HYLEBOS FISH INJURY STUDY
Round II

Part 1: Effects of Chemical Contaminants from the Hylebos Waterway on Disease Resistance of Juvenile Chinook Salmon

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS 4

LIST OF FIGURES 5

LIST OF TABLES AND APPENDICES 6

EXECUTIVE SUMMARY 7

INTRODUCTION 9

METHODS 11
- Exposure Assessment 13
- Bacterial lethal concentration curve 13
- Statistical methods - disease challenge 13

RESULTS 14
- Sediment Extract Chemistry 14
- Bacterial Growth and Concentration-Response Curve 17
- Disease Challenge Studies 17

DISCUSSION OF MAJOR FINDINGS 21

REFERENCES 24

EXECUTIVE SUMMARY

The Hylebos Waterway of Commencement Bay, an urban estuary in central Puget Sound in the state of Washington, is severely contaminated with a variety of organic and inorganic contaminants. Juvenile salmon inhabit this waterway in the late spring and early summer. In 1994, studies (Round I of the Hylebos Fish Injury Study) were initiated to determine contaminant exposure in juvenile salmonids which migrate through this waterway. The findings showed that juvenile chinook and chum salmon sampled from this site are being exposed to a wide range of chemical contaminants (Collier et al. 1998), and the levels of exposure are comparable to levels which have previously been shown to cause impaired growth (Casillas et al. 1995a), immunosuppression, and increased mortality following pathogen exposure in juvenile salmon (Arkoosh et al 1998). Whether juvenile salmon exposed specifically to chemical contaminants characteristic of the Hylebos Waterway suffer injurious biological effects as a result of the exposure was not determined in the Round I study.

The objective of the present study (Part 1 of Round II of the Hylebos Fish Injury Study) was to determine if contaminants specifically associated with the Hylebos Waterway can alter (increase) the disease susceptibility of juvenile chinook salmon to the bacterial pathogen, *Vibrio anguillarum*. Specifically, juvenile salmon were exposed for a 7 day period in Oct, 1995 to either: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) to a chlorinated-enriched fraction of a sediment extract made from Hylebos Waterway sediment (CHWSE), or 3) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to PAHs in a sediment sample from the Hylebos Waterway. The salmon were exposed to the chemicals by intraperitoneal injection to ensure consistent delivery of a specific dosage. Disease susceptibility was then assessed by exposing fish to *Vibrio anguillarum*, a known marine pathogen of salmon.

Significant ($p \leq 0.05$) differences in mortality (increased disease susceptibility) between experimentally exposed juveniles and juveniles administered the carrier compound were observed. Exposure to chlorinated hydrocarbons, characterized by HCBD and the CHWSE, which is composed primarily of polychlorobutadienes and certain aromatic hydrocarbons (e.g. phenanthrene, fluoranthene, pyrene), were associated with increased mortality induced by *Vibrio anguillarum*. These findings support the hypothesis that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway influences their physiology such that their survival potential may be reduced. Since recruitment of salmon appears to be strongly influenced by factors acting on the first year of ocean life (Pearcy 1992), these results suggest that the risk for

increased juvenile mortality and subsequent decreased adult recruitment is potentially greater for juveniles exposed to contaminants accumulated during their residence in the Hylebos Waterway.

INTRODUCTION

Estuaries are critical habitats for juveniles of several Pacific salmon species during their transition to life in the ocean (Levings and Bouillon 1997). Estuarine habitats provide refuge from predators, a rich food supply to support rapid growth, and are where juveniles make the transition from freshwater to marine conditions (Thorpe 1994). Urban estuaries, however, receive inputs of toxic anthropogenic substances from a variety of sources, and many of these chemicals can accumulate in sediments and thus can be retained in the estuary. There is concern that because juvenile salmon are undergoing numerous physiological adaptations during their residence in estuarine environments, any additional stresses such as exposure to toxic chemicals, may be injurious.

The Hylebos Waterway of Commencement Bay, in central Puget Sound in the state of Washington, is severely contaminated by a variety of organic and inorganic contaminants and juvenile chinook salmon inhabit this waterway in the late spring and early summer. In 1994, studies for Round I of the Hylebos Fish Injury Study were initiated to: 1) determine if contaminant source control had resulted in improvements in habitat quality, as determined by prevalences of liver disease in flatfish; 2) identify impaired reproductive function in flatfish; and 3) determine whether and to what degree there might be contaminant exposure of juvenile salmonids which migrate through this waterway. The findings showed (Collier et al. 1998)that there have been no appreciable changes in disease prevalences or levels of contaminant exposure in flatfish from this site since the 1970s. Moreover, female flatfish from the Hylebos Waterway are showing evidence of precocious sexual maturation in young animals and inhibited gonadal development in older fish. More importantly for the studies reported herein, the results also showed that two species chinook (*Oncorhynchus tshawytscha*) and chum (*Oncorhynchus keta*) of juvenile salmon sampled from this site are being exposed to a wide range of chemical contaminants and in particular showed increased exposure to chlorinated compounds (HCBD, HCB) that are elevated in Hylebos Waterway sediments compared to other sites in Puget Sound. The levels of exposure to CHs and PAHs are comparable to levels which have previously been shown to cause impaired growth, immunosuppression, and increased mortality following pathogen exposure in juvenile salmon from the contaminated Duwamish Waterway, Puget Sound. These studies provide the scientific rationale for determining if juvenile chinook salmon, exposed to chemical contaminants characteristic of the Hylebos Waterway, suffer injury from such exposure.

Immune dysfunction in mammals has been recognized as a serious sublethal effect of chemical contaminant exposure, affecting both cellular and humoral aspects of the immune

system (Dean et al., 1990). Laboratory studies have extended these findings to fish by demonstrating that their immune system is susceptible to specific contaminants, often resulting in a wide array of immunosuppressive effects (McLeay and Gordon 1977; Arkoosh and Kaattari 1987; Rice and Weeks 1989; Thuvander 1989). We have examined the immunocompetence of juvenile chinook salmon and found that juvenile chinook salmon from polluted environments are immunosuppressed (Arkoosh et al., 1991). In our studies with juvenile salmon from Puget Sound, WA, immunocompetence was evaluated by analyzing the functional ability of white blood cells to produce antibodies after either their first exposure to a new antigen (the primary response) or after re-exposure to the same antigen (the secondary or memory response) which is evaluated using a plaque-forming cell (PFC) assay. Such an approach has been shown to be sensitive in evaluating long-term chronic effects following short-term exposure to chemical contaminants (Arkoosh and Kaattari 1987). Previously, we showed that white blood cells (leukocytes) of juvenile chinook salmon collected from hatcheries and from a nonurban estuary (the Nisqually estuary in Puget Sound) were able to generate a significantly higher secondary PFC response to a foreign antigen than that produced during the primary PFC response, which is the normal and expected response (Arkoosh et al., 1991). In contrast, an enhanced secondary PFC response did not occur with leukocytes of juvenile chinook salmon exposed to pollution from the urban Duwamish Waterway estuary. Thus, this field study demonstrated that fish from the contaminated estuary were immunosuppressed and suggested that juvenile chinook salmon may be more susceptible to disease than juveniles from non-urban environments. This increased potential for disease increases the risk of mortality for juveniles in contaminant-exposed populations of chinook salmon.

In our previous study (Arkoosh et al. 1998), to determine if salmon from a contaminated environment may be more susceptible to disease, we collected juvenile fall chinook salmon from the urban Duwamish Waterway (Collier et al., 1998) estuary and from the nonurban Nisqually estuary and the respective releasing hatcheries, and exposed them in the laboratory to the marine pathogen, *Vibrio anguillarum*. *Vibrio anguillarum* is a fish pathogen known to cause disease when fish are under stress (Warren 1991). We found that juvenile chinook salmon from the contaminated estuary were more susceptible to *V. anguillarum* induced mortality than were fish from the hatchery on the river system. In contrast, juvenile fall chinook salmon from a nonurban estuary showed no increase in susceptibility to *V. anguillarum* induced mortality than did fish from the corresponding hatchery for this river system. These disease challenge studies indicate that juvenile chinook salmon exhibiting contaminant-associated immunodysfunction also exhibited increased susceptibility to pathogenesis by a virulent marine bacterium (Arkoosh et al., 1998). Further support for a linkage between contaminant exposure, immune function, and the potential for increased mortality is provided from recent laboratory studies. Two classes of

contaminants, PCBs and PAHs, found in the Hylebos Waterway are known to induce immunosuppression as well as to increase disease susceptibility in mammals (Ward et al., 1985; Dean et al., 1990). Our recent laboratory studies with juvenile chinook salmon administered sublethal doses of PCBs and PAHs showed suppression of their secondary PFC response (Arkoosh et al., 1994), as well as an increase in disease susceptibility (Arkoosh et al., 1998). These findings substantiate a linkage between immune dysfunction and increased disease susceptibility and support a probable causal relationship between impaired immunity, increased disease susceptibility and chemical contaminant exposure in juvenile fall chinook salmon from polluted urban estuaries. These findings also support the use of disease challenge experiments in linking contaminant exposure to a serious biological dysfunction. The ecotoxicological implications are for potentially increased mortality for juvenile salmon using contaminated estuaries.

Accordingly, having determined previously that juvenile fall chinook and chum salmon are exposed to contaminants during residence in the Hylebos Waterway (Collier et al. 1998), our aim in this study (Part 1 of Round II of the Hylebos Fish Injury Study) was to determine if contaminants specifically associated with the Hylebos Waterway can increase the disease susceptibility of juvenile chinook salmon to the bacterial marine pathogen, *Vibrio anguillarum*. Specifically, juvenile chinook salmon were exposed to one of the following for a 7-day period: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) to a chlorinated-enriched fraction of a sediment extract made from the Hylebos Waterway sediment, or 3) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to the level of PAHs in sediment from the Hylebos Waterway. The chemical contaminants were administered 7 day prior to being challenged with *Vibrio anguillarum* after which mortality was monitored for a 14 day period. The hypothesis being addressed was whether differences in mortality between experimentally exposed juveniles and juveniles administered the carrier compound (acetone:emulphor) and exposed to *Vibrio anguillarum* were evident. In this study reference extract was not used as a control.

METHODS

Details of the disease challenge study are described in detail in the SAP (Appendix 1). The chemicals identified in the CHWSE and their concentration are listed in Table 1. The composition of the PAH model mixture is listed in Table 2.

Table 1. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Concentrations of selected aromatic hydrocarbons, chlorinated butadienes, pesticides, and polychlorinated biphenyls in sediment extract from the pentane extraction of sediment from the Hylebos Waterway (HWSE-P) that was used in the laboratory experiment. Values are reported as ng/g sediment.

Analyte	ng/g sediment[a]
Aromatic Hydrocarbons	
naphthalene	26
2-methylnaphthalene	18
1-methylnaphthalene	10
biphenyl	6
2,6-dimethylnaphthalene	10
acenaphthylene	0.9
acenaphthene	28
2,3,5-trimethylnaphthalene	28
fluorene	28
phenanthrene	140
anthracene	47
1-methylphenanthrene	10
fluoranthene	170
pyrene	170
benz[a]anthracene	23
chrysene	33
benzo[b]fluoranthene	6
benzo[k]fluoranthene	5
benzo[e]pyrene	9
benzo[a]pyrene	10
perylene	2
indeno[1,2,3-cd]pyrene	0.3
dibenz[a,h]anthracene	< 0.02
benzo[ghi]perylene	1
dibenzothiophene	12
Chlorinated Butadienes[b]	
Trichlorobutadiene	480
Tetrachlorobutadiene	450
Pentachlorobutadiene	160
Hexachlorobutadiene	150

Analyte	ng/g sediment
Pesticides	
hexachlorobenzene	110
lindane (gamma-BHC)	2
heptachlor	0.8
aldrin	< 0.06
heptachlorepoxide	21
oxychlordane	< 0.07
trans-chlordane	< 0.06
nonachlor-III	< 0.06
alpha-chlordane	2
trans-nonachlor	5
cis-nonachlor	0.4
dieldrin	2
mirex	2
o,p'-DDE	1
p,p'-DDE	0.4
o,p'-DDD	20
p,p'-DDD	1
o,p'-DDT	< 0.1
p,p'-DDT	< 0.1
PCBs	
trichlorobiphenyl - 18	17
trichlorobiphenyl - 28	6
tetrachlorobiphenyl - 44	1
tetrachlorobiphenyl - 52	3
tetrachlorobiphenyl - 66	< 0.08
pentachlorobiphenyl - 101	3
pentachlorobiphenyl - 105	< 0.06
pentachlorobiphenyl - 118	< 0.07
hexachlorobiphenyl - 128	0.8
hexachlorobiphenyl - 138	2
hexachlorobiphenyl - 153	4
heptachlorobiphenyl - 170	1
heptachlorobiphenyl - 180	9
heptachlorobiphenyl - 187	2
octachlorobiphenyl - 195	2
nonachlorobiphenyl - 206	12
decachlorobiphenyl - 209	31

[a] Analyte concentrations calculated on a sediment wet weight basis.

[b] Concentrations of the butadienes were calculated using a response factor of 1 with GC/MS total ion current areas.

Table 2. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. High molecular weight hydrocarbons that comprise the PAH model mixture. The compounds were combined in acetone at the same ratios as they were present in the sediment at station 24 of the Hylebos Waterway to a concentration equivalent to 400 g sediment extracted per ml of acetone.

PAH Analytes	
Fluoranthene	Benz[k]fluoranthene
Pyrene	Benzo[a]pyrene
Benz[a]anthracene	Indeno[1,2,3-cd]pyrene
Chrysene,	Dibenz[a,h]anthracene
Benz[b]fluoranthene	Benzo[ghi]perylene

Exposure Assessment

Information on the level of exposure for the treatments used here will be generated in subsequent experiments as described in the SAP (Appendix 1).

Bacterial Lethal Concentration Curve

Juvenile chinook salmon from the Salmon River Hatchery, OR, were obtained to conduct exposure and challenge studies which were conducted at our field station at Newport, OR. The concentration-response curve relating the percent of fish killed with increasing concentration of *Vibrio anguillarum* after a 7 day exposure period was conducted to determine the LC_{30} and LC_{50} for the challenge studies. The 168 h lethal concentration response curve of juvenile chinook salmon exposed to *V. anguillarum* was fitted using logit regression analysis using DeltaGraph™ (Wulf et al. 1991).

Statistical methods - disease challenge

Statistical significance of the challenge studies were assessed using generalized linear models (GLM). The number of survivors and mortalities in a disease challenge study follow a binomial distribution (Hildén and Hirvi 1987). Statistical analyses to assess significance of difference in mortality among the experimental treatment groups compared to the control group were assessed using GLM, a general linear model, performed with the GLMStat computer application (Beath 1995). To define a generalized linear model, it is important to identify the error structure and the link function which relates the linear predictor derived from the GLM analysis to the expected survival/mortality probabilities (Baker and Nelder 1978).

The logistic generalized linear model was used for the analysis of data. For this model the error structure is binomial and the linear predictor was related to the expected value of the datum by the logit link function. This analysis was used to evaluate if survival/mortality of fish injected with contaminants was significantly different ($\alpha = 0.05$) from salmon injected with the carrier 7d post challenge as described in Arkoosh et al. (1998) although data is reported for a 14 day period to more easily visualize differences in mortality among the treatment groups. These analyses were conducted after correction for mortalities in the group of juvenile salmon that served as the non-*Vibrio* treated control for these experiments. The background mortality was determined during the same period (Figure 3). The net mortality was determined by subtracting background mortality observed in juvenile chinook salmon that received chemical contaminants but were not exposed to bacteria. Statistical analyses were conducted separately for each exposure concentration of the bacteria.

RESULTS

Sediment Extract Chemistry

The objective of administering HCBD, a chlorinated-enriched fraction of sediment from the Hylebos Waterway (CHWSE), and a model mixture of PAHs was to expose juvenile salmon to compounds representative of chemical contaminants in the Hylebos Waterway. HCBD is considered a marker chemical for the Hylebos Waterway. HCBD was administered at a dose of 21 mg/kg. CHWSE was prepared from sediment collected near the mouth of the Hylebos Waterway. Specifically, these sediments were taken at Stations HY-07, -08, and -09. These sediment sites were designated and analyzed during the sediment injury studies conducted during Phase 1 of the Commencement Bay Damage Assessment investigations. The objective of preparing the sediment extract was to obtain an extract of chemical contaminants that was representative and enriched in the less polar CHs. The composition of analytes in the extract is listed in Table 1. The extract is enriched in butadiene-like compounds although it still retains significant quantities of AHs. The PCBs present in the sediment were largely removed in the preparation. The final concentration of chemical contaminants in 1 µl of the CHWSE was equivalent to chemical contaminants in 0.2 gr of sediment. A model mixture of polycyclic aromatic hydrocarbons (PAHs) containing 10 high molecular weight PAHs was also prepared to reflect the same ratios of these analytes previously found in sediment during Phase I of the Commencement Bay Damage Assessment studies at Station HY-24. The final concentration of chemical contaminants in 1 µl of the PAH model mixture was equivalent to chemical contaminants in 0.2 gr of sediment. The chemical contaminants along with the acetone:emulphor carrier were each administered at a volume equivalent to 1.5 µl solution/g fish.

Bacterial Growth and Concentration-Response Curve.

The peak of the exponential growth phase of *V. anguillarum* grown in 500 mL of TSB supplemented with 0.5% NaCl and slowly agitated at 25°C occurred at approximately 14 h with an optical density of 1.1 (Figure 1). In subsequent challenge experiments, *V. anguillarum* cultures were grown until an optical density of approximately 1.1 for preparation of the bacterial culture dilutions used for juvenile salmon exposures. The 168 h (7 d) lethal response curve for Salmon River Hatchery salmon exposed to *V. anguillarum* was determined by a logit regression analysis (Figure 2). The dilutions of bacteria inducing 50% mortality in the Salmon River fish over a 7 d period were 6×10^{-5} ml stock bacterial culture/mL sea water; this dilution was equivalent to approximately 1×10^{7} bacteria/mL sea water.

Disease Challenge Studies.

