TOXICITY TESTING FOR GUIDELINE DEVELOPMENT OF SELECTED PULP MILL CHEMICALS THAT ARE PRIORITY SUBSTANCES IN THE FRASER RIVER.

DOE FRAP 1998-12

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June 1998

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ABSTRACT

Through the Fraser River Action Plan (FRAP), six pulp mill effluent (PME) chemicals were selected for toxicity evaluations. These were the chlorophenolics 4,5-dichloroguaiacol (4,5-DCG), 3,4,5-trichloroguaiacol (3,4,5-TCG), 4,5-dichlorocatechol (4,5-DCC) and 6-chlorovanillin (6-CV); the resin acid, abietic acid; and the PAH, retene. Tests using *Oncorhynchus mykiss*, *Pimephales promelas*, *Chironomus tentans* and *Ceriodaphnia dubia* with these chemicals found that 3,4,5-TCG, 4,5-DCC, 6-CV and abietic acid exhibited both chronic and acute toxic effects, while retene toxicity was generally chronic. Although 4,5-DCG was only tested for acute toxicity, behavioural effects in fish were observed in the non-lethal concentrations. Acute toxicity (expressed as LC_{50}) ranged from 0.33 to 13.9 mg/L, while chronic threshold effects concentrations (TEC) ranged from 0.051 to 6.9 mg/L.

Ambient levels of individual PME chemicals in the Fraser Basin were orders of magnitude below these levels, suggesting that these chemicals are not a concern. However, these short-term, acute, and single-chemical tests should be used with caution when assessing effects of PME on aquatic life because these tests do not address possible additive or synergistic effects of the many compounds found in PME.

Difficulties with chemical solubilities led to increased uncertainty concerning exposure concentrations. Further testing and data analysis is required to address the data gaps and uncertainty for all the chemicals. In an effort to make the most prudent and scientifically defensible use of existing data, the current toxicity data was used to develop effects-based reference values for the Fraser Basin based on the CCME WQG derivation procedure.

RÉSUMÉ

Dans le cadre du Plan d'action du Fraser (PAF), on a sélectionné six produits chimiques des effluents d'usines de pâtes pour en évaluer la toxicité. Ces substances étaient les produits chlorophénoliques 4,5-dichloroguaiacol (4,5-DCG), 3,4,5-trichloroguaiacol (3,4,5-TCG), 4,5-dichlorocatechol (4,5-DCC) et 6-chlorovanilline (6-CV); l'acide abiétique, un acide résinique; et le rétène, un HAP. Les tests utilisant *Oncorhynchus mykiss, Pimephales promelas, Chironomus tentans* et *Ceriodaphnia dubia* ont montré que le 3,4,5-TCG, le 4,5-DCC, la 6-CV et l'acide abiétique avaient des effets toxiques aigus et chroniques, tandis que la toxicité du rétène était généralement chronique. Bien qu'on ait seulement testé la toxicité aiguë du 4,5-DCG, des effets comportementaux chez les poissons ont été observés aux concentrations non létales. Les concentrations de toxicité aiguë (CL_{50}) variaient de 0,33 à 13,9 mg/L, tandis que les concentrations seuils d'effets chroniques variaient de 0,051 à 6,9 mg/L.

Les concentrations dans le milieu des produits chimiques des effluents d'usines de pâtes dans le bassin du Fraser étaient inférieures à ces niveaux de plusieurs ordres de grandeur, ce qui laisse penser que ces substances ne sont pas préoccupantes. Cependant, ces tests à court terme évaluant un seul produit à la fois et se limitant à la toxicité aiguë doivent être utilisés avec prudence quand on évalue les effets des produits chimiques des effluents des usines de pâtes sur la vie aquatique parce que ces tests ne mesurent pas les effets additifs et synergiques possibles des nombreux composés présents dans ces effluents.

