicates were initially stocked with $32-40$ fronds, with a mean dry weight of 5.4 mg . Over the $7-\mathrm{d}$ test, the number of fronds, and the total dry weight, in the river and ditch waters doubled. Increases in weight and frond number in the controls were $\leq 50 \%$. The only other difference of note was that the ditch waters produced a greater number of fronds than did the Upstream water. However, the number of green fronds, and their total dry weight, in the ditch waters was not significantly greater than in the Upstream water. Thus, the ditch waters were at best a marginally superior growth medium.

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As for Selenastrum, the most obvious explanation for the enhanced growth of duckweed in the ditch and river waters is that they contained more nutrients. The nutrient solution provided large excesses of some compounds ( $\mathrm{P}, \mathrm{K}$ ), but not others ( N ) (Table 11). It was no more effective as a growth medium at 1:100 dilution than at the $1: 10,000$ dilution used in the preliminary trial described in Paine (1990). The problem with using excess amounts of nutrient solutions is that the limiting factor is unknown, and the concentrations of other factors such as metals or ions may increase to toxic levels.
3.4

Field Monitoring

### 3.4.1 Artificial Substrates

The contributions of ten major taxa to the macroinvertebrate communities colonizing the substrates are shown in Figures 25 to 27; complete taxonomic lists are given in Volume II. Note that relative, rather than absolute, abundances are given in the figures, which standardizes the scale for all stations and seasons, but it may obscure some seasonal or spatial trends in absolute numbers. Oligochaeta (Figure 25) and Chironomidae (Figure 27) were the dominant taxa. They usually accounted for $>80 \%$ of the total numbers and were abundant throughout the year. The only other taxa to account for $>5 \%$ of the total were Amphipoda in February-April and Plecoptera in December-January.

The communities were unusual in that non-insect taxa were more abundant than in most other stream communities. There were several marine or estuarine taxa present, despite the low salinities recorded in water samples (Figure 9). All amphipods were estuarine or marine - Paramoera nr. carlottensis, Allorchestes sp., and Ramellogammarus ramellus (Kozloff, 1987). One of the two isopod taxa, Gnorimosphaeroma oregonense, was estuarine; the other, Asellus occidentalis, was freshwater. Rarer marine forms included polychaetes and the mysid shrimp Neomysis.

Results of the multivariate analyses (MANOVA) of the abundances of the ten taxa are given in Table 12. We examined differences among seasons, downstream trends, and the interaction between the two (i.e., changes in downstream trends with season). Univariate tests indicated that the abundance of every taxon except Aselhus differed significantly among the four seasons, largely because abundance was much greater in spring and summer than in fall or winter (Figure 28). The multivariate $P$ was $<0.0001$. The major vector describing seasonal differences was positively correlated with the abundance of every taxon except Plecoptera. Stoneflies are unusual among insects. Many species emerge as adults in late winter rather than in summer or fall (Hynes, 1970). The taxa most strongly correlated with the first seasonal vector were Oligochaeta, Amphipoda, and Plecoptera. Including or excluding November data had no effect on the analyses of seasonal trends, as the univariate and multivariate results did not change.

The abundances of most taxa also showed significant downstream trends (Table 12). The multivariate $P$ for the downstream contrast was $<0.0001$. Taxa which increased in abundance downstream (indicated by a
positive correlation with the downstream vector) were Nematoda, and the two dominant taxa, Oligochaeta and Chironomidae. All other taxa, except Amphipoda ánd Copepoda, declined in abundance downstream. Note that the estuarine Gnorimosphaeroma was abundant only at Stations 1 and 2 (Figure 26). We had separated the two isopod taxa in our analyses; on the assumption that the abundance of the marine species would increase downstream, whereas that of Asellus would decrease. However, the two species followed similar patterns. Although the broad downstream trend did not change if November data were included, and Station 1 excluded, there were some changes in the taxa contributing the most to the downstream trend. These changes were largely a function of seasonal effects - for exampie, Plecoptera were abundant in November, and if those data were included, then Plecoptera made a major contribution to the downstream vector.

The fact that including the November data could alter the downstream trend suggests that there was an interaction between seasonal and downstream effects, and there was (cf. univariate results in Table 12; multivariate $P$ was $<0.0001$ ). The interaction was significant for only a few taxa, including the two dominants, Oligochaeta and Chironomidae. For Gnorimosphaeroma, Plecoptera, and Ephemeroptera, the significance of the interaction depended on which stations or seasons were included. For all five taxa, the interaction meant that a downstream trend was present or strong in some months, usually when the taxon was most abundant, and absent or even reversed in others.

The results of the multivariate analyses, plus the chemical analyses (Section 3.2), suggested two variables and several hypotheses for further testing. The two variables were total abundance, which should reflect nutrient levels and food availability, and EP/C (the abundance of Ephemeroptera and Plecoptera divided by the abundance of Chironomidae). EP/C is similar to an index commonly used in macroinvertebrate monitoring studies - the ratio of EPT (Ephemeroptera, Plecoptera, Trichoptera; the abundance of Trichoptera was negligible in our study) and Chironomidae abundances (Plafkin et al., 1989). The EPT taxa are considered sensitive to impacts; Chironomidae are considered tolerant. Thus a low EP/C suggests negative impacts from chemical or physical factors.

Seasonal and spatial changes in total abundance and EP/C are shown in Figure 28. There were significant differences among seasons for both variables (Table 13). As noted earlier for individual taxa, abundance was. much greater in the spring and summer than in the fall and winter. EP/C was higher in winter and fall than at other times, because of the additional contribution of Plecoptera. Downstream trends were also significant for both variables (Table 13). Total abundance increased downstream, reflecting increases in Oligochaeta and Chironomidae. EP/C decreased downstream, paralleling the decrease in the relative abundance of the EP taxa (Figure 27). These results suggest a downstream deterioration associated with enrichment, and a decrease in the abundance of sensitive taxa.

The chemical analyses showed that concentrations of the target pesticides were greatest in February-March (Section 3.2.3). If these concentrations had an affect on the local biota, the downstream trends should be stronger at that time. This hypothesis was tested explicitly with contrasts. Downstream trends in total abundance, but not EP/C, were stronger in February to April than at other times (Table 13). However, the
trend was a downstream increase in abundance, which is an unusual response to an insecticide such as endosulfan. However, the increase was primarily restricted to the two tolerant taxa, Chironomidae and Oligochaeta. The downstream trends for each month are given in Table 14. The strong downstream increase in total abundance may be an artifact, reflecting the fact that statistically it is easier to detect such a trend when abundances are higher (February to April, April to July) than when abundances are lower (December to January, November). The downstream decrease in EP/C was significant in every month, but not strongest in February to April (Table 14).

Although not tested explicitly, a final hypothesis is that the downstream trends should be weakest in April to July, when ditches are much less likely to discharge to the river. This hypothesis assumes that the ditches are the source of a steady stream of a broad range of contaminants' and nutrients. The data in Table 14 do not support this hypothesis. During the April to July period, spraying of pesticides, although probably not the target pesticides, would have occurred. It could be argued that any acute effects from aerial drift of pesticides to the Nicomekl River should be evident from artificial substrates in place at the time. It is not clear that any acute effects on invertebrates on the substrates would persist, if invertebrates were continuously available for recolonization. It would also be naive to assume that drift and any associated effects would increase the strength of downstream trends. Any effects would depend on when and where the spraying occurred. Finally, while the effects of drift should not bé ignored, they are not necessary to explain the trends in EP/C and, to a lesser extent, total abundance in Table 14. A decrease in the abundance of EPT taxa relative to Chironomidae and other more tolerant taxa, is a classic response to organic enrichment, and the data on nutrient concentrations certainly indicate that enrichment was occurring.

### 3.4.2 Rainbow Trout Alevin in situ Bioassay

Table 15 provides mortality of alevins incubated in situ for four weeks, and in the laboratory for an additional 9 d . In the field, mortality was significantly lower in the ditches than in the river stations (Table 16); only $2 \%$ of alevins in Logging ditch survived. There was no evidence that mortality in the river increased downstream. Mortality in the laboratory was negligible, except for one case in which alevins experienced high accidental mortality.

Field and laboratory growth are shown in Figures 29 and 30. Alevins reared in Burrows ditch had significantly smaller bodies, and larger yolks than did alevins reared in the river (Table 16). The Burrows alevins were also less efficient at converting yolk to body tissue (Figure 29, Table 16). However, when the alevins were returned to the laboratory, growth and conversion efficiency were similar in ditch- and river-reared groups (Figure 30, table 16). Downstream trends were not evident for either field or laboratory growth.
 yolk. Conversion efficiency was analyzed by comparing body-yolk weight relationships by ANCOVA, but analysis of YCE values in an ANOVA provided identical conclusions. The slope of the body-yolk weight relationship was not significantly different from 0 because the range of data was limited.

## 4.0 <br> DISCUSSION

### 4.1 Dinoseb and Endosulfan

### 4.1.1 Seasonal Patterns

Concentrations of the two target pesticides exceeded CCREM water quality guidelines only in February and early March, 1990. Our initial expectation, and that of reviewers of the interim report (Paine, 1990), was that concentrations would be highest in November-December, following heavy fall rains. This expectation was not met in either 1989 or 1990. In a study of pesticides in southwestern B.C. drainage ditches, including one on 168th Street draining into the Nicomekl River (Figure 2), Wan (1989) also observed that maximum concentrations of dinoseb and endosulfan lagged one to several months behind fall rains. However, maxima in his study were generally observed in December, rather than in February. Wan (1989) also measured high levels ( $1500 \mu \mathrm{~g} / \mathrm{L}$ ) of endosulfan in ditch waters 30 min after spraying; concentrations immediately after spraying were not measured in the present study. The discussion below is largely restricted to longer term patterns of pesticide concentrations associated with runoff and ditch discharge, but transient higher concentrations after spraying should not be ignored.

Pesticides may take some time to move from fields into ditches. The elevated clay banks of the larger ditches, such as Burrows and Logging, would impede surface runoff and groundwater penetration. Certainly this is the case for pesticide entry into the river, which has clay banks elevated $2-3 \mathrm{~m}$ above ground level. Runoff can only enter the river via ditches. The banks must be impermeable to groundwater intrusion, since they are designed to prevent salt water intrusion into fields. However, pesticides do not need to enter the ditches via runoff or groundwater flow, as the farmers actively pump water into the ditches during heavy rains. We believe that pesticide entry into the ditches is a complex function of soil types, ditch construction, flood control practices, and the amount of rainfall at specific times. As a result, the timing of maximum concentrations in ditches and rivers may be unpredictable, except that it occurs in late fall to early winter. This has some implications for monitoring strategies (Section 4.3).

In this study, dinoseb concentrations in sediments were lower than those recorded by Wan (1989) and McLeay (1989) in ditches leading to the Nicomekl, and were detectable only in winter. Endosulfan, primarily $\beta$ endosulfan and endosulfan sulfate, persisted in sediments throughout the year, although concentrations were highest in winter and spring. From an environmental perspective, the effects of endosulfan in sediments are likely to be less seasonal than those of endosulfan in water, and persist for some time after termination of endosulfan use. Concentrations of both pesticides in the river sediments were low throughout the year. The endosulfan concentrations we observed in ditch sediments were lower than those observed by Wan (1989; >600 $\mathrm{ng} / \mathrm{g} \alpha$-, $\beta$-endosulfan, and endosulfan sulfate combined).

### 4.1.2 <br> Biological Effects

### 4.1.2.1 Laboratory Bioassays

With the possible exception of the Selenastrum bioassay, none of the bioassays conducted revealed any significant negative impacts of the ditch waters relative to the receiving (Upstream) water. Even in the Selenastrum bioassay, algal growth was only suppressed, in undiluted ditch water, but not at 10 or $30 \%$ dilutions. The dilutions were similar to those expected in the river, and growth in those dilutions may be more relevant to field conditions. Furthermore, the effects of the undiluted ditch water were probably not attributable to the target pesticides, which were present only at trace levels, but to other compounds (e.g., metals and other ions). The literature on these pesticides (Section 1.4) indicates that algae are only affected - at concentrations much higher than those affecting fish; and no effects on fish were observed.

Effects due to the target pesticides were only expected in February-March, as CCREM guidelines were not exceeded at other times. (The issue of effects due to other compounds is addressed in Section 4.2.) The rainbow trout alevin and the Ceriodaphnia bioassays were conducted at that time. There are several hypotheses that would explain the absence of any effects:

Hypothesis 1: The guidelines are poor predictors of the potential for effects.

If guidelines protect biota, this hypothesis must be true. Guidelines are concentrations at which effects should not occur, not concentrations at which effects should occur. Thus, there should be only a remote possibility of observing effects at concentrations only slightly in excess of the guidelines. This may explain why effects were not observed in some of the dilutions of ditch water. However, concentrations of dinoseb and endosulfan in the undiluted ditch waters, especially from Burrows ditch, exceeded the guidelines by one or more orders of magnitude, and were at levels at which effects have actually been observed. The concentration of $\alpha$-endosulfan in Burrows ditch on February 12 (170 $\mathrm{ng} / \mathrm{L}$ ) was one-half the average LC50 for rainbow trout ( $340 \mathrm{ng} / \mathrm{L}$; the LC50 actually refers to a 70:30 mix of the $\alpha$ - and $\beta$-isomers, which would contain $240 \mathrm{ng} / \mathrm{L} \alpha$-endosuifan). We doubt that doseresponse relationships are so steep that mortality would not be observed at $\geq 50 \%$ of the LC50; certainly, application factors are not so low that sublethal or chronic effects would not occur. The concentration of dinoseb ( $1600 \mathrm{ng} / \mathrm{L}$ ) was below the LC50 ( $>32,000 \mathrm{ng} / \mathrm{L}$ ) but was triple the concentration known to affect lake trout embryos/juveniles.