Background mortality and the percent cumulative mortality of the four different groups of juvenile chinook salmon exposed to *V. anguillarum* is shown in Figure 3 and 4, respectively. The cumulative mortality curves are reported for a 14 day period following exposure to the bacteria. Statistical testing was restricted to 7 days post exposure, as outlined in Arkoosh et al (1998). Recording mortality for a 14 day period allows observations for any unusual mortality events after the traditional 7 day observation period. The data in Figure 4 represent the net mortality attributed to exposure to the bacteria after subtracting background mortality (Figure 3) observed in juvenile chinook salmon that received chemical contaminants but were not exposed to bacteria. The net cumulative mortality of juvenile chinook salmon exposed to bacteria equivalent to an LC_{50} dose after receiving either CHWSE, HCBD, or the model mixture of PAHs ranged from 28 to 31% compared to the 17% observed in the acetone:emulphor control group at 7 days post bacterial challenge. The cumulative mortality was significantly higher in fish exposed to CHWSE, the PAH model mixture, or HCBD relative to fish receiving only the carrier. There was essentially no change in the net cumulative mortality of juvenile salmon from

the 7 to 14 day observational period and the magnitude of the differences in mortality between the carrier control and the three treatment groups remained relatively unchanged. Because the acetone: emulphor control group for the LC_{30} treatment group was lost, due to a procedural error (loss of air to the tanks), the significance of mortality due to exposure to chemical contaminants could not be determined for this treatment group; the data for LC_{30} - dose treatments are in the Appendix.

Table 3 shows the percentage of salmon carcasses examined that were infected with *V. anguillarum* on days 7 and 14 of the experiment using an LC_{50} exposure dose. Confirmation of the presence of *V. anguillarum* ranged from 44% to 100% in the subsamples tested. The agglutination test identifies bacteria subsampled from each fish that are able to agglutinate anti-*V. anguillarum* antiserum whereas the oxidase positive test identifies fish with bacteria which have cytochrome oxidase enzyme activity which is characteristic of *Vibrio* spp. *Pseudomonas* spp. and *Aeromonas* spp. bacteria, and includes *V. anguillarum*. The findings show that we were able to attribute a majority of the mortalities to *V. anguillarum*.

Table 3. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Ratio and proportion of juvenile salmon identified to be infected with *V. anguillarum* after exposure to waterborne *V. anguillarum* equivalent to the LC_{50} concentration.

	Positive Agglutination[a]	Agglutination (%)	Oxidase Positive[b]	Oxidase Positive (%)	Combined[c]
Day 7[d]					
acetone/emulphor	7/11	64%	11/11	100%	64%
HCBD	4/9	44%	9/10	90%	44%
CHWSE	12/12	100%	12/12	100%	100%
Model Mixture PAH	7/11	64%	11/11	100%	64%
Day 14					
acetone/emulphor	8/16	50%	12/16	75%	50%
HCBD	4/9	44%	9/10	90%	44%
CHWSE	14/14	100%	14/14	100%	100%
Model Mixture PAH	7/13	54%	13/13	100%	54%

[a] Number of fish examined which had bacteria that agglutinated with the anti-*V. anguillarum* antiserum/total number of fish examined

[b] Number of fish examined which were oxidase positive/ total number of fish examined.

[c] Percentage of fish that had bacteria that were both oxidase positive and agglutinated anti-*V. anguillarum* antiserum.

[d] The number of days after *V. anguillarum* challenge.

DISCUSSION OF MAJOR FINDINGS

Depressed disease resistance results from exposure of juvenile chinook salmon to contaminants specific to the Hylebos Waterway.

Juvenile chinook salmon exposed to contaminants associated with the Hylebos Waterway exhibited a higher susceptibility to mortality induced by the marine pathogen *V. anguillarum,* than did juvenile chinook salmon treated only with the carrier. These findings suggest that a higher predisposition to infection/disease may occur in salmon exposed to chemical contaminants specific to the Hylebos Waterway. At the doses tested, each of the treatment groups, which represent specific subsets of chemical contaminants characteristic of the Hylebos Waterway, increased the disease susceptibility of juvenile chinook salmon. Chlorinated hydrocarbons, characterized by HCBD and the CHWSE, which is composed primarily of HCBD-like compounds, and aromatic hydrocarbons, characterized by the model mixture of PAHs, affected the susceptibility of juvenile chinook salmon to mortality induced by a marine bacterial pathogen. The present findings support the hypothesis that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway influences their physiology such that their survival potential may be reduced. This conclusion is supported by studies in mammals, which have also demonstrated that immunoaltering chemicals can increase disease susceptibility (Friend and Trainer 1970; Thigpen et al. 1975; Loose et al. 1978).

The increased susceptibility of salmon first exposed to contaminants associated with the Hylebos Waterway and then challenged with a marine bacterial pathogen is consistent with our previous findings showing effects of chemical contaminants on immunocompetence and disease susceptibility of juvenile chinook salmon from the Duwamish Waterway in Elliott Bay also located in Puget Sound, WA. Arkoosh et al. (1991, 1998) showed a suppressed secondary immune response in anterior kidney white blood cells to specific model antigens and a greater percent cumulative mortality to *V. anguillarum* after natural exposure to chemical contaminants in the waterway, including PAHs and PCBs. Juvenile salmon exposed to a model PAH, dimethylbenzanthracene (DMBA) or the PCB mixture, Aroclor 1254, in the laboratory also exhibited a similar suppressed secondary immune response in anterior kidney white blood cells to model antigens (Arkoosh et al. 1994). Both PAHs and PCBs are known to induce immunosuppression in other species (Thomas and Hinsdill 1978; Ward et al. 1985).

The overall effects of exposure to chemical contaminants specific to the Hylebos Waterway on disease resistance in juvenile salmon could affect their survival potential in the estuary and early ocean environment.

Although the salmon in the present study were found to have an altered disease resistance shortly after exposure to toxic chemicals, previous data suggests that juvenile salmon may experience continued altered health for an extended period of time after exposure. We found that fish from the contaminated Duwamish estuary held for two months in noncontaminated sea water still exhibited increased mortality associated with the earlier exposure to chemical contaminants (Arkoosh et al. 1998). This finding is also supported by laboratory studies evaluating the duration of immunosuppression in salmonids after an initial exposure to other immunosuppressive chemical contaminants. A study (Arkoosh and Kaattari 1987) examining the effects of aflatoxin B_1, a mycotoxin, on the immune response of rainbow trout demonstrated that a brief exposure of embryos to this immunotoxin, at sublethal concentrations, can manifest its effects months later. The trout experienced immune dysfunction 5-6 months after a single exposure to aflatoxin B_1 (Arkoosh and Kaattari 1987). Our previous studies in conjunction with the study reported by Arkoosh and Kaattari (1987) suggest that although juvenile chinook salmon are only briefly exposed to contaminants in an urban estuary as they migrate to sea, immune altering events may be persistent, and a consequent increase in disease susceptibility in exposed juvenile salmon may extend into their early ocean life. Because recruitment of fish to the adult stages is considered to be dependent on factors acting during the first year of life (Sissenwine 1984), the potential for contaminants to potentiate disease outbreaks and influence mortality rates within the population is a possibility. The potential for disease to influence population structure is supported by studies showing that coho salmon infected with a parasite (*Nanophytes* sp.) as they leave freshwater and enter the ocean environment had much lower adult survival rates than coho with lower infection rates (Schroder and Fresh 1992). Although these findings do not directly reflect cause-and-effect, the results from other previous studies of coho on the Washington coast suggested that diseased fish were lost from the ocean population (Schroder and Fresh 1992), supporting an association between infection with a pathogen and lower survival potential.

The consequences of immunosuppression and an increase in disease susceptibility may be greater than are currently recognized. Immunosuppression has been suggested to play a major role in population regulation and disease itself has been shown to be an important factor in the extinction of several species (Sheldon and Verhulst 1996). The ramifications of disease on marine fish populations can be manifold (Sindermann 1990) including mass mortalities, impaired locomotion, delayed metamorphosis, and increased mortality from predation. In addition,

immunosuppressed animals may allocate greater energy and resources for defending themselves against infection, reducing resources for other vital physiologic functions, such as growth and reproduction (Sheldon and Verhulst 1996). In summary, the findings from Round I of the Hylebos Fish Injury Study (Collier et al 1998) show that the relatively brief residence of juvenile chinook and chum salmon in the Hylebos Waterway increased exposure to chemical contaminants and induced early biological alterations (elevated hepatic CYP1A and DNA damage). In the present study we demonstrated that a model suite of PAHs characteristic of sediments from the Hylebos Waterway, as well as specific Hylebos-associated chemicals (organic solvent extracts of Hylebos sediment), increased disease susceptibility. Because recruitment of salmon appears to be strongly influenced by factors acting on the first year of ocean life (Pearcy 1992), alterations in their overall fitness during this period could be detrimental. Although there is uncertainty in delineating the contribution of the many individual factors that affect survival of juvenile salmon in the estuarine and marine environment, disease susceptibility has consistently been linked to an increase in mortality (Grenfell and Dobson 1995) in natural populations. The findings from the present study showed that exposure to chemical contaminants associated with the Hylebos Waterway increases the susceptibility of juvenile chinook salmon to disease. Quantitating the level of increased risk of subsequent mortality, however, cannot be assessed from the current information. Additional studies to determine the relationship between level of exposure to chemical contaminants and the extent of disease susceptibility are needed to assess further the level of risk to juvenile salmon of increased disease susceptibility from exposure to toxic chemicals present in the Hylebos Waterway.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS 4

LIST OF FIGURES 5

LIST OF TABLES AND APPENDICES 6

EXECUTIVE SUMMARY 7

INTRODUCTION 9

METHODS 11
- Exposure Assessment 13
- Bacterial lethal concentration curve 13
- Statistical methods - disease challenge 13

RESULTS 14
- Sediment Extract Chemistry 14
- Bacterial Growth and Concentration-Response Curve 17
- Disease Challenge Studies 17

DISCUSSION OF MAJOR FINDINGS 21

REFERENCES 24

LIST OF ABBREVIATIONS

AHs	aromatic hydrocarbons
CHs	chlorinated hydrocarbons
CHWSE	chlorinated-enriched Hylebos Waterway extract
CYP1A	cytochrome P4501A
DMBA	dimethylbenzanthracene
DNA	deoxyribonucleic acid
GC/MS	gas chromatography/mass spectometry
GLM	generalized linear model
HCB	hexachlorobenzene
HCBD	hexachlorobutadiene
LC	lethal concentration
NOAA	National Oceanic and Atmospheric Administration
NMFS	National Marine Fisheries Service
NWFSC	Northwest Fisheries Science Center
PCBs	polychlorinated biphenyls
PFC	plaque-forming cell
PAHs	polycyclic aromatic hydrocarbons
SAP	Sampling and Analysis Plan
TSB	tryptocase soy broth

LIST OF FIGURES

Figure 1. Growth curve of *Vibrio anguillarum* 15

Figure 2. The 7 day lethal response curve of juvenile chinook salmon exposed to *Vibrio anguillarum* 16

Figure 3. Background cumulative mortality of juvenile chinook salmon 18

Figure 4. Percent cumulative mortality of juvenile chinook salmon exposed to *Vibrio anguillarum* 19

LIST OF TABLES

Table 1. Concentration of analytes in the chlorinated enriched fraction of a sediment extract from the Hylebos Waterway 12

Table 2. High molecular weight hydrocarbons that comprise the PAH model mixture 13

Table 3. Ratio and proportion of juvenile salmon identified to be infected with *Vibrio anguillarum* 20

LIST OF APPENDICES

Appendix 1. Case Narratives for the Hylebos Waterway Disease Resistance Laboratory Study.

Appendix 2. Results of the LC_{30} disease resistance experiment.

EXECUTIVE SUMMARY

The Hylebos Waterway of Commencement Bay, an urban estuary in central Puget Sound in the state of Washington, is severely contaminated with a variety of organic and inorganic contaminants. Juvenile salmon inhabit this waterway in the late spring and early summer. In 1994, studies (Round I of the Hylebos Fish Injury Study) were initiated to determine contaminant exposure in juvenile salmonids which migrate through this waterway. The findings showed that juvenile chinook and chum salmon sampled from this site are being exposed to a wide range of chemical contaminants (Collier et al. 1998), and the levels of exposure are comparable to levels which have previously been shown to cause impaired growth (Casillas et al. 1995a), immunosuppression, and increased mortality following pathogen exposure in juvenile salmon (Arkoosh et al 1998). Whether juvenile salmon exposed specifically to chemical contaminants characteristic of the Hylebos Waterway suffer injurious biological effects as a result of the exposure was not determined in the Round I study

The objective of the present study (Part 1 of Round II of the Hylebos Fish Injury Study) was to determine if contaminants specifically associated with the Hylebos Waterway can alter (increase) the disease susceptibility of juvenile chinook salmon to the bacterial pathogen, *Vibrio anguillarum*. Specifically, juvenile salmon were exposed for a 7 day period in Oct, 1995 to either: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) to a chlorinated-enriched fraction of a sediment extract made from Hylebos Waterway sediment (CHWSE), or 3) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to PAHs in a sediment sample from the Hylebos Waterway. The salmon were exposed to the chemicals by intraperitoneal injection to ensure consistent delivery of a specific dosage. Disease susceptibility was then assessed by exposing fish to *Vibrio anguillarum*, a known marine pathogen of salmon.

Significant ($p \leq 0.05$) differences in mortality (increased disease susceptibility) between experimentally exposed juveniles and juveniles administered the carrier compound were observed. Exposure to chlorinated hydrocarbons, characterized by HCBD and the CHWSE, which is composed primarily of polychlorobutadienes and certain aromatic hydrocarbons (e.g. phenanthrene, fluoranthene, pyrene), were associated with increased mortality induced by *Vibrio anguillarum*. These findings support the hypothesis that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway influences their physiology such that their survival potential may be reduced. Since recruitment of salmon appears to be strongly influenced by factors acting on the first year of ocean life (Pearcy 1992), these results suggest that the risk for

increased juvenile mortality and subsequent decreased adult recruitment is potentially greater for juveniles exposed to contaminants accumulated during their residence in the Hylebos Waterway.

INTRODUCTION

Estuaries are critical habitats for juveniles of several Pacific salmon species during their transition to life in the ocean (Levings and Bouillon 1997). Estuarine habitats provide refuge from predators, a rich food supply to support rapid growth, and are where juveniles make the transition from freshwater to marine conditions (Thorpe 1994). Urban estuaries, however, receive inputs of toxic anthropogenic substances from a variety of sources, and many of these chemicals can accumulate in sediments and thus can be retained in the estuary. There is concern that because juvenile salmon are undergoing numerous physiological adaptations during their residence in estuarine environments, any additional stresses such as exposure to toxic chemicals, may be injurious.

The Hylebos Waterway of Commencement Bay, in central Puget Sound in the state of Washington, is severely contaminated by a variety of organic and inorganic contaminants and juvenile chinook salmon inhabit this waterway in the late spring and early summer. In 1994, studies for Round I of the Hylebos Fish Injury Study were initiated to: 1) determine if contaminant source control had resulted in improvements in habitat quality, as determined by prevalences of liver disease in flatfish; 2) identify impaired reproductive function in flatfish; and 3) determine whether and to what degree there might be contaminant exposure of juvenile salmonids which migrate through this waterway. The findings showed (Collier et al. 1998)that there have been no appreciable changes in disease prevalences or levels of contaminant exposure in flatfish from this site since the 1970s. Moreover, female flatfish from the Hylebos Waterway are showing evidence of precocious sexual maturation in young animals and inhibited gonadal development in older fish. More importantly for the studies reported herein, the results also showed that two species chinook (*Oncorhynchus tshawytscha*) and chum (*Oncorhynchus keta*) of juvenile salmon sampled from this site are being exposed to a wide range of chemical contaminants and in particular showed increased exposure to chlorinated compounds (HCBD, HCB) that are elevated in Hylebos Waterway sediments compared to other sites in Puget Sound. The levels of exposure to CHs and PAHs are comparable to levels which have previously been shown to cause impaired growth, immunosuppression, and increased mortality following pathogen exposure in juvenile salmon from the contaminated Duwamish Waterway, Puget Sound. These studies provide the scientific rationale for determining if juvenile chinook salmon, exposed to chemical contaminants characteristic of the Hylebos Waterway, suffer injury from such exposure.

Immune dysfunction in mammals has been recognized as a serious sublethal effect of chemical contaminant exposure, affecting both cellular and humoral aspects of the immune

system (Dean et al., 1990). Laboratory studies have extended these findings to fish by demonstrating that their immune system is susceptible to specific contaminants, often resulting in a wide array of immunosuppressive effects (McLeay and Gordon 1977; Arkoosh and Kaattari 1987; Rice and Weeks 1989; Thuvander 1989). We have examined the immunocompetence of juvenile chinook salmon and found that juvenile chinook salmon from polluted environments are immunosuppressed (Arkoosh et al., 1991). In our studies with juvenile salmon from Puget Sound, WA, immunocompetence was evaluated by analyzing the functional ability of white blood cells to produce antibodies after either their first exposure to a new antigen (the primary response) or after re-exposure to the same antigen (the secondary or memory response) which is evaluated using a plaque-forming cell (PFC) assay. Such an approach has been shown to be sensitive in evaluating long-term chronic effects following short-term exposure to chemical contaminants (Arkoosh and Kaattari 1987). Previously, we showed that white blood cells (leukocytes) of juvenile chinook salmon collected from hatcheries and from a nonurban estuary (the Nisqually estuary in Puget Sound) were able to generate a significantly higher secondary PFC response to a foreign antigen than that produced during the primary PFC response, which is the normal and expected response (Arkoosh et al., 1991). In contrast, an enhanced secondary PFC response did not occur with leukocytes of juvenile chinook salmon exposed to pollution from the urban Duwamish Waterway estuary. Thus, this field study demonstrated that fish from the contaminated estuary were immunosuppressed and suggested that juvenile chinook salmon may be more susceptible to disease than juveniles from non-urban environments. This increased potential for disease increases the risk of mortality for juveniles in contaminant-exposed populations of chinook salmon.

In our previous study (Arkoosh et al. 1998), to determine if salmon from a contaminated environment may be more susceptible to disease, we collected juvenile fall chinook salmon from the urban Duwamish Waterway (Collier et al., 1998) estuary and from the nonurban Nisqually estuary and the respective releasing hatcheries, and exposed them in the laboratory to the marine pathogen, *Vibrio anguillarum*. *Vibrio anguillarum* is a fish pathogen known to cause disease when fish are under stress (Warren 1991). We found that juvenile chinook salmon from the contaminated estuary were more susceptible to *V. anguillarum* induced mortality than were fish from the hatchery on the river system. In contrast, juvenile fall chinook salmon from a nonurban estuary showed no increase in susceptibility to *V. anguillarum* induced mortality than did fish from the corresponding hatchery for this river system. These disease challenge studies indicate that juvenile chinook salmon exhibiting contaminant-associated immunodysfunction also exhibited increased susceptibility to pathogenesis by a virulent marine bacterium (Arkoosh et al., 1998). Further support for a linkage between contaminant exposure, immune function, and the potential for increased mortality is provided from recent laboratory studies. Two classes of

contaminants, PCBs and PAHs, found in the Hylebos Waterway are known to induce immunosuppression as well as to increase disease susceptibility in mammals (Ward et al., 1985; Dean et al., 1990). Our recent laboratory studies with juvenile chinook salmon administered sublethal doses of PCBs and PAHs showed suppression of their secondary PFC response (Arkoosh et al., 1994), as well as an increase in disease susceptibility (Arkoosh et al., 1998). These findings substantiate a linkage between immune dysfunction and increased disease susceptibility and support a probable causal relationship between impaired immunity, increased disease susceptibility and chemical contaminant exposure in juvenile fall chinook salmon from polluted urban estuaries. These findings also support the use of disease challenge experiments in linking contaminant exposure to a serious biological dysfunction. The ecotoxicological implications are for potentially increased mortality for juvenile salmon using contaminated estuaries.