Les difficultés liées aux solubilités des produits ont accru l'incertitude concernant les concentrations d'exposition. On doit faire de nouveaux tests et analyser plus avant les données pour corriger les lacunes et les incertitudes dans les données relatives à tous les produits chimiques. En s'efforçant de faire l'utilisation la plus prudente et la plus scientifiquement défendable des données existantes, on a utilisé les données actuelles de toxicité pour établir des valeurs de référence fondées sur les effets pour le bassin du Fraser, en se fondant sur la procédure d'établissement des recommandations pour la qualité des eaux du CCME.

ACKNOWLEDGEMENTS

This report was prepared through an agreement with Consulting and Audit Canada. Funding for this study was provided by Environment Canada through the Fraser River Action Plan (FRAP). The support and advice of Colin Gray during this study was invaluable and very much appreciated. This report is the result of collaboration between FRAP and other Environment Canada divisions, as well as consultants. In particular, water quality guideline development was done by Robert Kent, Pierre-Yves Caux and Lars Juergensen of Environment Canada's Guidelines Division in Hull, Quebec. Toxicity bioassays for 4,5-dichloroguaiacol were conducted by Graham van Aggelen of the Toxicology Section from Environment Canada's Pacific Environmental Science Centre (PESC). All other toxicity tests were conducted by Lesley Novak of B.A.R. Environmental Inc. Chemical analyses for B.A.R. toxicity tests were conducted by Klaus Kaiser and Virginia Palabrica of Environment Canada's National Water Research Institute (NWRI). Mark Servos, also of NWRI, was the Scientific Authority for the B.A.R. bioassays. The chemicals were selected based on a priority substance list developed by Roxanne Brewer of Aquatic and Atmospheric Sciences Division, Pacific and Yukon Region, Environment Canada in Vancouver, BC. Editorial comments were provided by David Dougherty of Consulting and Audit Canada.

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1.0 INTRODUCTION

The release of chemical contaminants from pulp mills into the aquatic environment is a concern globally. The complexity of the effluent makes it difficult to identify the source of toxicity in the mixture. In the past, the most serious toxic effects were due to the presence of dioxins and furans. The bleaching process has since been changed to eliminate the release of dioxins. However, pulp mill effluent is still toxic to aquatic organisms, and the source and nature of this toxicity are under investigation.

There are currently six pulp mills operating in the Fraser Basin, five of which are located on the mainstem of the Fraser River. Of the six mills, only one (Quesnel River Pulp Company) does not use any form of chlorine in the bleaching process. The rest all employ chlorine dioxide substitution in the bleaching process to produce bleached softwood kraft pulp. Prior to 1991, molecular chlorine was used in the bleaching process (Sekela et al., 1995). Chlorine bleaching leads to the release of chlorinated contaminants into the receiving environment.

The process of converting wood to pulp results in the release of a large number of by-product chemicals in liquid effluent discharged into receiving waters. The by-products are a combination of naturally occurring wood extracts and reaction products that are frequently chlorinated. There is a great deal of information available about the fate and effects of pulp mill effluent, but information regarding the toxicity of individual chemicals found in the effluent is limited. This applies particularly to certain groups of chlorophenolics, resin and fatty acids, and selected polycyclic aromatic hydrocarbons (PAHs) (LaFleur, 1996; Solomon et al., 1993).

The chlorinated guaiacols and catechols are chlorophenolics that are formed from lignin residue during the chlorine bleaching of wood pulps (LaFleur, 1996; Rosemarin et al., 1990). Chlorovanillins are very stable compounds in pulp mill effluent treatment systems and they are often formed in the treatment ponds from other phenolics (LaFleur, 1996). Resin acids are a group of diterpene acids extracted from wood fibre cellulose during processing. They are natural wood extracts that are considered waste by-products (Taylor et al., 1988 in Li et al., 1996). Resin acids, in turn are transformed to either oxidized forms or aromatic structures such as PAHs

(LaFleur, 1996). Retene is a PAH that is formed from abietic acid (a resin acid) and is frequently found in sediment downstream of pulp mills (Travendale et al., 1995 in Billiard and Hodson, 1997).