## Hypothesis 2: The bioassays were not sensitive to pesticide effects.

Sensitivity refers to biological, procedural, and statistical sensitivity. The lack of biological sensitivity may explain the absence of effects in the Ceriodaphnia test. Although concentrations of the pesticides in the ditch waters were, at the level at which effects on fish are expected, invertebrates are less likely to be affected, given their lower sensitivity (see Section 1.4). Furthermore, the positive effects of any
additional nutrition (i.e., bacteria) may have negated any slight negative effects from the pesticides. However, rainbow trout alevins represent the most sensitive stage of a sensitive group of fishes. We know from this study and others (e.g., Woodward, 1976) that effects on alevins occur at concentrations well below the LC50 for juvenile trout. EVS Consultants has been examining the effects of oil sands tailing pond and related waters for 10 years, and the alevin bioassay has proven to be the most sensitive in test batteries (Nix and Paine, 1990; Nix et al., 1990). The normal procedure has been to subject test waters to a rainbow trout pass/fail test. If no mortality occurs, the waters are then subjected to the alevin test. The alevin test often reveals lethal or sublethal effects of waters which had no lethal effects on juveniles.

There are two aspects of the alevin test protocol which may have reduced test sensitivity. First, the, fish were exposed for $3-4$ weeks, rather than the 80 d used by Woodward (1976). In general, a longer exposure time should yield a lower NOEC or LOEC, within certain limits. Thus, sublethal effects due to dinoseb might not be expected, especially if rainbow trout are less sensitive than are lake trout. However, this would not account for the absence of effects from endosulfan. Second, aeration of the test beakers, and the use of a static-replacement rather than flow-through test, probably reduced the concentration of the pesticides, especially endosulfan. A five-fold increase in LC50 values is the maximum expected (McLeay, 1989). It is not clear that this would have been sufficient to reduce endosulfan concentrations below the levels causing sublethal effects, but it is certainly a factor worth considering. However, $96-\mathrm{h}$ LC5Os as low as $300 \mathrm{ng} / \mathrm{L}$ endosulfan have been recorded in static bioassays (McLeay, 1989).

Statistical sensitivity refers to statistical power - the probability of detecting an effect of a given magnitude (Peterman, 1990). Power depends on variability, the number of replicates, and the statistical test used. The power of contrasts varies depending on the hypothesis tested. Therefore, we cannot give a blanket power estimate for the alevin test (see Appendix II for details). We were able to detect significant differences in yolk and body weight between ditches in the February-March tests (Table 8). These differences were $\approx 5 \%$ of mean body weight, and $35 \%$ of mean yolk weight. For comparison, the significant difference in body weight between controls and $500 \mathrm{ng} / \mathrm{L}$ in Woodward (1976) represented a $\mathbf{3 4 \%}$ reduction; in general, MATCs (maximum acceptable toxicant concentrations or the geometric mean of NOEC and LOEC) from chronic effects bioassays are equivalent to a $\mathbf{2 5 \%}$ difference from controls (Suter et al., 1987). Thus, we are confident that our test was statistically as powerful or more powerful than alternatives.

## Hypothesis 3: The pesticides were not available for uptake.

Pesticides in water samples may be in solution, available for uptake (bioavailable), or sorbed to particles or other compounds, and thus unavailable for uptake. The fraction in solution can be calculated from the total suspended solids, the organic carbon content of the particles, and the organic-carbon normalized solid-liquid partition coefficient ( $K_{\alpha c}$ ) (Oliver, 1987). Oliver also provides
a relationship for calculating $K_{\mathrm{oc}}$ from the octanol-water partition coefficient ( $K_{\text {ou }}$ ). Thus, the fraction in solution in our February samples can be calculated using the observed TSS of $30 \mathrm{mg} / \mathrm{L}$, a maximum organic carbon content of $40 \%$ (the organic carbon content of biological organisms such as bacteria and algae), and $K_{\text {ow }}$ values given in CCREM (1987) and MacDonald et al. (1990). The fraction of the pesticides in solution would then be $80 \%$ (or $20 \%$ sorbed). Thus sorption would not remove a significant amount of pesticides from solution. We emphasize that we tried several approaches to calculating the fraction in solution, including using the formula of DiToro (1985); which assumes that sorption to suspended particles is greater than to sediment particles. The approach discussed above gave the maximum fraction sorbed.

The effects of pesticides can also be altered by water quality parameters. For example, the toxicity of dinoseb increases with decreasing pH . Considering that the ditch waters in February were more acidic than at any other time of year (Figure 8), pH was unlikely to reduce toxicity. There is some evidence that contaminants may be associated with dissolved organic molecules and, thus, unavailable for uptake. Oikari and Kukkonen (1990) observed a 5 -10-fold reduction in bioaccumulation of benzo(a)pyrene by Daphnia as dissolved organic carbon (DOC) levels increased from 1 to $20 \mathrm{mg} / \mathrm{L}$ Assuming that bioaccumulation has some direct relationship with toxicity, and that most bioassays are conducted using water with low DOC, the high DOC in the ditch waters ( $=25 \mathrm{mg} / \mathrm{L}$ total organic carbon, which includes particulate as well as dissolved) may have reduced the availability and toxicity of the target pesticides. From Oikari and Kukkonen (1990), the maximum estimated reduction in effective concentration would be 5 -10-fold, probably sufficient to reduce or eliminate sublethal effects. However, Capel and Eisenreich (1990) estimate that DOC is $\approx 50 \%$ as effective as particulate O'C at removing contaminants. Assuming that DOC in the ditch waters was at least equal to particulate TOC ( $\{25-\{0.4 \times 30\}=\{0.4 \times 30\}$ ), an additional $20 \%$ of the pesticides would be associated with DOC, which leaves $60 \%$ in solution. Thus, it is unclear whether DOC could remove sufficient pesticide to eliminate effects, and the extent of removal probably depends on the type of DOC present.

Organic molecules are not the only molecules which can reduce the effective concentration of pesticides. Greve and Wit (1971) noted that iron in solution ( $14 \mathrm{mg} / \mathrm{LFe}$ ) effectively removed endosulfan, apparently by sorption and by acting as a catalyst for hydrolysis. Iron levels in the ditches and river were high (Figure 14), although not $>14 \mathrm{mg} / \mathrm{L}$. These authors also noted that suspended silt removed $80 \%$ of the endosulfan; their statement is misleading. After centrifuging water samples they found that $80 \%$ of the endosulfan was associated with the lower silt-containing fraction. However, that fraction was still liquid, with silt levels of only $25-50 \mathrm{mg} / \mathrm{L}$. Actual measurements of concentrations on silt particles indicated that solid-liquid partitioning coefficients were not greater than those we used in our calculations.

In conclusion, the concentrations of pesticides, especially endosulfan, in the ditch waters in February were high enough that biological effects should have been detected uniess the effective concentrations were somehow
reduced. Therefore, either the static-renewal procedure or removal by DOC or iron was responsible for the absence of effects; the data do not exist to decide which factor was responsible.

### 4.1.2.2 Field Monitoring

In contrast to laboratory bioassays, the artificial substrates and alevin in situ test provided some indications that discharges to the river, and the ditch waters themselves, had impacts on aquatic biota. The major question is whether these impacts can be attributed to the target pesticides or other contaminants.

The in situ test demonstrated that alevins performed poorly in the ditch waters relative to the river. It is unlikely that the target pesticides, or any other lipophilic and/or bioaccumulated compound, was responsible for the poor performance in the ditches. First, the pesticides were not present in detectable levels, and no effects were observed in laboratory bioassays conducted at the same time. However, our sampling could easily have missed a pulse of high pesticide levels during the 4 -week exposure period, and, the field tests may be more sensitive than the laboratory tests because they are flow-through. Second, we were unable to observe any reduction in performance downstream in the river, associated with the addition of ditch water. However, the dilution factor may have been sufficient to eliminate effects in the river. Also, survival was variable, and replication limited, which reduced statistical power. Third, the alevins from the ditch waters performed as well as those from the river when reared in the laboratory for 10 d . Lipophilic compounds; such as, the target pesticides should accumulate in the lipid-rich yolk, and result in delayed effects when the yolk is used and the compounds liberated into the body (e.g., Solbakken et al., 1984). (In Section 1.4, we indicated that the target compounds were eliminated rapidly, but "rapidly" refers to elimination in days, rather than months or years.) The evidence for delayed effects on juveniles briefly exposed to endosulfan and then transferred to uncontaminated water is admittedly equivocal (McLeay, 1989); these delayed effects have not been examined in larvae or alevins.

The most probable cause of the poor performance of alevins in ditch waters was low oxygen levels. The dissolved oxygen was low in the ditches on occasion (Table 1), and the cages and sandwiches in the ditches were more extensively clogged with particulate matter than those in the river. However, we cannot rule out the possibility that some non-accumulated compound such as nitrite or nitrate was responsible for the poor survival in the ditches.

The abundance of Ephemeroptera and Plecoptera, both absolute and relative to Chironomidae, declined from upstream to downstream, whereas total abundance (primarily Chironomidae and Oligochaeta) increased. Both trends have been associated with environmental stress or effluent discharges in previous studies (e.g., Gibbons, 1991; see Plafkin et al., 1989 for a review). Using the system of Biological Scoring Criteria in Plafkin et al. (1989, p. 6-27), the redụction in EP/C we observed from upstream to downstream ( $>90 \%$ except in DecemberJanuary) would result in the classification of the downstream areas as severely impaired. However, the scoring criteria should really be averaged over several indices, rather than derived from only one. We would have
preferred to use more indices, but the taxa present in the Nicomekl were not ones commonly used to calculate indices, which are primarily based on insects. The raw data in Volume II include calculation of some other indices for the interested reader.

It would be difficult to attribute the downstream changes in EP/C and abundance specifically to the target pesticides. First, downstream changes such as those observed can occur naturally, although usually over a much lònger stretch than our study reach (e.g., Munkittrick et al., 1990; Gibbons et al., 1991; Kilgour and Gibbons, 1991). Because downstream drift would be an important source of colonizing invertebrates, the community changes we observed may reflect impacts further upstream, rather than in our study area. Second, other compounds which could have produced the observed effects were present. The EP taxa, for example, are especially intolerant of metals relative to Chironomidae (Plafkin et al., 1989). Nutrient addition would be an obvious potential cause of increased abundance downstream. Third, there was no evidence that the downstream trends were strongest when pesticide levels were highest (February-April). Abundance actually increased downstream, which would be difficult to reconcile with insecticide use. The contrast used was statistically less powerful than other contrasts, and if the pesticides affected young and small invertebrates $(<250 \mu \mathrm{~m})$, the effects might not be apparent until later when the affected taxa were large enough to retain in sieves.

### 4.1.3 Status of the Target Pesticides

We conclude that dinoseb and endosulfan had no measurable chronic effects on the biota of the Nicomekl River. The pesticides may have contributed to some overall degradation of macroinvertebrate communities from upstream to downstream, but their contribution was minor. Concentrations of the pesticides in the Nicomekl River were generally low and below CCREM guideline values except in February, 1990. Dissolved organic molecules and iron in the river water may further reduce the effects of these low concentrations. Concentrations in ditch sediments are lower than those recorded by Wan (1989) in a nearby ditch, suggesting that use has been declining. Certainly our interviews indicated that dinoseb should not be a pollutant of concern after recent and proposed future restrictions. The CCREM guidelines appear adequate in terms of protecting aquatic biota; although we argue elsewhere that, in general, guidelines have very little predictive value; and their derivation does not maximize the use of available information (Section 4.3.5).

Dinoseb and especially endosulfan levels in ditches in winter may be high enough to affect ditch biota, if our failure to detect effects of ditch water in February-March can be attributed to the use of a static-renewal rather than flow-through bioassay. Reviewers of our interim report suggested that we should also monitor pesticide levels in the ditches immediately after application in spring-summer, 1990 [this would account for aerial pesticide drift only, as Wan (1989) demonstrates that runoff is negligible until fall]. This proved to be impossible as no farmer would admit to using endosulfan, and dinoseb should not have been in use. In retrospect, acute effects immediately after spraying should have been measured, especially in the Nicomekl River (i.e., as opposed to the ditches only), even if pesticides other than the target pesticides were being

[^1]sprayed. There is no question that concentrations of pesticides in nearby waters can reach lethal levels immediately after spraying (e.g., Wan, 1989; Ernst et al., 1991). However, the effects may be severely restricted in time and space. Their importance should be evaluated relative to more persistent (chronic) effects from factors such as nutrient addition, channelization and siltation.

Our major concerns with respect to chronic effects are effects of endosulfan (and other pesticides) in sediments, and in the marine environment of Boundary Bay. The results of this study and Wan (1989), plus the review in MacDonald et al. (1990), indicate that dinoseb will disappear rapidly from sediments following restrictions on use. Endosulfan, particularly $\beta$-endosulfan and endosulfan sulfate, are much more likely to persist in the sediments. In both this study, and Wan (1989), endosulfan was present in sediments throughout the year, even though levels did increase in the wet season. The compounds in the sediments can be released into the interstitial and bottom water after use has ceased. Ditch dredging may also liberate sediment-bound contaminants. Our residual oxygen bioassays with elutriate suggested that these sorts of effects were unlikely to be toxic, but we did not test for sublethal effects in elutriate or sediment bioassays. Direct accumulation of sediment-bound endosulfan by organisms feeding on detritus is unlikely to be a problem, given the rapid elimination of this compound.

