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A Study of the Lethal and Sublethal Toxicity of Polyphase P-100, an Antisapstain Fungicide Containing 3-Iodo-2-Propynyl Butyl Carbamate (IPBC), on Fish and Aquatic Invertebrates

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Abstract. The acute toxicity of Polyphase P-100, an antisapstain wood preservative that contains 97% 3-iodo-2-propynyl butyl carbamate (IPBC), was determined for three species of fish (coho salmon, rainbow trout, and starry flounder) and three species of aquatic invertebrates (*Daphnia magna, Hyalella azteca,* and *Neomysis mercedis*). The 96-h LC_{50} values for the various fish species exposed to Polyphase P-100 ranged from 95 ppb for coho smolts (*Oncorhynchus kisutch*) to 370 ppm for juvenile starry flounder (*Platichthys stellatus*). The sensitivity of coho to Polyphase P-100 was altered by their developmental stage. Coho embryos were six to nine times more tolerant of Polyphase P-100 than coho alevins, which were twice as tolerant as coho smolts. The 48-h LC_{50} values for the invertebrates *D. magna, H. azteca,* and *N. mercedis* were 40 ppb, 500 ppb, and 2,920 ppb, respectively. In addition to a wider range of sensitivity to Polyphase P-100 compared with the fish species, the invertebrate species were characterized by a shallower concentration-response. In acute, 24-h sublethal tests with juvenile starry flounder and rainbow trout, there was no primary or secondary stress response (changes in hematocrit, leucocrit, hemoglobin concentration, plasma lactate concentration, and plasma cortisol concentration) at concentrations up to 50% of the 96-h LC_{50} value. The acute toxicity of a 1:8 mixture of Polyphase P-100 and Bardac 2280 (another antisapstain compound that contains didecyldimethylammonium chloride [DDAC] as the active ingredient) was close to additive for fish, but not for invertebrate species. The acute toxicity of the mixture was seven to eight times more than additive for *H. azteca,* but two to three times less than additive for *D. magna.* Some sublethal stress responses were revealed with the mixture that were not observed with the test chemicals alone.

To maintain lumber marketability, the forest products industry in the northwestern regions of the United States and Canada has come to rely heavily on the use of antisapstain products to prevent the growth of molds and fungi that stain milled lumber. Historically, environmental concerns (*e.g.,* NRCC 1982; Kennedy and Law 1986; Krahn *et al.* 1987; MacKinnon and Farrell 1992; Nikl and Farrell 1993) over the use of both first-generation antisapstain products (chlorinated phenolic compounds) and second-generation antisapstain chemicals (*e.g.,* 2-(thiocyanomethylthio)-benzothiazole [TCMTB] and copper-8-quinolate) have resulted in a switch to a third generation of antisapstain chemicals that predominantly utilize didecyldimethylammonium chloride (DDAC) and 3-iodo-2-propynyl butyl carbamate (IPBC) as active ingredients. Information on the toxicity of both of these compounds to aquatic organisms is extremely limited in the refereed literature, even though proprietary information has been reviewed (Henderson 1992a, 1992b; Envirochem 1992). Moreover, recent studies on the acute and sublethal toxicity of DDAC (Wood *et al.* 1996; Farrell *et al.* 1998) have raised a concern about the adequacy of present regulatory levels for storm water runoff from mill sites into the Fraser River, British Columbia, where a substantial proportion of the annual provincial usage of approximately 400 tons of DDAC takes place. Information on the toxicity of IPBC is even more scant than that for DDAC. In the absence of a reliable, refereed data base, the regulatory limit for IPBC is presently set at 120 ppb for storm water runoff from mill sites in British Columbia (Henderson 1992a).

The aim of the present research was to substantially improve the baseline data on the aquatic toxicology of IPBC-based antisaptain formulations. Polyphase P-100 (Kop-Coat Inc., Pittsburgh, PA), which contains 97% IPBC, was tested either alone or in combination with Bardac 2280 (principal active ingredient 80% didecyldimethylammonium chloride, DDAC). Acute and sublethal toxicity tests were performed using a suite of aquatic organisms under laboratory test conditions. These baseline data should provide for more reliable evaluations of the risk to aquatic organisms of stormwater runoff containing antisapstain chemicals.