Accordingly, having determined previously that juvenile fall chinook and chum salmon are exposed to contaminants during residence in the Hylebos Waterway (Collier et al. 1998), our aim in this study (Part 1 of Round II of the Hylebos Fish Injury Study) was to determine if contaminants specifically associated with the Hylebos Waterway can increase the disease susceptibility of juvenile chinook salmon to the bacterial marine pathogen, *Vibrio anguillarum*. Specifically, juvenile chinook salmon were exposed to one of the following for a 7-day period: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) to a chlorinated-enriched fraction of a sediment extract made from the Hylebos Waterway sediment, or 3) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) in proportion to the level of PAHs in sediment from the Hylebos Waterway. The chemical contaminants were administered 7 day prior to being challenged with *Vibrio anguillarum* after which mortality was monitored for a 14 day period. The hypothesis being addressed was whether differences in mortality between experimentally exposed juveniles and juveniles administered the carrier compound (acetone:emulphor) and exposed to *Vibrio anguillarum* were evident. In this study reference extract was not used as a control.

METHODS

Details of the disease challenge study are described in detail in the SAP (Appendix 1). The chemicals identified in the CHWSE and their concentration are listed in Table 1. The composition of the PAH model mixture is listed in Table 2.

Table 1. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Concentrations of selected aromatic hydrocarbons, chlorinated butadienes, pesticides, and polychlorinated biphenyls in sediment extract from the pentane extraction of sediment from the Hylebos Waterway (HWSE-P) that was used in the laboratory experiment. Values are reported as ng/g sediment.

Analyte	ng/g sediment[a]
Aromatic Hydrocarbons	
naphthalene	26
2-methylnaphthalene	18
1-methylnaphthalene	10
biphenyl	6
2,6-dimethylnaphthalene	10
acenaphthylene	0.9
acenaphthene	28
2,3,5-trimethylnaphthalene	28
fluorene	28
phenanthrene	140
anthracene	47
1-methylphenanthrene	10
fluoranthene	170
pyrene	170
benz[a]anthracene	23
chrysene	33
benzo[b]fluoranthene	6
benzo[k]fluoranthene	5
benzo[e]pyrene	9
benzo[a]pyrene	10
perylene	2
indeno[1,2,3-cd]pyrene	0.3
dibenz[a,h]anthracene	< 0.02
benzo[ghi]perylene	1
dibenzothiophene	12
Chlorinated Butadienes[b]	
Trichlorobutadiene	480
Tetrachlorobutadiene	450
Pentachlorobutadiene	160
Hexachlorobutadiene	150

Analyte	ng/g sediment
Pesticides	
hexachlorobenzene	110
lindane (gamma-BHC)	2
heptachlor	0.8
aldrin	< 0.06
heptachlorepoxide	21
oxychlordane	< 0.07
trans-chlordane	< 0.06
nonachlor-III	< 0.06
alpha-chlordane	2
trans-nonachlor	5
cis-nonachlor	0.4
dieldrin	2
mirex	2
o,p'-DDE	1
p,p'-DDE	0.4
o,p'-DDD	20
p,p'-DDD	1
o,p'-DDT	< 0.1
p,p'-DDT	< 0.1
PCBs	
trichlorobiphenyl - 18	17
trichlorobiphenyl - 28	6
tetrachlorobiphenyl - 44	1
tetrachlorobiphenyl - 52	3
tetrachlorobiphenyl - 66	< 0.08
pentachlorobiphenyl - 101	3
pentachlorobiphenyl - 105	< 0.06
pentachlorobiphenyl - 118	< 0.07
hexachlorobiphenyl - 128	0.8
hexachlorobiphenyl - 138	2
hexachlorobiphenyl - 153	4
heptachlorobiphenyl - 170	1
heptachlorobiphenyl - 180	9
heptachlorobiphenyl - 187	2
octachlorobiphenyl - 195	2
nonachlorobiphenyl - 206	12
decachlorobiphenyl - 209	31

[a] Analyte concentrations calculated on a sediment wet weight basis.

[b] Concentrations of the butadienes were calculated using a response factor of 1 with GC/MS total ion current areas.

Table 2. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. High molecular weight hydrocarbons that comprise the PAH model mixture. The compounds were combined in acetone at the same ratios as they were present in the sediment at station 24 of the Hylebos Waterway to a concentration equivalent to 400 g sediment extracted per ml of acetone.

PAH Analytes	
Fluoranthene	Benz[k]fluoranthene
Pyrene	Benzo[a]pyrene
Benz[a]anthracene	Indeno[1,2,3-cd]pyrene
Chrysene,	Dibenz[a,h]anthracene
Benz[b]fluoranthene	Benzo[ghi]perylene

Exposure Assessment

Information on the level of exposure for the treatments used here will be generated in subsequent experiments as described in the SAP (Appendix 1).

Bacterial Lethal Concentration Curve

Juvenile chinook salmon from the Salmon River Hatchery, OR, were obtained to conduct exposure and challenge studies which were conducted at our field station at Newport, OR. The concentration-response curve relating the percent of fish killed with increasing concentration of *Vibrio anguillarum* after a 7 day exposure period was conducted to determine the LC_{30} and LC_{50} for the challenge studies. The 168 h lethal concentration response curve of juvenile chinook salmon exposed to *V. anguillarum* was fitted using logit regression analysis using DeltaGraph™ (Wulf et al. 1991).

Statistical methods - disease challenge

Statistical significance of the challenge studies were assessed using generalized linear models (GLM). The number of survivors and mortalities in a disease challenge study follow a binomial distribution (Hildén and Hirvi 1987). Statistical analyses to assess significance of difference in mortality among the experimental treatment groups compared to the control group were assessed using GLM, a general linear model, performed with the GLMStat computer application (Beath 1995). To define a generalized linear model, it is important to identify the error structure and the link function which relates the linear predictor derived from the GLM analysis to the expected survival/mortality probabilities (Baker and Nelder 1978).

The logistic generalized linear model was used for the analysis of data. For this model the error structure is binomial and the linear predictor was related to the expected value of the datum by the logit link function. This analysis was used to evaluate if survival/mortality of fish injected with contaminants was significantly different ($\alpha = 0.05$) from salmon injected with the carrier 7d post challenge as described in Arkoosh et al. (1998) although data is reported for a 14 day period to more easily visualize differences in mortality among the treatment groups. These analyses were conducted after correction for mortalities in the group of juvenile salmon that served as the non-*Vibrio* treated control for these experiments. The background mortality was determined during the same period (Figure 3). The net mortality was determined by subtracting background mortality observed in juvenile chinook salmon that received chemical contaminants but were not exposed to bacteria. Statistical analyses were conducted separately for each exposure concentration of the bacteria.

RESULTS

Sediment Extract Chemistry

The objective of administering HCBD, a chlorinated-enriched fraction of sediment from the Hylebos Waterway (CHWSE), and a model mixture of PAHs was to expose juvenile salmon to compounds representative of chemical contaminants in the Hylebos Waterway. HCBD is considered a marker chemical for the Hylebos Waterway. HCBD was administered at a dose of 21 mg/kg. CHWSE was prepared from sediment collected near the mouth of the Hylebos Waterway. Specifically, these sediments were taken at Stations HY-07, -08, and -09. These sediment sites were designated and analyzed during the sediment injury studies conducted during Phase 1 of the Commencement Bay Damage Assessment investigations. The objective of preparing the sediment extract was to obtain an extract of chemical contaminants that was representative and enriched in the less polar CHs. The composition of analytes in the extract is listed in Table 1. The extract is enriched in butadiene-like compounds although it still retains significant quantities of AHs. The PCBs present in the sediment were largely removed in the preparation. The final concentration of chemical contaminants in 1 µl of the CHWSE was equivalent to chemical contaminants in 0.2 gr of sediment. A model mixture of polycyclic aromatic hydrocarbons (PAHs) containing 10 high molecular weight PAHs was also prepared to reflect the same ratios of these analytes previously found in sediment during Phase I of the Commencement Bay Damage Assessment studies at Station HY-24. The final concentration of chemical contaminants in 1 µl of the PAH model mixture was equivalent to chemical contaminants in 0.2 gr of sediment. The chemical contaminants along with the acetone:emulphor carrier were each administered at a volume equivalent to 1.5 µl solution/g fish.

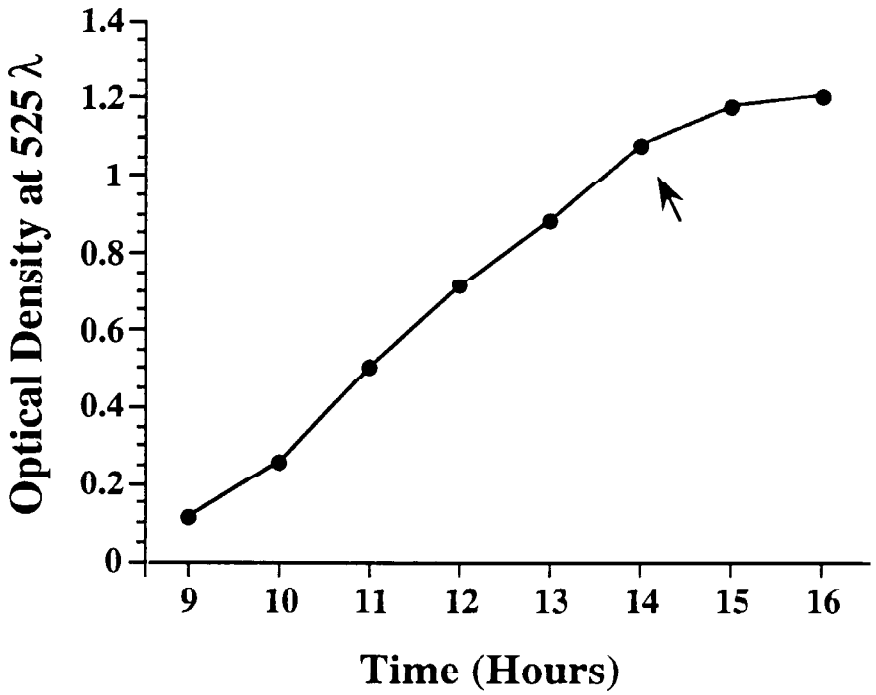

Figure 1. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Growth curve of *Vibrio anguillarum*, expressed as the change in optical density of the culture media with incubation time. The arrow indicates the peak of the exponential growth phase of the bacteria.

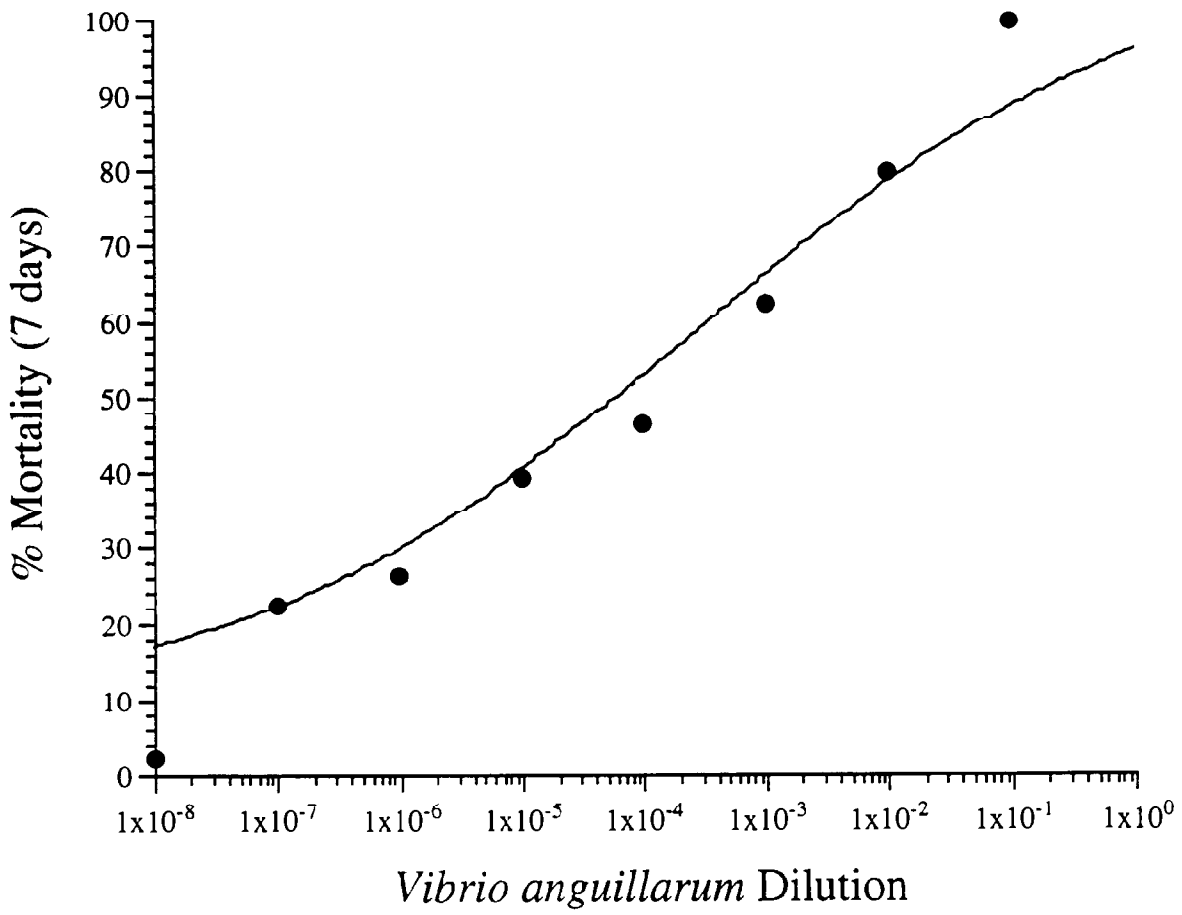

Figure 2. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. The 168 h (7 day) lethal response curve of juvenile chinook salmon exposed to *Vibrio anguillarum*. The *Vibrio anguillarum* dilution represents the volume (ml) of stock bacterial culture diluted per ml of sea water used for exposure of the pathogen to juvenile chinook salmon. The lethal reponse curve was used to select concentrations of *Vibrio anguillarum* for the disease challenge. See the Hylebos Phase II SAP for more details (Appendix 1).

Bacterial Growth and Concentration-Response Curve.

The peak of the exponential growth phase of *V. anguillarum* grown in 500 mL of TSB supplemented with 0.5% NaCl and slowly agitated at 25°C occurred at approximately 14 h with an optical density of 1.1 (Figure 1). In subsequent challenge experiments, *V. anguillarum* cultures were grown until an optical density of approximately 1.1 for preparation of the bacterial culture dilutions used for juvenile salmon exposures. The 168 h (7 d) lethal response curve for Salmon River Hatchery salmon exposed to *V. anguillarum* was determined by a logit regression analysis (Figure 2). The dilutions of bacteria inducing 50% mortality in the Salmon River fish over a 7 d period were 6×10^{-5} ml stock bacterial culture/mL sea water; this dilution was equivalent to approximately 1×10^{7} bacteria/mL sea water.

Disease Challenge Studies.

Background mortality and the percent cumulative mortality of the four different groups of juvenile chinook salmon exposed to *V. anguillarum* is shown in Figure 3 and 4, respectively. The cumulative mortality curves are reported for a 14 day period following exposure to the bacteria. Statistical testing was restricted to 7 days post exposure, as outlined in Arkoosh et al (1998). Recording mortality for a 14 day period allows observations for any unusual mortality events after the traditional 7 day observation period. The data in Figure 4 represent the net mortality attributed to exposure to the bacteria after subtracting background mortality (Figure 3) observed in juvenile chinook salmon that received chemical contaminants but were not exposed to bacteria. The net cumulative mortality of juvenile chinook salmon exposed to bacteria equivalent to an LC_{50} dose after receiving either CHWSE, HCBD, or the model mixture of PAHs ranged from 28 to 31% compared to the 17% observed in the acetone:emulphor control group at 7 days post bacterial challenge. The cumulative mortality was significantly higher in fish exposed to CHWSE, the PAH model mixture, or HCBD relative to fish receiving only the carrier. There was essentially no change in the net cumulative mortality of juvenile salmon from

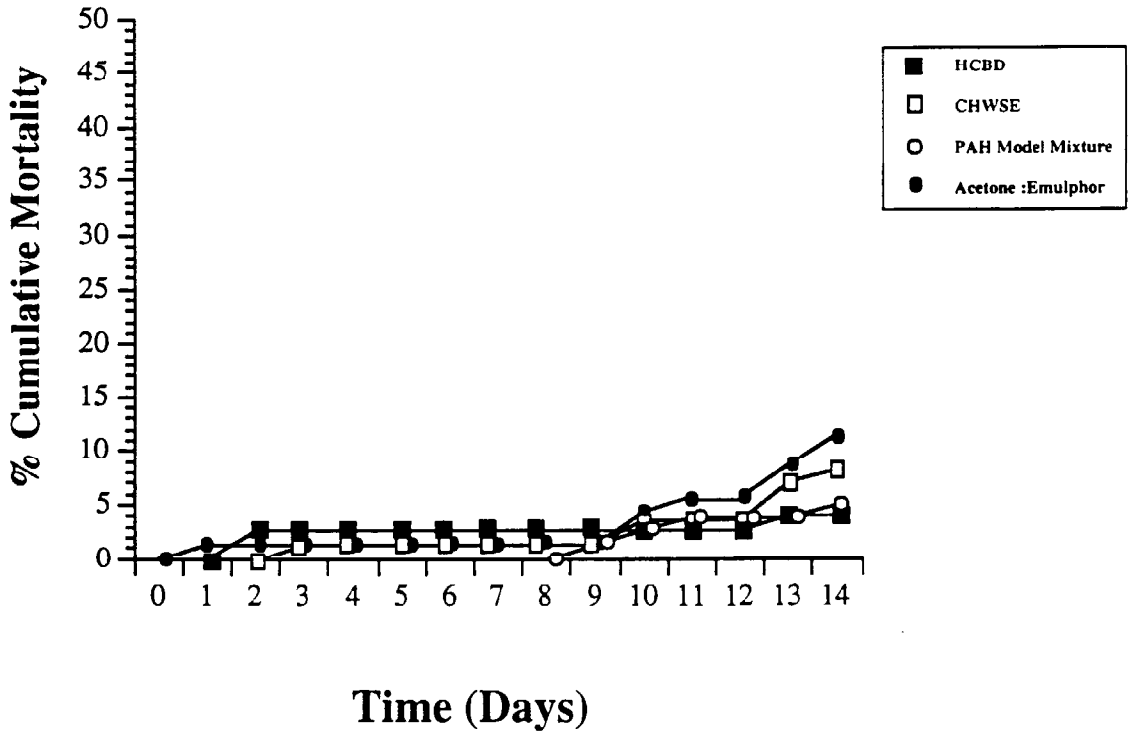

Figure 3. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Background percent cumulative mortality of juvenile chinook salmon injected with either HCBD, a chlorinated-enriched sediment extract from the Hylebos Waterway (CHWSE), a model mixture of PAHs, or the carrier control (acetone:emulphor) but not exposed to *Vibrio anguillarum*.