Problems with pesticides in the Nicomekl River are probably transitory and largely restricted to the wet season or immediately after spraying. However, the river has been adding pesticides to Boundary Bay for years and much may still reside in the Bay sediments. These sediment loads may pose problems in the long term. Short term exposures of estuarine organisms to pesticides in the water column may also represent an environmental hazard, because marine organisms may be more sensitive than are freshwater organisms. The EPA (1986) marine acute criterion for endosulfan at $8.7 \mathrm{ng} / \mathrm{L}$ is almost an order of magnitude below the freshwater criterion of $56 \mathrm{ng} / \mathrm{L}$. Inflow from the Nicomekl is diluted in Boundary Bay, but the diluent is in part inflow from other rivers and streams carrying pesticides from agricultural areas.

### 4.2 Status of Nicomeld River

The Nicomekl River has been subjected to many physical, biological, and chemical impacts (this study; Swain and Holms, 1988a,b; McLeay, 1989). Discharge into the river is regulated by pumphouses; tidal flux is regulated by tide gates. Vegetation has been removed from the banks which have been built up to form dykes. Tributaries have been channelized into drainage or irrigation ditches, and these ditches and roadside ditches probably contribute, a substantial silt load. The flap gates on the ditch outlets restrict access to former salmonid spawning areas. 'The water is highly coloured so that visibility is limited. The only fish we observed during our field work were introduced carp (Cyprinus carpio), which used our anchor ropes as spawning substrate while we were unsuccessfully trying to locate a source of salmonid eggs to use for in situ bioassays. Faecal coliforms from dairy cattle and poultry farms enter the river and, presumably, Boundary Bay. Temperatures rise in the summer in the absence of shade, and dissolved oxygen levels decline to unacceptable levels. The list of water quality parameters exceeding criteria or guidelines was limited largely by the number
Discharge in the Nicomekl River $\mathbf{3 k m}$ upstream of the study area (from Water Survey Canada, Environment Canada).
Figure 7.

$$
\begin{gathered}
\text { Nickomekl River at } 203 \text { rd Street, Langley } \\
\text { January to December } 1990
\end{gathered}
$$


(s/\& $\varepsilon^{\text {(1) }) ~ ә б д е ч о s!ด ~}$

## Basic Water Quality



Figure 8. Basic water quality of Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $B=$ Burrows, $L=$ Logging).


Figure 9. Ion concentrations in the Nicomekl River (bars; Stations 1-4), and in the study ditches (triangles; $B=$ Burrows, $L=$ Logging).

## Physical Parameters



Figure 10. Suspended solids and colour for the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $\mathrm{B}=$ Burrows, $\mathrm{L}=$ Logging).
Nutrients




Metal Concentrations in Water


.......Water Quality Criterion (chronic effects)

- Water Quality Criterion (acute effects)

Figure 12. Cadmium and aluminum concentrations in the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $B=B u r r o w s, ~ L=L o g g i n g$ ). Cadmium was not detected in sediment samples; aluminum was not measured.

## Copper

Water Concentrations


Sediment Concentrations


Figure 13. Copper concentrations in water and sediments from the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $\mathrm{B}=$ Burrows, $\mathrm{L}=$ Logging).


Water Quality Criterion (chronic effects)

Sediment Concentrations


Figure 14. Iron concentrations in water and sediments from the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $\mathrm{B}=$ Burrows, $\mathrm{L}=$ Logging).


Figure 15. Lead concentrations in water and sediments from the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $\mathrm{B}=\mathrm{Bu}$ urrows, $\mathrm{L}=\mathrm{Logging}$ ).

## Manganese

Water Concentrations


Water Quality Criterion (chronic effects)

Sediment Concentrations


Figure 16. Manganese concentrations in water and sediments from the Nicomekl River (bars; Stations $1-4$ ), and the study ditches (triangles; $\mathrm{B}=$ Burrows, $\mathrm{L}=$ Logging).

## Mercury

Water Concentrations


Sediment Concentrations


Figure 17. Mercury concentrations in water and sediments from the Nicomekl River (bars; Stations 14), and the study ditches (triangles; $\mathrm{B}=\mathrm{Bu}$ (rrows, $\mathrm{L}=\mathrm{L}$ ogging).

.-.-Water Quallty Criterion (chronic elfects)

## Sediment Concentrations



Figure 18. Zinc concentrations in water and sediments from the Nicomekl River (bars; Stations 1-4), and the study ditches (triangles; $B=B u r r o w s, L=L o g g i n g$ ).


Figure 19. Results of rainbow trout alevin bioassay, December, 1989 - January, 1990. Values are means $\pm 1$ SE. Regression line on bottom graph excludes controls.

Rainbow Trout Alevin Bioassay February to March, 1990


Figure 20. Results of rainbow trout alevin bioassay, February - March, 1990. Values are means $\pm 1$ SE. Regression line on bottom graph excludes controls.

## Rainbow Trout Alevin Bioassay November, 1990



Figure 21. Results of rainbow trout alevin bioassay, November, 1990. Values are means $\pm 1$ SE. There was no significant relationship between body and yolk weight.

## CERIODAPHNIA

December, 1989


February, 1990

$\square$ Control ZZ Burrows Upstream

Figure 22.
Results of Ceriodaphnia 7-d bloassays. Bars are 1 SE:


## Duckweed -- April to May, 1990




Figure 24. Results of 7-d duckweed bioassay. Values are means $\pm$ i SE.

Nicomekl River Macroinvertebrates -Miscellaneous Taxa




Figure 25. Relative abundance of miscellaneous invertebrate taxa colonizing artificial substrates in the Nicomekl River. Values are means of 4-6 substrates.
Figure 26. Relative abundance of crustacean taxa colonizing articial substrates in the Nicomekl River. Values are means of 4-6 substrates.

Nicomekl River Macroinvertebrates -- Crustaceans

Relative

## Nicomekl River Macroinvertebrates -- Insects



Figure 27. . Relative abundance of insect taxa colonizing artificial substrates in the Nicomekl River. Values are means of 4-6 substrates.

## Nicomekl River -- Macroinvertebrates




Figure 28. . Total abundance (no. per substrate), and the abundance of Ephemeroptera plus Plecoptera relative to Chironomidae (EP/C as \%), for substrates from the Nicomek! River. Values are means of 4-6 substrates.

## Rainbow Trout Alevin, In Situ Bioassay: Field Growth



Figure 29. Growth of rainbow trout embryos/alevins reared for 4 weeks in the Nicomekl river and in Burrows ditch. Values are means $\pm 1$ SE.

## Rainbow Trout Alevin In Situ Bioassay -- Lab Growth




Figure 30. Growth of rainbow trout alevins reared in the laboratory for $9 \mathbf{d}$ after being reared in the field for 4 weeks. All values are means $\pm 1 \mathrm{SE}$, and refer to the change in weight in the laboratory. There was no relationship between change in body weight and change in yolk weight.


## APPENDIX I

## Chemical QA/QC

## 1. First Round

Sediment and water collected from upstream of Station 1 were spiked with dinoseb and endosulfan by Environment Canada laboratory personnel. Subsamples were shipped to Zenon for analysis. Environment Canada also analyzed subsamples using the same procedures. The results revealed that Zenon was using a defective Florisil column and were thus unable to recover $\beta$-endosulfan and endosulfan sulfate. This problem was rectified after the first round of QA/QC. The results for water samples were:

| Analyst |  |  | \% Recovery |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | $\alpha$-endosuifan |  |  |  |
|  |  |  | $100 \mathrm{mg} / \mathrm{L}$ | $5170 \mathrm{mg} / \mathrm{L}$ |  |  |
| Zenon | 20 | 570 | 45 | 104 |  |  |
|  | 11 | 460 | 69 | 103 |  |  |
| Environment Canada | $<24$ | 114 | $<20$ | 12 |  |  |

Both Environment Canada and Zenon did not detect dinoseb or endosulfan in the original matrix which was the only point of agreement. The high spike solution was kept in storage ( $4^{\circ} \mathrm{C}$, dark) for an additional week, and then another sample sent to Zenon. (We were interested in loss during storage.) They measured dinoseb and endosulfan concentrations within $15 \%$ of their initial measurements. We suspect this is evidence that pesticide loss was minimal during storage; but under the circumstances, it was impossible to be confident.

Obviously, the results for dinoseb were unacceptable; the low recovery of endosulfan from the low spike was disturbing. The sediment spike results were even worse as recovery for low and high spikes was $1-2 \%$ for both analysts. Zenon argued that the spike levels had been miscalculated as their recoveries were a reasonably consistent $1-2 \%$ for both dinoseb and endosulfan (and $\beta$-endosulfan and endosulfan sulfate when samples of the remaining extract were run through a functioning Forisil column).

The obvious solution was to conduct another round of analyses, and Environment Canada prepared and shipped water and sediment samples in February, 1990.

## 2. Second Round (February, 1990)

Environment Canada apparently provided a second set of spiked sampled to Zenon in February, 1990. However, no results were ever received by EVS Consultants from either Environment Canada or Zenon despite repeated requests. Either the samples were never sent or one or both parties misplaced the samples or the results. Zenon, therefore, offered to conduct a third round of sample spiking in the spring of 1991 after the study had been completed.

## 3. Third Round (May - June, 1991)

EVS Consultants provided samples of water and sediment from the Upstream reference station; Zenon spiked these samples with various concentrations of dinoseb and endosulfan. Results are provided in Tables 1-1 and

I-2. Recoveries of $\alpha$ - and $\beta$-endosulfan in water samples centred around $100 \%$ ( $92-115 \%$ ), but recoveries of dinoseb and endosulfan sulfate were lower ( $72-92 \%$ for dinoseb, $67-70 \%$ for endosulfan sulfate). Surrogate (various brominated phenols) recoveries ranged from $60-127 \%$; the range included the full range of recoveries for the target compounds. The target compounds were not detected in blanks.

Recoveries of the target compounds from sediments were all $<100 \%$ (Table 1-2). Recoveries of dinoseb (72$88 \%$ ) were greater than recoveries of the endosulfan compounds ( $50-72 \%$ ). Surrogate recoveries ranged from $60-140 \%$ and centred around $100 \%$ suggesting that recovery of the surrogates was more complete than recovery of the target compounds. The target compounds were not detected in blanks.

These results suggest that the recoveries of the target compounds were generally good. Concentrations may have been underestimated for dinoseb and endosulfan sulfate in water, and dinoseb and especially the endosulfan compounds in sediments. Also the concentrations in the spiked samples were 25 times detection limits so the conclusions may not extend to samples with lower concentrations. The spiked samples were also prepared and analyzed after the study finished, and the samples were not spiked by an independent laboratory (e.g., Environment Canada). Thus, the results may not be applicable to concentrations measured during the study. However, Zenon did ensure that the same methods and technicians used during the study were also used in the analysis of the spiked samples.

Table 1-1. Recovery of dinoseb and endosulfan in spiked water samples, May, 1991.

| Compound | High Concentration |  | Low Concentration |  |
| :--- | :---: | :---: | :---: | :---: |
|  | Added (ng/L) | \% Recover | Added (ng/L) | \% Recovery |
| Dinoseb | 1,000 | $79-85$ | 250 | $72-92$ |
| Endosulfan |  |  |  |  |
| $\alpha-$ endosulfan | 630 | $103-105$ | 130 | $92-115$ |
| B-endosulfan | 630 | $106-110$ | 130 | $92-115$ |
| Endosulfan sulfate | 630 | 65.70 | 130 | 69 |
| Surrogates |  |  |  |  |
| Br2Pehnol | - | $76-86$ | - | $60-77$ |
| BrPhenol | - | $94-104$ | - | $66-90$ |
| Br2Pehnol2 |  | 127 | - | $120-122$ |

NOTE: Recoveries are based on two replicate extractions

Table 1-2. Recovery of dinoseb and endosulfan in spiked sediment samples, June, 1991.

| Compound | High Concentration |  | Low Concentration |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Added (ng/L) | \% Recover | Added ( $\mathrm{ng} / \mathrm{L}$ ) | \% Recovery |
| Dinoseb | 125 | 88 | 25 | 72-80 |
| Endosulfan $\alpha$-endosulfan $\beta$-endosulfan Endosulfan sulfate | $\begin{aligned} & 62.5 \\ & 62.5 \\ & 62.5 \end{aligned}$ | $\begin{aligned} & 59-64 \\ & 53-54 \\ & 56-59 \end{aligned}$ | $\begin{aligned} & 12.5 \\ & 12.5 \\ & 12.5 \end{aligned}$ | $\begin{aligned} & 67-72 \\ & 50-51 \\ & 50-51 \end{aligned}$ |
| Surrogates Br 2 Pehnol Br3Phenol Br2Pehnol2 | - | $\begin{gathered} 102-105 \\ 116-140 \\ 89-92 \end{gathered}$ | $\stackrel{-}{-}$ | $\begin{aligned} & 60-78 \\ & 82-90 \\ & 88-95 \end{aligned}$ |

NOTE: Recoveries are based on two replicate extractions.


## APPENDIX II

## Contrasts and Hypothesis Testing

A contrast $(L)$ is a linear combination of treatment means $\left(M_{1}\right)$ :

$$
L=c_{1} M_{1}+c_{2} M_{2}+\ldots c_{r} M_{1}
$$

The coefficients ( $c_{i}$ ) usually sum to 0 , and are set up so that $L$ is some difference of interest. For example, we might compare a control ( $M_{1}$ ) to some treatment (say $M_{4}$ ):

$$
L=(1)\left(M_{\Lambda}\right)+(-1)\left(M_{1}\right)
$$

We then test the hypothesis that $L=0$ to determine if there is a significant difference. For any study with $a$ treatment means, there are ( $a-1$ ) independent (orthogonal) 1 -degree-of-freedom (d.f.) contrasts.

