Studies of the lethal and sublethal toxicity of antisapstain fungicide formulations containing didecydimethylammonium chloride (DDAC) and 3-iodo-2-propynyl butyl carbamate (IPBC) to fishes and aquatic invertebrates

DOE FRAP Report

Prepared for:

Environment Canada
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June 1999

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This report was funded by Environment Canada under the Fraser River Action Plan through the Environmental Quality Technical Working Group. The views expressed herein are those of the authors and do not necessarily state or reflect the policies of Environment Canada

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Abstract

The acute lethal and sublethal toxicity of antifungal agents Bardac 2280 (containing 80% didecyldimethylammonium chloride, DDAC) and Polyphase P-100 (containing 97% 3-iodo-2-propynyl butyl carbamate, IPBC) were determined for four fish and 5 aquatic invertebrate species. Several species were of ecological relevance to the lower Fraser River and its estuary: *Platichthys stellatus* (starry flounder), *Oncorhynchus kisutch* (coho salmon), *Acipenser transmontanus* (white sturgeon) and *Neomysis mercedis*. The remainder were commonly used test species: *O. mykiss* (rainbow trout), *Pimephales promelas* (fathead minnow), *Hyalella azteca*, *Daphnia magna*, and *Mysidopsis bahia*.

Bardac 2280 was more toxic to invertebrates (48-h LC_{50} values from 37 ppb for *D. magna* to 972 ppb for *N. mercedis)* than fish with the exception of white sturgeon fry, the most sensitive species tested. The 96-h LC_{50} values for fish exposed to Bardac 2280 ranged from 2.5 ppb for white sturgeon fry to 2,000 ppb for juvenile starry flounder. Sublethal stress was observed as an increase in plasma lactate in juvenile starry flounder after 24-h exposure to 50% of the 96-h LC_{50} value and swimming performance decreased in juvenile rainbow trout after 12-h exposure to 50% of the 96-h LC_{50} value.

Polyphase P-100 exhibited both the highest and lowest toxicity in invertebrates (48-h LC_{50} values from 40 ppb for *D. magna* to 2,920 ppb for *N. mercedis).* The 96-hour LC_{50} values for fish exposed to Polyphase P-100 ranged from 95 ppb in 10-month old coho smolts to 370 in juvenile starry flounder. Acute 24-hour sublethal exposure of rainbow trout and starry flounder did not elicit strong primary stress responses.

The acute toxicity of a 1:8 mixture of Polyphase P-100 and Barbac 2280 was greater for invertebrates (48-h LC_{50} values from 26 ppb in *H. azteca* to 770 ppb in *N. mercedis)* than fish (96-h LC_{50} values from 430 ppb in coho smolts to 1,200 ppb in starry flounder). Acute 24-hour sublethal exposure of starry flounder to concentrations as low as 25% of the 96-h LC_{50} value resulted in significant differences in levels of plasma lactate, glucose, hematocrit and leucocrit.

Rainbow trout in the current study appear more sensitive (96-h LC_{50} of 328 ppb DDAC based on 80% Bardac 2280) than those reported in the proprietary literature (440 to 2,800 ppb DDAC). The acute toxicity of IPBC to rainbow trout in this study (96-h LC_{50} of 97 ppb IPBC based on 97% Polyphase P-100) was consistent with previous reports (67-310 ppb IPBC).

These findings suggest that current regulatory limits for DDAC and IPBC (700 ppb and 120 ppb, respectively) may not be sufficient for the protection of all fish and invertebrate species found in the Fraser River. Further field and lab studies are recommended to consider how the toxicity of these substances may be affected by suspended sediment, interactions with other contaminants, and fluctuations in salinity, temperature and mixing capacity.

Résumé

La toxicité aiguë létale et sublétale des antifongiques Bardac 2280 [renfermant 80 % de chlorure de didécyldiméthylammonium (DDAC)] et Polyphase P-100 (renfermant 97 % de carbamate 3-iodo-2-propynylbutyle (IPBC) a été établie pour quatre espèces de poisson et cinq espèces d'invertébré aquatique. Plusieurs espèces choisies avaient une importance écologique dans le cours inférieur du Fraser et son estuaire : *Platichthys stellatus* (flet étoilé), *Oncorhynchus kisutch* (coho), *Acipenser transmontanus* (esturgeon blanc) et *Neomysis mercedis*. Dans les autres cas, il s'agissait d'espèces couramment étudiées : *O. mykiss* (truite arc-en-ciel), *Pimephales promelas* (tête-de-boule), *Hyalella azteca*, *Daphnia magna* et de *Mysidopsis bahia*.

Le Bardac 2280 était plus toxique pour les invertébrés (CL50 à 48 h comprise entre 37 parties x 10^{-9} chez *D. magna* et 972 parties x 10^{-9} chez *N. mercedis*) que pour les poissons, sauf chez les alevins d'esturgeon blanc, espèce à l'étude la plus sensible. La CL50 à 96 h chez les poissons exposés au Bardac 2280 était comprise entre 2,5 parties x 10^{-9} chez les alevins d'esturgeon blanc et 2 000 parties x 10^{-9} chez les flets étoilés juvéniles. Le stress sublétal a été observé sous forme d'augmentation du lactate dans le plasma chez les flets étoilés juvéniles après une exposition de 24 heures à la moitié de la CL50 à 96 h et la performance natatoire diminuait chez les truites arc-en-ciel juvéniles après une exposition de 12 heures à la moitié de la CL50 à 96 h.

Dans le cas du Polyphase P-100, on a relevé la toxicité la plus élevée et la toxicité la plus faible chez les invertébrés (CL50 à 48 h comprise entre 40 parties x 10^{-9} chez *D. magna* et 2 920 parties x 10^{-9} chez *N. mercedis*). La CL50 à 96 h pour les poissons exposés au Polyphase P-100 était comprise entre 95 parties x 10^{-9} chez des smolts de coho âgés de 10 mois et 370 parties x 10^{-9} chez des flets étoilés juvéniles. Chez la truite arc-en-ciel et le flet étoilé, une exposition aiguë sublétale de 24 heures n'a pas déclenché de fortes réactions primaires de stress.

La toxicité aiguë d'un mélange 1:8 de Polyphase P-100 et de Bardac 2280 était plus élevée chez les invertébrés (CL50 à 48 h comprise entre 26 parties x 10^{-9} chez *H. azteca* et 770 parties x 10^{-9} chez *N. mercedis*) que chez les poissons (CL50 à 96 h comprise entre 430 parties x 10^{-9} chez les smolts de coho et 1 200 parties x 10^{-9} chez le flet étoilé). Une exposition aiguë de 24 heures de flets étoilés à des concentrations correspondant à peine à 25 % de la CL50 à 96 h a produit des différences importantes au niveau des taux de lactate et de glucose dans le plasma, de l'hématocrite et du leucocrite.