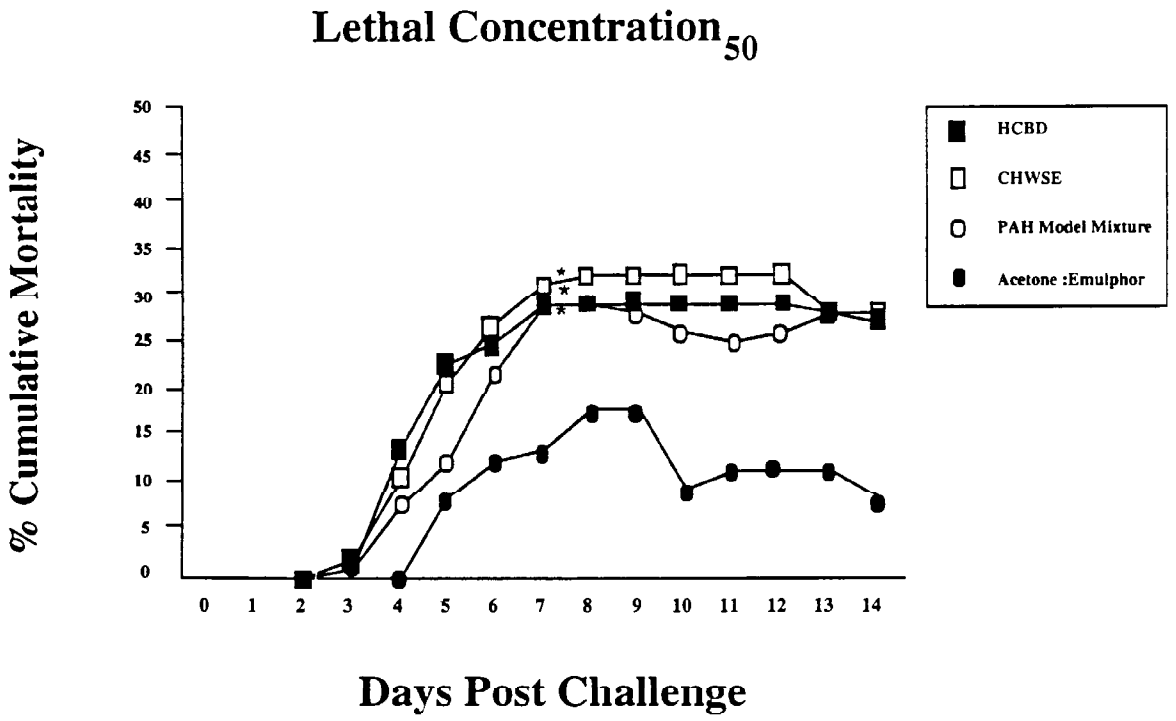

Figure 4. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Percent cumulative mortality of juvenile chinook salmon injected with either HCBD, a chlorinated-enriched sediment extract from the Hylebos Waterway (CHWSE), a model mixture of PAHs, or the carrier control (acetone:emulphor) during a 14 day exposure to 6×10^{-5} ml bacterial solution/ml seawater (LC_{50}). The net cumulative mortalities due to the bacteria are corrected by subtracting mortalities observed in juvenile chinook salmon injected with either HCBD, CHWSE, the model mixture of PAHs, or the carrier control (acetone:emulphor) but not exposed to *Vibrio anguillarum*. NA signifies data not available. * indicates significantly ($p \leq 0.05$) different from acetone:emulphor control group.

the 7 to 14 day observational period and the magnitude of the differences in mortality between the carrier control and the three treatment groups remained relatively unchanged. Because the acetone: emulphor control group for the LC_{30} treatment group was lost, due to a procedural error (loss of air to the tanks), the significance of mortality due to exposure to chemical contaminants could not be determined for this treatment group; the data for LC_{30} - dose treatments are in the Appendix.

Table 3 shows the percentage of salmon carcasses examined that were infected with *V. anguillarum* on days 7 and 14 of the experiment using an LC_{50} exposure dose. Confirmation of the presence of *V. anguillarum* ranged from 44% to 100% in the subsamples tested. The agglutination test identifies bacteria subsampled from each fish that are able to agglutinate anti-*V. anguillarum* antiserum whereas the oxidase positive test identifies fish with bacteria which have cytochrome oxidase enzyme activity which is characteristic of *Vibrio* spp. *Pseudomonas* spp. and *Aeromonas* spp. bacteria, and includes *V. anguillarum*. The findings show that we were able to attribute a majority of the mortalities to *V. anguillarum*.

Table 3. Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Ratio and proportion of juvenile salmon identified to be infected with *V. anguillarum* after exposure to waterborne *V. anguillarum* equivalent to the LC_{50} concentration.

	Positive Agglutination[a]	Agglutination (%)	Oxidase Positive[b]	Oxidase Positive (%)	Combined[c]
Day 7[d]					
acetone/emulphor	7/11	64%	11/11	100%	64%
HCBD	4/9	44%	9/10	90%	44%
CHWSE	12/12	100%	12/12	100%	100%
Model Mixture PAH	7/11	64%	11/11	100%	64%
Day 14					
acetone/emulphor	8/16	50%	12/16	75%	50%
HCBD	4/9	44%	9/10	90%	44%
CHWSE	14/14	100%	14/14	100%	100%
Model Mixture PAH	7/13	54%	13/13	100%	54%

[a] Number of fish examined which had bacteria that agglutinated with the anti-*V. anguillarum* antiserum/total number of fish examined

[b] Number of fish examined which were oxidase positive/ total number of fish examined.

[c] Percentage of fish that had bacteria that were both oxidase positive and agglutinated anti-*V. anguillarum* antiserum.

[d] The number of days after *V. anguillarum* challenge.

DISCUSSION OF MAJOR FINDINGS

Depressed disease resistance results from exposure of juvenile chinook salmon to contaminants specific to the Hylebos Waterway.

Juvenile chinook salmon exposed to contaminants associated with the Hylebos Waterway exhibited a higher susceptibility to mortality induced by the marine pathogen *V. anguillarum,* than did juvenile chinook salmon treated only with the carrier. These findings suggest that a higher predisposition to infection/disease may occur in salmon exposed to chemical contaminants specific to the Hylebos Waterway. At the doses tested, each of the treatment groups, which represent specific subsets of chemical contaminants characteristic of the Hylebos Waterway, increased the disease susceptibility of juvenile chinook salmon. Chlorinated hydrocarbons, characterized by HCBD and the CHWSE, which is composed primarily of HCBD-like compounds, and aromatic hydrocarbons, characterized by the model mixture of PAHs, affected the susceptibility of juvenile chinook salmon to mortality induced by a marine bacterial pathogen. The present findings support the hypothesis that chemical contaminant exposure of juvenile salmon in the Hylebos Waterway influences their physiology such that their survival potential may be reduced. This conclusion is supported by studies in mammals, which have also demonstrated that immunoaltering chemicals can increase disease susceptibility (Friend and Trainer 1970; Thigpen et al. 1975; Loose et al. 1978).

The increased susceptibility of salmon first exposed to contaminants associated with the Hylebos Waterway and then challenged with a marine bacterial pathogen is consistent with our previous findings showing effects of chemical contaminants on immunocompetence and disease susceptibility of juvenile chinook salmon from the Duwamish Waterway in Elliott Bay also located in Puget Sound, WA. Arkoosh et al. (1991, 1998) showed a suppressed secondary immune response in anterior kidney white blood cells to specific model antigens and a greater percent cumulative mortality to *V. anguillarum* after natural exposure to chemical contaminants in the waterway, including PAHs and PCBs. Juvenile salmon exposed to a model PAH, dimethylbenzanthracene (DMBA) or the PCB mixture, Aroclor 1254, in the laboratory also exhibited a similar suppressed secondary immune response in anterior kidney white blood cells to model antigens (Arkoosh et al. 1994). Both PAHs and PCBs are known to induce immunosuppression in other species (Thomas and Hinsdill 1978; Ward et al. 1985).

The overall effects of exposure to chemical contaminants specific to the Hylebos Waterway on disease resistance in juvenile salmon could affect their survival potential in the estuary and early ocean environment.

Although the salmon in the present study were found to have an altered disease resistance shortly after exposure to toxic chemicals, previous data suggests that juvenile salmon may experience continued altered health for an extended period of time after exposure. We found that fish from the contaminated Duwamish estuary held for two months in noncontaminated sea water still exhibited increased mortality associated with the earlier exposure to chemical contaminants (Arkoosh et al. 1998). This finding is also supported by laboratory studies evaluating the duration of immunosuppression in salmonids after an initial exposure to other immunosuppressive chemical contaminants. A study (Arkoosh and Kaattari 1987) examining the effects of aflatoxin B_1, a mycotoxin, on the immune response of rainbow trout demonstrated that a brief exposure of embryos to this immunotoxin, at sublethal concentrations, can manifest its effects months later. The trout experienced immune dysfunction 5-6 months after a single exposure to aflatoxin B_1 (Arkoosh and Kaattari 1987). Our previous studies in conjunction with the study reported by Arkoosh and Kaattari (1987) suggest that although juvenile chinook salmon are only briefly exposed to contaminants in an urban estuary as they migrate to sea, immune altering events may be persistent, and a consequent increase in disease susceptibility in exposed juvenile salmon may extend into their early ocean life. Because recruitment of fish to the adult stages is considered to be dependent on factors acting during the first year of life (Sissenwine 1984), the potential for contaminants to potentiate disease outbreaks and influence mortality rates within the population is a possibility. The potential for disease to influence population structure is supported by studies showing that coho salmon infected with a parasite (*Nanophytes* sp.) as they leave freshwater and enter the ocean environment had much lower adult survival rates than coho with lower infection rates (Schroder and Fresh 1992). Although these findings do not directly reflect cause-and-effect, the results from other previous studies of coho on the Washington coast suggested that diseased fish were lost from the ocean population (Schroder and Fresh 1992), supporting an association between infection with a pathogen and lower survival potential.

The consequences of immunosuppression and an increase in disease susceptibility may be greater than are currently recognized. Immunosuppression has been suggested to play a major role in population regulation and disease itself has been shown to be an important factor in the extinction of several species (Sheldon and Verhulst 1996). The ramifications of disease on marine fish populations can be manifold (Sindermann 1990) including mass mortalities, impaired locomotion, delayed metamorphosis, and increased mortality from predation. In addition,

immunosuppressed animals may allocate greater energy and resources for defending themselves against infection, reducing resources for other vital physiologic functions, such as growth and reproduction (Sheldon and Verhulst 1996). In summary, the findings from Round I of the Hylebos Fish Injury Study (Collier et al 1998) show that the relatively brief residence of juvenile chinook and chum salmon in the Hylebos Waterway increased exposure to chemical contaminants and induced early biological alterations (elevated hepatic CYP1A and DNA damage). In the present study we demonstrated that a model suite of PAHs characteristic of sediments from the Hylebos Waterway, as well as specific Hylebos-associated chemicals (organic solvent extracts of Hylebos sediment), increased disease susceptibility. Because recruitment of salmon appears to be strongly influenced by factors acting on the first year of ocean life (Pearcy 1992), alterations in their overall fitness during this period could be detrimental. Although there is uncertainty in delineating the contribution of the many individual factors that affect survival of juvenile salmon in the estuarine and marine environment, disease susceptibility has consistently been linked to an increase in mortality (Grenfell and Dobson 1995) in natural populations. The findings from the present study showed that exposure to chemical contaminants associated with the Hylebos Waterway increases the susceptibility of juvenile chinook salmon to disease. Quantitating the level of increased risk of subsequent mortality, however, cannot be assessed from the current information. Additional studies to determine the relationship between level of exposure to chemical contaminants and the extent of disease susceptibility are needed to assess further the level of risk to juvenile salmon of increased disease susceptibility from exposure to toxic chemicals present in the Hylebos Waterway.

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Hylebos Fish Injury Study - Round II, Part 1
Individual Data and Quality Assurance Results
CASE NARRATIVE

Effects of Chemical Contaminants from the Hylebos Waterway on Disease Resistance of Juvenile Chinook Salmon

Calibrations

Prior to initiation of the study, balances were calibrated to insure their accuracy. Balances were re-zeroed between weight determinations.

Quality control

A growth curve for *Vibrio anguillarum* was generated to identify the peak of exponential growth for the culture of *V. anguillarum* used in these studies. The optical density of the culture over time was the measure of growth of *V. anguillarum.* The optical density that replicated the peak of exponential growth of *V. anguillarum* identified previously was used on the day of the challenge studies since the culture may be growing at a different rate of speed than previously observed. The *V. anguillarum* growth curve is reported in the Interpretive Report.

A lethal response curve was generated to identify the concentrations of *V. anguillarum* that would likely allow for a protracted death of juvenile salmon over a seven day period. The lethal concentration whereby 30% and 50% of the fish died after a 7 day challenge to *V. anguillarum* was calculated from the lethal reponse curve. These concentrations of *V. anguillarum* were not likely to overwhelm the fish to the point a difference in treatment mortality as a result of the disease challenge could not be detected. The lethal response curve is reported in the Interpretive Report.

Mortalities from each tank were collected on a daily basis for two weeks and recorded by the sampler. Data from each of the tanks are shown in the following tables. The data from the necropsy are also presented in table form. As stated in the Sampling and Analysis Plan two controls groups were used. These include fish injected only with the carrier (acetone:emuulphor) and exposed to one of two concentrations of *V. anguillarum* (LC_{30}, LC_{50}) and fish injected with either acetone:emulphor, HCBD, the model mixture of PAHs, or CHWSE, but not subject to *V. anguillarum* challenge. The acetone:emulphor control groups exposed to the various concentrations of *V. anguillarum* were used for comparison to the contaminant-injected groups that were exposed to *V. anguillarum* to identify treatment differences in susceptibility to a disease challenge whereas fish injected with either acetone:emulphor, HCBD, the model mixture of PAHs, or CHWSE, but not subject to *V. anguillarum* challenge were conducted to identify the average number of fish that were dying directly from toxic exposure. Mortalities derived from this latter case were used to correct for contaminant derived mortalities in the treatment groups exposed to contaminants and challenged to *V. anguillarum.* The data summarizing the correction for mortalities attributable solely to exposure to each treatment are also included. This data is presented in two tables, one describing the average mortality for juvenile salmon over the same 7 day period that had received acetone:emulphor (the carrier), HCBD, the model mixture of PAHs, of CHWSE, but were exposed to *V. anguillarum*, the other table summarizes the uncorrected and corrected mortalities attributable to each treatment in each tank. The corrected mortalities were achieved by using the average mortality observed solely due to acetone:emulphor (the carrier), HCBD, the model mixture of PAHs, or CHWSE exposure and converting mortalities as survivors to an equivalent level in the groups receiving acetone:emulphor (the carrier), HCBD, the model mixture of PAHs, or CHWSE and then exposed to *V. anguillarum.* For example, if the average mortality in fish in all tanks receiving acetone:emulphor (the carrier) only was one and the mortality in a tank of fish receiving

acetone:emulphor (the carrier) and exposed to *V. anguillarum* over the same 7 day period was 5, then the corrected mortality due to exposure to *V. anguillarum* exposure for this partcular tank of fish was recorded as 4, achieved by subtracting 1 from 5. Also, fish which died on the day of challenge were considered to have died from handling stresses due to the act of challenging and were not considered as mortalities due to the bacterial challenge.

Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon.

Data tables of daily mortality and cumulative survival of juvenile salmon exposed to acetone:emulphor, HCBD, A PAH model mixture, or CHWSE for a 8 day period and then challenged to *Vibrio anguillarum* via waterborne exposure.

Tank #: 49 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	1	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	20[a]	10	ERC
21-Oct	1	9	ERC
22-Oct	0	9	ANK
23-Oct	0	9	PH
24-Oct	0	9	ERC
25-Oct	0	9	ANK
26-Oct	0	9	ANK
27-Oct	0	9	PH
28-Oct	0	9	ERC
29-Oct	0	9	ERC
30-Oct	2	7	ERC
31-Oct	0	7	PH
1-Nov	0	7	ANK
2-Nov	0	7	PH
3-Nov	1	6	ANK

[a] Fish which died on the day of challenge were considered to have died from handing stresses due to the act of challenging and were not considered as mortalities due to the bacterial challenge.

Tank #: 50 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	0	30	ERC
25-Oct	0	30	ANK
26-Oct	0	30	ANK
27-Oct	0	30	PH
28-Oct	0	30	ERC
29-Oct	0	30	ERC
30-Oct	0	30	ERC
31-Oct	1	29	PH
1-Nov	0	29	ANK
2-Nov	2	27	PH
3-Nov	0	27	ANK

Tank #: 51 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	0	30	ERC
25-Oct	0	30	ANK
26-Oct	0	30	ANK
27-Oct	0	30	PH
28-Oct	0	30	ERC
29-Oct	0	30	ERC
30-Oct	0	30	ERC
31-Oct	0	30	PH
1-Nov	0	30	ANK
2-Nov	0	30	PH
3-Nov	1	29	ANK

Tank #: 52 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	1	29	PH
14-Oct	0	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	NO AIR FISH DEAD[a]		
21-Oct			
22-Oct			
23-Oct			
24-Oct			
25-Oct			
26-Oct			
27-Oct			
28-Oct			
29-Oct			
30-Oct			
31-Oct			
1-Nov			
2-Nov			
3-Nov			

[a] All fish died from lack of oxygen as a result of air supply inadvertantly shut off during exposure to *Vibrio anguillarum*.

Tank #: 53 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	1	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	NO AIR FISH DEAD[a]		
21-Oct			
22-Oct			
23-Oct			
24-Oct			
25-Oct			
26-Oct			
27-Oct			
28-Oct			
29-Oct			
30-Oct			
31-Oct			
1-Nov			
2-Nov			
3-Nov			

[a] All fish died from lack of oxygen as a result of air supply inadvertantly shut off during exposure to *Vibrio anguillarum*.