If a set of $j$ contrasts is orthogonal, then:

$$
\Sigma \dot{c}_{1}=0
$$

For each contrast; and

$$
\Sigma\left(c_{h_{1}}\right)\left(c_{c_{2}}\right) \ldots\left(c_{l_{2}}\right)=0
$$

Consider the example of the contrasts used in laboratory bioassays (Section 3.3). The $c_{\mathrm{i}}$ were:

| Contrast | Treatment |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | C | U | B |  |  | L |  |  | $\Sigma$ |
|  |  |  | 10\% | 30\% | 100\% | 10\% | 30\% | 100\% |  |
| C vs others | -1 | $1 / 7$ | $1 / 7$ | 1/7 | $1 / 7$ | 1/7 | $1 / 7$ | $1 / 7$ | 0 |
| U vs ditches | 0 | -1 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| Between ditches | 0 | 0 | 1/3 | 1/3 | 1/3 | -1/3 | -1/3 | -1/3 | 0 |
| Product | 0 | 0 | 1/126 | 1/126 | 1/126 | -1/126 | -1/126 | -1/126 | 0 |

For each contrast $\boldsymbol{\Sigma} c_{\mathrm{i}}=0$, and the sum of the products of the $c_{\mathrm{i}}$ for each treatment is also 0 .

It is also possible to test multiple d.f. contrasts; for example, the seasonal differences among macroinvertebrate abundances (see Sokal and Rohif, 1981). In a two-factor ANOVA, it is also possible to use contrasts to test interactions of interest; for example, seasonal changes in downstream trends. The appropriate $c_{\mathrm{i}}$ are determined by multiplying $c_{i}$ for the main effects. Consider the simplified case of four river stations and two seasons:

| Season | Station | Contrast |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  | Season | Downstream (DS) | 8 DS |
| 1 | 1 | 1/4 | -3 | -3/4 |
|  | 2 | $1 / 4$ | -1 | -1/4. |
|  | 3 | 1/4 | 1 | 1/4 |
|  | 4. | 1/4 | 3 | 3/4 |
| 2 | 1 | -1/4 | -3 | 3/4 |
|  | 2 | $-1 / 4$ | -1 | - $1 / 4$ |
|  | 3 | -1/4 | 1 | -1/4 |
|  | 4 | -1/4 | 3 | -3/4 |

Why use contrasts instead of multiple comparison procedures such as Dunnett's Test? Multiple comparison procedures provide protection against making Type I errors (declaring a difference significant when it is not) when making comparison which are not independent. Consider the case of the $c_{\mathrm{i}}$ for all comparisons of 3 treatment means with a control:

| Contrast | C | 1 | 2 | 3 | $\Sigma$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| 1 vs C | -1 | 1 | 0 | 0 | 0 |
| 2 vs C | -1 | 0 | 1 | 0 | 0 |
| 3 vs C | -1 | 0 | 0 | 1 | 0 |
| Product | 1 | 0 | 0 | 0 | 1 |

The sum of the products is not 0 ; the comparisons are not independent. Multiple comparison procedures are always too conservative (declaring a difference not significant when it is real) when the comparison are independent. Furthermore, if an investigator cannot construct several specific independent hypotheses to test prior to an experiment, then perhaps the experiment should not be conducted. Most agricultural journals no longer accept multiple comparison procedures, and contrasts are widely used in agricultural experiments. It should be noted that linear and quadratic contrasts, coefficients for which can be found in almost any statistical text, are ideal for examining dose-response relationships in toxicological experiments.

The drawbacks to using contrasts are that an LOEC or NOEC cannot be established, and that a surprising result cannot be tested by contrast specified a priori. The first drawback would not exist if dose-response relationships-were used to establish EC25s or some other effect percentile as Suter et al., (1987) suggest. It should be noted that NOECs established in different experiments depend on the number of treatments compared, and the sample size so that comparison of NOECs from different studies is a dubious exercise. The second drawback can always be eliminated by using a posteriori multiple comparison procedures to test
surprising results. Designing experiments specifically testing a priori for the existence of these surprising results would be a wise follow-up.

Two final points should be made. First, using contrasts (and multiple comparison procedures) becomes more complicated when sample sizes are unequal. Most statistical programs (e.g., SAS, SYSTAT) have general linear model packages that will ensure contrasts are independent even if sample sizes are unequal. Second statistical power calculations are possible for contrasts. ,The basic formula for $t$ tests is (Sokal and Rohlf, 1980):

$$
n \geq 2(s / \delta)^{2}\left(t_{e}+t_{2(1-\mu)}\right)^{2}
$$

where:
$n$ = sample size
$s=$ standard deviation
$\delta=$ size of difference to be detected
$t$ = student $t$-value
$\alpha=$ significance level (usually 0.05 )
$P=$ probability of detecting the difference
Usually the investigator wants to find the appropriate $n$ to have a high probability ( $P$ ) of detecting some difference $\delta$, but the formula can be rearranged to solve for $P$ or $\delta$. For contrasts, one should substitute $\Sigma c_{i}^{2}$ for 2 (which is $\Sigma c_{1}^{2}$ for comparing two means) and use $t$-values for the total error d.f. For multiple comparisons, the appropriate test statistic (e.g. Dunnett's $t$ ) is substituted for Student's $t$. The formula for $n$ must be solved iteratively as the error d.f. change with $n$. (Approximations and computer packages are available to eliminate iteration by hand.)

Two cautions should be noted when calculating sample sizes or power for contrasts. First, unequal sample sizes will complicate the calculations if power is calculated after the fact. It is also possible to increase power a priori by using deliberately unequal sample sizes when, for example, there are more treatments than controls or more stations upstream of an effluent discharge than downstream.

Second, investigators should take care to calculate exactly what $L$ is when using linear or interaction contrasts. The significance of $L$ does not change if the coefficients are multiplied by a constant but, obviously, the value of the difference will depend on the coefficients. In other words, testing $10\left(M_{1}-M_{2}\right)=0$ is the same as testing $\left(M_{1}-M_{2}\right)=0$, but $10\left(M_{1}-M_{2}\right) \neq\left(M_{1}-M_{2}\right)$.

Finally, an example of the power of the alevin bioassay to detect differences between upstream and ditch waters using the contrast given earlier. The calculations were based on 3 reps for each of 7 treatments (controls excluded) and the variance ( $s^{2}$ ) for the February - March tests. Values given are 8 (mg) or the differences that could be detected with a given probability of $P$.

| Variable | $s$ | $\boldsymbol{P}$ |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  |  | 0.95 | 0.80 | 0.50 |
| Body weight | 7.3 | 17.0 | 13.1 | 9.3 |
| Yolk weight | 2.0 | 4.6 | 3.6 | 2.6 |

With an overall mean of 120 mg for body weight and 28 mg for yolk weight, we had $=50 \%$ probability of detecting a $10 \%$ difference and $\mathbf{> 9 5 \%}$ probability of detecting a $20 \%$ difference. The contrast testing differences between ditches was even more powerful (compare $\Sigma \boldsymbol{c}_{\mathrm{i}}^{2}$ ).
of criteria available and the number of parameters measured. Although endosulfan and dinoseb may not be major concerns, other pesticides have replaced them. The record of past use of pesticides, including DDT, still remains in the sediments. The oil spill in June, 1990 served as an example of the river polluting the drainage ditchés.

Despite these impacts, laboratory bioassays indicated only enhanced growth and/or reproduction due to nutrient addition (algae, duckweed, Ceriodaphnia), or water hardness (alevins). The suitability of control waters for the bioassays is questionable. We also note that the bioassays cannot test the effects of prolonged exposure, and the effects of factors such as physical alterations to habitat. The downstream decline in the relative abundance of Ephemeroptera and Plecoptera, and the increase in abundance of tolerant taxa such as Chironomidae and Oligochaeta, are probably a more accurate reflection of impacts on biota.: Even the estuarine isopod Gnorimosphaeroma successfully colonized substrates at the upstream sites, rather than those at the downstream sites closer to the estuary. The introduced carp seem to be thriving in the area, whereas the salmonids, especially cutthroat trout, may be declining. Any young salmonids migrating downstream will experience problems seeing prey, feeding efficiency and growth decline rapidly with increasing turbidity (Everest et al., 1985). The recreational fishery is limited, perhaps because fish cannot see lures or flies.

The various impacts on the river may be antagonistic to some extent. For example, high iron levels may reduce the effects of endosulfan. The potential for increased algal growth from elevated nutrient levels is probably cancelled by the negative effects of reduced light penetration in the coloured waters, and the absence of a suitable substrate for attached algae. The hard water and, perhaps, the high DOC, may limit the effects of metals. These sorts of interactions may explain the absence of effects in laboratory bioassays. The balance ' between positive and negative impacts should not be a reason for complacency.

Given the large number of impacts in the Nicomekl River, and the altered nature of the habitat, remediation may seem an impossible task. The local farmers were strongly opposed to any attempt to restore streamside vegetation to the river, as the roots tend to crack the dyke walls resulting in salt water intrusion. As long as the area is used for agriculture, there will be a drainage/irrigation system-in place, and the river will never revert to its original state.(probably lowland river/marsh). Some of the compounds present at elevated levels, such as iron, may have natural sources (Siwain and Holms, 1988a,b). Others, such as aluminum, may originate upstream of the study area, and remediation would involve control at the source. The primary compounds. of concern in the study area would be nutrients, ions, metals, and pesticides.

Fertilizers, commercial or manure, are the most obvious source of phosphorous, nitrogen, perhaps organic carbon, ions (especially potassium), and trace elements (e.g., copper, manganese, zinc). This is why the ditch. waters were such effective nutrient sources for algae and duckweed. There is also nutrient addition from cattle and chicken manure. Runoff from these latter sources can be controlled by good farming practice, and we question the need for fertilizer addition to the extent that occurs. Pesticide use has been curtailed, especially by Surrey Municipality, and the farmers have switched to less toxic pesticides. The installation of pumphouses; with pumps drawing water from near the surface, reduces the silt load. However, the flow and water level in

[^2]the river fluctuates more with the sporadic ditch discharge. Small deeper ponds on roadside ditches might settle silt and metals but would probably not be effective during flood conditions. Diffuse contamination from agricultural runoff will continue to be the major problem in the study area for some time, but the problem could be reduced. The best way to limit the impacts of the many users may be to include environmentally sound practices as part of the extension services offered by Agriculture Canada and the provincial Ministry of Agriculture, Food and Fisheries (B.C. MAFF). The federal and provincial focus on Integrated Pest Management (IPM) should reduce pesticide effects, although a similar emphasis on reducing nutrient and other effects may be more beneficial to aquatic environments in agricultural areas.

### 4.3 Monitoring Pesticides in Other Areas

A good environmental monitoring program integrates chemical analyses with laboratory bioassays and field monitoring of resident biota (Chapman et al., 1987; 1991; Power et al., 1991). The objectives should be to estimate the effects of the target pesticides on the resident biota, and to evaluate the effectiveness of water quality guidelines based on laboratory tests for protecting field populations. The present study attempted to meet these objectives by using chemical analyses, bioassays, and field monitoring. There is clearly room for improvement in any subsequent pesticide monitoring programs Environment Canada undertakes. Below, the three major components - chemical analyses, laboratory bioassays, and field monitoring - as well as overall strategy, are discussed. We then discuss methods of developing and assessing guidelines which have predictive. as well as regulatory (protective) value.

### 4.3.1 Chemical Analyses

The major concerns with chemical analyses are technical (lowering detection limits, ensuring that procedures are accurate and precise), and strategical (sampling on the appropriate temporal and spatial scale, measuring the appropriate compounds). Technical concerns receive a disproportionate share of attention, and monitoring, studies may be distorted as a result. Chemical data should be treated in the same way as any other variable in a study or experiment. ${ }^{-}$

Pesticides monitoring programs should include QA/QC analyses of spiked samples, as described in Appendix I. However, this is a small part of calibrating chemical analyses, and is the equivalent of trying to minimize measurement error for rulers or balances' used in a fish growth study. Measurement error is, or should be, a trivial source of variance relative to spatial or temporal heterogeneity among samples (discussed below), and variance among laboratories. Another distinction which should be made is between the MDL (method detection limit) and the PQL (practical quantitation level). The MDL is the lowest level which is distinguishable from zero (i.e., a "plus-minus" distinction); the PQL is the lowest level at which quantitative comparisons should be made (Federal Register, 1985). The PQL should really be estimated from interlaboratory comparisons, but can be roughly approximated by $5-10$ times the MDL. The MDL is only
useful for stating whether a compound is or is not present in a particular sample. The PQL is the appropriate lower limit for comparisons among studies and comparisons with guidelines. Thus, in our study, it.might have been more appropriate to set the PQL, rather than the MDL, at the guideline levels, so that we could be reasonably confident that exceedances were real. However, even that does not help comparisons with past studies, as we cannot change PQLs from the past (i.e., the PQL for our study would only apply to comparisons with studies using the same procedures).