Materials and Methods

Experimental Organisms

The following fish were used for flow-through, acute toxicity testing: juvenile rainbow trout (*Oncorhynchus mykiss,* 6 to 8 g), juvenile starry

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flounder (*Platichthys stellatus*, 3 to 30 g), and coho salmon (*Oncorhynchus kisutch*). Information on the various test species, as well as their holding and testing conditions are summarized in Table 1. Various developmental stages of coho salmon were tested: 11-day-old embryos, 34-day-old embryos, 67-day-old alevins, 86-day-old alevins, 120-day-old fry, and 10-month-old smolts with visible characteristics. Juvenile starry flounder were caught by beach seine at MacDonald Beach in the Fraser River estuary, BC, at least 3 weeks before use. Feeding was daily, but was stopped 1–2 days before testing. Acute, 96-h toxicity tests were performed only on healthy fish taken from populations in which there was either little (<0.05% per week) or no mortality for 2 weeks prior to testing.

The invertebrate species used for acute, static-replacement toxicity tests were: *Daphnia magna*, *Hyalella azteca*, and *Neomysis mercedis*. *D. magna* and *H. azteca* were obtained from a commercial supplier (Aquatic Research Organisms, Hampton, NH) and cultures were kept until a stable, reproducing population was attained (US EPA 1985, 1994). *D. magna* neonates (<24 h old) were used for acute toxicity tests. A mixture of adult and juvenile *H. azteca* were used for acute toxicity tests. Adult *N. mercedis* were caught by dip net on Iona Beach in the Fraser River estuary, BC, and held in the laboratory in 35-L glass aquariums containing diluted (15‰) seawater. *N. mercedis* were fed ground trout chow until one day before an experiment.

Fish Toxicity Tests

The flow-through dosing apparatus used for the exposure of rainbow trout, coho salmon (alevin, fry, and smolt stages), and juvenile starry flounder has been described in detail previously (*e.g.*, Johansen and Geen 1990; Janz *et al.* 1991; Kennedy *et al.* 1995). Briefly, groups of at least 10 fish were placed in one of six 8-L glass exposure vessels and allowed a 24-h habituation period before dosing was started. At the beginning of the exposure period, each vessel was manually injected and mixed with the appropriate quantity of stock solution to immediately attain the appropriate concentration. The water in each exposure vessel was replaced hourly via computer-controlled pumps and valves that regulated the amount of stock solution and replacement water pumped into each vessel. Initial range-finding tests (data not reported) were used to set the test concentrations. The acute toxicity tests were performed in duplicate, except for those with coho salmon embryos, which were conducted in triplicate using 500-ml glass beakers with test water replacement every 24 h. Mortality of eggs was recorded when they turned opaque. Mortality of fish was recorded when opercular movements stopped.

The 24-h sublethal exposure tests with juvenile starry flounder and rainbow trout were performed with the computer-controlled exposure apparatus described above, but using six to 10 fish in each 65-L glass aquarium. The test concentrations were 25%, 50%, and 100% of the 96-h LC_{50} value. Aquariums were isolated from each other and from outside disturbances with a black plastic blind. At the end of the exposure period, fish were anesthetized by injecting buffered MS 222 (Sigma Chemical Co., St. Louis, MO) into the water (Iwama *et al.* 1989). Anesthetized fish were quickly removed from the test chamber, weighed, measured, and the tail severed for blood sampling according to Janz *et al.* (1991). Analysis of blood samples for the indices of primary and secondary stress variables followed established methods (Janz *et al.* 1991; Johansen *et al.* 1994; Kennedy *et al.* 1995; Wood *et al.* 1996; Farrell *et al.* 1998). Blood samples were collected into hematocrit tubes and blood hemoglobin concentration was measured immediately. After centrifugation at 10,000× *g* for 2 min, hematocrit (Hct) and leucocrit (Lct) were measured immediately. Plasma was stored at −80°C until glucose, cortisol, and lactate concentrations were measured. These variables are regarded as indicators of primary (plasma cortisol) and secondary (Hct, hemoglobin, Lct, plasma lactate, and plasma glucose) stress (Adams 1990; Schreck 1990).