This project was initiated to fill specific data needs that have been identified over the course of Environment Canada's Fraser River Action Plan (FRAP). The purpose of this project was to provide scientific effects-based reference values (water quality guidelines or WQGs) to aid in the interpretation of assessment data collected under FRAP. Canadian WQGs were selected as the reference values of choice in this case because of their recognised scientific defensibility, established use and national acceptance. Chemicals produced by the pulp and paper industry were the focus for this study, as this industry is a major activity in the basin.

2.0 SELECTION OF PULP MILL EFFLUENT CHEMICALS

The effluent released by the six mills in the Fraser Basin contains many chemicals whose toxicity is not well understood. The FRAP Environmental Quality Workgroup identified a set of 11 chemicals of concern (COCs) from pulp mill effluent (PME) present in the Fraser Basin (Table 1). The COCs were detected in Fraser River samples taken throughout the basin at sites downstream of pulp mills. The range of levels measured in Fraser basin sites are given in Table 2. All chemicals were identified through analysis in suspended and bed sediments. Contaminants were deemed to be 'of concern' when no criteria or guidelines were available and either one of the following conditions were met:

I. levels at downstream locations were 100x greater than at reference sites, or

II. levels at downstream locations were 10x greater than at reference sites; and either one of the two following conditions are met:

   A) the substance was concluded to be toxic under CEPA, or

   B) the substance is currently on CEPA's Priority Substance List#2 (PSL2)

Suspended sediment data were used for selection of COCs since bed sediment data were preliminary and incomplete at the time. The bed sediment values have since become available, along with whole water (total of clarified and suspended sediment values), and are included in

Table 2 for comparison. Contaminant levels in the suspended sediment and water are discussed fully in Sekela et al. (1995).

Of these COCs, six were chosen for national WQG development because they required the least additional data to meet the national guideline development criteria. These were 4,5-dichloroguaiacol (4,5-DCG), 3,4,5-trichloroguaiacol (3,4,5-TCG), 4,5-dichlorocatechol (4,5-DCC), 6-chlorovanillin (6-CV), abietic acid and retene. Their chemical structures are shown in Figure 1.

In an effort to establish WQGs for the protection of freshwater life in the ambient receiving environment, toxicological data requirements had to be met. A database existed for most of the selected COCs, however gaps were identified that required additional studies prior to guideline development. A minimum set of toxicity data was required for the development of Canadian WQGs (CCME, 1991).

3.0 FRESHWATER TOXICITY STUDIES

Toxicity tests were conducted based on the data requirements for developing WQGs (Table 3). Bioassays and associated chemical analyses were conducted by B.A.R. Environmental Ltd., Environment Canada's National Water Research Institute (NWRI), and Environment Canada's Pacific Environmental Science Centre (PESC).

3.1 Methodology

3.1.1 Test Organisms

Test organisms were selected based on the recommendations of Guidelines Division to meet the data requirements for guidelines development (Table 3). Juvenile *Oncorhynchus mykiss* (rainbow trout) was selected as a native North American (and Fraser Basin) cold-water fish species. This species is commonly used for toxicological testing following established protocols. Larval *Pimephales promelas* (fathead minnows) were selected as warm-water fish species native to

North America since they are commonly used and have established protocols for sub-lethal toxicity tests. However, although *P. promelas* have been introduced into the Fraser Basin, they are not a native species. The water flea, *Ceriodaphnia dubia*, was selected as the planktonic invertebrate since this species is commonly used for sub-lethal toxicity tests in Canada, following established protocols. Although recommended, *Daphnia magna* was not selected since *C. dubia* is preferable for sub-lethal testing, which are more sensitive than acute toxicity tests. The freshwater midge, *Chironomus tentans* was selected as the non-planktonic invertebrate. This species is primarily used for sediment toxicity testing, however, protocols were modified for a water only acute toxicity test.

Two tests were conducted at PESC. Juvenile *O. mykiss* were purchased from Fraser River Trout Hatchery and acclimated for at least 14 days in laboratory dilution water prior to testing. *C. tentans* were obtained from an in-house culture. No water adjustments were required since laboratory culture and dilution waters were moderately hard (80-100 mg/L as $CaCO_3$).