Dans le cadre de la présente étude, la truite arc-en-ciel serait moins sensible (CL50 à 96 h pour une dose de 328 parties x 10^{-9} de DDAC établie à partir du Bardac 2280 à 80 %) que les autres espèces signalées dans les documents présentés par les détenteurs de brevets (440 à 2 800 parties x 10^{-9} de DDAC). Dans la présente étude, la toxicité aiguë de l'IPBC chez la truite arc-en-ciel (CL50 à 96 h de 97 parties x 10^{-9} d'IPBC établie à partir du Polyphase P-100 à 97 %) était conforme aux données des rapports précédents (67 à 310 parties x 10^{-9} d'IPBC).

D'après ces résultats, les limites réglementaires obligatoires pour le DDAC et l'IPBC (respectivement de 700 parties x 10^{-9} et 120 parties x 10^{-9}) ne permettraient pas d'assurer la protection de toutes les espèces de poissons et d'invertébrés dans le Fraser. On recommande d'effectuer d'autres études sur le terrain et en laboratoire afin de déterminer de quelle manière la toxicité de ces substances peut être touchée par les sédiments en suspension, les interactions avec d'autres contaminants et les variations de salinité, de température et de la capacité de mélange.

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Introduction

The forest products industry in the northwestern regions of Canada relies heavily on the use of antisapstain products to prevent the growth of moulds and fungi and to maintain lumber marketability. Sawmills on the Fraser River, BC predominantly utilize formulations containing didecyldimethylammonium chloride (DDAC) and 3-iodo-2-propynyl butyl carbamate (IPBC) as active ingredients. In fact, a substantial proportion of the annual provincial usage of around 400 tonnes of DDAC takes place at sites adjoining the Fraser River. Whereas the proprietary literature on the toxicity for both of these compounds to aquatic organisms has been reviewed (Hendersen 1992a,b; Envirochem 1992), information contained in the refereed literature is very limited. As such, it is extremely difficult for the government of Canada to set reliable Water Quality Criteria with such a paltry data base from which to work.

In the absence of Water Quality Criteria, the regulatory limits for DDAC and IPBC in British Columbia are presently set at 700 ppb and 120 ppb, respectively, for storm water runoff from mill sites. However, the adequacy of these regulatory levels for storm water can be challenged even using proprietary information, because certain organisms have acute toxicity values lower than the regulatory level. Also, non-compliance has been reported (Envirochem 1992).

The aim of the present research program was to place baseline aquatic toxicity data for DDAC and IPBC in the refereed literature. The test organisms used were fishes and aquatic invertebrates that either were relevant to the Fraser River or could be used for broader comparison with standard test organisms. Bardac 2280, containing 80% DDAC, and Troysan Polyphase P-100, containing 97% IPBC, were used either singly or in a 8:1 mixture for the toxicity tests.

Materials and Methods

Information on the fish and aquatic invertebrate species used in this study, as well as their holding and testing conditions, is summarized in Table 1. Acute lethality studies were performed, as well as acute sublethal studies that measured indicators of stress (biochemical and physiological changes in tissues) and indicators of performance (swimming speed and disease resistance) (Adams 1990; Schreck 1990). The methodologies for these tests have been published previously (Johansen and Geen 1990; Janz et al. 1991; MacKinnon and Farrell 1992; Nikl and Farrell 1993; Weber 1993; Johansen et al. 1994; Kennedy et al. 1995; Bennett 1996).

Bardac 2280 (Lonza Inc., Fair Lawn, NJ) contained 80-82% didecyldimethylammonium chloride (DDAC), as the principal active ingredient, 10% ethanol, 7-10 % water, and <1 % amine chloride. Dilutions of Bardac 2280 were made with double distilled water to make stock solutions. Technical grade Troysan Polyphase P-100 (Kop-Coat Inc., Pittsburgh, PA) contained 97% IPBC, <0.9% NaCl and <0.1% tri-iodo-allyl butyl carbamate. Dilutions of Polyphase P-100 were made with double distilled water to make stock solutions. Polyphase P-100 was also tested in combination with Bardac 2280 at a ratio of 1:8. This resulted in a nominal 1.0:6.4 ratio for the active ingredients (IPBC:DDAC). Quality assurance and quality control was confirmed with high resolution gas chromatography (Nitrogen-Phosphorus detection) analysis of representative 1 L samples of the stock and experimental solutions of Bardac 2280 and Polyphase P-100 (Canadian Organic Chemistry

Analytical Laboratory, Pacific Environmental Science Centre, Environment Canada, North Vancouver, BC). Recovery of both chemicals was typically greater than 85%. Expected concentrations of DDAC and IPBC alone were within 30% of measured concentrations. Concentrations reported are nominal.

Assessment of additive toxicity of the chemical mixture of IPBC and DDAC was performed according to the linear additive index method of Marking (1977), where an additive index greater than zero indicates a "greater than additive effect" and an index less than zero indicates a "less than additive effect. Zero represents a simple additive effect.

Results

Fish toxicity tests with Bardac 2280

The acute toxicity of Bardac 2280 to fish species (96-h LC_{50}) varied by about 10-fold, from 330 ppb for fathead minnows to 2,000 ppb for starry flounder, with the exception of white sturgeon fry (Table 2). The acute toxicity of Bardac 2280 was similar for juvenile rainbow trout, fathead minnows, coho alevins and coho fry tested in freshwater, whereas coho smolts and starry flounder were between 3- and 5-times more tolerant of Bardac 2280 (Table 2, Figure 1a). White sturgeon fry were an exceptionally sensitive fish species, being 10- to 100-times more sensitive to Bardac 2280 than the other species tested. All sturgeon fry died with exposures to 10, 50, 100 and 500 ppb Bardac 2280. The 96-h LC_{50} value for white sturgeon fry was 2.5 ppb Bardac 2280.

The sensitivity of coho salmon to Bardac 2280 was significantly altered by their developmental stage (Table 2, Figure 1a). Tests performed with coho smolts in either 15‰ or 30‰ seawater had no significant effect on the acute toxicity of Bardac 2280 (LC_{50} = 950 ppb and 850 ppb, respectively) compared with those performed in fresh water (Table 2, Figure 1a).

An acute, 24-h sublethal exposure to Bardac 2280 caused only a limited stress response in both rainbow trout and starry flounder (Table 3). Among the suite of primary and secondary stress indicators, plasma glucose, lactate and cortisol levels increased significantly, but the changes were not large even with a 24-h exposure to 100% of the LC_{50} concentration. Similarly for starry flounder, only plasma lactate was significantly elevated at 50% of the LC_{50} concentration. Despite the absence of a major stress response, the maximum prolonged swimming performance of rainbow trout was reduced significantly (up to 25%) with 12-h and 24-h exposures to 50% and 100% of the LC_{50} concentration of Bardac 2280 (Table 3). The reduced swimming performance was not related to external gill damage (inspected by scanning electron microscopy) as has been the case with toxic exposure to certain metals (Waiwood and Beamish 1978), pulpmill effluent (Howard 1975; McLeay and Brown 1979) and TCMTB (Nikl and Farrell 1993). The resistance of juvenile trout to a disease challenge by *Vibrio anguillarum* was significantly improved with exposure to 50% and 100% of the LC_{50} concentration for Bardac 2280 (data not shown).