Invertebrate Toxicity Testing

Acute, 48-h toxicity tests with invertebrates were performed in duplicate using static systems with a single replacement every 24 h (US EPA 1985, 1994). For *D. magna* and *H. azteca*, groups of 10 animals were placed into 50-ml glass beakers. For *N. mercedis*, groups of 10 animals were placed in 500-ml beakers containing 15‰ seawater.

Test Chemical and Water Analysis

The technical product Troysan Polyphase P-100 (Kop-Coat Inc., Pittsburgh, PA), containing 97% IPBC, <0.9% NaCl, and <0.1% tri-iodo-allyl butyl carbamate, was used in all toxicity tests. Dilutions of Polyphase P-100 were made with double-distilled water to give appropriate nominal concentrations of Polyphase P-100 in stock solutions. Polyphase P-100 was also tested in combination with Bardac 2280 (Lonza Inc., Fair Lawn, NJ) at a ratio of 1:8. This resulted in a 1.0:6.4 ratio for the active ingredients (IPBC:DDAC). Bardac 2280 contained 80–82% 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. All stock solutions were made up immediately before use. Several blank test runs were performed with extensive water quality analysis of a full range of chemical concentrations to confirm the accuracy of the computer-controlled dosing apparatus and the preparation and dilution of stock solutions. In addition, the dosing apparatus was precalibrated and periodically checked with a spectrophotometer dye dilution technique. Typically chemical analysis was performed on one 1-L water sample taken at the end of a test. The water samples were collected and preserved (5 ml Rexonic N25-7 solution and 10 ml formaldehyde) according to the procedures laid out by the Canadian Organic Chemistry Analytical Laboratory (Pacific Environmental Science Centre, Environment Canada, North Vancouver, BC), where chemical analysis was performed. IPBC and DDAC were analyzed separately with high-resolution gas chromatography using Nitrogen-Phosphorus detection. Recovery was typically greater than 85% for IPBC and 92% for DDAC. Expected concentrations were linearly related to ($R^2 > 0.85$) and within 30% of the measured concentration. Results are reported as nominal concentrations.

Data Analysis

LC_{50} values were based on the cumulative mortality observed at the end of any exposure period. 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 (Abbott 1925). We also report the lowest test concentration at which 100% mortality was observed (LC_{100}) and the highest test concentration at which mortality was identical to the control (NOEC). If no test concentration resulted in zero mortality, then the NOEC is reported as less than the lowest concentration tested. The indices of sublethal toxicity were compared with ANOVA-SNK using a significance level of $p < 0.05$. Assessment for additive toxicity of the chemical mixture of IPBC and DDAC was performed according to the linear additive index method of Marking (1977) with the formula $S = (A_m/A_i) + (B_m/B_i)$, where S is the sum of the activities, A and B are the two test chemicals, and i and m are the LC_{50}s of the individual chemicals and mixtures, respectively. By correcting S values that were <1 (*i.e.*, greater than additive toxicity) by $S(-1) + 1$ and by correcting S values that were >1 (*i.e.*, less than additive toxicity) by $1/S - 1$, a corrected sum-additive index greater than zero indicated a greater than additive effect and an index less than