Tank #: 54 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	1	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	NO AIR FISH DEAD[a]		
21-Oct			
22-Oct			
23-Oct			
24-Oct			
25-Oct			
26-Oct			
27-Oct			
28-Oct			
29-Oct			
30-Oct			
31-Oct			
1-Nov			
2-Nov			
3-Nov			

[a] All fish died from lack of oxygen as a result of air supply inadvertantly shut off during exposure to *Vibrio anguillarum*.

Tank #: 46 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	1	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	1	28	ANK
26-Oct	2	26	ANK
27-Oct	0	26	PH
28-Oct	2	24	ERC
29-Oct	0	24	ERC
30-Oct	0	24	ERC
31-Oct	0	24	PH
1-Nov	0	24	ANK
2-Nov	0	24	PH
3-Nov	0	24	ANK

Tank #: 47 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	1	29	PH
14-Oct	0	29	PH
15-Oct	0	29	PH
16-Oct	1	28	ANK
17-Oct	0	28	PH
18-Oct	0	28	ANK
19-Oct	0	28	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	28	ERC
21-Oct	0	28	ERC
22-Oct	0	28	ANK
23-Oct	0	28	PH
24-Oct	0	28	ERC
25-Oct	6	22	ANK
26-Oct	1	21	ANK
27-Oct	1	20	PH
28-Oct	0	20	ERC
29-Oct	0	20	ERC
30-Oct	0	20	ERC
31-Oct	1	19	PH
1-Nov	0	19	ANK
2-Nov	1	18	PH
3-Nov	1	17	ANK

Tank #: 48 **Injection date:** 10/12/95

Injected with: Acetone:Emulphor **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	1	29	ERC
25-Oct	2	27	ANK
26-Oct	1	26	ANK
27-Oct	0	26	PH
28-Oct	1	25	ERC
29-Oct	0	25	ERC
30-Oct	0	25	ERC
31-Oct	1	24	PH
1-Nov	0	24	ANK
2-Nov	1	23	PH
3-Nov	0	23	ANK

Tank #: 25 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	4	26	ERC
21-Oct	0	26	ERC
22-Oct	2	24	ANK
23-Oct	0	24	PH
24-Oct	0	24	ERC
25-Oct	0	24	ANK
26-Oct	0	24	ANK
27-Oct	0	24	PH
28-Oct	0	24	ERC
29-Oct	0	24	ERC
30-Oct	0	24	ERC
31-Oct	0	24	PH
1-Nov	0	24	ANK
2-Nov	0	24	PH
3-Nov	0	24	ANK

Tank #: 28 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	0	30	ERC
25-Oct	0	30	ANK
26-Oct	0	30	ANK
27-Oct	0	30	PH
28-Oct	0	30	ERC
29-Oct	0	30	ERC
30-Oct	0	30	ERC
31-Oct	0	30	PH
1-Nov	0	30	ANK
2-Nov	0	30	PH
3-Nov	0	30	ANK

Tank #: 37 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	19[a]	
13-Oct	0	19	PH
14-Oct	0	19	PH
15-Oct	0	19	PH
16-Oct	0	19	ANK
17-Oct	0	19	PH
18-Oct	0	19	ANK
19-Oct	0	19	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	19	ERC
21-Oct	0	19	ERC
22-Oct	0	19	ANK
23-Oct	0	19	PH
24-Oct	0	19	ERC
25-Oct	0	19	ANK
26-Oct	0	19	ANK
27-Oct	0	19	PH
28-Oct	0	19	ERC
29-Oct	0	19	ERC
30-Oct	0	19	ERC
31-Oct	0	19	PH
1-Nov	0	19	ANK
2-Nov	1	18	PH
3-Nov	0	18	ANK

[a] Only 19 fish available for this treatment and tank, not allowing us to inject 30 fish as described in the SAP.

Tank #: 13 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	0	30	ERC
25-Oct	0	30	ANK
26-Oct	0	30	ANK
27-Oct	0	30	PH
28-Oct	0	30	ERC
29-Oct	0	30	ERC
30-Oct	0	30	ERC
31-Oct	0	30	PH
1-Nov	0	30	ANK
2-Nov	0	30	PH
3-Nov	0	30	ANK

Tank #: 19 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	1	28	ANK
27-Oct	0	28	PH
28-Oct	1	27	ERC
29-Oct	1	26	ERC
30-Oct	0	26	ERC
31-Oct	3	23	PH
1-Nov	0	23	ANK
2-Nov	3	20	PH
3-Nov	2	18	ANK

Tank #: 22 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	1	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	0	29	ERC
29-Oct	0	29	ERC
30-Oct	0	29	ERC
31-Oct	0	29	PH
1-Nov	0	29	ANK
2-Nov	0	29	PH
3-Nov	0	29	ANK

Tank #: 7 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	2	28	PH
24-Oct	5	23	ERC
25-Oct	3	20	ANK
26-Oct	1	19	ANK
27-Oct	0	19	PH
28-Oct	0	19	ERC
29-Oct	0	19	ERC
30-Oct	0	19	ERC
31-Oct	0	19	PH
1-Nov	0	19	ANK
2-Nov	0	19	PH
3-Nov	0	19	ANK

Tank #: 10 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	1	29	PH
14-Oct	0	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	6	23	ERC
25-Oct	4	19	ANK
26-Oct	1	18	ANK
27-Oct	1	17	PH
28-Oct	0	17	ERC
29-Oct	0	17	ERC
30-Oct	0	17	ERC
31-Oct	0	17	PH
1-Nov	0	17	ANK
2-Nov	0	17	PH
3-Nov	0	17	ANK

Tank #: 16 **Injection date:** 10/12/95

Injected with: HCBD **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	9	20	ERC
21-Oct	0	20	ERC
22-Oct	0	20	ANK
23-Oct	0	20	PH
24-Oct	0	20	ERC
25-Oct	1	19	ANK
26-Oct	0	19	ANK
27-Oct	2	17	PH
28-Oct	0	17	ERC
29-Oct	0	17	ERC
30-Oct	0	17	ERC
31-Oct	0	17	PH
1-Nov	0	17	ANK
2-Nov	0	17	PH
3-Nov	0	17	ANK

Tank #: 26 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	3	26	ERC
21-Oct	0	26	ERC
22-Oct	0	26	ANK
23-Oct	1	25	PH
24-Oct	0	25	ERC
25-Oct	0	25	ANK
26-Oct	0	25	ANK
27-Oct	0	25	PH
28-Oct	0	25	ERC
29-Oct	0	25	ERC
30-Oct	2	23	ERC
31-Oct	0	23	PH
1-Nov	0	23	ANK
2-Nov	2	21	PH
3-Nov	1	20	ANK

Tank #: 29 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	1	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	0	29	ERC
29-Oct	0	29	ERC
30-Oct	0	29	ERC
31-Oct	0	29	PH
1-Nov	0	29	ANK
2-Nov	1	28	PH
3-Nov	0	28	ANK

Tank #: 38 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	1	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	0	29	ERC
29-Oct	0	29	ERC
30-Oct	0	29	ERC
31-Oct	0	29	PH
1-Nov	0	29	ANK
2-Nov	0	29	PH
3-Nov	0	29	ANK

Tank #: 14 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	1	29	PH
14-Oct	0	29	PH
15-Oct	0	29	PH
16-Oct	2	27	ANK
17-Oct	1	26	PH
18-Oct	0	26	ANK
19-Oct	0	26	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	26	ERC
21-Oct	0	26	ERC
22-Oct	0	26	ANK
23-Oct	0	26	PH
24-Oct	0	26	ERC
25-Oct	1	25	ANK
26-Oct	0	25	ANK
27-Oct	2	23	PH
28-Oct	0	23	ERC
29-Oct	0	23	ERC
30-Oct	1	22	ERC
31-Oct	0	22	PH
1-Nov	0	22	ANK
2-Nov	0	22	PH
3-Nov	1	21	ANK

Tank #: 20 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	3	27	ERC
21-Oct	0	27	ERC
22-Oct	0	27	ANK
23-Oct	0	27	PH
24-Oct	0	27	ERC
25-Oct	0	27	ANK
26-Oct	0	27	ANK
27-Oct	0	27	PH
28-Oct	1	26	ERC
29-Oct	0	26	ERC
30-Oct	1	25	ERC
31-Oct	0	25	PH
1-Nov	0	25	ANK
2-Nov	1	24	PH
3-Nov	3	21	ANK

Tank #: 23

Injection date: 10/12/95

Injected with: CHWSE

Challenge date: 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	2	28	PH
15-Oct	0	28	PH
16-Oct	2	26	ANK
17-Oct	0	26	PH
18-Oct	0	26	ANK
19-Oct	0	26	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	1	25	ERC
21-Oct	0	25	ERC
22-Oct	0	25	ANK
23-Oct	0	25	PH
24-Oct	0	25	ERC
25-Oct	0	25	ANK
26-Oct	0	25	ANK
27-Oct	1	24	PH
28-Oct	1	23	ERC
29-Oct	1	22	ERC
30-Oct	0	22	ERC
31-Oct	1	21	PH
1-Nov	0	21	ANK
2-Nov	0	21	PH
3-Nov	0	21	ANK

Tank #: 8 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	1	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC 50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	2	27	PH
24-Oct	5	22	ERC
25-Oct	2	20	ANK
26-Oct	3	17	ANK
27-Oct	1	16	PH
28-Oct	0	16	ERC
29-Oct	0	16	ERC
30-Oct	1	15	ERC
31-Oct	0	15	PH
1-Nov	0	15	ANK
2-Nov	0	15	PH
3-Nov	0	15	ANK

Tank #: 17 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	1	28	PH
16-Oct	1	27	ANK
17-Oct	2	25	PH
18-Oct	0	25	ANK
19-Oct	0	25	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	25	ERC
21-Oct	0	25	ERC
22-Oct	0	25	ANK
23-Oct	0	25	PH
24-Oct	2	23	ERC
25-Oct	2	21	ANK
26-Oct	1	20	ANK
27-Oct	1	19	PH
28-Oct	0	19	ERC
29-Oct	0	19	ERC
30-Oct	1	18	ERC
31-Oct	0	18	PH
1-Nov	0	18	ANK
2-Nov	0	18	PH
3-Nov	0	18	ANK

Tank #: 11 **Injection date:** 10/12/95

Injected with: CHWSE **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	1	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	5	24	ANK
26-Oct	1	23	ANK
27-Oct	1	22	PH
28-Oct	1	21	ERC
29-Oct	0	21	ERC
30-Oct	0	21	ERC
31-Oct	0	21	PH
1-Nov	0	21	ANK
2-Nov	0	21	PH
3-Nov	0	21	ANK

Tank #: 27 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	2	27	PH
18-Oct	0	27	ANK
19-Oct	0	27	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	7	20	ERC
21-Oct	0	20	ERC
22-Oct	0	20	ANK
23-Oct	0	20	PH
24-Oct	0	20	ERC
25-Oct	0	20	ANK
26-Oct	0	20	ANK
27-Oct	0	20	PH
28-Oct	0	20	ERC
29-Oct	0	20	ERC
30-Oct	1	19	ERC
31-Oct	1	18	PH
1-Nov	0	18	ANK
2-Nov	0	18	PH
3-Nov	1	17	ANK

Tank #: 30 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	1	29	PH
14-Oct	0	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	0	29	ERC
29-Oct	1	28	ERC
30-Oct	0	28	ERC
31-Oct	0	28	PH
1-Nov	0	28	ANK
2-Nov	0	28	PH
3-Nov	0	28	ANK

Tank #: 39 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC0

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	1	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	0	29	ERC
29-Oct	0	29	ERC
30-Oct	0	29	ERC
31-Oct	0	29	PH
1-Nov	0	29	ANK
2-Nov	0	29	PH
3-Nov	0	29	ANK

Tank #: 15 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	3	26	PH
18-Oct	0	26	ANK
19-Oct	0	26	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	26	ERC
21-Oct	0	26	ERC
22-Oct	0	26	ANK
23-Oct	0	26	PH
24-Oct	0	26	ERC
25-Oct	0	26	ANK
26-Oct	0	26	ANK
27-Oct	2	24	PH
28-Oct	1	23	ERC
29-Oct	0	23	ERC
30-Oct	0	23	ERC
31-Oct	0	23	PH
1-Nov	0	23	ANK
2-Nov	0	23	PH
3-Nov	0	23	ANK

Tank #: 21 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	1	29	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	29	ERC
21-Oct	0	29	ERC
22-Oct	0	29	ANK
23-Oct	0	29	PH
24-Oct	0	29	ERC
25-Oct	0	29	ANK
26-Oct	0	29	ANK
27-Oct	0	29	PH
28-Oct	2	27	ERC
29-Oct	0	27	ERC
30-Oct	0	27	ERC
31-Oct	1	26	PH
1-Nov	1	25	ANK
2-Nov	0	25	PH
3-Nov	0	25	ANK

Tank #: 24 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC30

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	4	26	ERC
21-Oct	0	26	ERC
22-Oct	0	26	ANK
23-Oct	0	26	PH
24-Oct	0	26	ERC
25-Oct	0	26	ANK
26-Oct	0	26	ANK
27-Oct	0	26	PH
28-Oct	0	26	ERC
29-Oct	2	24	ERC
30-Oct	0	24	ERC
31-Oct	1	23	PH
1-Nov	0	23	ANK
2-Nov	0	23	PH
3-Nov	0	23	ANK

Tank #: 9 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	0	30	PH
24-Oct	2	28	ERC
25-Oct	4	24	ANK
26-Oct	3	21	ANK
27-Oct	2	19	PH
28-Oct	0	19	ERC
29-Oct	0	19	ERC
30-Oct	0	19	ERC
31-Oct	0	19	PH
1-Nov	0	19	ANK
2-Nov	0	19	PH
3-Nov	0	19	ANK

Tank #: 12 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	0	30	PH
15-Oct	0	30	PH
16-Oct	0	30	ANK
17-Oct	0	30	PH
18-Oct	0	30	ANK
19-Oct	0	30	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	0	30	ERC
21-Oct	0	30	ERC
22-Oct	0	30	ANK
23-Oct	1	29	PH
24-Oct	1	28	ERC
25-Oct	1	27	ANK
26-Oct	3	24	ANK
27-Oct	2	22	PH
28-Oct	0	22	ERC
29-Oct	0	22	ERC
30-Oct	0	22	ERC
31-Oct	0	22	PH
1-Nov	0	22	ANK
2-Nov	2	20	PH
3-Nov	0	20	ANK

Tank #: 18 **Injection date:** 10/12/95

Injected with: PAH model mix **Challenge date:** 10/20/95

Date	Mortalities	Cumulative Survival	Sampler
12-Oct	-	30	
13-Oct	0	30	PH
14-Oct	1	29	PH
15-Oct	0	29	PH
16-Oct	0	29	ANK
17-Oct	0	29	PH
18-Oct	0	29	ANK
19-Oct	0	29	ANK

Vibrio Treatment: LC50

Date	Mortalities	Cumulative Survival	Sampler
20-Oct	1	28	ERC
21-Oct	0	28	ERC
22-Oct	0	28	ANK
23-Oct	0	28	PH
24-Oct	2	26	ERC
25-Oct	0	26	ANK
26-Oct	2	24	ANK
27-Oct	2	22	PH
28-Oct	0	22	ERC
29-Oct	0	22	ERC
30-Oct	0	22	ERC
31-Oct	0	22	PH
1-Nov	1	21	ANK
2-Nov	0	21	PH
3-Nov	0	21	ANK

Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon.

Data table documenting the average number of salmon deaths in non-vibrio exposed fish after a 7 day period. This number is used as a correction factor to correct for mortalities not attributable to exposure to *Vibrio anguillarum* in the challenge study where juvenile salmon are exposed to *V. anguillarum* via waterborne exposure.

Treatment	Tank #s	Average Mortality[a]
Acetone:Emulphor	49, 50, 51	1
HCBD	25, 28, 37	1
CHWSE	26, 29, 38	1
PAH model mixture	27, 30, 39	0

[a] The value represents the average mortality of juvenile chinook salmon in all tanks for fish receiving either the carrier compound (acetone:emulphor), HCBD, CHWSE, or the PAH model mixture but was not exposed to *Vibrio anguillarum*. The average values were rounded up to the next whole number.

Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon.

Data table documenting the number of juvenile salmon mortalities and survivors before and after correcting for the background mortalities not attributable to *Vibrio anguillarum* exposure after a 7 day period.

Treatment	Tank #	LC_{50}[a]			
		Uncorrected[b]		Corrected[c]	
		survivors	mortalities	survivors	mortalities
Acetone:Emulphor	46	26	3	27	2
	47	21	8	22	7
	48	26	4	27	3
	Total	73	14	76	12
HCBD	7	19	11	20	10
	10	18	12	19	11
	16	17	3	18	2
	Total	54	53	57	23
CHWSE	8	16	13	17	12
	11	22	7	21	6
	17	19	6	20	5
	Total	57	26	58	23
PAH model mixture	9	18	11	18	11
	12	22	8	22	8
	18	22	6	22	6
	Total	62	25	62	25

[a] Represents the *Vibrio anguillarum* exposure concentration. Uncorrected and corrected survival and mortalities for juvenile salmon exposed to the LC_{30} *V. anguillarum* exposure concentration were not reported because the loss of all control fish at this *V. anguillarum* exposure level.

[b] Represents the cumulative raw data of mortality and survival of juvenile salmon exposed to *Vibrio anguillarum* after a 7 day period after preexposure to either the carrier compound (acetone:emulphor), HCBD, CHWSE, or the PAH model mixture.

[c] Represents the cumulative corrected data (corrected for mortalities not attributable to exposure to *Vibrio anguillarum)* of mortality and survival of juvenile salmon exposed to *Vibrio anguillarum* after a 7 day period after preexposure to either the carrier compound (acetone:emulphor), HCBD, CHWSE, or the PAH model mixture.

Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon.

Data table documenting test results to confirm the presence of *Vibiro anguillarum* in juvenile salmon previously exposed to *V. anguillarum* via waterborne exposure.