Invertebrate Toxicity Tests

The acute toxicity of Bardac 2280 to invertebrate species (48-h LC_{50}) varied by about 30-fold, from 37 ppb for *Daphnia magna* to 972 ppb for *Neomysis mercedis* (Table 2, Figure 1a).

Fish Toxicity Tests with Polyphase P-100

The acute toxicity of Polyphase P-100 to the fish species (96-h LC_{50}) varied by 30-fold, from 95 ppb for coho smolts to 1,900 ppb for coho embryos (Table 4, Figure 1b). Juvenile rainbow trout and coho fry showed a similar sensitivity to Polyphase P-100, but starry flounder were almost 4-times more tolerant of Polyphase P-100 (Table 4, Figure 1b).

Acute (24-h) sublethal exposure to Polyphase P-100 did not elicit a strong primary stress response in either rainbow trout or starry flounder. The plasma variables were unchanged in rainbow trout and only leucocrit decreased significantly in starry flounder after exposure to 100% of the LC_{50} concentration for Polyphase P-100 (Table 5).

Invertebrate Toxicity Tests with Polyphase P-100

The acute toxicity of Polyphase P-100 to the invertebrate species (48-h LC_{50}) varied by 70-fold, from 40 ppb for *D. magna* to 2,920 ppb for *N. mercedis* (Table 4, Figure 1b).

Toxicity Tests with a mixture of Polyphase P-100 and Bardac 2280

The acute toxicity of a 1:8 v/v mixture of Polyphase P-100 and Bardac 2280 to the fish species (96-h LC_{50}) varied three-fold, from 430 ppb for coho smolts to 1,280 ppb for juvenile starry flounder (Table 6, Figure 1c). The acute toxicity (48-h) of this mixture for the invertebrate species varied by 30-fold, from 26 ppb for *H. azteca* to 770 ppb for *N. mercedis* (Table 6, Figure 1c). The additive indices for fish acute toxicity (Table 6) indicated that Polyphase P-100 and Bardac 2280 were marginally, but consistently less than additive for rainbow trout and coho, and marginally additive for flounder. For the invertebrates, Polyphase P-100 and Bardac 2280 were less than additive for *D. magna*, marginally more than additive for *N. mercedis*, and considerably more than additive for *H. azteca* (Table 6).

An acute, 24-h sublethal exposures to the mixture of Polyphase P-100 and Bardac 2280 caused little change in most of the measured stress variables, even at a concentration of 100% of the 96-h LC_{50} concentration (Table 7). However, plasma cortisol levels in rainbow trout were significantly elevated in a concentration-dependent manner (a primary stress response), beginning with lowest concentration tested. Juvenile starry flounder responded at 100% of the 96-h LC_{50} value with elevated plasma glucose and decreased leucocrit, both of which indicate a secondary stress response. However, plasma lactate was significantly decreased at all concentrations tested, a response that indicates an anaesthetic/analgesic action.

Discussion

This study provides comprehensive information on the acute toxicity of Bardac 2280 and Polyphase P-100 to aquatic organisms. These data allow for a better assessment of the toxicity of these antisapstains in relation to their entry into the Fraser River as leachate from treated wood.

To provide some idea of the data quality generated in the present study, comparisons are made with the propriety information previously reviewed by Henderson (1992a) for DDAC-based antisapstain chemicals. Although the comparisons are hampered by the lack of details in these reviews on test conditions and the restriction of comparing similar formulations, there is reasonable agreement, as shown below. Notable exceptions in the present study show a higher level of sensitivity of certain aquatic organisms.

For Bardac 2280, the acute toxicity of the most sensitive fish tested in the proprietary literature (320 ppb DDAC for bluegill sunfish; unpublished data, Springborn Laboratories, Inc.) is similar to that reported here for coho alevins, coho fry and juvenile rainbow trout (310-450 ppb DDAC). Species that were found in the current study to be more sensitive than test species in the literature included juvenile fathead minnow and white sturgeon fry (264 ppb and 2.0 ppb DDAC, respectively). The acute toxicity for coho in the proprietary literature (1,000 ppb DDAC; unpublished data, Springborn Laboratories, Inc.) was less than that reported in this study for all juvenile stages of coho (312 to 880 ppb DDAC). The acute toxicity for rainbow trout varies considerably in the proprietary literature (440 to 2,800 ppb DDAC), whereas our LC_{50} value lies at the low end of this range (328 ppb DDAC). The exceptionally high sensitivity of white sturgeon fry to Bardac 2280 is novel, and is discussed later in this report.

The acute toxicity of Bardac 2280 can be compared in a similar way for invertebrate species. For *Mysidopsis bahia*, the acute toxicity was similar to that of an unspecified mysid shrimp reported in the proprietary data (69 ppb DDAC; unpublished data, Springborn Laboratories, Inc.). However, we found that *Daphnia magna* and *Mysidopsis bahia* were over twice as sensitive to Bardac 2280 compared with the proprietary data (48-h LC_{50} value of 94 ppb DDAC for *Daphnia magna;* unpublished data, Springborn Laboratories, Inc.).

For all fish and aquatic invertebrates tested here, the acute toxicity ranged from approximately 2 ppb to 1,600 ppb Bardac 2280. This range is not too different from the more general finding that the acute toxicity of better studied quaternary ammonium compounds is between 100 and 1,000 ppb for aquatic invertebrates and fishes (Cooper 1998). However, we have either identified some of the more sensitive aquatic organisms, or Bardac 2280 is one of the more acutely toxic quaternary ammonium compounds.

For Polyphase P-100, again there is consistency between our acute toxicity data and the proprietary information.. Henderson (1992b) reported LC_{50} values for rainbow trout that ranged from 67 ppb IPBC for a 24-h flow-through bioassay to 310 ppb IPBC for an unspecified bioassay. In our study, juvenile rainbow trout and coho smolts had a 96-h LC_{50} value of approximately 100 ppb. Henderson (1992b) also reported that rainbow trout were about 2-times more sensitive to IPBC than bluegill sunfish. We found that rainbow trout (and coho salmon) were almost 4-times more sensitive to Polyphase P-100

than starry flounder. Invertebrates represented the most sensitive species (*Daphnia magna;* LC_{50} value of 39 ppb) and the most tolerant species (*Neomysis mercedis;* LC_{50} value of 2,832 ppb) to Polyphase P-100. In contrast to our findings, Henderson (1992b) reported 48-h LC_{50} value for *D. magna* (645 ppb) that was almost 15-times higher than the value obtained here.

The present studies were intended to be useful in developing Water Quality Criteria for antisapstains. In this regard it is important to comment on three other aspects of our study: (1) test species that are relevant to the Fraser River, BC; (2) the relevance of the present tests to the environmental conditions that exist in the Fraser River; and (3) the relevance of using acute testing for Water Quality Criteria.