All four species were used for tests conducted by B.A.R. Environmental. *C. dubia* and *C. tentans* were obtained from in-house cultures, while *P. promelas* and *O. mykiss* were purchased from commercial suppliers. All organisms used for testing were acclimatised to the 100 mg/L hardness adjusted dilution water prior to testing since B.A.R.'s standard laboratory dilution water had a hardness of 250-280 mg/L as $CaCO_3$.

P. promelas were acclimated over a period of 5 to 7 days and started with newly fertilised eggs. These eggs were collected and transferred to adjusted water, and then used in toxicity testing.

C. dubia were acclimated as neonates less than 24 hours old. The neonates were collected and placed in adjusted dilution water. Culturing in the adjusted water continued until first generation adults were obtained (1 week). Toxicity tests were performed with the third brood of neonates from the first generation culture. Organisms were cultured for a maximum of 3 weeks.

Acclimation of *O. mykiss* started with juveniles from the standard B.A.R. culture. The fish were placed in adjusted dilution water. Culturing continued for 3 weeks prior to testing.

Acclimation of *C. tentans* started with egg ropes. They were allowed to hatch in the adjusted dilution water. Culturing continued until the animals were 10 to 12 days old (second instar). Toxicity tests were performed with 10 to 12 day old larvae.

3.1.2 Dilution water

Water quality of the dilution water was required to be representative of conditions in the Fraser Basin. This was an important consideration since factors such as hardness and pH would affect toxicity of certain chemicals. Moderately hard water (80-100 mg/L as $CaCO_3$) was used for all toxicity tests. Dilution water used for bioassays conducted at PESC were not adjusted. Laboratory water used as dilution water at B.A.R. Environmental was hardness adjusted in all tests. Standard laboratory dilution water (hardness 250-280 mg/L as $CaCO_3$) was diluted with reverse osmosis treated water to produce a hardness adjusted dilution water of 100 ±10 mg/L as $CaCO_3$. The hardness of all adjusted water used for culturing or testing was checked prior to use and adjusted if necessary. The concentration of dissolved oxygen in the water was maintained at >80% of the air saturation value and pH was in the range of 7.1 to 8.5.

3.1.3 Chemicals and Preparation of Test Solutions

All chemicals were purchased from chemical suppliers. Helix Biotech Ltd. (Richmond, BC) provided supplies of 4,5-DCC, 4,5-DCG, 3,4,5-TCG, 6-CV and abietic acid. Retene was purchased from ICN Biomedicals (Costa Mesa, California). Chemical identification details of the compounds is provided in Appendix 1. The purity for all compounds was guaranteed over 90% and confirmed by analysis at NWRI. Quantitative analyses and stated purities of standard concentrations are shown in Appendix 2.

Prior to testing, all chemicals were stored according to directions provided with the Material Safety Data Sheets. Prior to testing, samples of 6-CV, 4,5-DCC, 4,5-DCG and 3,4,5-TCG were refrigerated at 6^{o}C. Abietic acid was stored in a freezer at -4^{o}C and retene was stored at room temperature (20^{o}C).

Concerns over solubility of the chemicals led to the use of solvent carriers for all tests. Carrier selection was based on previous toxicity studies with these compounds and standard protocol requirements (Bennett, 1996; Billiard and Hodson, 1997). Results from the initial range finding tests suggested that some of the solvent carriers were toxic to both *P. promelas* and *C. dubia*. This necessitated further testing to resolve the issue of a suitable carrier. Ethanol was observed to be highly toxic to *C. dubia* and was therefore excluded as a possible carrier. Acetone had no effect on *P. promelas* survival or growth, or on *C. dubia* survival or reproduction at 0.5 mg/L (the highest concentrations tested). In addition, a white filamentous growth was observed in all exposure vessels containing methanol. With three exceptions, acetone was selected as the solvent carrier for all tests. Tests with *P. promelas* exposed to 3,4,5-TCG using methanol as the solvent carrier were initiated prior to the solvent tests. Both tests conducted at PESC with 4,5-DCG (*O. mykiss* and *C. tentans*) used methanol as the solvent carrier.