Date of Mortality	Tank#	Injected Compound	*Vibrio* Treatment	[a]Fish tested (Total Morts)	Agglutination Test (+/-)	Oxidase Test (+/-)	*Vibrio* (yes/no)
21-Oct	54	AE	0	1(1)	No plate	No plate	No
22-Oct	25	HCBD	0	1(2)	-	+	No
24-Oct	7	HCBD	50	1(5)	-	+	No
24-Oct	10	HCBD	50	1(6)	+	+	Yes
24-Oct	10	HCBD	50	1(6)	-	+	No
24-Oct	8	CHWSE	50	1(5)	+	+	Yes
24-Oct	17	CHWSE	50	1(2)	+	+	Yes
24-Oct	9	PAH	50	1(2)	+	+	Yes
24-Oct	12	PAH	50	1(1)	-	+	No
24-Oct	18	PAH	50	1(2)	+	+	Yes
24-Oct	48	AE	50	1(1)	+	+	Yes
25-Oct	7	HCBD	50	1(3)	-	+	No
25-Oct	10	HCBD	50	1(4)	-	+	No
25-Oct	16	HCBD	50	1(1)	+	+	Yes
25-Oct	8	CHWSE	50	1(2)	+	+	Yes
25-Oct	11	CHWSE	50	1(5)	+	+	Yes
25-Oct	17	CHWSE	50	1(2)	+	+	Yes
25-Oct	9	PAH	50	1(4)	+	+	Yes
25-Oct	12	PAH	50	1(1)	+	+	Yes
25-Oct	46	AE	50	1(1)	-	+	No
25-Oct	47	AE	50	1(6)[b]	+	+	Yes
25-Oct	48	AE	50	1(2)	-	+	No
26-Oct	7	HCBD	50	1(1)	+	+	Yes
26-Oct	10	HCBD	50	1(1)	+	+	Yes
26-Oct	8	CHWSE	50	1(3)	+	+	Yes
26-Oct	11	CHWSE	50	1(1)	+	+	Yes
26-Oct	17	CHWSE	50	1(1)	+	+	Yes
26-Oct	9	PAH	50	1(3)	+	+	Yes
26-Oct	12	PAH	50	1(1)	+	+	Yes
26-Oct	18	PAH	50	1(2)	-	+	No
26-Oct	46	AE	50	1(2)	+	+	Yes
26-Oct	47	AE	50	1(1)	+	+	Yes
26-Oct	48	AE	50	1(2)	+	+	Yes

Cont.

Date of Mortality	Tank#	Injected Compound	*Vibrio* Treatment	[a]Fish tested (Total Morts)	Agglutination Test (+/-)	Oxidase Test (+/-)	*Vibrio* (yes/no)
27-Oct	8	CHWSE	50	1(1)	+	+	Yes
27-Oct	11	CHWSE	50	1(1)	+	+	Yes
27-Oct	17	CHWSE	50	1(1)	+	+	Yes
27-Oct	9	PAH	50	1(2)	+	+	Yes
27-Oct	12	PAH	50	1(2)	-	+	No
27-Oct	18	PAH	50	1(2)	+	+	Yes
27-Oct	10	HCBD	50	1(1)	+	+	Yes
27-Oct	16	HCBD	50	1(2)	-	+	No
27-Oct	47	AE	50	1(1)	+	+	Yes
28-Oct	11	CHWSE	50	1(1)	+	+	Yes
28-Oct	46	AE	50	1(2)	-	+	No
28-Oct	48	AE	50	1(1)	+	+	Yes
29-Oct	30	PAH	0	1(1)	+	-	Yes
30-Oct	8	CHWSE	50	1(1)	+	+	Yes
30-Oct	17	CHWSE	50	1(1)	+	+	Yes
30-Oct	26	CHWSE	0	1(2)	-	+	No
30-Oct	49	AE	0	1(2)	-	+	No
31-Oct	27	PAH	0	1(1)	-	+	No
31-Oct	47	AE	50	1(1)	-	-	No
31-Oct	48	AE	50	1(1)	+	+	Yes
31-Oct	50	AE	0	1(1)	-	+	No
1-Nov	18	PAH	50	1(1)	-	+	No
2-Nov	12	PAH	50	1(2)	-	+	No
2-Nov	37	HCBD	0	1(1)	-	+	No
2-Nov	47	AE	50	1(1)	-	+	No
2-Nov	48	AE	50	1(1)	-	+	No
2-Nov	50	AE	0	1(2)	+	+	Yes
3-Nov	26	CHWSE	0	1(1)	-	+	No
3-Nov	27	PAH	0	1(1)	-	+	No
3-Nov	47	AE	50	1(1)	-	+	No
3-Nov	49	AE	0	1(1)	-	+	No
3-Nov	51	AE	0	1(1)	-	+	No

[a] Repesents the result for one fish subsampled from the group of fish that died on one day for each tank. The value in parentheses represents the total number of fish that died on the particular day that a subsample was assessed to confirm the presence of Vibrio anguillarum.

[b] Only 1 of 6 fish subsampled to identify and confirm the presence of Vibrio anguillarum rather than 1 of 3 as stated in the SAP.

Calculation of the GLM statistic

As described in the Sampling and Analysis Plan, the GLMStat computer application was used to generate the information needed to assess the statistical significance of the challenge studies at day 7 of the challenge as follows:

Acetone:Emulphor-injected fish vrs HCBD-injected fish

Source	Degrees of freedom (DF)	Deviance	mean square (dev/DF)	F Value	a = 0.05	significantly different?
Total	167	127.5				
Ace:emulphor vrs HCBD inj.	1	5.179	5.179	$F_{1,166}$ = 7.03	3.90	yes
residual	166	122.3	0.737			

Acetone:Emulphor-injected fish vrs CHWSE-injected fish

Source	Degrees of freedom (DF)	Deviance	mean square (dev/DF)	F Value	a = 0.05	significantly different?
Total	170	128.8				
Ace:Emul vrs CHWSE-inj.	1	4.662	4.662	$F_{1,169}$ = 6.349	3.90	yes
residual	169	124.1	0.7343			

Acetone:Emulphor-injected fish vrs PAH model mixture-injected fish

Source	Degrees of freedom (DF)	Deviance	mean square (dev/DF)	F Value	a = 0.05	significantly different?
Total	174	13306				
Ace:Emul vrs PAH-inj.	1	5.374	5.374	$F_{1,173}$ = 7.24	3.89	yes
residual	173	128.3	0.742			

Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon.

Data tables documenting the GLMStat data files used to assess the significance of exposure to *Vibrio anguillarum* after exposure to acetone:emulphor (carrier), HCBD, CHWSE, or a PAH model mixture. Data for the exposure using a *V. anguillarum* exposure challenge equivalent to the LC_{30} dose was not included in this data file as statistical testing of this group was not evaluated due to loss of the corresponding acetone:emulphor control group during the actual *Vibrio* challenge.

The key to the data table is as follows:

Column 1 = Round: An internal record of the challenge experiment

Column 2 = Contaminant: Treatment group
1 = acetonne:emulphor
2 = HCBD
3 = CHWSE
4 = PAH model mixture

Column 3 = Treatment: *Vibrio anguillarum* exposure group
1 = LC_0
2 = LC_{50}

Column 4 = Tank #: Tank number

Column 5 = D/A(4day): a fish that is dead or alive after 4 days exposure to *V. anguillarum*
1 = dead
2 = alive

Column 6 – D/A(7day): a fish that is dead or alive after 7 days exposure to *V. anguillarum*
1 = dead
2 = alive

Column 7 = D/A(14day): a fish that is dead or alive after 14 days exposure to *V. anguillarum*
1 = dead
2 = alive

Column 8 = D/A(4day)-BKD: a fish that is dead or alive after 4 days exposure to *V. anguillarum*, corrected for background mortality
1 = dead
2 = alive

Column 9 = D/A(7day)-BKD: a fish that is dead or alive after 7 days exposure to *V. anguillarum*, corrected for background mortality
1 = dead
2 = alive

Column 10 = D/A(14day)-BKD: a fish that is dead or alive after 14 days exposure to *V. anguillarum*, corrected for background mortality
1 = dead
2 = alive

Column 11 = binomialn: The binomial n value of 2 used for derivation of the statistic based on the binomial distribution.

	Round	Contaminant [4]	Treatment [2]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomial/n
1	5.000	2	2	7	1	1	1	1	1	1	2.000
2	5.000	2	2	7	1	1	1	1	1	1	2.000
3	5.000	2	2	7	1	1	1	1	1	1	2.000
4	5.000	2	2	7	1	1	1	1	1	1	2.000
5	5.000	2	2	7	1	1	1	1	1	1	2.000
6	5.000	2	2	7	1	1	1	1	1	1	2.000
7	5.000	2	2	7	1	1	1	2	1	1	2.000
8	5.000	2	2	7	2	1	1	2	1	1	2.000
9	5.000	2	2	7	2	1	1	2	1	1	2.000
10	5.000	2	2	7	2	1	1	2	1	1	2.000
11	5.000	2	2	7	2	1	1	2	2	2	2.000
12	5.000	2	2	7	2	2	2	2	2	2	2.000
13	5.000	2	2	7	2	2	2	2	2	2	2.000
14	5.000	2	2	7	2	2	2	2	2	2	2.000
15	5.000	2	2	7	2	2	2	2	2	2	2.000
16	5.000	2	2	7	2	2	2	2	2	2	2.000
17	5.000	2	2	7	2	2	2	2	2	2	2.000
18	5.000	2	2	7	2	2	2	2	2	2	2.000
19	5.000	2	2	7	2	2	2	2	2	2	2.000
20	5.000	2	2	7	2	2	2	2	2	2	2.000
21	5.000	2	2	7	2	2	2	2	2	2	2.000
22	5.000	2	2	7	2	2	2	2	2	2	2.000
23	5.000	2	2	7	2	2	2	2	2	2	2.000
24	5.000	2	2	7	2	2	2	2	2	2	2.000
25	5.000	2	2	7	2	2	2	2	2	2	2.000
26	5.000	2	2	7	2	2	2	2	2	2	2.000
27	5.000	2	2	7	2	2	2	2	2	2	2.000
28	5.000	2	2	7	2	2	2	2	2	2	2.000
29	5.000	2	2	7	2	2	2	2	2	2	2.000
30	5.000	2	2	7	2	2	2	2	2	2	2.000
31	5.000	3	2	8	1	1	1	1	1	1	2.000
32	5.000	3	2	8	1	1	1	1	1	1	2.000
33	5.000	3	2	8	1	1	1	1	1	1	2.000
34	5.000	3	2	8	1	1	1	1	1	1	2.000
35	5.000	3	2	8	1	1	1	1	1	1	2.000
36	5.000	3	2	8	1	1	1	1	1	1	2.000
37	5.000	3	2	8	1	1	1	2	1	1	2.000
38	5.000	3	2	8	2	1	1	2	1	1	2.000
39	5.000	3	2	8	2	1	1	2	1	1	2.000
40	5.000	3	2	8	2	1	1	2	1	1	2.000
41	5.000	3	2	8	2	1	1	2	1	1	2.000
42	5.000	3	2	8	2	1	1	2	1	2	2.000
43	5.000	3	2	8	2	1	1	2	2	2	2.000
44	5.000	3	2	8	2	2	1	2	2	2	2.000
45	5.000	3	2	8	2	2	2	2	2	2	2.000
46	5.000	3	2	8	2	2	2	2	2	2	2.000
47	5.000	3	2	8	2	2	2	2	2	2	2.000
48	5.000	3	2	8	2	2	2	2	2	2	2.000
49	5.000	3	2	8	2	2	2	2	2	2	2.000
50	5.000	3	2	8	2	2	2	2	2	2	2.000
51	5.000	3	2	8	2	2	2	2	2	2	2.000
52	5.000	3	2	8	2	2	2	2	2	2	2.000
53	5.000	3	2	8	2	2	2	2	2	2	2.000
54	5.000	3	2	8	2	2	2	2	2	2	2.000
55	5.000	3	2	8	2	2	2	2	2	2	2.000
56	5.000	3	2	8	2	2	2	2	2	2	2.000
57	5.000	3	2	8	2	2	2	2	2	2	2.000
58	5.000	3	2	8	2	2	2	2	2	2	2.000
59	5.000	3	2	8	2	2	2	2	2	2	2.000
60	5.000	4	2	9	1	1	1	1	1	1	2.000
61	5.000	4	2	9	1	1	1	1	1	1	2.000
62	5.000	4	2	9	2	1	1	2	1	1	2.000
63	5.000	4	2	9	2	1	1	2	1	1	2.000
64	5.000	4	2	9	2	1	1	2	1	1	2.000
65	5.000	4	2	9	2	1	1	2	1	1	2.000
66	5.000	4	2	9	2	1	1	2	1	1	2.000
67	5.000	4	2	9	2	1	1	2	1	1	2.000
68	5.000	4	2	9	2	1	1	2	1	1	2.000
69	5.000	4	2	9	2	1	1	2	1	2	2.000
70	5.000	4	2	9	2	1	1	2	1	2	2.000
71	5.000	4	2	9	2	2	2	2	2	2	2.000
72	5.000	4	2	9	2	2	2	2	2	2	2.000
73	5.000	4	2	9	2	2	2	2	2	2	2.000
74	5.000	4	2	9	2	2	2	2	2	2	2.000
75	5.000	4	2	9	2	2	2	2	2	2	2.000
76	5.000	4	2	9	2	2	2	2	2	2	2.000
77	5.000	4	2	9	2	2	2	2	2	2	2.000
78	5.000	4	2	9	2	2	2	2	2	2	2.000
79	5.000	4	2	9	2	2	2	2	2	2	2.000
80	5.000	4	2	9	2	2	2	2	2	2	2.000
81	5.000	4	2	9	2	2	2	2	2	2	2.000
82	5.000	4	2	9	2	2	2	2	2	2	2.000
83	5.000	4	2	9	2	2	2	2	2	2	2.000
84	5.000	4	2	9	2	2	2	2	2	2	2.000
85	5.000	4	2	9	2	2	2	2	2	2	2.000
86	5.000	4	2	9	2	2	2	2	2	2	2.000
87	5.000	4	2	9	2	2	2	2	2	2	2.000
88	5.000	4	2	9	2	2	2	2	2	2	2.000

	Round	Contaminant [4]	Treatment [3]	Tank4	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomial n
89	5.000	2	2	10	1	1	1	1	1	1	2.000
90	5.000	2	2	10	1	1	1	1	1	1	2.000
91	5.000	2	2	10	1	1	1	1	1	1	2.000
92	5.000	2	2	10	1	1	1	1	1	1	2.000
93	5.000	2	2	10	1	1	1	1	1	1	2.000
94	5.000	2	2	10	1	1	1	2	1	1	2.000
95	5.000	2	2	10	2	1	1	2	1	1	2.000
96	5.000	2	2	10	2	1	1	2	1	1	2.000
97	5.000	2	2	10	2	1	1	2	1	1	2.000
98	5.000	2	2	10	2	1	1	2	1	1	2.000
99	5.000	2	2	10	2	1	1	2	1	1	2.000
100	5.000	2	2	10	2	1	1	2	2	2	2.000
101	5.000	2	2	10	2	2	2	2	2	2	2.000
102	5.000	2	2	10	2	2	2	2	2	2	2.000
103	5.000	2	2	10	2	2	2	2	2	2	2.000
104	5.000	2	2	10	2	2	2	2	2	2	2.000
105	5.000	2	2	10	2	2	2	2	2	2	2.000
106	5.000	2	2	10	2	2	2	2	2	2	2.000
107	5.000	2	2	10	2	2	2	2	2	2	2.000
108	5.000	2	2	10	2	2	2	2	2	2	2.000
109	5.000	2	2	10	2	2	2	2	2	2	2.000
110	5.000	2	2	10	2	2	2	2	2	2	2.000
111	5.000	2	2	10	2	2	2	2	2	2	2.000
112	5.000	2	2	10	2	2	2	2	2	2	2.000
113	5.000	2	2	10	2	2	2	2	2	2	2.000
114	5.000	2	2	10	2	2	2	2	2	2	2.000
115	5.000	2	2	10	2	2	2	2	2	2	2.000
116	5.000	2	2	10	2	2	2	2	2	2	2.000
117	5.000	2	2	10	2	2	2	2	2	2	2.000
118	5.000	2	2	10	2	2	2	2	2	2	2.000
119	5.000	3	2	11	2	1	1	2	1	1	2.000
120	5.000	3	2	11	2	1	1	2	1	1	2.000
121	5.000	3	2	11	2	1	1	2	1	1	2.000
122	5.000	3	2	11	2	1	1	2	1	1	2.000
123	5.000	3	2	11	2	1	1	2	1	1	2.000
124	5.000	3	2	11	2	1	1	2	1	2	2.000
125	5.000	3	2	11	2	1	1	2	2	2	2.000
126	5.000	3	2	11	2	2	1	2	2	2	2.000
127	5.000	3	2	11	2	2	2	2	2	2	2.000
128	5.000	3	2	11	2	2	2	2	2	2	2.000
129	5.000	3	2	11	2	2	2	2	2	2	2.000
130	5.000	3	2	11	2	2	2	2	2	2	2.000
131	5.000	3	2	11	2	2	2	2	2	2	2.000
132	5.000	3	2	11	2	2	2	2	2	2	2.000
133	5.000	3	2	11	2	2	2	2	2	2	2.000
134	5.000	3	2	11	2	2	2	2	2	2	2.000
135	5.000	3	2	11	2	2	2	2	2	2	2.000
136	5.000	3	2	11	2	2	2	2	2	2	2.000
137	5.000	3	2	11	2	2	2	2	2	2	2.000
138	5.000	3	2	11	2	2	2	2	2	2	2.000
139	5.000	3	2	11	2	2	2	2	2	2	2.000
140	5.000	3	2	11	2	2	2	2	2	2	2.000
141	5.000	3	2	11	2	2	2	2	2	2	2.000
142	5.000	3	2	11	2	2	2	2	2	2	2.000
143	5.000	3	2	11	2	2	2	2	2	2	2.000
144	5.000	3	2	11	2	2	2	2	2	2	2.000
145	5.000	3	2	11	2	2	2	2	2	2	2.000
146	5.000	3	2	11	2	2	2	2	2	2	2.000
147	5.000	3	2	11	2	2	2	2	2	2	2.000
148	5.000	4	2	12	1	1	1	1	1	1	2.000
149	5.000	4	2	12	1	1	1	1	1	1	2.000
150	5.000	4	2	12	2	1	1	2	1	1	2.000
151	5.000	4	2	12	2	1	1	2	1	1	2.000
152	5.000	4	2	12	2	1	1	2	1	1	2.000
153	5.000	4	2	12	2	1	1	2	1	1	2.000
154	5.000	4	2	12	2	1	1	2	1	1	2.000
155	5.000	4	2	12	2	1	1	2	1	1	2.000
156	5.000	4	2	12	2	2	1	2	2	2	2.000
157	5.000	4	2	12	2	2	1	2	2	2	2.000
158	5.000	4	2	12	2	2	2	2	2	2	2.000
159	5.000	4	2	12	2	2	2	2	2	2	2.000
160	5.000	4	2	12	2	2	2	2	2	2	2.000
161	5.000	4	2	12	2	2	2	2	2	2	2.000
162	5.000	4	2	12	2	2	2	2	2	2	2.000
163	5.000	4	2	12	2	2	2	2	2	2	2.000
164	5.000	4	2	12	2	2	2	2	2	2	2.000
165	5.000	4	2	12	2	2	2	2	2	2	2.000
166	5.000	4	2	12	2	2	2	2	2	2	2.000
167	5.000	4	2	12	2	2	2	2	2	2	2.000
168	5.000	4	2	12	2	2	2	2	2	2	2.000
169	5.000	4	2	12	2	2	2	2	2	2	2.000
170	5.000	4	2	12	2	2	2	2	2	2	2.000
171	5.000	4	2	12	2	2	2	2	2	2	2.000
172	5.000	4	2	12	2	2	2	2	2	2	2.000
173	5.000	4	2	12	2	2	2	2	2	2	2.000
174	5.000	4	2	12	2	2	2	2	2	2	2.000
175	5.000	4	2	12	2	2	2	2	2	2	2.000
176	5.000	4	2	12	2	2	2	2	2	2	2.000