Relevant Species

The test species that were relevant to receiving waters in BC included *Neomysis mercedis*, starry flounder, early life stages of coho salmon and juvenile white sturgeon. Under the present regulatory limit of 700 ppb DDAC for stormwater discharge and using 50% lethality as the measure of a deleterious effect, the most tolerant of the invertebrate or fish species we tested (i.e., adult *N. mercedis* and juvenile starry flounder) are possibly protected as specified by the Canadian Federal Fisheries Act, which requires the absence of a deleterious effect. However, since these animals were collected in the estuarine area of the Fraser River, where they normally live and breed, it could be argued that our testing used only a selected sub-population already exposed to and tolerant of numerous toxicants that potentially included DDAC.

Our studies suggest that, if exposed, juvenile coho, and especially juvenile white sturgeon would not be adequately protected by the regulatory limit for DDAC, even using 50% lethality as the measure of a deleterious effect. It is important, therefore, to determine the likelihood of DDAC exposure for these species and whether or not the high sensitivity of these species is characteristic of other aquatic organisms that were not tested here, but nonetheless at risk of DDAC exposure. The extreme sensitivity of juvenile white sturgeon is of particular concern. Further work should be done to consolidate this finding. Foremost, the acute toxicity tests should be corroborated in other laboratories (even though the tests were reproduced in consecutive years using different egg supplies) since these were the first ever toxicity tests, to our knowledge, on early life stages of white sturgeon (see Farrell and Bennett, this volume). We also used a Californian, rather than Fraser River fish stock. in these studies. Second, efforts should be made to describe the ecology of white sturgeon in the Fraser River to identify their probability of exposure. White sturgeon are, after all, a valued indicator species of large river systems such as the Fraser River.

Under the present regulatory limit of 120 ppb IPBC and using 50% lethality as the measure of a deleterious effect, the most tolerant of the invertebrate or fish species we tested (i.e., *N. mercedis* and juvenile starry flounder) appear to be protected, as specified by the Canadian Federal Fisheries Act. Protection of juvenile coho, if exposed, would be marginal.

Measured IPBC concentrations in stormwater runoff from sawmills on the lower Fraser River in British Columbia have ranged from non-detectable to as high as 370 ppb, whereas DDAC has been measured at levels as high as 6,000 ppb (Envirochem 1992). Therefore, given the present range of acute toxicity, compliance to regulations is very much a significant concern.

Relevance of Test Conditions to the Field

To obtain a reasonable measure of confidence in the data, the present suite of testing was performed under controlled laboratory conditions. Therefore, extrapolations from the present data to field situations, such as those made above, are made with some caution. Foremost, the water quality conditions that exist in the Fraser River differ considerably from those used in the laboratory. The Fraser River contains a high sediment load and contains other toxicants. Furthermore, the sawmills mostly operate in the lower, estuarine reaches where the receiving water is subjected to tidal fluctuations in salinity and temperature, and there can be considerable limitations on mixing, depending on the daily tidal cycle and the seasonal river flow.

Ideally, some of the acute toxicity testing should be repeated under more relevant field conditions, perhaps using mesocosms containing some of the more sensitive species. In the absence of such information, we must draw what we can from existing data.

With regard to a possible confounding effect of salinity, the two most tolerant species tested were the euryhaline species (starry flounder and *Neomysis*). However, this tolerance may simply reflect species-specific variability because salinity per se had no significant effect acute toxicity of Bardac 2280 in juvenile coho salmon. Different species were tested at different acclimation temperatures, but no experiments specifically examined the effect of temperature. Therefore, comments on the possible confounding effect of temperature are not possible. However, all of the test temperatures used were relevant to the seasonal fluctuation in water temperature in the lower Fraser River.

With regard to a possible confounding effect from the suspended sediment and organic matter load in the river, it is well established that chemicals can bind to sediments. In fact, quaternary ammonium halides are characterized by strong sorption to sediments (Lewis and Wee 1983; Lewis 1991; Versteeg and Shorter 1992). While it is likely that DDAC shows strong sorption properties, the prediction that DDAC sorption to sediments would reduce toxicity in sediment-laden water, while likely needs to be quantified. There is a danger of making generalizations that are too broad, especially given the absence of specific information for DDAC toxicity under conditions resembling the Fraser River. For example, on one hand, Lewis and Wee (1983) showed that, in tests using river water, acute and chronic toxicity and bioconcentration of three dialkyl (C15 and C17) dimethyl ammonium halides were considerably less (10-fold) than those in corresponding tests conducted with filtered laboratory water. These differences were largely attributed to the strong adsorption and aqueous insolubility of these compounds. On the other hand, Versteeg and Shorter (1992) found that alkyl chain length significantly altered this sorption effect. For example, humic acid reduced the acute toxicity of the fathead minnow to quaternary compounds with an chain length of 16-18, but not to those with a alkyl chain length of 8 to 14. DDAC has a chain length of 10 and therefore sorption may not affect DDAC toxicity as much, if at all, but we simply do not know. In addition to chemical structure, the sorptive capacity of sediment-laden water is highly correlated to its organic carbon content. Thus, the sorptive capacity of Fraser River sediments is likely to be lower than many lakes because of the lower organic carbon content. In fact, in the only study we are aware of to specifically examine the effect of Fraser River sediments on chemical bioavailability, uptake of hydophobic biphenyl toxicants across fish gills was

increased (rather than decreased) even though there was considerable chemical sorption to the suspended sediments (Qiao and Farrell 1996).

Relevance of Acute Lethality For Deriving Water Quality Criteria

Acute toxicity tests are the first and often the only types of toxicity tests performed on new compounds. Thus, acute toxicity values represent a large and useful comparative data base, around which Water Quality Criteria are typically built. Similarly, our above predictions about protection of relevant Fraser River aquatic organisms were based on the assumption that acute toxicity data are useful in this regard. Some of the work performed here allows us to examine this assumption.

A number of factors are critical to using acute toxicity to protect aquatic life. One factor is the dilution of the chemical after discharge. However, stormwater typically is not well mixed with the receiving environment of the Fraser River (G. Kruzynski pers. comm.). Therefore, even though the potential for dilution is large in the Fraser River, stormwater containing antisapstains entrains to the river banks. In the lower Fraser River, tidal activity exacerbates this entrainment of storm water because current reversals and salt water wedges limit horizontal and vertical mixing. Therefore, definitive information on the three-dimensional dispersal pattern of stormwater discharge is needed to identify 10-fold and 100-fold dilution zones. The importance of knowing these dilution zones relates to (1) the extensive usage of the near shore environment by fishes and aquatic invertebrates, and (2) the relationship between chemical concentration and toxicity. It has been suggested that sublethal toxicity is not apparent for many chemicals at 1% and even 10% of LC_{50} concentrations (i.e., a 10- to 100-fold dilution). Thus, the slope of the concentration-response relationship for acute lethality provide valuable insights into the impact of dilution on toxicity.