Once technical variability has been accounted for by QA/QC analyses of spiked samples, and establishment of PQLs and MDLs, the next step is to account for spatial and temporal heterogeneity in sample collection. Assessing temporal heterogeneity usually involves sampling on a monthly or seasonal basis. Based on the discussion in Section 4.1.1, sampling should be concentrated in the wet season, which would be October to March in the Fraser Valley. Investigators must accept that the timing of maximum concentrations within this period will be unpredictable. They should also be aware that if short-term variability is high, spot samples taken monthly may not reflect real seasonal patterns. Thus, it is possible that brief exposures to high concentrations may be missed (a problem of estimating extremes), and that the spot samples will not represent average conditions over longer term exposures (a problem of estimating means without replication). As discussed in Haith (1987), a statistical approach to these issues would require long-term sampling and frequent expensive analyses. From a more practical perspective, investigators may wish to focus sampling on days immediatèly after heavy rains, and to composite samples over time. The first solution requires a flexible work schedule; the second solution may be expensive. If pesticide loss during storage in the cold and dark were minimal, samples could be composited over time by sampling frequently, and adding those samples to a large composite sample. This would be very costly if the sample site were far from the laboratory; there are automated devices used for effluent monitoring that might be adaptable for continuous field monitoring.

Ideally, spatial variability, especially in sediment samples, should be estimated from a pilot study examining variation at different level (among grabs, among composites from the same site, among different sites). This step, which measures an important source of variability, is often overlooked in monitoring program design. We urge Environment Canada and other interested parties to incorporate estimates of sampling variability into interlaboratory comparisons by requesting the participating laboratories to collect their own samples from a reference site, rather than simply analyzing a reference sample sent to them (see Hamilton, 1991, for a good discussion of the issue). In any specific study, if funds cannot cover the costs of estimating spatial heterogeneity in a pilot study, a practical compromise is to composite samples over an appropriate scale. The scale would depend on the objectives. In our study, we were interested in effects at specific river stations, and our composite samples included súbsamples from the sides and middle of the river. In general, taking a thorough composite of many subsamples over a broad area adds little to sampling costs, as the major costs are associated with equipment and getting to the study site.

The discussion above refers to measurement of pesticide concentrations from runoff or ditch discharge. Concentrations should also be monitored immediately after spraying. If appropriate arrangements can be made with local farmers, the time of sampling is predictable. Sampling should be extensive enough to measure
the temporal and spatial extent of expected high concentrations from aerial drift. The data can then be used to evaluate the longer term implications of any transitory lethal concentrations, to establish appropriate setback distances (i.e., the minimum distance between the area sprayed and the nearest water course), and to compare various methods of application (e.g., on foot, from a vehicle, from an aircraft).
'Planning monitoring studies also requires identifying which compounds should be measured. In the case of a pesticide study, this means identifying the most toxic widely used pesticides. In our study, we were fortunate that preliminary surveys had been conducted (e.g., Moody, 1989; McLeay, 1989; Wan, 1989). We were also fortunate that Environment Canada laboratories conducted analyses of other water quality parameters. In any system with numerous and diffuse sources, such as the Nicomekl River, these additional analyses are invaluable. They also serve to put the effects of pesticides in perspective.

The major problem we encountered was the rapid decrease in use of the target pesticides, especially dinoseb. We might have decided on other target compounds after more extensive interviews. However, the presence of dinoseb and endosulfan in the winter of $1989-90$ did not support the farmers' assertions that they no longer used these compounds. The farmers in the study area face the conflicting pressures of expanding suburb development, and environmentalist efforts to preserve or restore fisheries and wetland habitat. Hence, they are often resistant to questions about their practices. Moody (1989) also experienced problems obtaining accurate information from distributors, and went so far as to recommend that the interviewees be forced to comply with interviewers' requests. We suggest that surveys of pesticide use might best be conducted in conjunction with Agriculture Canada or provincial extension services.

### 4.3.2 Laboratory Bioassays

Laboratory tests can conveniently be separated into standardized bioassays (e.g., Ceriodaphnia, Selenastrum, rainbow trout juvenile LC50) and experiments (e.g., the studies of Woodward, 1976). The duckweed and alevin bioassays probably fall between these two types, as they have been conducted in similar fashion by others, but do not yet have standardized protocols. The advantage of the standardized bioassays is that results from different studies are comparable, and they provide a good relative measure of toxicity. Thus they can be used to monitor effluents or river sites over time to determine changes in toxicity. However, the tests or the organisms may not be particularly sensitive, and the standardized protocols (statistical tests, control waters) may not be appropriate for specific studies. In the case of pesticide studies, it is important that the tests control the effects of excess nutrients, which will be present in agricultural areas. Using the receiving water (e.g., Upstream) as a diluent and control or reference may solve this problem, but it removes the advantage. of comparability with other studies. The insensitivity of the test organisms to pesticide effects suggests that the bioassays should only be used when effects are expected to occur, and that the results should never be used as an indication of the magnitude of effects expected on resident or more sensitive biota.

Carefully conducted experiments can be used to match exposure conditions to those expected in the field, and to test hypotheses and species of specific interest. The advantage of these experiments is that they are more realistic, and usually more sensitive, than standardized bioassays. It would be very interesting to survey the lowest LOECs for a broad range of compounds; we suspect that these LOECs would come primarily from experiments conducted in the 1960 s and 1970 s, before the widespread use of standardized bioassays. Note that this is the case for dinoseb, with the lowest LOEC coming from Woodward (1976; his study also provides the lowest LOEC for picloram that we have seen). The experiments may not be comparable with those conducted by other investigators, and the costs of developing and conducting long-term flow-through tests can be high.

The duckweed and alevin bioassays represented an attempt to combine the best features of standardized bioassays and specific experiments. The duckweed bioassay probably combined the worst of the two test types - the organisms may be insensitive, the effects of excess nutrients were not removed, the test was expensive (more than the price actually charged), and the results were not really comparable with those of other investigators using somewhat different protocols. The only advantage was that the duckweed is a native vascular plant, which has some merit in a study of herbicide effects. The alevin bioassay; in contrast, seems much more promising. The test' species is native to the area, and sensitive to contaminant (especially pesticide) effects; the confounding effects of the presence of food sources in the test waters is a non-issue for the non-feeding alevins. The test can be shortened to reduce costs to a reasonable level; and the problems caused by soft water controls are easily solved. Although other studies may not follow our protocol exactly, and yolk conversion efficiency may not be calculated directly; yolk and body weight or length are commonly measured in early life stage tests (e.g., Woodward, 1976; the studies reviewed in Hodson and Blunt, 1981, 1986; Paine et al., 1988, 1990, 1991). The test is easily adapted to other species, which makes it useful for in situ tests of native species. The major disadvantage is that eggs are not available in late spring and summer.

The best approach to laboratory testing for pesticide monitoring would be to use some simple, fairly common experimental approach, with a sensitive species (preferably native). In most cases, this would mean an alevin, larval, or juvenile growth/development study with a salmonid species. Flexibility in experimental design and test species should probably be encouraged. Standardized bioassays should be used for screening or if a reasonable probability exists that effects will occur. Sediment bioassays may also be useful, although freshwater tests are not well-developed. We suggested a crayfish bioassay in our interim report (Paine, 1990), but that would be feasible only in the field. Recent experience in our laboratory with Daphnia magna bioassays with sediment look promising.

### 4.3.3

Field Monitoring

Macroinvertebrate community studies and in situ bioassays are probably the best methods for assessing pesticide effects in the field. Monitoring studies often assess fish populations as well, but the only fish in many lower Fraser Valley rivers may be anadromous salmonids and stickleback (Gasterosteus), or exotics such as carp. The advantage of field monitoring is that it provides a more direct measure of effects than do laboratory
tests, and may, be more sensitive. Control of factors other than pesticide effects is more difficult than in the laboratory, and it is very difficult to determine if effects are related to elevated concentrations of the target pesticides. In a system with multiple stresses, such as the Nicomekl, it is not clear that this is'a disadvantage if the effects really are due to the combined effects of the stressors. A potential disadvantage of field monitoring is that the results are rarely comparable with other studies, except in broad and relative terms. Therefore, a satisfactory internal control or reference site must be used, and hypotheses must be tailored to identify important patterns (e.g., by testing for downstream trends rather than simply comparing one or more impacted sites with the reference).

Our in situ test successfully documented significant differences between ditch and river waters, 'although the effects werealmost certainly not due to the target pesticides. Rearing the alevins in the laboratory after field exposure may be a promising means of separating the effects of accumulated compounds such as pesticides from those of nonaccumulated compounds or low oxygen. The approach would be verified if tissue levels of the target pesticides were measured before and after laboratory rearing. In sifu tests can be conducted with almost any species and life stage. Tests can be shorter with alevins and larvae, as weight-specific growth is faster. However, these stages are only seasonally available. Juveniles and adults are available year-round, and have broader temperature tolerances than do younger stages. Thus, the time of year would determine the best life stage to use.

In situ tests are also the best means of assessing effects immediately after spraying. Transporting samples to the laboratory would be logistically difficult in such cases, and problems in the field with vandalism, clogging of test containers, etc. would be largely eliminated with the investigators on site. Monitoring natural invertebrate or fish communities might not reveal transient acute effects if these communities were able to recover rapidly.

Macroinvertebrate communities are sensitive to pollutants and other stressors, and provide an almost continuous record of effects. This study was unusual, as the natural substrate/was not very suitable for macroinvertebrates, and there were significant'numbers of marine organisms colonizing the artificial substrates. As a result many of the indices given in Plafkin et al. (1989) could not be used. In other areas, the natural substrate may be more suitable for sampling, and insects may be more dominant. Then investigators can eliminate the extra costs associated with artificial substrates, and use a broader range of indices. We would, however, recommend that artificial substrates be used wherever natural substrates change from station to station, and that multivariate analyses be used as an objective method to establish which indices are most suitable. Our experience has been that the abundance of EPT taxa relative to Chironomidae and Oligochaeta, species richness, and total abundance are usually sufficient to assess impacts. Investigators should be wary of complicated derived indices, such as diversity. The component parts (evenness, richness) are better analyzed separately. Other indices, such as the abundance of various feeding groups or Hilsenhoffs Biotic Index, may require expensive identification to species, especially of Chironomidae, or may apply only to specific types of communities (e.g., rock-riffle).

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One addition we would like to see in macroinvertebrate monitoring is the use of total and mean biomass for the entire community, or for individual dominant taxa. The results could then be placed in the context of the extensive literature on the ecological importance of body size (see Peters, 1983 for a review). Biomass, rather than numbers, may be a better measure of nutrient effects, and we suspect that a decline in mean biomass would be a useful indicator of environmental stress. Minimal effort and cost would be required to dry and weigh entire samples, or subsamples of the dominant taxa:

Investigators will always find it difficult to relate effects on macroinvertebrates to/target pesticides, and to assess the effects of short-term "slugs" of high concentrations. The macroinvertebrates provided an indication of overall effects of all stressors over longer periods of time (probably equal to the average life cycle of the organisms) - they are integrators. If factors other than target pesticides are responsible for effects, or if the invertebrates recover quickly from the effects of short-term "slugs", then perhaps we should not focus on the pesticides.

However, it is necessary to relate effects to specific causes, in order to implement effective remediation. We attempted to do so by examining time-site interactions which test whether the greatest difference among sites occurred when pesticide concentrations were highest. This is the generally recommended approach for environmental impact studies (Green, 1989). It works well when assessing the effects of improved effluent treatment using years rather than seasons within years as the time periods (Gibbons, 1991). However, the tests used are statistically less powerful than are other simpler tests of time or site effects, and the pesticide effects, may not be immediately apparent if they are greatest on early instars. In situ tests could be used, if the high concentrations were predictable. Unfortunately, these maxima will probably occur in winter, when growth of any test organism will be negligible. Thus, it may only be posssible to test effects on survival.

### 4.3.4 Overall Monitoring Strategy

There are two important points to be made with respect to overall strategy:

1. the effort expended on the various components (chemical analyses; laboratory bioassays, field monitoring) should be consistent with the objectives;
2. some screening procedures can be used to limit costs, but all three components are necessary for assessing pesticide effects.

The first point may seem obvious but is usually violated (and was in this study). In this study, approximately one-half of the budget was spent on costs common to all three components - transportation and labour for field trips, project management, report writing, data analysis, interviews with farmers and other individuals. Of the remaining one-half, $50 \%$ went to chemical analyses (not including the costs of the chemical analyses conducted by Environment Canada), $\mathbf{2 5 \%}$ went to laboratory bioassays, and $25 \%$ went to field monitoring.

The focus of preliminary phases was almost exclusively chemistry (Wan, 1989), or split between chemistry and a literature review of pesticide toxicity in laboratory bioassays (McLeay, 1989). We appreciate that pesticide concentrations must be measured in pesticide monitoring studies, that the high cosis of these analyses are unavoidable, and that there probably was no literature on effects on actual biological communities to review. However, not one single biological test was conducted in four years of preliminary studies. A more effective and logical distribution of costs and effort would be an equal split between the three components, which would be consistent with the objectives stated in Section 1.2.

Sampling and testing strategy in a monitoring program must also match objectives. If chronic effects are of interest, chronic tests should be used, and if possible, chemical concentrations should be averages over the appropriate exposure period. If the effects of short-term "slugs" are of interest, then some means of predicting these "slugs" must be developed, and acute tests and short exposures must be used. If effects on river biota, rather than ditch biota, are of interest, then samples should be taken predominantly from the river, and the river biota should be sampled. Again, in the preliminary studies (Wan, 1989; McLeay, 1989) samples were taken almost exclusively from ditches, rather than receiving waters. If the variation of interest is between times, or between different sites, then temporal and spatial variation, not instrument error, should be the focus of QA/QC programs. If effective remediation is to be implemented, then the stressors of concern should be identified and receive priority, even if these stressors are not the target pesticides.

There are some screening procedures which could be used to reduce the costs of monitoring programs. Several reviewers of the interim report noted that we should not have been conducting laboratory bioassays on samples in which pesticide concentrations did not exceed CCREM guidelines. This criticism was warranted, but we would recommend using some level higher than the CCREM guidelines as the level at which laboratory testing would be initiated ( $=$ screening level). The probability of observing effects at the guideline levels in laboratory tests should be remote (see Section 4.3.5), and many "no effect" results would be generated if the guidelines were used as a screening levél.