Table 1. Rearing and test conditions of test organisms

Species	Age	Source of Organisms	Feed Type	Water Source	Temperature (°C)		Photo Period (light:dark) (h)		Water	
					Rearing	Testing	Rearing	Testing	Rearing	Testing
Fishes										
Coho salmon	embryo	Capilano Hatchery	N/A	MW	8	8	12:12	12:12	F	S
Coho salmon	alevin	Capilano Hatchery	Trout chow	MW	8–10	10	14:10	14:10	F	F
Coho salmon	fry	Capilano Hatchery	Trout chow	MW	12–16	12	14:10	14:10	F	F
Coho salmon	smolt	Capilano Hatchery	Trout chow	MW/SW	10–12	12	14:10	14:10	F	F
Rainbow trout	juvenile	West Creek Trout Farm	Trout chow	MW	12–13	12	14:10	14:10	F	F
Starry flounder	juvenile	Wild—Fraser River	Chironomid larvae	SW	12	12	14:10	14:10	R	F
Invertebrates										
Hyalella azteca	2–9 days	Aquatic Research Organisms	Trout chow	MHS	25	25	16:8	16:8	SR	SR
Daphnia magna	<1 day	Aquatic Research Organisms	Trout chow, algae	MHS	20	20	16:8	16:8	SR	SR
Neomysis mercedis	adult	Wild—Fraser River	Trout chow	MW/SW	12–15	12	12:12	12:12	SR	SR

All temperatures ±1°C except where range is given. Water quality parameters were measured daily and remained within the stated ranges. 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. Capilano Hatchery, North Vancouver, BC; West Creek Trout Farm, Langley, BC; Aquatic Research Organisms, Hampton, NH

zero indicated a less than additive effect. LC_{50} values and 95% CI from data for Polyphase P-100 presented here and from previously published data for Bardac 2280 (Farrell *et al.* 1998) were used for this analysis.

Results

Fish Toxicity Tests with Polyphase P-100

The acute toxicity values for the fish species (96-h LC_{50}) ranged from 95 ppb for coho smolts to 1,900 ppb for coho embryos (Table 2). Starry flounder were over three times more tolerant of Polyphase P-100 compared with coho smolts. In contrast, juvenile rainbow trout showed a similar sensitivity to Polyphase P-100 as coho fry.

The sensitivity of coho salmon to Polyphase P-100 was significantly altered by their developmental stage (Table 2). Coho smolts were over 10 times more sensitive to Polyphase P-100 than embryos. Fry and alevin sensitivity to Polyphase P-100 was similar to that of smolts.

The slope of the concentration-response relationship for fishes was unusually steep (Figure 1). The Polyphase P-100 concentrations for the NOEC and 100% mortality were never more than an order of magnitude apart. Consistent with this steep concentration-response relationship was the absence of primary and secondary stress responses for most of the blood parameters measured after a 24-h exposure to Polyphase P-100 at concentrations between 25% and 100% of the 96-h LC_{50} value. For juvenile rainbow trout, hematocrit, leucocrit, plasma glucose, plasma cortisol, plasma lactate, and hemoglobin were unchanged by exposures up to a concentration of 140 ppb Polyphase P-100 (100% of the 96-h LC_{50}) (Table 3). Similar results were found for starry flounder regarding these sublethal indices. Polyphase P-100 concentrations up to 180 ppb (50% of the 96-h LC_{50} value) had no significant effect on the measured variables (Table 3). However, at a concentration of 360 ppb, plasma cortisol variability increased and Lct decreased significantly in starry flounder.

Invertebrate Toxicity Tests with Polyphase P-100

The acute toxicity values for invertebrate species ranged from 40 ppb for *D. magna* to 2,920 ppb for *N. mercedis* (Table 2), spanning the LC_{50} values for fish. In addition, the slopes for the concentration-response relationship for invertebrates were much shallower than those for fishes (Figure 1).

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

Fish exposed to a 1:8 (v/v) mixture of Polyphase P-100 and Bardac 2280 had acute toxicity values ranging from 420 ppb for coho smolts to 1,280 ppb for juvenile starry flounder (Table 4). The additive indices for acute toxicity are also presented in Table 4. For flounder, the toxicity of Polyphase P-100 and Bardac 2280 were marginally additive (*i.e.*, additive index = 0.06). For rainbow trout and coho, the toxicities of the mixture were marginally, but consistently less than additive (*i.e.*, additive indices from −0.27 to −0.38). Invertebrates exposed to the mixture of Polyphase P-100 and Bardac 2280 had acute toxicity values ranging from 26 ppb for *H. azteca* to 770 ppb for *N. mercedis* (Table 4). The additive indices (Table 4) showed that Polyphase P-100 and Bardac 2280 were less than additive for *D. magna* (*i.e.*, additive index = −1.77), marginally more than additive for *N. mercedis* (*i.e.*, additive index = 0.37), and considerably more than additive for *H. azteca* (*i.e.*, additive index = 7.47).