We consistently discovered unusually steep concentration-response relationships for Bardac 2280 with fish and aquatic invertebrates (Figure 1). The same occurred for Polyphase P-100 with fish, but not invertebrates. This steep concentration-response relationship is in keeping with the more general finding for quaternary ammonium halides (Cooper 1988). There are several implications of this relationship. First, little to no overlap could exist between the concentrations of antisapstains producing lethality in one species versus another. This finding suggests that for a wide range of aquatic species, there is a fairly well defined, species-specific threshold concentration above which Bardac 2280 is toxic. Second, the concentrations for the NOEC and 100% mortality were rarely more than an order of magnitude apart. Thus, a 10-fold dilution from the LC_{50} value would easily prevent acute lethality. Third, if acute lethality is reduced so dramatically by dilution, then sublethal toxicity is less likely. Sublethal effects are now discussed further because, in a natural setting, the resultant decrease in physiological fitness may decrease survival or reproductive success. [Exposure to an end-of-pipe concentration equivalent to an LC_{50} value theoretically means that 50% of organisms would die there if the exposure duration as long enough. However, in reality in a river setting, water currents would wash away the animals as they became progressively incapacitated and could not swim well enough to hold station.]

Novel information on the sublethal toxicity antisapstains was generated in this study. The sublethal exposure period was limited to 24-h to better simulate a stormwater runoff situation. Test concentrations were set at a proportion of the LC_{50} value to assist comparisons. In general, a primary

stress response was not observed in either rainbow trout or starry flounder at an exposure concentration lower than 50% of the 96-h LC_{50} concentration. Likewise, in unspecified studies with bluegill sunfish, coho salmon, *Daphnia magna*, and a mysid shrimp, the reported NOEC was always within 50% of the LC_{50} value for DDAC (unpublished data, Springborn Laboratories, Inc.; as quoted in Henderson 1992a). In view of this, acute toxicity endpoints may be a reasonable starting point for the development of Water Quality Criteria for short exposures to antisapstain fungicides. However, at this time we do not know what a relevant exposure period might be. The precise nature, extent and timing of the sublethal response will depend on the mechanism of action of the chemical, of which we know little for these antisapstain fungicides in aquatic organisms (Johnston et al. 1997).

The likelihood of aquatic organisms being challenged with only either DDAC or IPBC in the Fraser River is unlikely. There are many other toxicants, pathogens and water quality conditions (e.g., hypoxia) that collectively tax the overall tolerance of these organisms, perhaps increasing their sensitivity to DDAC and IPBC. Also, there are various antisapstain formulations in use that incorporate both IPBC and DDAC (Henderson 1992b). For example, the formulation NP-2 contains a 1:7 mixture of the two antisapstain compounds IPBC and DDAC. The present study provided new information on the interactions of a mixture of IPBC and DDAC.

Additive toxicity indices for fish species deviated very little from a simple additive effect of IPBC and DDAC. However, the findings for the invertebrate species varied considerably and were less predictable. Although the combined effects of IPBC and DDAC on *N. mercedis* were nearly additive, simple addition would overestimate by more than 2-fold the toxicity of the mixture to *D. magna*. In contrast, simple addition would underestimate by 16-fold the acute toxicity of the mixture to *H. azteca*. Of further concern were the sublethal stress effects that were revealed with the mixture but not with the individual chemicals. A primary stress response (elevated cortisol) occurred in rainbow trout at a much lower concentration of Bardac 2280 when it was mixed with Polyphase P-100. Also, we have no explanation for (but are concerned about) the lowered plasma lactate in starry flounder when exposed to the mixture. Since the possibility sublethal effects of mixtures cannot be simply excluded, even when the sublethal effects are shown to be absent for the principal components tested alone and when there are steep concentration-response curves for acute toxicity, the sublethal effects of chemical mixtures relevant to the Fraser River require greater attention.

A major contribution of these acute toxicity studies is that we have (1) identified some of the more sensitive aquatic organisms, (2) confirmed some of the proprietary literature, and (3) placed a suite of information into the refereed literature which can be used for developing Water Quality Criteria for these fungicides in Canada. We conclude that, until more work is performed to better define species variability to these mixtures in relation to species and water quality conditions relevant to the receiving environments, it is perhaps prudent to use a precautionary principle and adopt the most sensitive fish and/or invertebrate species to develop regulatory guidelines.

Acknowledgements

This work was funded by Environment Canada through the Fraser River Action Plan. The support and advice of Colin Gray and Fred Mah during this study were welcomed and appreciated. The technical support of Antony Wood, Eric Stockner, Blair Johnston, Joanne Scherba and Keith Tierney during this project was invaluable. The test chemicals used in this study were kindly donated by Kop-Coat Inc., Pittsburgh, PA (Troysan Polyphase P-100) and Lonza Inc., Fair Lawn, NJ (Bardac 2280)

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Table 1a. Rearing and Test Conditions of Fishes

Species	Age	Source of Organisms	Feed Type	Water Source	Temperature		Photoperiod		Water	
					Rearing	Testing	Rearing	Testing	Rearing	Testing
Oncorhynchus kisutch (Coho salmon)	embryo	Capilano Hatchery	N/A	MW	8°C	8°C	12:12	12:12	F	S
O. *kisutch* (Coho salmon)	alevin	Capilano Hatchery	Trout chow	MW	8-10°C	10°C	14:10	14:10	F	F
O. *kisutch* (Coho salmon)	fry	Capilano Hatchery	Trout chow	MW	12-16°C	12°C	14:10	14:10	F	F
O. *kisutch* (Coho salmon)	smolt	Capilano Hatchery	Trout chow	MW / SW	10-12°C	12°C	14:10	14:10	F	F
O. mykiss (Rainbow trout)	juvenile	West Creek Trout Farm	Trout chow	MW	12-13°C	12°C	14:10	14:10	F	F
Platichthys stellatus (Starry flounder)	juvenile	Wild - Fraser River	Chironomid larvae	SW	12°C	12°C	14:10	14:10	R	F
Pimephales promelas (Fathead minnow)	fry	Aquatic Research Organisms	*Artemia* nauplii	MHS	25°C	25°C	14:10	14:10	SR	SR
Acipenser transmontanus (White sturgeon)	fry	UC Davis	Trout chow	M	15 °C	15 °C	14:10	14:10	R	S

All temperatures ± 1°C except where range is given. Photoperiod recorded in hours (light:dark). MW = dechlorinated municipal tapwater (pH 6.1 - 6.7, hardness 6.0 mg/L $CaCO_3$); SW = salt water (pH 7.9 - 8.2, salinity 27%0); MHS = moderately hard synthetic water (pH 8.1 - 8.3, hardness 180 mg/l $CaCO_3$); F = flow through; S = static; SR = static with replacement; R = recirculated with filtration.