The appropriate screening level can be established in two ways. The first would be to use the regression approach described in Section 4.3 .5 to calculate the concentration at which there is a high probability (e.g., 25 or $50 \%$ ) of detecting effects. A better approach would be to establish NOECs by spiking samples of the receiving water with known quantities of the target compounds; the spiked samples could also be used as part of the chemical QA/QC program. The sensitivities of potential tests could easily be established using this approach and, then, the most sensitive test selected for future use. If tests conducted on actual samples with levels greater than this screening level subsequently revealed no effects, then it would be reasonable to conclude that there were some antagonistic factors present.

We would still recommend conducting some tests on samples with levels below the NOEC, in order to determine if other factors, or those factors combined with low pesticide levels, had effects. Chemical analyses results would also not be available until several weeks after sample collection. If the screening level were exceeded, the sample might not be available, and concentrations in ditches or rivers might have declined by
the time a sample is collected for laboratory bioassays. Thus, investigators would probably begin testing, or intensify the frequency of testing, when concentrations exceeded the screening level, or start by automatically conducting tests during the wet season, and terminating the testing when concentrations declined to beiow the screening level. If samples were composited over time, or if macroinvertebrate communities were also monitored, the consequences of not being able to react quickly enough to test during short-term maxima would be minimized.

A screening approach should not be used to determine whether to conduct field monitoring. One of the purposes of field monitoring is to determine if experimentally guidelines derived are low enough to protect real aquatic communities. Thus, the monitoring should be conducted at lower concentrations than are laboratory bioassays. Macroinvertebrate communities will also reflect exposure conditions over the last year. Therefore, the pesticide concentrations measured at a particular time may not be good predictors of effects to be found in communities sampled concurrently.

From the perspective of costs, it would be best to develop some screening procedure to limit chemical analyses. One could, for example, not conduct chemical analyses unless effects on macroinvertebrates were detected. However, there would be no way to evaluate guidelines unless concentrations were measured during laboratory bioassays and field monitoring. All three components are required in pesticide monitoring studies, as they provide different types of information. In most cases, screening procedures cannot work because analysis of data takes too long to provide meaningful feedback.

Based on the above discussion, the ideal pesticide monitoring study would proceed as follows (any implied criticism of previous studies should be tempered by the fact that we have the enormous advantage of retrospect). First, the contract would be tendered and awarded in spring, not fall, to allow time for necessary preliminary steps. Farmers and agricultural officials would be interviewed to determine the target pesticides. Sediment samples from the ditches and receiving river or stream would be analyzed to verify the interview results, and provide a record of past use of persistent pesticides. Suitable study ditchès and river stations would be selected during these preliminary visits.' During the spring or summer, the effects of post-spray drift could be assessed in a small study, using in situ bioassays. Screening levels, and the most sensitive test, would be established by conducting laboratory bioassays with spiked samples. Appropriate PQLs would be established, and a chemical QA/QC program using spiked samples conducted. In late August, macroinvertebrate communities from natural substrates would be sampled, or artificial substrates would be placed in the river (abundances are highest in fall, and the insects are present as late instars which makes identification easier). If possible, both artificial and natural substrates would be used for the remainder of the study. The natural substrates would be sampled once (fall) or twice (fall, spring) a year, the artificial substrates would be deployed in September-November, December-February, and March-May. Sample collection for chemical analyses would be conducted approximately every 4-8 weeks, and samples would be composited over the appropriate space and, if possible, time. A broad range of chemical and physical parameters, not just the target pesticides, would be measured on these samples. Sampling effort would be split evenly between the study ditches and the river. Laboratory bioassays would be conducted only once or twice
over the wet season, unless concentrations were above the screening level'on a sustained basis. In situ bioassays would be conducted as required. Alevin tests could be used when temperatures were $210^{\circ} \mathrm{C}$; juveniles growth/survival tests-might be useful in fall, or late winter/early spring.

### 4.3.5 Developing and Assessing Guidelines

Guidelines are developed from laboratory bioassays. Their adequacy in protecting aquatic biota can only be assessed by comparing effects on real communities (e.g., macroinvertebrates) with measured concentrations. However, there are several problems with the existing procedures for developing guidelines, which we feel could be partially eliminated by using the regression approach we describe below. The problems are:

- procedures for developing guidelines are not consistent (see Section 1.4)
- an application factor (LOEC/LC50) of 0.05 seems reasonable, but should be verified
- guidelines are based on extremes, and do not take advantage of the available data
- safety factors should not be arbitrary, but should be related to estimates of probability or risk
- guidelines are not useful for working environmental biologists (arguably, that may not be their function).

We suggest that guidelines should be developed from regressions similar to those in Suter et al. (1987). Those authors provide regressions of MATCs on LC50s fot several types of contaminants and tests. Most of the chronic effects studies reviewed in CCREM (1987) would also include LC50s for the compound and species tested. An alternative would be to use the rainbow trout LC 50 as the predictor ( X ) variable, since these data are available for most compounds. However, its use would probably increase the scatter about the regression line. A regression of MATC, LOEC, or NOEC on LC50 defines the application factor. From Suter et al. (1987), it appears that an application factor of 0.05 is reasonable. If their geometric mean MATC is divided by the geometric mean LC50, the application factor is 0.05 ; if the slope of the log-log regression is assumed to be 1 (it is 1.07), the antilog of the intercept is the application factor and that is 0.03 . These application factors will differ slightly, if subsets of tests or compoinds are analyzed. Thus, for guideline development, separate regressions might be used for different classes of compounds - pesticides, narcotics, metals, etc.

If the regression approach only verified that the application factor currently used was correct, it would not be an improvement over present procedures. However, the application factor is not consistently applied to guideline development (note difference between derivation of endosulfan and dinoseb guidelines). Furthermore, it might be more appropriate to use the lower $95 \%$ prediction limit of the MATC as the guideline, rather than the mean or 50th percentile. If the mean is used, there would be a $50 \%$ probability of
observing an effect at the guideline level, which may be too high a risk (the risk can be lowered by using the LC50 from the most sensitive species to develop the guideline, although it might be better to standardize the procedure by using the rainbow trout LC50). Using the lower $95 \%$ prediction interval is the equivalent of using a safety factor, except that safety factors are not associated with specific risks.

Using a safety factor of 10 is a reasonable procedure, as $95 \%$ prediction intervals from Suter et al. (1987) cluster around $1(\log 10)$. However, that safety factor should not be'applied in the way it was in the derivation of the dinoseb guideline (MacDonald et al, 1990). The guideline was derived by dividing the lowest LOEC by 10 ; we would argue that lowest LOEC already represented the lower $95 \%$ (or some other percentage) prediction limit. The guideline should have been $200 \mathrm{ng} / \mathrm{L}$ - the lake trout LC50 ( $\approx 40 \mu \mathrm{~g} \mathrm{~L}$ ) multiplied by the application factor of 0.05 , then divided by 10 to give the lower $95 \%$ prediction limit. The endosulfan guideline would be $2 \mathrm{ng} / \mathrm{L}$ - the rainbow trout $-\mathrm{LC50}(340 \mathrm{ng} / \mathrm{L})$ multiplied by 0.05 , then divided by 10 . These two guidelines would express approximately equal risks, given the fact that endosulfan is 10 times as toxic as dinoseb, despite the similarity of the existing guidelines. Of course, the actual regressions for pesticides, and not those from Suter et al. (1987), would be used to calculate the prediction interval and risk.

Note that if the regression approach is used, the data from the excellent and sensitive studies of Woodward (1976) would play a role in defining the lower prediction limit for all pesticides. As Peters (1986) points out, biologists conduct research as if only they knew what the "perfect" test was, and their failure to use existing data represents a real misuse of funds. We have suggested the $95 \%$ prediction limit as an apprópriate risk level ( $2.5 \%$ ), but regulatory agencies would be free to use whatever risk they felt was appropriate. These risks would be stated explicitly and would be calculated from all available data rather than from just a few extremes for individual compounds. The working biologist could calculate the concentration at which there was a reasonable ( $25 \%$ or $50 \%$ ) probability of observing effects in laboratory tests, and structure testing programs around that value. Depending on the amount of data available, multiple regression analysis could indicate whether other variables (hardness, pH , test species, endpoint used, exposure duration) were also useful predictors of NOECs or MATCs.

There are limitations to the regression approach. The basic assumption is that the relationship between MATCs and LC50s is similar across broad groups of compounds, and across species. The width of the prediction intervals probably depends as much on differences among investigators as it does on differences among test species or some other factor of interest. These assumptions are also behind the use of application and safety factors associated with current procedures. The real limitation, of any method of guideline development is that it is based on laboratory bioassays, not field monitoring. Ideally, a regression between existing guidelines, LC50s, or MATCs, and concentrations causing effects in the field would be used to develop more refined guidelines. That ideal is unlikely to be realized in the near future.

### 5.0 CONCLUSIONS/RECOMMENDATIONS

This study indicated that the Nicomekl River was suffering from physical, biological, and chemical stresses, which appeared to impact macroinvertebrate communities. No evidence was found that these impacts were due to the target pesticides, even though concentrations in February significantly exceeded CCREM guidelines. The absence of pesticide effects may have resulted from removal by iron or dissolved organic molecules. In general, laboratory tests were unable to demonstrate any effects other than those of nutrient addition (algae, duckweed, Ceriodaphnia) and water hardness (alevins). Remediation in the study area will require control of diffuse sources, and probably some effort to integrate environmental awareness into Agriculture Canada extension services. Our general recommendations for future studies are that funds and effort be distributed more equitably between chemical analyses, laboratory bioassays, and field monitoring, and that sampling and testing approaches match objectives more closely than they have in past studies. Specific recommendations are given below.

### 5.1 Dinoseb and Endosulfan

Use of endosulfan appears to be declining, and dinoseb use should be completely eliminated soon. The priority areas for study would be the effects of endosulfan in sediments, and in the marine environment (Boundary Bay). Current guidelines appear adequate for the protection of aquatic biota, although the endosulfan guideline may be too liberal in areas with low iron and dissolved organic carbon levels.

### 5.2 Status of Nicomekl River

The Nicomekl River could be described as an environmental disgrace. The primary factors of concern are metals, nutrients, and physical alterations associated with drainage control. These concerns were identified by Swain and Holms (1988a,b) and confirmed in this study. Remediation will require control of upstream sources and diffuse agricultural sources. As an agricultural area suffering from creeping suburbanization, the study area is unlikely to ever revert to its natural state. However, some alteration in farming practices would limit nutrient and silt lọads.

### 5.3 Monitoring Pesticides in Other Areas

Based on this study we offer the following recommendations for future monitoring studies:

1. Combine chemical analyses, laboratory bioassays, and field monitoring in approximately equal proportions.
2. Interlaboratory comparisons conducted by Environment Canada should include estimates of variance associated with sampling procedures, rather than just instrument error. This can be accomplished by having participating laboratories collect their own samples from a common reference site.
3. PQLs (practical quantitation limits), rather than MDLs (method detection limits), should probably be established to meet objectives, and used when making comparisons with other studies.
4. Collection of samples for chemical analyses must be conducted on appropriate spatial and temporal scales. Usually this will mean compositing samples over space, and time (if possible). In the past, sampling effort has been almost exclusively restricted to ditches, rather than receiving 'waters (rivers or streams).
5. Standardized laboratory bioassays are likely to be insensitive to pesticide effects, and provide an inexpensive relative measure of toxicity. Experiments designed to use longer exposures, and test hypotheses of interest, are likely to be more sensitive, but also more expensive. A suitable compromise would be tests such as the alevin bioassay, which are (or should be) sensitive, relatively inexpensive, and comparable with similar studies conducted in the past. Spiked samples can be used to assess the sensitivity of potential tests, and establish a screening level at which laboratory testing is initiated or intensified.
6. Assessment of macroinvertebrate communities, from natural and/or artificial substrates, should be the main focus of field monitoring. These, and fish, are the organisms Environment Canada and other agencies are charged with protecting. It will always be difficult to separate the effects of pesticides from those of other factors, because the system is subject to multiple stressors and macroinvertebrate communities are integrators. In situ tests may partially solve this problem, if conducted at times when pesticide effects are expected to be maximal (i.e., after spraying).
7. Regulatory agencies should consider using a regression approach (discussed in Section 4.3.5) to develop guidelines. The approach would make maximal use of available data, associate guidelines with specific risk levels, remove inconsistencies in current approaches, and be much more useful to working biologists than are existing guidelines. However, the adequacy of guidelines can only be assessed by comparing effects on natural communities with measured field concentrations.