	Round	Contaminent [4]	Treatment [2]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomial
177	5.000	4	2	12	2	2	2	2	2	2	2.000
178	5.000	2	2	16	2	1	1	2	1	1	2.000
179	5.000	2	2	16	2	1	1	2	1	1	2.000
180	5.000	2	2	16	2	1	1	2	2	2	2.000
181	5.000	2	2	16	2	2	2	2	2	2	2.000
182	5.000	2	2	16	2	2	2	2	2	2	2.000
183	5.000	2	2	16	2	2	2	2	2	2	2.000
184	5.000	2	2	16	2	2	2	2	2	2	2.000
185	5.000	2	2	16	2	2	2	2	2	2	2.000
186	5.000	2	2	16	2	2	2	2	2	2	2.000
187	5.000	2	2	16	2	2	2	2	2	2	2.000
188	5.000	2	2	16	2	2	2	2	2	2	2.000
189	5.000	2	2	16	2	2	2	2	2	2	2.000
190	5.000	2	2	16	2	2	2	2	2	2	2.000
191	5.000	2	2	16	2	2	2	2	2	2	2.000
192	5.000	2	2	16	2	2	2	2	2	2	2.000
193	5.000	2	2	16	2	2	2	2	2	2	2.000
194	5.000	2	2	16	2	2	2	2	2	2	2.000
195	5.000	2	2	16	2	2	2	2	2	2	2.000
196	5.000	2	2	16	2	2	2	2	2	2	2.000
197	5.000	2	2	16	2	2	2	2	2	2	2.000
198	5.000	3	2	17	1	1	1	1	1	1	2.000
199	5.000	3	2	17	1	1	1	2	1	1	2.000
200	5.000	3	2	17	2	1	1	2	1	1	2.000
201	5.000	3	2	17	2	1	1	2	1	1	2.000
202	5.000	3	2	17	2	1	1	2	1	2	2.000
203	5.000	3	2	17	2	1	1	2	2	2	2.000
204	5.000	3	2	17	2	2	1	2	2	2	2.000
205	5.000	3	2	17	2	2	2	2	2	2	2.000
206	5.000	3	2	17	2	2	2	2	2	2	2.000
207	5.000	3	2	17	2	2	2	2	2	2	2.000
208	5.000	3	2	17	2	2	2	2	2	2	2.000
209	5.000	3	2	17	2	2	2	2	2	2	2.000
210	5.000	3	2	17	2	2	2	2	2	2	2.000
211	5.000	3	2	17	2	2	2	2	2	2	2.000
212	5.000	3	2	17	2	2	2	2	2	2	2.000
213	5.000	3	2	17	2	2	2	2	2	2	2.000
214	5.000	3	2	17	2	2	2	2	2	2	2.000
215	5.000	3	2	17	2	2	2	2	2	2	2.000
216	5.000	3	2	17	2	2	2	2	2	2	2.000
217	5.000	3	2	17	2	2	2	2	2	2	2.000
218	5.000	3	2	17	2	2	2	2	2	2	2.000
219	5.000	3	2	17	2	2	2	2	2	2	2.000
220	5.000	3	2	17	2	2	2	2	2	2	2.000
221	5.000	3	2	17	2	2	2	2	2	2	2.000
222	5.000	3	2	17	2	2	2	2	2	2	2.000
223	5.000	4	2	18	1	1	1	1	1	1	2.000
224	5.000	4	2	18	1	1	1	1	1	1	2.000
225	5.000	4	2	18	2	1	1	2	1	1	2.000
226	5.000	4	2	18	2	1	1	2	1	1	2.000
227	5.000	4	2	18	2	1	1	2	1	1	2.000
228	5.000	4	2	18	2	1	1	2	1	2	2.000
229	5.000	4	2	18	2	2	1	2	2	2	2.000
230	5.000	4	2	18	2	2	2	2	2	2	2.000
231	5.000	4	2	18	2	2	2	2	2	2	2.000
232	5.000	4	2	18	2	2	2	2	2	2	2.000
233	5.000	4	2	18	2	2	2	2	2	2	2.000
234	5.000	4	2	18	2	2	2	2	2	2	2.000
235	5.000	4	2	18	2	2	2	2	2	2	2.000
236	5.000	4	2	18	2	2	2	2	2	2	2.000
237	5.000	4	2	18	2	2	2	2	2	2	2.000
238	5.000	4	2	18	2	2	2	2	2	2	2.000
239	5.000	4	2	18	2	2	2	2	2	2	2.000
240	5.000	4	2	18	2	2	2	2	2	2	2.000
241	5.000	4	2	18	2	2	2	2	2	2	2.000
242	5.000	4	2	18	2	2	2	2	2	2	2.000
243	5.000	4	2	18	2	2	2	2	2	2	2.000
244	5.000	4	2	18	2	2	2	2	2	2	2.000
245	5.000	4	2	18	2	2	2	2	2	2	2.000
246	5.000	4	2	18	2	2	2	2	2	2	2.000
247	5.000	4	2	18	2	2	2	2	2	2	2.000
248	5.000	4	2	18	2	2	2	2	2	2	2.000
249	5.000	4	2	18	2	2	2	2	2	2	2.000
250	5.000	4	2	18	2	2	2	2	2	2	2.000
251	5.000	2	1	25	1	1	1	1	1	1	2.000
252	5.000	2	1	25	1	1	1	2	2	2	2.000
253	5.000	2	1	25	2	2	2	2	2	2	2.000
254	5.000	2	1	25	2	2	2	2	2	2	2.000
255	5.000	2	1	25	2	2	2	2	2	2	2.000
256	5.000	2	1	25	2	2	2	2	2	2	2.000
257	5.000	2	1	25	2	2	2	2	2	2	2.000
258	5.000	2	1	25	2	2	2	2	2	2	2.000
259	5.000	2	1	25	2	2	2	2	2	2	2.000
260	5.000	2	1	25	2	2	2	2	2	2	2.000
261	5.000	2	1	25	2	2	2	2	2	2	2.000
262	5.000	2	1	25	2	2	2	2	2	2	2.000
263	5.000	2	1	25	2	2	2	2	2	2	2.000
264	5.000	2	1	25	2	2	2	2	2	2	2.000

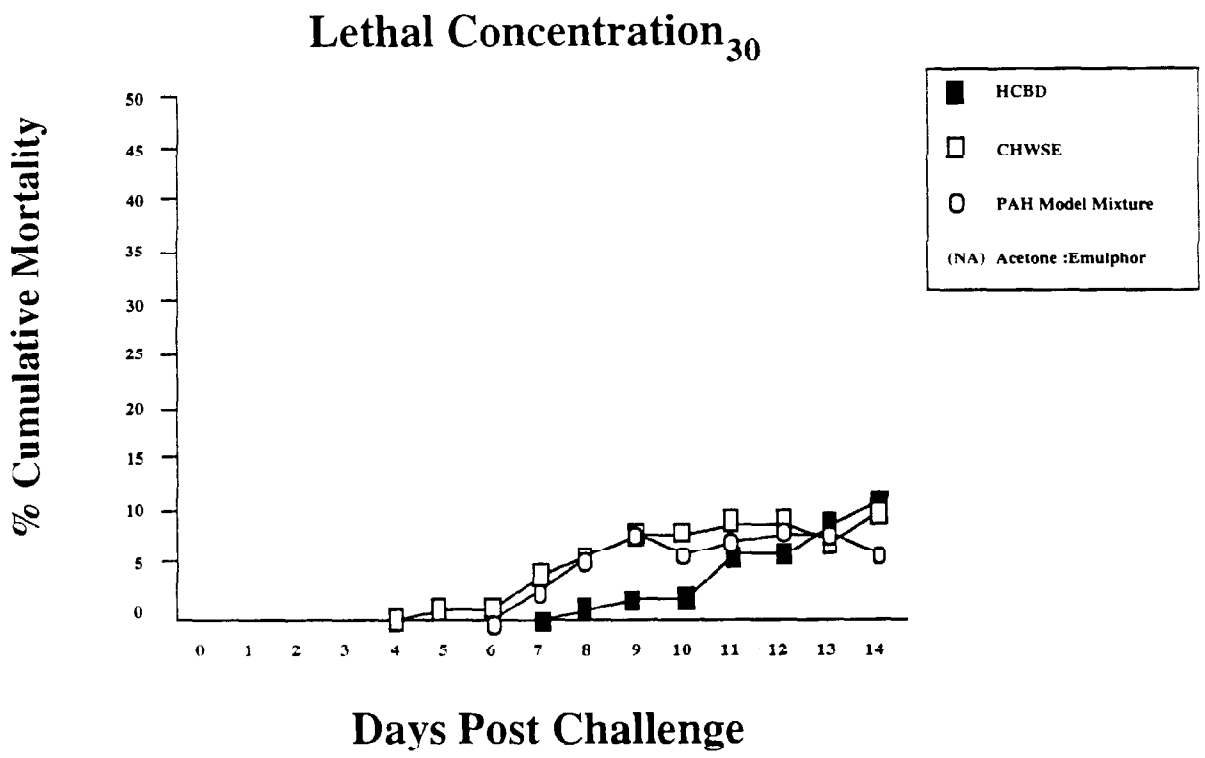
	Round	Contaminant [4]	Treatment [2]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomial
177	5.000	4	2	12	2	2	2	2	2	2	2.000
178	5.000	2	2	16	2	1	1	2	1	1	2.000
179	5.000	2	2	16	2	1	1	2	1	1	2.000
180	5.000	2	2	16	2	1	1	2	2	2	2.000
181	5.000	2	2	16	2	2	2	2	2	2	2.000
182	5.000	2	2	16	2	2	2	2	2	2	2.000
183	5.000	2	2	16	2	2	2	2	2	2	2.000
184	5.000	2	2	16	2	2	2	2	2	2	2.000
185	5.000	2	2	16	2	2	2	2	2	2	2.000
186	5.000	2	2	16	2	2	2	2	2	2	2.000
187	5.000	2	2	16	2	2	2	2	2	2	2.000
188	5.000	2	2	16	2	2	2	2	2	2	2.000
189	5.000	2	2	16	2	2	2	2	2	2	2.000
190	5.000	2	2	16	2	2	2	2	2	2	2.000
191	5.000	2	2	16	2	2	2	2	2	2	2.000
192	5.000	2	2	16	2	2	2	2	2	2	2.000
193	5.000	2	2	16	2	2	2	2	2	2	2.000
194	5.000	2	2	16	2	2	2	2	2	2	2.000
195	5.000	2	2	16	2	2	2	2	2	2	2.000
196	5.000	2	2	16	2	2	2	2	2	2	2.000
197	5.000	2	2	16	2	2	2	2	2	2	2.000
198	5.000	3	2	17	1	1	1	1	1	1	2.000
199	5.000	3	2	17	1	1	1	2	1	1	2.000
200	5.000	3	2	17	2	1	1	2	1	1	2.000
201	5.000	3	2	17	2	1	1	2	1	1	2.000
202	5.000	3	2	17	2	1	1	2	1	2	2.000
203	5.000	3	2	17	2	1	1	2	2	2	2.000
204	5.000	3	2	17	2	2	1	2	2	2	2.000
205	5.000	3	2	17	2	2	2	2	2	2	2.000
206	5.000	3	2	17	2	2	2	2	2	2	2.000
207	5.000	3	2	17	2	2	2	2	2	2	2.000
208	5.000	3	2	17	2	2	2	2	2	2	2.000
209	5.000	3	2	17	2	2	2	2	2	2	2.000
210	5.000	3	2	17	2	2	2	2	2	2	2.000
211	5.000	3	2	17	2	2	2	2	2	2	2.000
212	5.000	3	2	17	2	2	2	2	2	2	2.000
213	5.000	3	2	17	2	2	2	2	2	2	2.000
214	5.000	3	2	17	2	2	2	2	2	2	2.000
215	5.000	3	2	17	2	2	2	2	2	2	2.000
216	5.000	3	2	17	2	2	2	2	2	2	2.000
217	5.000	3	2	17	2	2	2	2	2	2	2.000
218	5.000	3	2	17	2	2	2	2	2	2	2.000
219	5.000	3	2	17	2	2	2	2	2	2	2.000
220	5.000	3	2	17	2	2	2	2	2	2	2.000
221	5.000	3	2	17	2	2	2	2	2	2	2.000
222	5.000	3	2	17	2	2	2	2	2	2	2.000
223	5.000	4	2	18	1	1	1	1	1	1	2.000
224	5.000	4	2	18	1	1	1	1	1	1	2.000
225	5.000	4	2	18	2	1	1	2	1	1	2.000
226	5.000	4	2	18	2	1	1	2	1	1	2.000
227	5.000	4	2	18	2	1	1	2	1	1	2.000
228	5.000	4	2	18	2	1	1	2	1	2	2.000
229	5.000	4	2	18	2	2	1	2	2	2	2.000
230	5.000	4	2	18	2	2	2	2	2	2	2.000
231	5.000	4	2	18	2	2	2	2	2	2	2.000
232	5.000	4	2	18	2	2	2	2	2	2	2.000
233	5.000	4	2	18	2	2	2	2	2	2	2.000
234	5.000	4	2	18	2	2	2	2	2	2	2.000
235	5.000	4	2	18	2	2	2	2	2	2	2.000
236	5.000	4	2	18	2	2	2	2	2	2	2.000
237	5.000	4	2	18	2	2	2	2	2	2	2.000
238	5.000	4	2	18	2	2	2	2	2	2	2.000
239	5.000	4	2	18	2	2	2	2	2	2	2.000
240	5.000	4	2	18	2	2	2	2	2	2	2.000
241	5.000	4	2	18	2	2	2	2	2	2	2.000
242	5.000	4	2	18	2	2	2	2	2	2	2.000
243	5.000	4	2	18	2	2	2	2	2	2	2.000
244	5.000	4	2	18	2	2	2	2	2	2	2.000
245	5.000	4	2	18	2	2	2	2	2	2	2.000
246	5.000	4	2	18	2	2	2	2	2	2	2.000
247	5.000	4	2	18	2	2	2	2	2	2	2.000
248	5.000	4	2	18	2	2	2	2	2	2	2.000
249	5.000	4	2	18	2	2	2	2	2	2	2.000
250	5.000	4	2	18	2	2	2	2	2	2	2.000
251	5.000	2	1	25	1	1	1	1	1	1	2.000
252	5.000	2	1	25	1	1	1	2	2	2	2.000
253	5.000	2	1	25	2	2	2	2	2	2	2.000
254	5.000	2	1	25	2	2	2	2	2	2	2.000
255	5.000	2	1	25	2	2	2	2	2	2	2.000
256	5.000	2	1	25	2	2	2	2	2	2	2.000
257	5.000	2	1	25	2	2	2	2	2	2	2.000
258	5.000	2	1	25	2	2	2	2	2	2	2.000
259	5.000	2	1	25	2	2	2	2	2	2	2.000
260	5.000	2	1	25	2	2	2	2	2	2	2.000
261	5.000	2	1	25	2	2	2	2	2	2	2.000
262	5.000	2	1	25	2	2	2	2	2	2	2.000
263	5.000	2	1	25	2	2	2	2	2	2	2.000
264	5.000	2	1	25	2	2	2	2	2	2	2.000