Table 1b. Rearing and Test Conditions of Aquatic Invertebrates

Species	Age	Source of Organisms	Feed Type	Water Source	Temperature		Photoperiod		Water	
					Rearing	Testing	Rearing	Testing	Rearing	Testing
Hyalella azteca	2-9 days	Aquatic Research Organisms	Trout chow	MHS	25°C	25°C	16:8	16:8	SR	SR
Daphnia magna	<1 day	Aquatic Research Organisms	Trout chow, algae	MHS	20°C	20°C	16:8	16:8	SR	SR
Mysidopsis bahia	-	Aquatic Research Organisms	*Artemia* nauplii	SW	25°C	25°C	16:8	16:8	SR	SR
Neomysis mercedis	adult	Wild - Fraser River	Trout chow	MW / SW	12-15°C	12°C	12:12	12:12	SR	SR

All temperatures ± 1°C except where range is given. Photoperiod recorded in hours (light:dark). MW = dechlorinated municipal tapwater (pH 6.1 - 6.7, hardness 6.0 mg/L $CaCO_3$); SW = salt water (pH 7.9 - 8.2, salinity 27‰); MHS = moderately hard synthetic water (pH 8.1 - 8.3, hardness 180 mg/l $CaCO_3$); F = flow through; S = static; SR = static with replacement; R = recirculated with filtration.

Table 2. Acute toxicity of Bardac 2280 to fishes and aquatic invertebrates.

Test Species	Exposure Duration	NOEC	LC_{50} of Bardac (95% CI)	LC_{50} of DDAC (based on 80% a.i.)	100% mortality
Fishes					
Coho embryo (4-day old)	96-h	150 ppb	570 ppb (400-920)	456 ppb	1,200 ppb
Coho eyed-embryo (42-day old)	96-h	600 ppb	1,100 ppb (600-1,200)	880 ppb	1,200 ppb
Coho alevin (67-day old)	96-h	320 ppb	420 ppb (320-560)	336 ppb	560 ppb
Coho alevin (76-day old)	96-h	320 ppb	390 ppb (350-430)	312 ppb	560 ppb
Coho alevin (86-day old)	96-h	400 ppb	460 ppb (430-580)	368 ppb	560 ppb
Coho swim-up fry (104-day old)	96-h	420ppb	490 ppb (460-540)	392 ppb	560 ppb
Coho smolt (10-month old)	96-h	500 ppb	950 ppb (810-1,100)	760 ppb	1,200 ppb
Rainbow trout juvenile	96-h	200 ppb	410 ppb (330-510)	328 ppb	500 ppb
Starry Flounder juvenile	96-h	1,500 ppb	2,000 ppb (1,500-2,200)	1600 ppb	2,200 ppb
Fathead minnow (7-day old)	96-h	50 ppb	330 ppb (300-500)	264 ppb	500 ppb
White sturgeon fry (42-day old)	96-h	1 ppb	2.5 ppb (1-10)	2.0 ppb	10 ppb
Invertebrates					
Hyalella azteca	48-h	75 ppb	110 ppb (93-120)	88 ppb	240 ppb
Daphnia magna	48-h	30 ppb	37 ppb (28-48)	30 ppb	75 ppb
Mysidopsis bahia	48-h	20 ppb	39 ppb (20-40)	31 ppb	40 ppb
Neomysis mercedis	48-h	420 ppb	972 ppb (720-1,100)	778 ppb	1,400 ppb

Concentrations are reported as nominal concentrations.
Age of coho salmon is in days or months post-fertilisation.
LC_{50} values and 95% confidence intervals (CI) were calculated using probit analysis, based on the pooled data set for a given test organism. There was no mortality observed in any fish control groups. Mortality in invertebrate control groups was rare and never exceeded 10% in a given test, in which case, the adjusted mortality was calculated according to Abbott's formula. LC_{100} is the lowest test concentration at which 100% mortality was observed.
NOEC is the highest test concentration at which mortality was identical to the control. If no test concentration resulted in zero mortality, then the NOEC is reported as less than the lowest concentration tested.
LC_{50} values of DDAC were calculated from the LC_{50} value of Barbac 2280 which contains 80% DDAC and given the assumption that no interactions occur among all ingredients of Bardac 2280.

Table 3. The sublethal response of juvenile rainbow trout and starry flounder to a 24-h exposure to Bardac 2280.

	Trout				Flounder			
Conc. % LC_{50}	0.0 ppb control	100 ppb 25%	200 ppb 50%	400 ppb 100%	0.0 ppb control	500 ppb 25%	1,000 ppb 50%	2,000 ppb 100%
Lactate (mg/dL)	49.2 (4.79)	42.5 (2.50)	45.8 (4.55)	61.7* (3.02)	6.23 (0.47)	6.30 (0.18)	8.45* (1.07)	7.79* (0.68)
Glucose (mg/dL)	65.5 (3.2)	62,7 (2.72)	63.1 (2.36)	81.5* (3.02)	43.6 (2.54)	48.9 (2.54)	50.8 (3.57)	46.7 (2.05)
Hemoglobin (g/dL)	7.48 (0.53)	7.42 (0.16)	7.39 (0.23)	8.35 (0.23)	5.38 (0.45)	6.07 (0.44)	5.24 (0.39)	6.28 (1.01)
Cortisol (µg/dL)	1.88 (0.44)	3.19 (0.92)	0.81 (0.11)	8.17* (2.16)	- -	- -	- -	- -
Hematocrit (%)	46.0 (1.29)	46.3 (1.45)	42.9 (1.82)	48.9 (1.48)	24.2 (1.45)	26.7 (1.05)	23.2 (1.51)	23.6 (1.32)
Leucocrit (%)	0.67 (0.07)	1.02 (0.10)	0.88 (0.10)	0.78 (0.10)	0.75 (0.15)	0.93 (0.13)	0.59 (0.16)	0.51 (0.07)
Liver:somati index	1.10 (0.20)	1.10 (0.40)	1.20 (0.30)	1.10 (0.20)	-	-	-	-
U_{crit} (12-h) (bl/s)	6.64 (0.14)	6.73 (0.28)	5.46* (0.28)	6.02* (0.15)	-	-	-	-
U_{crit} (24-h) (bl/s)	6.62 (0.24)	7.32* (0.14)	5.04* (0.12)	5.15* (0.14)	-	-	-	-
U_{crit} (48-h) (bl/s)	6.54 (0.20)	6.34 (0.32)	5.99 (0.19)	5.78* (0.11)	-	-	-	-

*mean value for n=10 (SEM in parentheses).

* denotes a significant difference from the control value ($P<0.05$; ANOVA-SNK)

Table 4. Acute toxicity of Polyphase P-100 to fishes and aquatic invertebrates.

Test Species	Exposure Duration	NOEC	LC_{50} of Polyphase (95% CI)	LC_{50} of IPBC (based on 97% a.i.)	100% mortality
Fishes					
Coho embryo (11-day old)	96-h	<1,000 ppb	1,320 ppb (1,200-1,440)	1280 ppb	4,600 ppb
Coho eyed-embryo (34-day	96-h	<1,000 ppb	1,900 ppb (1,700-2,100)	1843 ppb	3,200 ppb
Coho alevin (67-day old)	96-h	<180 ppb	210 ppb (200-230)	204 ppb	320 ppb
Coho alevin (86-day old)	96-h	120 ppb	166 ppb (120-200)	161 ppb	200 ppb
Coho fry (120-day old)	96-h	100 ppb	130 ppb (100-160)	126 ppb	160 ppb
Coho smolt (10-month old)	96-h	<70 ppb	95 ppb (86-100)	92 ppb	100 ppb
Rainbow trout juvenile	96-h	70 ppb	100 ppb (124-140)	97 ppb	180 ppb
Starry Flounder juvenile	96-h	320 ppb	370 ppb (320-420)	359 ppb	420 ppb
Invertebrates					
Hyalella azteca	48-h	100 ppb	500 ppb (380-650)	485 ppb	2,200 ppb
Daphnia magna	48-h	<10 ppb	40 ppb (28-55)	39 ppb	>220 ppb
Neomysis mercedis	48-h	<1,000 ppb	2,920 ppb (2,470-3,520)	2832 ppb	6,800 ppb

Concentrations are reported as nominal concentrations.