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Table 1. Temperature ( ${ }^{\circ} \mathrm{C}$ ) and dissolved oxygen ( $\mathrm{mg} / \mathrm{L}$ ) for the four Nicomekl River stations, and Burrows (B) and Logging (L) ditches.

| Date | Variable | Station |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 1 | 2 | B | 3 | $\cdots$ | $\therefore 4$ |
| 14; 16 Nov. 1989 | Temperature Dissolved Oxygen | $\begin{array}{r} 8.0 \\ \\ \hline 10.0 \end{array}$ | $\begin{array}{r} 8.5 \\ 9.2 \end{array}$ | $\begin{aligned} & 9.0 \\ & 7.0 \end{aligned}$ | $\begin{aligned} & 8.0 \\ & 8.7 \end{aligned}$ | $\begin{array}{r}7.5 \\ \hline 8.4\end{array}$ | $\begin{aligned} & 8.0 \\ & 9.0 \end{aligned}$ |
| 14 Dec. 1989 | Temperature Dissolved Oxygen | $\begin{array}{r} 2.0 \\ 11.8 \end{array}$ | $\begin{array}{r} 2.5 \\ 10.0 \end{array}$ | $\begin{array}{r} 2.0 \\ \therefore \quad 8.2 \end{array}$ | $\begin{array}{r} 3.0 \\ 10.2 \end{array}$ | $\begin{aligned} & 2.0 \\ & 8.6 \end{aligned}$ | $\begin{array}{r} 3.0 \\ \\ \\ \hline \end{array}$ |
| 12 Feb. 1990 | Temperature Dissolved Oxygen | $\because 4.0$ $\therefore \quad 11.2$ | $\begin{array}{r} 4.0 \\ 11.8 \\ \hline \end{array}$ | $\begin{array}{r} 3.0 \\ \therefore \quad 10.2 \\ \hline \end{array}$ | $\begin{array}{r} 4.0 \\ 11.2 \\ \hline \end{array}$ | $\begin{array}{r} 4.0 \\ \quad 10.2 \end{array}$ | $\therefore$ 4.0 <br>  12.2 |
| 27. Mar. 1990 | Temperature Dissolved Oxygen | $\begin{array}{r} 6.5 \\ 11.6 \end{array}$ | $\begin{array}{r} 7.2 \\ 11.7 \end{array}$ | 5.5 9.4 | $\begin{array}{r} 7.8 \\ 12.8 \end{array}$ | $\begin{array}{r} 8.2 \\ -10.3 \end{array}$ | $\begin{array}{r} 8.2 \\ 11.5 \end{array}$ |
| 12 Apr. 1990 | Temperature Dissolved Oxygen | $\begin{array}{r} 11.8 \\ -10.6 \end{array}$ | $\begin{aligned} & 13.0 \\ & 10.6 \end{aligned}$ | ㄱ 15.0 <br> +12.3 | $\begin{array}{r} 13.0 \\ \\ \hline 12.2 \end{array}$ | . 15.0 | 13.0 $\therefore \quad 11.0$ |
| 25 Apr. 1990 :- | Temperature Dissolved Oxygen | $\begin{array}{r} 11.3 \\ 8.6 \end{array}$ | $\begin{array}{r} 11.7 \\ 8.7 \end{array}$ | $\begin{array}{r} 11.8 \\ 9.4 \end{array}$ | $\begin{array}{r} 11.5 \\ 9.0 \end{array}$ | $\begin{array}{r} 13.0 \\ \times \quad 9.8 \\ \hline \end{array}$ | $\begin{array}{r} 11.5 \\ 9.2 \end{array}$ |
| 3 July 1990 | Temperature Dissolved Oxygen | $\begin{array}{r} 15.2 \\ 9.0 \\ \hline \end{array}$ | $\begin{aligned} & 16.5 \\ & 10.7 \end{aligned}$ | $\begin{array}{r} 20.0 \\ 7.6 \\ \hline \end{array}$ | $\begin{array}{r} 19.0 \\ -\quad 9.6 \\ \hline \end{array}$ | $\begin{array}{r} 20.4 \\ \times \quad 7.8 \\ \hline \end{array}$ | $\begin{array}{r} \therefore 9.5 \\ \\ \hline \end{array}$ |
| 8 Nov. 1990 | Temperature Dissolved Oxygen | $\begin{array}{r} 8.0 \\ 11.7 \end{array}$ | $\begin{array}{r} 8.0 \\ \quad 11.4 \end{array}$ | $\begin{array}{r} 9.0 \\ 7.4 \end{array}$ | $\begin{array}{rr} \therefore & 8.0 \\ & 10.8 \end{array}$ | $\begin{aligned} & 9.0 \\ & 8.8 \end{aligned}$ | $\begin{array}{r} 8.0 \\ 11.0 \end{array}$ |
| 15 Nov. 1990 | Temperature Dissolved Oxygen | $\begin{aligned} & \mathrm{NM} \\ & \mathrm{NM} \end{aligned}$ | $\begin{aligned} & 8.1 \\ & 7.6 \end{aligned}$ | $\begin{aligned} & 9.2 \\ & 4.2 \end{aligned}$ | $\begin{array}{r} 8.0 \\ \quad 7.7 \\ \hline \end{array}$ | $\begin{aligned} & 9.2 \\ & 6.5 \end{aligned}$ | $\begin{array}{r}8.0 \\ 7.4 \\ \hline\end{array}$ |

NM = not measured.
Water quality guidelines - federal (CCREM, 1987) and B.C. MOE (Pommen, 1989). Unless otherwise indicated, guidelines are for chronic exposure.
Table 2.

| Parameter | B.C. MOE | CCREM |
| :---: | :---: | :---: |
| Aluminum | Acute @ $\mathrm{pH}=6.4: 0.07 \mathrm{mg} / \mathrm{L}$ <br> Acute@ $\mathrm{pH} 2.6 .5: 0.1 \mathrm{mg} /$ <br> Chronic (30 day) @ $\mathrm{pH}=6.4$ : $0.04 \mathrm{mg} / \mathrm{L}$ <br> Chronic @ pH $26.5: 0.05 \mathrm{mg} / \mathrm{L}$. | $0.1 \mathrm{mg} / \mathrm{L}$ |
| Barium | $1 \mathrm{mg} / \mathrm{L}$ | . . . . . . - - |
| Beryllium : $\quad$ : $\quad$. | If hardness > $75 \mathrm{mg} / \mathrm{L} \mathrm{CaCO}_{3}$, criterion is $\mathbf{1 . 1}$ to $1.5 \mathrm{mg} / \mathrm{L}$. |  |
| Cadmium | If hardness is $0-60 \mathrm{mg} / \mathrm{L} \mathrm{CaCO}_{3}$, criterion is $0.2 \mu \mathrm{gL}$. <br> For $60-120 \mathrm{mg} / \mathrm{L} \mathrm{CaCO}_{3}$, criterion is $0,8 \mu \mathrm{~g} / \mathrm{L}$. <br> For $120-180 \mathrm{mg} / \mathrm{LaCO}_{3}$, criterion is 1.3 $\mu \mathrm{g} / \mathrm{L}$. | If hardness is: $0-60 \mathrm{mg} / \mathrm{CaCO}_{3}$, guideline is $0.2 \mu \mathrm{~g} /$. <br> For $\mathbf{6 0 - 1 2 0} \mathrm{mg} / \mathrm{L} \mathrm{CaCO}_{3}$, guideline is 0.8 $\mu \mathrm{g} / \mathrm{L}$. <br> For $\mathbf{1 2 0 - 1 8 0 ~} \mathrm{mg} / \mathrm{L} \mathrm{CaCO}_{3}$, guideline is 1.3 $\mu \mathrm{g} / \mathrm{L}$. |
| Cobalt | $50 \mu \mathrm{~g} / \mathrm{L}$ | : . |
| Chromium | $0.02 \mathrm{mg} / \mathrm{L}$ (for fish) | $0.02 \mathrm{mg} / \mathrm{L}$. - |
| Copper | Acute: $[0.094 \times$ hardness $(\mathrm{mg} / \mathrm{L} \mathrm{CaCO} 3)-2]$. <br> Chronic: ( 30 day): $\leq 2 \mu \mathrm{~g} / \mathrm{L}$, if average water hardness is $\leq 50 \mathrm{mg} / \mathrm{CaCO}_{3} . \leq[0.04 \mathrm{x}$ average hardness], if hardness is $>50 \mathrm{mg} / \mathrm{L}$ $\mathrm{CaCO}_{3}$. | If hardness is: $0-60 \mathrm{mg} / \mathrm{LaCO}_{3}$, guideline is $2 \mu \mathrm{~g} / \mathrm{L}$ 。 <br> For $60-120 \mathrm{mg} / \mathrm{LCaCO}_{3}$, guideline is $2 \mu \mathrm{~g} / \mathrm{L}$ : <br> For $120-180 \mathrm{mg} / \mathrm{LaCO}_{3}$, guideline is $3 \mu \mathrm{~g} / \mathrm{L}$. |
| Iron |  | $0.3 \mathrm{mg} / \mathrm{L}$ |

[^3]Table 2. Continued


[^4] distinguishable from 0).
$$
\text { DINOSEB (Detection limit }=50 \mathrm{ng} / \mathrm{L} \text { ) }
$$

| Station | Sample Date (day/month/year) |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 11/12/89 | 14/12/89 | 27/12/89 | 12/02/90 | 01/03/90 | 27/03/90 | 26/04/90 | 02/07/90 | 02/11/90 |
| River 1 | 16 T |  | ND |  |  |  | ND | T | ND | ND |
| River 2. | 13T |  | ND | - - | $170^{\circ}$ |  | ND | ND | ND | ND |
| Burrows | 27T | ND | ND : | ND | 1600 | 100 | ND | ND | ND | ND |
| River 3 | 15T |  | ND |  |  |  | ND | T | ND | ND |
| Logging | $36 T$ | 25T | 39 T | ND | 490 | 110 | ND | ND | ND | ND |
| River 4 | 24 T |  | ND |  | 310, |  | ND | T | ND | ND |



| Station | Sample Date (day/month/year) |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 11/12/89 | 14/12/89 | 27/12/89 | 12/02/90 | 01/03/90 | 27/03/90 | 26/04/90 | 02/07/90 | 02/11/90 |
| River 1 | ND ${ }^{\text {' }}$ |  | ND |  | - |  | ND | ND | ' $\because$ ND | ND |
| River 2 | 4 T |  | ND |  | 25 |  | ND | ND | ND | ND |
| Burrows | 9 T | ND | ND | ND | 170 | 42 | ND | T | ND | ND |
| River 3 | $4 \mathrm{~T}^{-}$ |  | ND : |  |  |  | ND | ND | ND | ND |
| Logging | 6 T | ND | ND | ND | 40 | 23 | ND. | ND | ND | ND |
| River 4 | 4 T |  | ND |  | 23 |  | ND | ND | ND | ND |

3/047-24
9.03 .28
Continued.
Table 3.
$\beta-$ ENDOSULTFAN (Detection limit $=20 \mathrm{ng} /$ )

| Station | Sample Date (day/mionth/year) |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 11/12/89 | 14/12/89 | 27/12/89 | 12/02/90 | 01/03/90 | 27/03/90 | 26/04/90 | 02/07/90 | 02/11/90 |
| River 1 |  |  |  |  |  |  | ND | ND | ND | ND |
| River 2 |  |  |  |  | 63 |  | ND | ND | ND | ND |
| Burrows |  |  |  |  | 280 | 340 | 11 T | T | ND | ND |
| River 3. |  |  |  |  |  |  | 4 T | ND | ND | ND |
| Logging |  |  |  |  | 230 | 18T | 5 T . | T | ND | ND |
| River 4 |  |  |  |  | 150 |  | 3 T | ND | ND | ND |

ENDOSULFAN SULFATE (Detection limit $=\mathbf{2 0} \mathbf{n g} / \mathrm{L}$ )

| Station | Sample Date (day/month/year) |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 11/12/89 | 14/12/89 | 27/12/89 | 12/02/90 | 01/03/90 | 27/03/90 | 26/04/90 | 02/07/90 | 02/11/90 |
| River 1 |  |  |  |  |  |  | ND | ND | 15T | ND |
| River 2. | . - |  |  |  | 190 |  | 4 T | ND | ND | ND |
| Burrows |  |  |  |  | 800 | 780 | 10 T | 20 | 31 | ND |
| River 3 |  |  |  |  |  |  | 5 T | ND | ND | ND |
| Logging |  |  |  |  | 680 | 18 T | 9 T | T | 16 T | 'ND |
| River 4 |  |  |  |  | 380 |  | 4 T | ND | ND | ND |

## $3 / 2047.24$ 91.03 .28

DINOSEB (Detection limit $=\mathbf{5} \mathbf{n g} / \mathbf{g}$ )

| Station | Sample Date (day/month/year) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 14/12/89 | 12/02/90 | '27/03/90' | 26/04/90 | 02/11/90 |
| River 1 | - - | - ND | $\because \mathrm{ND}$ | ND | ND | ND |
| River 2 | ND | 2T | ND | ND | $\because$ ND | ND |
| Burrows | ND | 5 | 5 | $\therefore \mathrm{ND}$ | , ND | ND |
| River 3 | 4T | 2T | ND | - ND | ND | ND |
| Logging | ND | 13 | ND | 5 | ND | ND |
| River 4 | ND | $\cdots \mathrm{ND}$ | ND | $\cdots$ ND | ND | ND |

$\alpha \cdot$ ENDOSULFAN (Detection limit $=2 \mathbf{n g} / \mathbf{g}$ )

| Station | Sample Date (day/month/year) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 14/12/89 | 12/02/90 | 27/03/90 | 26/04/90 | 02/11/90 |
| River 1 |  | ND | ND | ND | ND | ND |
| River 2 | ND | 0.6T | ND | ND | ND | ND |
| Burrows | 10 | 11 | 14 | ND. | 2 | ND |
| River 3 | 1T | ND | ND | ND | ND | ND |
| Logging | 6 | $\because N D$ | 7 | ND | ND | ND |
| River 4 | 1 T | 1T | ND | ND | ND | ND |

Continued. | Station |
| :--- | :--- |
| River 1 |
| River 2 |
| Burrows |
| River 3 |
| Logging |
| River 4 |

ENDOSULFAN SULFATE (Detection limit $=\mathbf{2} \mathbf{n g} / \mathbf{g}$ )

| Station | Sample Date (day/month/year) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 14,16/11/89 | 14/12/89 | 12/02/90 | 27/03/90 | 26/04/90 | 02/11/90 |
| River 1 |  |  | - ND | 11 | ! ND | ND |
| River 2 |  |  | ND | ND | ND | ND |
| Burrows | , |  | 58 | 124 | . 23 | 70 |
| River 3 | - |  | ND | 3 | ND | ND |
| Logging | . |  | 16 | 18 | ND | 21 |
| River 4 |  |  | ND | 2 | ND | ND |

Table 5. Precipitation (mm) on, and prior to, sampling dates. Data are for Surrey Municipal Hall, provided by Environment Canada, Atmospheric Environment Services.