An acute, 24-h sublethal exposure of rainbow trout to the mixture of Polyphase P-100 and Bardac 2280 had little effect on most of the measured variables, except for plasma cortisol levels that were significantly elevated in a concentration-dependent manner (Table 5). This primary stress response was observed at the lowest concentration tested. Starry flounder responded differently to the mixture. At 100% of the 96-h LC_{50} value, plasma glucose was significantly elevated and leucocrit was significantly decreased, both of which can indicate a

Table 2. Acute toxicity of Polyphase P-100 to fishes and aquatic invertebrates

Test Species	Exposure Duration (h)	NOEC (ppb)	LC_{50} (ppb) (95% CI)	100% Mortality (ppb)
Fishes				
Coho; embryo (11-day old)	96	<1,000	1,320 (1,200–1,440)	4,600
Coho; eyed-embryo (34-day old)	96	<1,000	1,900 (1,700–2,100)	3,200
Coho; alevin (67-day old)	96	<180	210 (200–230)	320
Coho; alevin (86-day old)	96	120	166 (120–200)	200
Coho; fry (120-day old)	96	100	130 (100–160)	160
Coho smolt; juvenile (10-month old)	96	<70	95 (86–100)	100
Rainbow trout; juvenile (O^+)	96	70	100 (124–140)	180
Starry flounder; juvenile	96	320	370 (320–420)	420
Invertebrates				
Hyalella azteca	48	100	500 (380–650)	2,200
Daphnia magna	48	<10	40 (28–55)	>220
Neomysis mercedis	48	<1,000	2,920 (2,470–3,520)	6,800

Concentrations are reported as nominal concentrations. Age of coho salmon is in days or months postfertilization. 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

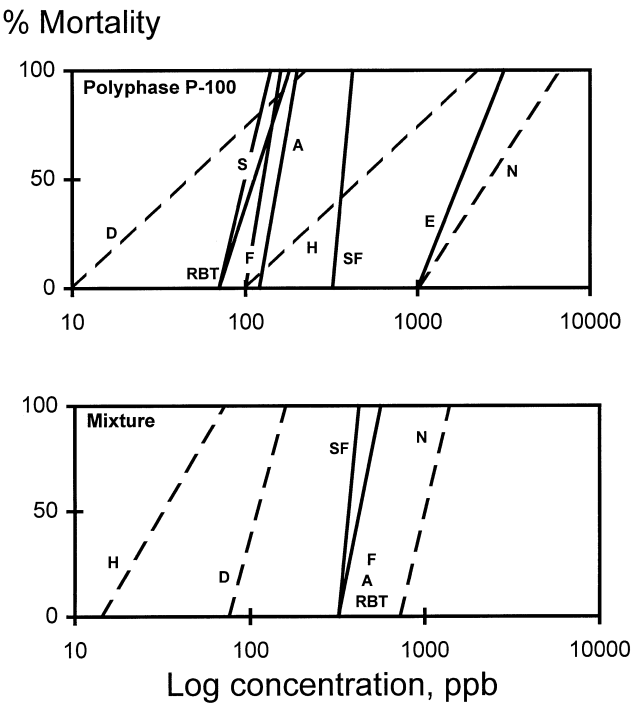

Fig. 1. A comparison of the concentration-response relationships for Polyphase P-100 alone and a mixture containing one part Polyphase P-100 and eight parts Bardac 2280. Each line represents one test organism and connects the concentration causing no mortality with the concentration producing 100% mortality. 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*)

secondary stress response. In contrast, plasma lactate was significantly decreased at all test concentrations. This may indicate an anesthetic/analgesic action.