Age of coho salmon is in days or months post-fertilisation.

LC_{50} values and 95% confidence intervals (CI) are presented.

LC_{50} values of IPBC were calculated from the LC_{50} value of Polyphase P-100 which contains 97% IPBC and given the assumption that no interactions occur among all ingredients of Polyphase P-100.

Table 5. The sublethal response of juvenile rainbow trout and starry flounder to a 24-h exposure to Polyphase P-100.

	Trout				Flounder			
Conc. % LC_{50}	0.0 ppb control	35 ppb 25%	70 ppb 50%	140 ppb 100%	0.0 ppb control	90 ppb 25%	180 ppb 50%	360 ppb 100%
Lactate (mg/dL)	22.4 (6.3)	16.9 (2.6)	12.0 (2.6)	13.5 (2.5)	10.4 (3.3; 5)	11.2 (1.6; 7)	9.0 (1.0; 8)	8.8 (1.3; 7)
Glucose (mg/dL)	126.8 (7.7)	109.5 (6.2)	124.2 (12.6)	133.6 (8.5)	47.0 (5.1; 5)	57.1 (17.7; 5)	53.1 (4.9; 7)	62.4 (5.5; 6)
Hemoglobin (g/dL)	8.82 (0.30)	7.83 (0.94)	7.86 (0.60)	8.70 (0.44)	5.90 (0.53; 9)	5.32 (0.30; 11)	6.73 (0.32; 11)	6.10 (0.36; 12)
Cortisol (μg/dL)	3.65 (0.85)	3.33 (2.60)	3.46 (1.04)	3.94 (2.05)	4.38 (1.91; 5)	13.4 (7.11; 4)	5.20 (2.45; 6)	20.9 (9.74; 5)
Hematocrit (%)	46.6 (1.7)	48.2 (1.9)	42.4 (2.7)	47.0 (2.6)	25.1 (1.0; 7)	22.6 (1.2; 7)	26.9 (1.5; 8)	27.0 (1.4; 7)
Leucocrit (%)	1.51 (0.09)	1.23 (0.09)	1.34 (0.15)	1.25 (0.09)	1.47 (0.15; 5)	1.00 (0.07; 7)	1.01 (0.11; 7)	0.71* (0.15; 7)
Liver:somati index	0.80 (0.02)	0.85 (0.03)	0.96[a] (0.03)	0.90[a] (0.04)	1.54 (0.30; 6)	3.70 (2.51; 6)	1.32 (0.23; 6)	1.44 (0.15; 6)

Mean value for n=10 (SEM in parentheses). The exposure periods for the swimming performance tests are indicated in parentheses. N values less than 10 are indicated as the second value in parentheses.

* denotes significant difference from control value ($P<0.05$, ANOVA; SNK).

Table 6. Acute (96-h exposure) toxicity of a mixture (1:8) of Polyphase P-100 and Bardac 2280 to fishes and invertebrates.

Test Species	Exposure Duration	NOEC	LC_{50} (95% CI)	100% mortality	Additive Index (95% CI)
Fishes					
Coho alevin (53-day old)	96-h	320 ppb	490 pp (450 to 520)	>560 ppb	-0.37 (-0.39 to -0.33)
Coho juvenile (7-month old)	96-h	320 ppb	430 ppb (380 to 490)	560 ppb	-0.27 (-0.33 to -0.17)
Rainbow trout juvenile	96-h	320 ppb	460 ppb (430 to 490)	560 ppb	-0.38 (-0.52 to -0.24)
Starry Flounder juvenile	96-h	700 ppb	1,280 ppb (1,200 to 1,370)	>1,500 ppb	0.06
Invertebrates					
Hyalella azteca	48-h	14 ppb	26 ppb (17 to 40)	72 ppb	7.47 (8.34 to 29.4)
Daphnia magna	48-h	<75 ppb	110 ppb (95 to 120)	160 ppb	-1.77 (-2.32 to -1.37)
Neomysis mercedis	48-h	<720 ppb	770 ppb (650 to 850)	1,400 ppb	0.37 (0.20 to 0.39)

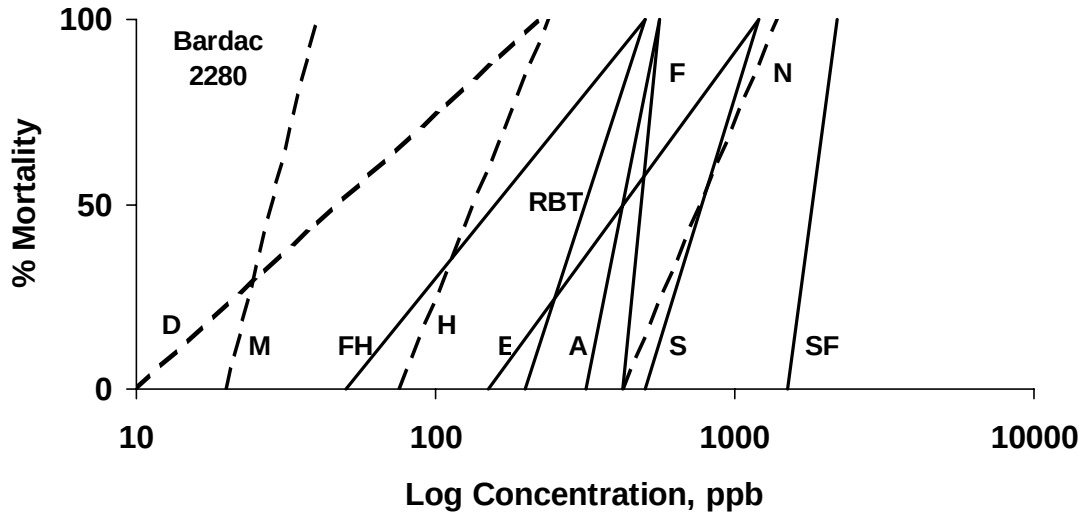
Nominal concentrations of the formulation (8 parts Bardac 2280 and 1 part Polyphase P-100) are presented. Nominal concentrations of the active ingredients are calculated using 0.71 times the formulation concentration for DDAC and 0.065 times the formulation concentration for IPBC.