Table 6. Residual oxygen bioassay results. Values are mean final dissolved oxygen (D.O.) concentrations $\pm 1 \mathrm{SE}$; initial concentrations were approximately $10 \mathrm{mg} / \mathrm{L}$ for all replicates.

| 18 November, 1989 |  |  | 8 March 1990 |  |
| :---: | :---: | :---: | :---: | :---: |
| Test Water | D.O | (mg/L) | Test Water | D.O. (mg/L) |
| Control | 2.0 | $\pm 0.40$ | Control | $1.60 \pm 0.00$ |
| Burrows - water <br> - elutriate | $\begin{aligned} & 1.30 \\ & 1.00 \end{aligned}$ | $\begin{array}{ll}  \pm & 0.30 \\ \pm & 0.00 \end{array}$ | Upstream | $1.33 \pm 0.07$ |
| Logging - water <br> - elutriate | $\begin{aligned} & 1.50 \\ & 1.15 \end{aligned}$ | $\begin{array}{ll}  \pm & 0.30 \\ \pm & 0.05 \end{array}$ | Burrows | $1.47 \pm 0.18$ |
| $\begin{array}{rll}\text { Erickson } & \text { water } \\ \text { - } & \text { elutriate }\end{array}$ | $\begin{aligned} & 1.10 \\ & 1.35 \end{aligned}$ | $\begin{aligned} & \pm 0.10 \\ & \pm 0.05 \end{aligned}$ | Logging | $1.20 \pm 0.12$ |

Table 7. Mean ( $\pm 1 \mathrm{SE}$ ) \% mortality for embryos and alevins in rainbow trout alevin bioassays. Except as noted, there were $\mathbf{3}$ replicates of 20 individuals each for each test water.

| Test Water: | December 1989 - January 1990 |  |  |  | February --March 1990 |  |  |  |  | November 1990 |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Embryo |  | Alevin |  | Embryo |  | Alevin |  |  | Embryo |  |  | Alevin |  |  |
| Control | 13.3 | $\pm .4 .4$ | 9.2 | $\pm .4 .7$ | 1.7 | $\pm 1.7$ | 10.2 | $\pm$ | 2.9 | 5.0 | $\pm$ | 0.0 | 5.3 | $\pm$ | 3.1 |
| Upstream |  | 土. 4.4 | 10.1 | $\pm 7.6$ | 5.0 | $\pm 2.9$ |  | $\pm$ | 4.5 | 5.0 | $\pm$ | 0.0 | 5.3 | $\pm$ | 0.0 |
| Burrows $10 \%$ <br> $30 \%$ <br>  <br>  <br>  <br>  $\mathbf{1 0 0 \%}$ | 8.3 23.3 11.7 | $\begin{array}{ll}  \pm & 3.3 \\ \pm & 8.8 \\ \pm & 3.3 \end{array}$ | 3.5 16.7 12.7 | $\begin{array}{ll}  \pm & 3.5 \\ \pm & 8.3 \\ \pm & 7.6 \end{array}$ | 8.3 3.3 1.7 | $\begin{array}{ll}  \pm & 3.3 \\ \pm & 1.7 \\ \pm & 1.7 \end{array}$ | 3.5 5.3 2.2 | $\pm$ $\pm$ $\pm$ | $\begin{aligned} & 1.8 \\ & 5.3 \\ & 2.2^{2} \end{aligned}$ | 3.3 0.0 5.0 | $\begin{array}{ll}  \pm & 1 \\ \pm & 0 \\ \pm & 2 \end{array}$ | $\begin{aligned} & 1.7 \\ & 0.0 \\ & 2.9 \end{aligned}$ | $\begin{array}{r} 3.5 \\ 10.0 \\ 10.6 \end{array}$ | $\pm$ $\pm$ $\pm$ | $\begin{aligned} & 3.5 \\ & 5.0 \\ & 3.3 \end{aligned}$ |
| Logging $10 \%$ <br>  <br>  <br>  <br> $30 \%$ <br>  <br>  $\mathbf{1 0 0 \%}$ |  | $\begin{array}{ll}  \pm & 0.0^{1} \\ \pm & 1.7 \\ \pm & 4.4 \end{array}$ | 7.9 14.8 9.1 | $\begin{aligned} & \pm \quad 7.9^{1} \\ & \pm \quad 9.8 \\ & \pm \quad 4.9 \end{aligned}$ | 3.3 10.0 0.0 | $\begin{aligned} & \pm \\ & \pm \\ & \pm .7 \\ & \pm \end{aligned}$ | 3.5 7.8 .1 .7 | $\pm$ $\pm$ $\pm$ | $\begin{aligned} & 3.5 \\ & 5.2 \\ & 1.7 \end{aligned}$ | 5.0 1.7 6.7 | $\pm$ $\pm$ $\pm$ | $\begin{aligned} & 2.9 \\ & 1.7 \\ & 1.7 \end{aligned}$ | 3.4 0.0 7.1 | $\pm$ $\pm$ $\pm$ | 1.7 0.0 1.7 |

In one replicate, 4 alevins died when they were trapped between the glass petri dish and the net. These individuals were excluded from analyses.

Table 8. Statistical analyses of rainbow trout alevin bioassays. Contrasts are defined in the text and in Appendix II.


```
* = P s 0.05
** = P\leq0.01
*** = P < 0.001
NS = not significant
```



Table 10. Nutrient content of Selenastrum culture medium and test waters collected 14 December, 1989. All values are $\mathrm{mg} / \mathrm{L}$.

| Element | Culture Medium | Upstream |
| :---: | :---: | :---: | :---: | :---: |

1. Chemical results are from Station 1.

2 NaOH is often added later to raise pH to 7.5 , so values given are minima.

Table 11. Nutrient contents of commercial nutrient solution used in duckweed bioassay, and test waters collected 26 April, 1990. All values are mg/L.


1. Chemical results are from Station 1.
Results of multivariate (MANOVA) and univariate (ANOVA) analyses of the abundance of macroinvertebrate taxa colonizing artificial substrates in the Nicomekl River. The MANOVA r gives the correlation between the taxon abundance and the first canonical vector for the contrast indicated; the univariate $P$ gives the $P$ value for the contrast for each taxon analyzed separately. The three highest $r$ values for each contrast have been underlined. No MANOVA $r$ values are given for the interaction between downstream trends and season because the vector is not particularly informative.

|  | Seasonal Changes |  |  |  | Downstream Trend |  |  |  | Interaction |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nov. excluded |  | Nov. included |  | Nov. excluded |  | Nov. included |  | Nov. excluded <br> Univariate | $\frac{\text { Nov. included }}{\text { Univariate }}$ |
|  | MANOVA | Univariate | MANOVA | Univariate | MANOVA | Univariate | MANOVA | Univariate |  |  |
|  | - r | $P$ | $r$ | $P$ | $r{ }^{1}$ | $P$ | $r^{1}$ | $P$ | $P$ | $P$ |
| Nematoda | 0.06 | -** | 0.18 | ** | 0.23 | *** | 0.33 | -** | NS | NS |
| Oligochaeta | 0.41 | *** | $0.44{ }^{\wedge}$ | ** | 0.37 | -** | 0.34 | -•• | ** | ** |
| Gastropoda | 0.09 | -* | 0.08 | ** | -0.20 | -9 | 0.40 | *** | NS | NS |
| Copepoda | 0.18 | *** | 0.17 | ** | 0.02 | NS | 0.24 | * | NS | - |
| Amphipoda | 0.59 | ** | 0.60 | -* | -0.11 | NS | -0.06 | NS | NS | NS |
| Gnorimosphacroma | 0.25 | *** | 0.06 | $\bullet$ | 0.64 | *** | -0.22 | - | *** | NS |
| Asellus . - | 0.06 | NS | 0.06 | NS | -0.12 | - | -0.26 | - | - NS | NS |
| Ephemeroptera | 0.04 | * | 0.04 | *** | -0.53 | *** | $\underline{0.39}$ | ** | ** | NS |
| Plecoptera | $\underline{-0.60}$ | *** | 0.42 | $\because \because \bullet$ | -0.24 | *** | -0.58 | -** | NS | -** |
| Chironomidae | 0.23 | . $\cdot \bullet \cdot$ | 0.33 | -** | $\bigcirc 0.30$ | -** | 0.39 | *** | -** | -* |

Table 13. .. Spatial and temporal changes in the total abundance of invertebrates, and in EP/C (\{Ephemeroptera + Plecoptera\}/Chironomidae). Values are P-values for hypotheses tested using contrasts. Contrasts are defined in the text and Appendix II.

A larger mean square (MS) indicates a stronger downstream trend. A positive trend indicates a downstream increase; a negative trend indicates a downstream decrease:
Table 14.

| Variable | Season | Trend | Stations 1 - 4 |  | Stations 2-4 |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | MS | $P$ | MS | $P$ |
| Abundance (log) | Dec. - Jan. | none. | 0.017 | 0.48 | 0.000 | 0.97 |
|  | Feb. - Apr. | + | 1.735 | < 0.0001 | $\cdots 0.946$ | < 0.0001 |
|  | Apr. - July | + | 0.312 | 0.003 | 0.624 | 0.0003 |
|  | Nov. | - |  |  | 0.093 | 0.15 |
| EP/C $(\arcsin \sqrt{ })$ | Dec. - Jan. | - | 0.036 | 0.03 | 0.045 | 0.02 |
|  | Feb. - Apr. | - | 0.321 | < 0.0001 | 0.169 | < 0.0001 |
|  | Apr. - July | - | 0.343 | < 0.0001 | 0.058 | 0.01. |
|  | Nov. |  |  |  | 0.315 | < 0.0001 |

Table 15. Mortality of alevins reared in situ for $\mathbf{3}$ weeks (field), then transferred to the lab for 9 days (lab). Values are means $\pm 1 \mathrm{SE}$.

| 1. <br> Station | Mortality |  |  | $\frac{2}{n^{2}}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Field | $n^{1}$ | Lab |  |  |
| River 2 | $27 \pm 12$ | 3 | $7.0 \pm 4.5$ | 3 |  |
| Burrows | $56 \pm 5$ | 3 | $5.6 \pm 5.6$ | 3 |  |
| River 3 | $0 \pm 0$ | 2 | $35.0 \pm 35.0^{3}$ | 2 |  |
| Logging | $98 \pm 2^{*}$ | 3 | N |  |  |
| River 4 | $56 \pm 29$ | 3 | $2.5 \pm 2.5$ | - 2 |  |

number of replicates ( 2 "sandwiches" of 20 fish each per replicate).
number of beakers ( $\leq 20$ fish each); each beaker held fish from one sandwich.
in one beaker, $70 \%$ of the fish died in the last two days, and may have been damaged when trapped between net and petri dish.

Table 16. Rainbow trout alevin field bioassay - results of statistical analyses. Contrasts are defined in text and Appendix II.

| Variable ${ }^{\prime \prime}$ | Contrast |  |  |
| :---: | :---: | :---: | :---: |
|  | Among Stations | River vs. Ditch(es). | Downstream Trend |
| Field Mortality | * | ** | NS |
| Field Growth <br> - Yolk weight <br> - Body weight <br> - adjusted for yolk weight | *** |  | $\begin{aligned} & \text { NS } \\ & \text { NS } \\ & \text { NS } \end{aligned}$ |
| Lab Groẃth <br> - Yolk weight <br> - Body weight <br> - adjusted for yolk weight | NS NS NS | NS NS NS | $\begin{aligned} & \text { NS } \\ & \text { NS } \\ & \text { NS } \end{aligned}$ |

$$
\begin{aligned}
& * \quad=P \leq 0.05 \\
& * * \quad=P \leq 0.01 \\
& * * * P \leq 0.001 \\
& \text { NS }=P \text { not significant }
\end{aligned}
$$



[^5]176th St. 184th St.

Analysis of Water Samples For Dinoseb, Endosulfan 1 , Endosulfan 2 , and Endosulfan Sulfate.

Surrogates:2,4,6-Tribromophenol(Dinoseb);4,4'-Dibromobiphenyl(Endosulfans)
Detection Limits: 0.02 ug/L,Endosulfans ; 0.05 ug/L,Dinoseb.
Figure 3. $\because \quad$ Protocol for analyses of water samples (provided by Zenon Environmental).

Surrogates: 2,4,6-Tribromophenol(Dinoseb);4,4'-Dibromoblphenyl(Endosulfans)
Detection Limits: $2 \mathrm{ug} / \mathrm{Kg}$, Endosulfans ; $5 \mathrm{ug} / \mathrm{Kg}$, Dinoseb:
Figure 4. Protocol for analyses of sediment samples (provided by Zenon Environmental).
LIGHT CRATING


## Surrey Municipal Hall Climate Summary November 1989 to October 1990



Figure 6. Daily temperature and precipitation during the study period (from Atmospheric Environment Service, Environment Canada).


[^0]:    3047.24

    930415

[^1]:    3047.24

    930415

[^2]:    3047.24

    930415

[^3]:    3.1017 .24
    91.03 .28

[^4]:    Formula 1:
    Formula 2:

[^5]:    Location of study area. The distribution of salmonid habitat in the Nicomekl and adjacent watersheds is also shown