Discussion

This study provides the first comprehensive information in the open literature on the acute toxicity of IPBC to aquatic organisms. Below, we make comparisons between the acute toxicity data in this study and the proprietary information reviewed by Henderson (1992a). While such comparisons are possible, they should be treated with caution because the information available to Henderson on test conditions was very limited. Henderson (1992a) 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, as well as a 96-h NOEC of 49 ppb IPBC. These values do not vary greatly from the values generated in the present study. For example, juvenile rainbow trout had a 96-h LC_{50} value of 130 ppb and a NOEC of 70 ppb (Table 2). Similarly, coho smolts had a 96-h LC_{50} value of 95 ppb and a NOEC of <70 ppb (Table 2). Thus, there seems to be some consistency between the IPBC acute toxicity data for salmonids.

The tolerance of coho salmon to Polyphase P-100 was affected by life stage. Smolts were the most sensitive life stage tested, and embryos were more tolerant than either alevins or fry. Why smolts are more sensitive is unclear at this time. However, the dramatic physiological and biochemical changes that occur in the gills during smoltification may prove to be important in this regard, especially if the gills are found to be a target site of action for IPBC. The tolerance to Polyphase P-100 also varied among fish species. Both salmonid species (rainbow trout and coho salmon) were almost four times more sensitive to Polyphase P-100 than starry flounder. Similarly, Henderson (1992a) reported that rainbow trout were about twice as sensitive to IPBC than bluegill sunfish. We discovered an interesting aspect of this species sensitivity among fish—a consistently steep concentration-response relationship (see Figure 1). This meant that overlap between the concentrations of Polyphase P-100 producing lethality in one fish species versus another could be minimal.

Table 3. 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 ppb Control	35 ppb 25%	70 ppb 50%	140 ppb 100%	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:somatic 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)

N values less than 10 are indicated as the second value in parentheses
[a] Mean value for n = 10 (SEM in parentheses)
* Denotes significant difference from control value ($p < 0.05$, ANOVA; SNK)

Table 4. Acute toxicity of a mixture (1:8) of Polyphase P-100 and Bardac 2280 to fishes and invertebrates

Test Species	Exposure Duration (h)	NOEC (ppb)	LC_{50} (ppb) (95% CI)	100% Mortality (ppb)	Additive Index (95% CI)
Fishes					
Coho; alevin (53-day old)	96	320	490 (450 to 520)	>560	−0.37 (−0.39 to −0.33)
Coho smolt; juvenile (7-month old)	96	320	430 (380 to 490)	560	−0.27 (−0.33 to −0.17)
Rainbow trout; juvenile (0^+)	96	320	460 (430 to 490)	560	−0.38 (−0.52 to −0.24)
Starry flounder; juvenile	96	320	370 (320 to 420)	420	0.06
Invertebrates					
Hyalella azteca	48	14	26 (17 to 40)	72	7.47 (8.34 to 29.4)
Daphnia magna	48	<75	110 (95 to 120)	160	−1.77 (−2.32 to −1.37)
Neomysis mercedis	48	<720	770 (650 to 850)	1,400	0.37 (0.20 to 0.39)

Nominal concentrations of the formulation (eight parts Bardac 2280 and one 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

The species variability for the acute toxicity of Polyphase P-100 was greater among the invertebrate species. As a result, an invertebrate represented the most sensitive species (*D. magna;* LC_{50} value of 40 ppb) as well as the most resistant species (*N. mercedis;* LC_{50} value of 2,920 ppb) tested. In addition, the concentration-response relationships were more shallow than those for fish. In contrast to our finding, Henderson (1992a) reported a 48-h LC_{50} value for *D. magna* (645 ppb) that was almost 15 times higher than the value obtained here. However, the reported data for a 21-day *D. magna* test were much closer to our data. In fact, the 7-day, 14-day, and 21-day EC_{50} values for *D. magna* (142 ppb, 136 ppb, and 133 ppb) showed little change. This means that while a portion of the test population died early on in the exposure period, as much as a threefold increase in exposure time had little effect on mortality. Further studies with *D. magna* are needed to understand this variability between studies.