	Round	Contaminant [4]	Treatment [2]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomialn
353	5.000	3	1	29	2	2	1	2	2	2	2.000
354	5.000	3	1	29	2	2	2	2	2	2	2.000
355	5.000	3	1	29	2	2	2	2	2	2	2.000
356	5.000	3	1	29	2	2	2	2	2	2	2.000
357	5.000	3	1	29	2	2	2	2	2	2	2.000
358	5.000	3	1	29	2	2	2	2	2	2	2.000
359	5.000	3	1	29	2	2	2	2	2	2	2.000
360	5.000	3	1	29	2	2	2	2	2	2	2.000
361	5.000	3	1	29	2	2	2	2	2	2	2.000
362	5.000	3	1	29	2	2	2	2	2	2	2.000
363	5.000	3	1	29	2	2	2	2	2	2	2.000
364	5.000	3	1	29	2	2	2	2	2	2	2.000
365	5.000	3	1	29	2	2	2	2	2	2	2.000
366	5.000	3	1	29	2	2	2	2	2	2	2.000
367	5.000	3	1	29	2	2	2	2	2	2	2.000
368	5.000	3	1	29	2	2	2	2	2	2	2.000
369	5.000	3	1	29	2	2	2	2	2	2	2.000
370	5.000	3	1	29	2	2	2	2	2	2	2.000
371	5.000	3	1	29	2	2	2	2	2	2	2.000
372	5.000	3	1	29	2	2	2	2	2	2	2.000
373	5.000	3	1	29	2	2	2	2	2	2	2.000
374	5.000	3	1	29	2	2	2	2	2	2	2.000
375	5.000	3	1	29	2	2	2	2	2	2	2.000
376	5.000	3	1	29	2	2	2	2	2	2	2.000
377	5.000	3	1	29	2	2	2	2	2	2	2.000
378	5.000	3	1	29	2	2	2	2	2	2	2.000
379	5.000	3	1	29	2	2	2	2	2	2	2.000
380	5.000	3	1	29	2	2	2	2	2	2	2.000
381	5.000	3	1	29	2	2	2	2	2	2	2.000
382	5.000	4	1	30	2	2	1	2	2	2	2.000
383	5.000	4	1	30	2	2	2	2	2	2	2.000
384	5.000	4	1	30	2	2	2	2	2	2	2.000
385	5.000	4	1	30	2	2	2	2	2	2	2.000
386	5.000	4	1	30	2	2	2	2	2	2	2.000
387	5.000	4	1	30	2	2	2	2	2	2	2.000
388	5.000	4	1	30	2	2	2	2	2	2	2.000
389	5.000	4	1	30	2	2	2	2	2	2	2.000
390	5.000	4	1	30	2	2	2	2	2	2	2.000
391	5.000	4	1	30	2	2	2	2	2	2	2.000
392	5.000	4	1	30	2	2	2	2	2	2	2.000
393	5.000	4	1	30	2	2	2	2	2	2	2.000
394	5.000	4	1	30	2	2	2	2	2	2	2.000
395	5.000	4	1	30	2	2	2	2	2	2	2.000
396	5.000	4	1	30	2	2	2	2	2	2	2.000
397	5.000	4	1	30	2	2	2	2	2	2	2.000
398	5.000	4	1	30	2	2	2	2	2	2	2.000
399	5.000	4	1	30	2	2	2	2	2	2	2.000
400	5.000	4	1	30	2	2	2	2	2	2	2.000
401	5.000	4	1	30	2	2	2	2	2	2	2.000
402	5.000	4	1	30	2	2	2	2	2	2	2.000
403	5.000	4	1	30	2	2	2	2	2	2	2.000
404	5.000	4	1	30	2	2	2	2	2	2	2.000
405	5.000	4	1	30	2	2	2	2	2	2	2.000
406	5.000	4	1	30	2	2	2	2	2	2	2.000
407	5.000	4	1	30	2	2	2	2	2	2	2.000
408	5.000	4	1	30	2	2	2	2	2	2	2.000
409	5.000	4	1	30	2	2	2	2	2	2	2.000
410	5.000	4	1	30	2	2	2	2	2	2	2.000
411	5.000	2	1	37	2	2	1	2	2	2	2.000
412	5.000	2	1	37	2	2	2	2	2	2	2.000
413	5.000	2	1	37	2	2	2	2	2	2	2.000
414	5.000	2	1	37	2	2	2	2	2	2	2.000
415	5.000	2	1	37	2	2	2	2	2	2	2.000
416	5.000	2	1	37	2	2	2	2	2	2	2.000
417	5.000	2	1	37	2	2	2	2	2	2	2.000
418	5.000	2	1	37	2	2	2	2	2	2	2.000
419	5.000	2	1	37	2	2	2	2	2	2	2.000
420	5.000	2	1	37	2	2	2	2	2	2	2.000
421	5.000	2	1	37	2	2	2	2	2	2	2.000
422	5.000	2	1	37	2	2	2	2	2	2	2.000
423	5.000	2	1	37	2	2	2	2	2	2	2.000
424	5.000	2	1	37	2	2	2	2	2	2	2.000
425	5.000	2	1	37	2	2	2	2	2	2	2.000
426	5.000	2	1	37	2	2	2	2	2	2	2.000
427	5.000	2	1	37	2	2	2	2	2	2	2.000
428	5.000	2	1	37	2	2	2	2	2	2	2.000
429	5.000	2	1	37	2	2	2	2	2	2	2.000
430	5.000	2	1	37	2	2	2	2	2	2	2.000
431	5.000	3	1	38	2	2	2	2	2	2	2.000
432	5.000	3	1	38	2	2	2	2	2	2	2.000
433	5.000	3	1	38	2	2	2	2	2	2	2.000
434	5.000	3	1	38	2	2	2	2	2	2	2.000
435	5.000	3	1	38	2	2	2	2	2	2	2.000
436	5.000	3	1	38	2	2	2	2	2	2	2.000
437	5.000	3	1	38	2	2	2	2	2	2	2.000
438	5.000	3	1	38	2	2	2	2	2	2	2.000
439	5.000	3	1	38	2	2	2	2	2	2	2.000
440	5.000	3	1	38	2	2	2	2	2	2	2.000

	Round	Contaminant [4]	Treatment [2]	Tank4	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomial n
441	5.000	3	1	38	2	2	2	2	2	2	2.000
442	5.000	3	1	38	2	2	2	2	2	2	2.000
443	5.000	3	1	38	2	2	2	2	2	2	2.000
444	5.000	3	1	38	2	2	2	2	2	2	2.000
445	5.000	3	1	38	2	2	2	2	2	2	2.000
446	5.000	3	1	38	2	2	2	2	2	2	2.000
447	5.000	3	1	38	2	2	2	2	2	2	2.000
448	5.000	3	1	38	2	2	2	2	2	2	2.000
449	5.000	3	1	38	2	2	2	2	2	2	2.000
450	5.000	3	1	38	2	2	2	2	2	2	2.000
451	5.000	3	1	38	2	2	2	2	2	2	2.000
452	5.000	3	1	38	2	2	2	2	2	2	2.000
453	5.000	3	1	38	2	2	2	2	2	2	2.000
454	5.000	3	1	38	2	2	2	2	2	2	2.000
455	5.000	3	1	38	2	2	2	2	2	2	2.000
456	5.000	3	1	38	2	2	2	2	2	2	2.000
457	5.000	3	1	38	2	2	2	2	2	2	2.000
458	5.000	3	1	38	2	2	2	2	2	2	2.000
459	5.000	3	1	38	2	2	2	2	2	2	2.000
460	5.000	4	1	39	2	2	2	2	2	2	2.000
461	5.000	4	1	39	2	2	2	2	2	2	2.000
462	5.000	4	1	39	2	2	2	2	2	2	2.000
463	5.000	4	1	39	2	2	2	2	2	2	2.000
464	5.000	4	1	39	2	2	2	2	2	2	2.000
465	5.000	4	1	39	2	2	2	2	2	2	2.000
466	5.000	4	1	39	2	2	2	2	2	2	2.000
467	5.000	4	1	39	2	2	2	2	2	2	2.000
468	5.000	4	1	39	2	2	2	2	2	2	2.000
469	5.000	4	1	39	2	2	2	2	2	2	2.000
470	5.000	4	1	39	2	2	2	2	2	2	2.000
471	5.000	4	1	39	2	2	2	2	2	2	2.000
472	5.000	4	1	39	2	2	2	2	2	2	2.000
473	5.000	4	1	39	2	2	2	2	2	2	2.000
474	5.000	4	1	39	2	2	2	2	2	2	2.000
475	5.000	4	1	39	2	2	2	2	2	2	2.000
476	5.000	4	1	39	2	2	2	2	2	2	2.000
477	5.000	4	1	39	2	2	2	2	2	2	2.000
478	5.000	4	1	39	2	2	2	2	2	2	2.000
479	5.000	4	1	39	2	2	2	2	2	2	2.000
480	5.000	4	1	39	2	2	2	2	2	2	2.000
481	5.000	4	1	39	2	2	2	2	2	2	2.000
482	5.000	4	1	39	2	2	2	2	2	2	2.000
483	5.000	4	1	39	2	2	2	2	2	2	2.000
484	5.000	4	1	39	2	2	2	2	2	2	2.000
485	5.000	4	1	39	2	2	2	2	2	2	2.000
486	5.000	4	1	39	2	2	2	2	2	2	2.000
487	5.000	4	1	39	2	2	2	2	2	2	2.000
488	5.000	4	1	39	2	2	2	2	2	2	2.000
489	5.000	1	2	46	2	1	1	2	1	1	2.000
490	5.000	1	2	46	2	1	1	2	1	1	2.000
491	5.000	1	2	46	2	1	1	2	2	2	2.000
492	5.000	1	2	46	2	2	1	2	2	2	2.000
493	5.000	1	2	46	2	2	1	2	2	2	2.000
494	5.000	1	2	46	2	2	2	2	2	2	2.000
495	5.000	1	2	46	2	2	2	2	2	2	2.000
496	5.000	1	2	46	2	2	2	2	2	2	2.000
497	5.000	1	2	46	2	2	2	2	2	2	2.000
498	5.000	1	2	46	2	2	2	2	2	2	2.000
499	5.000	1	2	46	2	2	2	2	2	2	2.000
500	5.000	1	2	46	2	2	2	2	2	2	2.000
501	5.000	1	2	46	2	2	2	2	2	2	2.000
502	5.000	1	2	46	2	2	2	2	2	2	2.000
503	5.000	1	2	46	2	2	2	2	2	2	2.000
504	5.000	1	2	46	2	2	2	2	2	2	2.000
505	5.000	1	2	46	2	2	2	2	2	2	2.000
506	5.000	1	2	46	2	2	2	2	2	2	2.000
507	5.000	1	2	46	2	2	2	2	2	2	2.000
508	5.000	1	2	46	2	2	2	2	2	2	2.000
509	5.000	1	2	46	2	2	2	2	2	2	2.000
510	5.000	1	2	46	2	2	2	2	2	2	2.000
511	5.000	1	2	46	2	2	2	2	2	2	2.000
512	5.000	1	2	46	2	2	2	2	2	2	2.000
513	5.000	1	2	46	2	2	2	2	2	2	2.000
514	5.000	1	2	46	2	2	2	2	2	2	2.000
515	5.000	1	2	46	2	2	2	2	2	2	2.000
516	5.000	1	2	46	2	2	2	2	2	2	2.000
517	5.000	1	2	46	2	2	2	2	2	2	2.000
518	5.000	1	2	47	2	1	1	2	1	1	2.000
519	5.000	1	2	47	2	1	1	2	1	1	2.000
520	5.000	1	2	47	2	1	1	2	1	1	2.000
521	5.000	1	2	47	2	1	1	2	1	1	2.000
522	5.000	1	2	47	2	1	1	2	1	1	2.000
523	5.000	1	2	47	2	1	1	2	1	1	2.000
524	5.000	1	2	47	2	1	1	2	1	1	2.000
525	5.000	1	2	47	2	1	1	2	2	1	2.000
526	5.000	1	2	47	2	2	1	2	2	2	2.000
527	5.000	1	2	47	2	2	1	2	2	2	2.000
528	5.000	1	2	47	2	2	1	2	2	2	2.000

	Round	Contaminant [4]	Treatment [2]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomialn
529	5.000	1	2	47	2	2	2	2	2	2	2.000
530	5.000	1	2	47	2	2	2	2	2	2	2.000
531	5.000	1	2	47	2	2	2	2	2	2	2.000
532	5.000	1	2	47	2	2	2	2	2	2	2.000
533	5.000	1	2	47	2	2	2	2	2	2	2.000
534	5.000	1	2	47	2	2	2	2	2	2	2.000
535	5.000	1	2	47	2	2	2	2	2	2	2.000
536	5.000	1	2	47	2	2	2	2	2	2	2.000
537	5.000	1	2	47	2	2	2	2	2	2	2.000
538	5.000	1	2	47	2	2	2	2	2	2	2.000
539	5.000	1	2	47	2	2	2	2	2	2	2.000
540	5.000	1	2	47	2	2	2	2	2	2	2.000
541	5.000	1	2	47	2	2	2	2	2	2	2.000
542	5.000	1	2	47	2	2	2	2	2	2	2.000
543	5.000	1	2	47	2	2	2	2	2	2	2.000
544	5.000	1	2	47	2	2	2	2	2	2	2.000
545	5.000	1	2	47	2	2	2	2	2	2	2.000
546	5.000	1	2	47	2	2	2	2	2	2	2.000
547	5.000	1	2	48	1	1	1	2	1	1	2.000
548	5.000	1	2	48	2	1	1	2	1	1	2.000
549	5.000	1	2	48	2	1	1	2	1	1	2.000
550	5.000	1	2	48	2	1	1	2	2	1	2.000
551	5.000	1	2	48	2	2	1	2	2	2	2.000
552	5.000	1	2	48	2	2	1	2	2	2	2.000
553	5.000	1	2	48	2	2	1	2	2	2	2.000
554	5.000	1	2	48	2	2	2	2	2	2	2.000
555	5.000	1	2	48	2	2	2	2	2	2	2.000
556	5.000	1	2	48	2	2	2	2	2	2	2.000
557	5.000	1	2	48	2	2	2	2	2	2	2.000
558	5.000	1	2	48	2	2	2	2	2	2	2.000
559	5.000	1	2	48	2	2	2	2	2	2	2.000
560	5.000	1	2	48	2	2	2	2	2	2	2.000
561	5.000	1	2	48	2	2	2	2	2	2	2.000
562	5.000	1	2	48	2	2	2	2	2	2	2.000
563	5.000	1	2	48	2	2	2	2	2	2	2.000
564	5.000	1	2	48	2	2	2	2	2	2	2.000
565	5.000	1	2	48	2	2	2	2	2	2	2.000
566	5.000	1	2	48	2	2	2	2	2	2	2.000
567	5.000	1	2	48	2	2	2	2	2	2	2.000
568	5.000	1	2	48	2	2	2	2	2	2	2.000
569	5.000	1	2	48	2	2	2	2	2	2	2.000
570	5.000	1	2	48	2	2	2	2	2	2	2.000
571	5.000	1	2	48	2	2	2	2	2	2	2.000
572	5.000	1	2	48	2	2	2	2	2	2	2.000
573	5.000	1	2	48	2	2	2	2	2	2	2.000
574	5.000	1	2	48	2	2	2	2	2	2	2.000
575	5.000	1	2	48	2	2	2	2	2	2	2.000
576	5.000	1	2	48	2	2	2	2	2	2	2.000
577	5.000	1	1	49	1	1	1	2	2	1	2.000
578	5.000	1	1	49	2	2	1	2	2	2	2.000
579	5.000	1	1	49	2	2	1	2	2	2	2.000
580	5.000	1	1	49	2	2	1	2	2	2	2.000
581	5.000	1	1	49	2	2	2	2	2	2	2.000
582	5.000	1	1	49	2	2	2	2	2	2	2.000
583	5.000	1	1	49	2	2	2	2	2	2	2.000
584	5.000	1	1	49	2	2	2	2	2	2	2.000
585	5.000	1	1	49	2	2	2	2	2	2	2.000
586	5.000	1	1	49	2	2	2	2	2	2	2.000
587	5.000	1	1	50	2	2	1	2	2	2	2.000
588	5.000	1	1	50	2	2	1	2	2	2	2.000
589	5.000	1	1	50	2	2	1	2	2	2	2.000
590	5.000	1	1	50	2	2	2	2	2	2	2.000
591	5.000	1	1	50	2	2	2	2	2	2	2.000
592	5.000	1	1	50	2	2	2	2	2	2	2.000
593	5.000	1	1	50	2	2	2	2	2	2	2.000
594	5.000	1	1	50	2	2	2	2	2	2	2.000
595	5.000	1	1	50	2	2	2	2	2	2	2.000
596	5.000	1	1	50	2	2	2	2	2	2	2.000
597	5.000	1	1	50	2	2	2	2	2	2	2.000
598	5.000	1	1	50	2	2	2	2	2	2	2.000
599	5.000	1	1	50	2	2	2	2	2	2	2.000
600	5.000	1	1	50	2	2	2	2	2	2	2.000
601	5.000	1	1	50	2	2	2	2	2	2	2.000
602	5.000	1	1	50	2	2	2	2	2	2	2.000
603	5.000	1	1	50	2	2	2	2	2	2	2.000
604	5.000	1	1	50	2	2	2	2	2	2	2.000
605	5.000	1	1	50	2	2	2	2	2	2	2.000
606	5.000	1	1	50	2	2	2	2	2	2	2.000
607	5.000	1	1	50	2	2	2	2	2	2	2.000
608	5.000	1	1	50	2	2	2	2	2	2	2.000
609	5.000	1	1	50	2	2	2	2	2	2	2.000
610	5.000	1	1	50	2	2	2	2	2	2	2.000
611	5.000	1	1	50	2	2	2	2	2	2	2.000
612	5.000	1	1	50	2	2	2	2	2	2	2.000
613	5.000	1	1	50	2	2	2	2	2	2	2.000
614	5.000	1	1	50	2	2	2	2	2	2	2.000
615	5.000	1	1	50	2	2	2	2	2	2	2.000
616	5.000	1	1	50	2	2	2	2	2	2	2.000

	Round	Contaminant [4]	Treatment [9]	Tank#	D/A(4day)	D/A(7day)	D/A(14day)	D/A(4)-Bkd	D/A(7)-Bkd	D/A(14)-Bkd	binomialn
617	5.000	1	1	51	2	2	1	2	2	2	2.000
618	5.000	1	1	51	2	2	2	2	2	2	2.000
619	5.000	1	1	51	2	2	2	2	2	2	2.000
620	5.000	1	1	51	2	2	2	2	2	2	2.000
621	5.000	1	1	51	2	2	2	2	2	2	2.000
622	5.000	1	1	51	2	2	2	2	2	2	2.000
623	5.000	1	1	51	2	2	2	2	2	2	2.000
624	5.000	1	1	51	2	2	2	2	2	2	2.000
625	5.000	1	1	51	2	2	2	2	2	2	2.000
626	5.000	1	1	51	2	2	2	2	2	2	2.000
627	5.000	1	1	51	2	2	2	2	2	2	2.000
628	5.000	1	1	51	2	2	2	2	2	2	2.000
629	5.000	1	1	51	2	2	2	2	2	2	2.000
630	5.000	1	1	51	2	2	2	2	2	2	2.000
631	5.000	1	1	51	2	2	2	2	2	2	2.000
632	5.000	1	1	51	2	2	2	2	2	2	2.000
633	5.000	1	1	51	2	2	2	2	2	2	2.000
634	5.000	1	1	51	2	2	2	2	2	2	2.000
635	5.000	1	1	51	2	2	2	2	2	2	2.000
636	5.000	1	1	51	2	2	2	2	2	2	2.000
637	5.000	1	1	51	2	2	2	2	2	2	2.000
638	5.000	1	1	51	2	2	2	2	2	2	2.000
639	5.000	1	1	51	2	2	2	2	2	2	2.000
640	5.000	1	1	51	2	2	2	2	2	2	2.000
641	5.000	1	1	51	2	2	2	2	2	2	2.000
642	5.000	1	1	51	2	2	2	2	2	2	2.000
643	5.000	1	1	51	2	2	2	2	2	2	2.000
644	5.000	1	1	51	2	2	2	2	2	2	2.000
645	5.000	1	1	51	2	2	2	2	2	2	2.000
646	5.000	1	1	51	2	2	2	2	2	2	2.000



Effects of chemical contaminants from the Hylebos Waterway on disease resistance of juvenile chinook salmon. Percent cumulative mortality of juvenile chinook salmon injected with either HCBD, a chlorinated-enriched sediment extract from the Hylebos Waterway (CHWSE), a model mixture of PAHs, or the carrier control (acetone:emulphor) during a 14 day exposure to 1×10^{-6} ml bacterial solution/ml seawater (LC_{30}). The net cumulative mortalities due to the bacteria are corrected by subtracting mortalities observed in juvenile chinook salmon injected with either HCBD, CHWSE, the model mixture of PAHs, or the carrier control (acetone:emulphor) but not exposed to *Vibrio anguillarum*. NA signifies data not available. * indicates significantly ($p \leq 0.05$) different from acetone:emulphor control group.