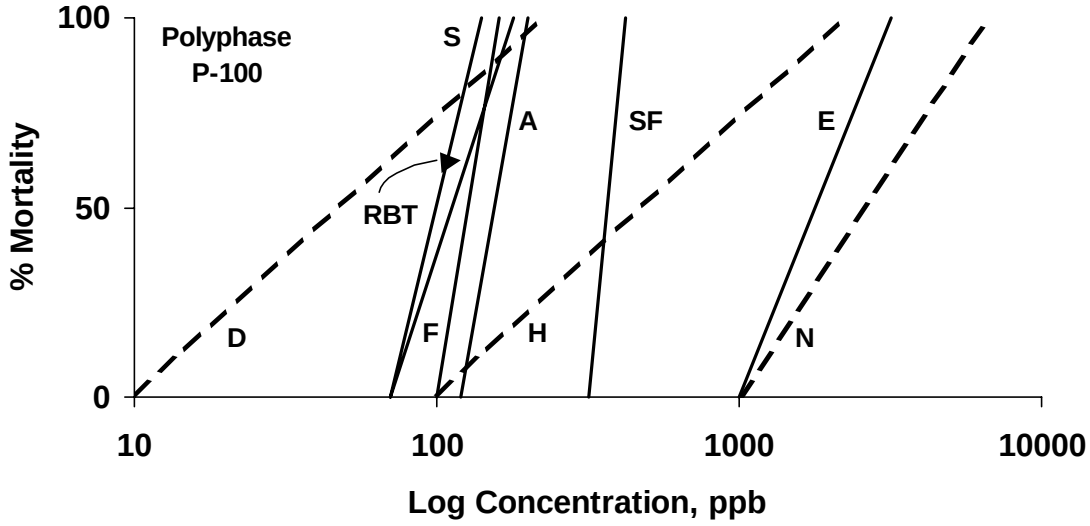
Table 7. The sublethal response of juvenile rainbow trout to a 24-h exposure to a mixture (1:8) of Polyphase P-100 and Bardac 2280.

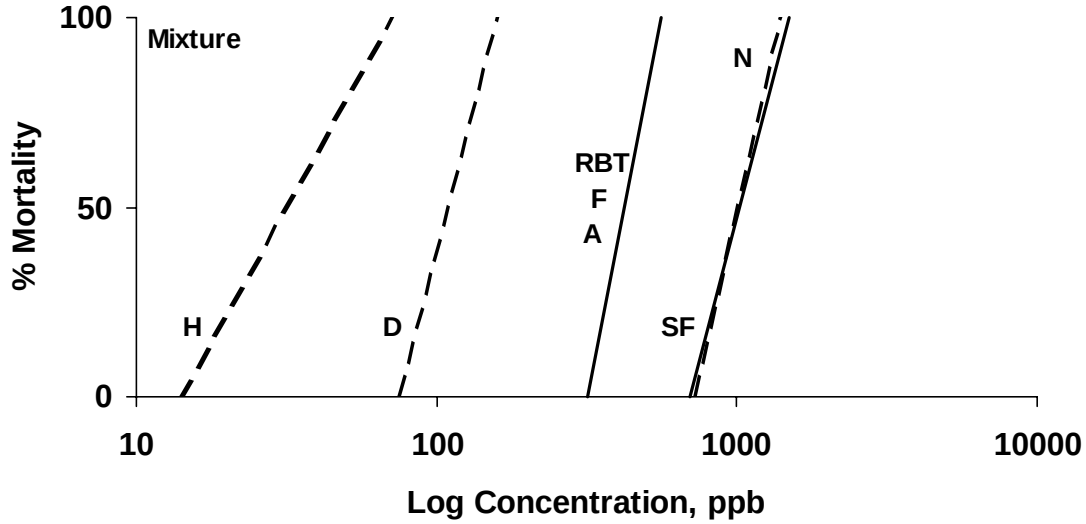
	Trout				Flounder			
Conc. % LC_{50}	0.0 ppb control	110 ppb 25	220 ppb 50	440 ppb 100	0.0 ppb control	300 ppb 25	600 ppb 50	1200 ppb 100
Lactate (mg/dL)	11.8 (2.2; 10)	19.3 (2.1; 10)	17.3 (2.9; 10)	16.4 (3.4; 10)	12.8^{abc} (13.2; 10)	5.7^{ad} (0.5; 10)	8.0^{bde} (1.6; 10)	5.5^{ce} (0.4; 10)
Glucose (mg/dL)	111.1 (7.0; 9)	104.4 (7.4; 10)	105.7 (4.0; 10)	127.1 (7.3; 10)	59.7^{o} (8.5; 4)	58.5^{p} (7.9; 5)	59.1^{q} (10.8; 6)	159.1^{opq} (29.8; 8)
Hemoglobin (g/dL)	8.75 (0.32; 10)	8.63 (0.35; 9)	8.66 (0.34; 10)	9.30 (0.36; 10)	6.47 (1.27; 8)	6.37 (0.93; 8)	6.54 (1.09; 8)	6.21 (1.05; 8)
Cortisol (µg/dL)	8.4^{jm} (4.13; 10)	28.3^{kn} (11.9; 8)	370.5^{lmn} (130; 8)	1000.0^{jkl} (501; 10)	31.7 (0.93; 6)	286.8 (1.76; 6)	67.0 (2.32; 6)	60.8 (1.92; 4)
Hematocrit (%)	43.4 (2.2; 10)	48.3 (1.9; 10)	45.6 (2.0; 10)	47.7 (3.3; 10)	25.4^{f} (1.1; 9)	24.6^{g} (0.6; 9)	28.7^{fg} (1.1; 9)	27.7 (1.0; 9)
Leucocrit (%)	0.84 (0.08; 10)	0.78 (0.14; 11)	0.74 (0.10; 10)	0.78 (0.09; 10)	0.99^{h} (0.15; 10)	1.03^{i} (0.16; 10)	0.67 (0.10; 10)	0.50^{hi} (0.09; 10)
Liver:somatic index	0.79 (0.03; 10)	0.80 (0.12; 11)	0.76 (0.03; 10)	0.81 (0.03; 10)	- -	- -	- -	- -

Mean values with SEM and N value present in parentheses.
Values sharing a common alphabetical superscript differ significantly ($P<0.05$, ANOVA; SNK).

Figure 1. A comparison of the concentration-response relationships for Bardac 2280 alone, Polyphase P-100 alone and a mixture containing 8 parts Bardac 2280 and 1 part Polyphase P-100. Each line represents one test organism and connects the concentration causing no mortality with the concentration producing 100% mortality. In general, the gradient of these lines is steep, indicating a narrow concentration range over which the chemical is acutely toxic. For comparison, fishes are presented with solid lines and invertebrates with broken lines. (Abbreviations: RBT = rainbow trout; FH = fathead minnow; E = coho salmon embryo; A = coho salmon alevin; F = coho salmon fry; S = coho salmon smolt; SF = starry flounder; D = *Daphnia magna*; H = *Hyalella azteca*; N = *Neomysis mercedis*; M = *Mysidopsis bahia*).






100
50
0
% Mortality
Mixture
H
D
RBT
F
A
N
SF
10
100
1000
10000
Log Concentration, ppb