The technical product Polyphase P-100 is used to formulate antisaptain chemical mixtures (Henderson 1992a). For example, the product used for the preparation of NP-1 contains 40% IPBC, in addition to 25% naphthalene, 17.5% dipropylene glycol, and 17.5% dimethyl sulfoxide. Thus, the NP-1 formulation, which has IPBC and DDAC as active ingredients, contains 64.8% DDAC, 7.6% IPBC, 8.1% ethyl alcohol, 4.8% petroleum naphtha, 2.8% dimethyl sulfoxide, 1.0% octyldecyl glycidyl ether, 1.0% dipropylene glycol, and 8.0% water (Envirochem 1992). The present study provides new information on the interactions of a mixture of IPBC and DDAC. Additive indices were calculated using the acute toxicity for DDAC generated previously in our laboratory under similar experimental condi-

Table 5. 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 ppb Control	110 ppb 25%	220 ppb 50%	440 ppb 100%	0 ppb Control	300 ppb 25%	600 ppb 50%	1,200 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)

tions (Farrell *et al.* 1998) and the findings for the fish species deviated only in a minor way from a simple additive effect of IPBC and DDAC. However, the findings for the invertebrate species varied considerably. Although the combined effects of IPBC and DDAC on *N. mercedis* were nearly additive, simple addition would overestimate by more than twofold 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.* Generalizations regarding the acute toxicity of antisapstain chemicals appear to be less predictable for invertebrates compared with fishes.

Sublethal stress effects were revealed with the mixture that were not observed with Polyphase P-100 alone. In an earlier study, Wood *et al.* (1996) reported modest primary and secondary stress responses (*i.e.*, elevated plasma cortisol, lactate, and glucose) in rainbow trout exposed for 24 h to 400 ppb Bardac 2280 alone, but not at lower concentrations. Thus, an elevated cortisol response occurs in rainbow trout at a much lower concentration of Bardac 2280 when it is mixed with Polyphase P-100. We have no explanation for this effect because little is known concerning the target sites of these chemicals. Also, we have no explanation for (but are concerned about) the depression of plasma lactate observed in starry flounder when exposed to the mixture. This anesthetic/analgesic type of response may be initiated by Polyphase P-100 alone (*i.e.*, plasma lactate tended to decrease in both rainbow trout and starry flounder; Table 3) and, in starry flounder at least, is accentuated by Bardac 2280. Interestingly, bluegill sunfish that survived 210 ppb IPBC for 96 h had darkened pigmentation and exhibited lethargic behavior (Donald Nye, Troy Corp., personal communication). The sublethal effects of fish exposed to mixtures clearly require greater attention. The present data show that the possibility sublethal effects of mixtures cannot be excluded simply because sublethal effects are absent for the principal components when tested alone.

Water quality criteria for IPBC-containing antisapstains are lacking in Canada. Measured IPBC concentrations in stormwater runoff from sawmills on the lower Fraser River, BC, have ranged from nondetectable to as high as 370 ppb (Envirochem 1992). Therefore, noncompliance with the regulatory level of 120 ppb IPBC has existed. Based on the acute toxicity data generated here, which is not at variance with proprietary data, the present regulatory level in British Columbia may be too liberal according to the Canadian Fisheries Act, which requires no deleterious effects on fish. Examination of Figure 1 shows that end-of-pipe concentrations at the regulatory level have the potential cause mortality among a variety of species. Even so, bioassays that are relevant to specific receiving environments are needed to validate this concern. Moreover, the added complexity of chemical mixtures will need much closer attention. Although only small errors would be expected using simple addition of component concentrations to assess the acute toxicity of a mixture of IPBC and DDAC to the fish species tested here, such would not be the case for *H. azteca.* Consideration of sublethal effects to fishes adds further complications. Therefore, until more work is performed to better define variability 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 invertebrate species to develop regulatory guidelines.

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