3.1.4 Chemical Analyses

For all chemicals except 4,5-DCG, concentrations were verified for each stock solution at the start of each test and at selected renewal periods (old solutions) from the highest test concentrations. Samples from the start of each test were taken from a thoroughly mixed stock solution. Equal sub-samples from each replicate were taken at selected renewal periods. All analyses, except for those with 4,5-DCG, were conducted by NWRI in Burlington, Ontario. All samples for chemical analysis were stored in the dark at 4^{o}C without preservation.

6-CV, 3,4,5-TCG, and 4,5-DCC were acetylated prior to analysis. Derivation and extraction were conducted based on the method by Lee et al. (1989). Retene was extracted with methylene chloride and abietic acid was derivatized following Lee et al. (1990). All extracts were analysed by gas chromatography with an electron capture detector (GC-ECD), except retene, which was analysed by gas chromatography with a mass spectrometric detector (GC-MS). Each sample was only processed and analysed once.

Concentrations of 4,5-DCG were verified for each test concentration on Day 0 and at the end of the test, on Day 4, for both bioassays. All analyses were conducted at PESC. Samples were

acetylated using potassium carbonate/acetic anhydride and then extracted with hexane and concentrated to volume. Extracts were analysed with GC-ECD.

3.1.5 Bioassays

All bioassays were conducted in glass containers to minimise adsorption of chemicals to the container walls.

Oncorhynchus mykiss 96-hour acute lethality bioassays were used to test 4,5-DCG, 4,5-DCC, and 6-CV. Bioassay methods followed Environment Canada Report EPS 1/RM/9 (Environment Canada, 1996), except for the following variances. Since the chemicals required a solvent carrier, a solvent control was run concurrently. The concentration of solvent (methanol or acetone) in the control was set to match the highest concentration seen in the test series. All tests were static, with the exception of a 48-h renewal during the 6-CV and 4,5-DCC testing.

Chironomus tentans water-only acute lethality bioassays were used to test all six chemicals. The standard test duration was 48 hours except the 4,5-DCG bioassay, which ran for 96 hours. The bioassay followed the ASTM(E729) standard protocol for midge reference toxicant testing (ASTM, 1990) and included the same solvent control test variance as in the *O. mykiss* bioassay. The health and sensitivity of the midges were assessed and deemed acceptable for testing by way of a copper reference toxicant test that was run concurrently with the 4,5-DCG bioassay. All tests were static.

Larval *Pimephales promelas* 7-day sub-lethal bioassays for survival and growth were conducted with 3,4,5-TCG, 4,5-DCC, 6-CV, abietic acid and retene. The bioassays followed standard protocol according to Environment Canada Report EPS 1/RM/22 (Environment Canada, 1992a). All test solutions were daily renewal.

Ceriodaphnia dubia 7-day sub-lethal bioassays for survival and reproduction were conducted with 4,5-DCC, 6-CV, abietic acid and retene. All tests were daily renewal and followed the Environment Canada Report EPS 1/RM/21 for standard protocols (Environment Canada, 1992b).

3.2 Results and Discussion

For all chemicals except 4,5-DCG, chemical analyses of the stock solutions and selected exposure concentrations at the end of certain renewal periods indicated a discrepancy between nominal and actual measured values. Solubility was a concern throughout the study for these chemicals. Solvent carriers were used to aid in dissolving the chemicals in water for testing. However, more serious solubility problems were encountered throughout the study for all the chemicals, except 4,5-DCG. Obvious problems were not observed when preparing solutions of 3,4,5-TCG or 4,5-DCC, however, chemical analyses to verify test concentrations found the concentrations were much less than expected. Differences between nominal and verified concentrations ranged from 3% to 98%. Retene appeared to dissolve in the solvent carrier, however, very fine particles were observed to form when the chemical/solvent mixture was added to the dilution water. Similarly, abietic acid also appeared to dissolve in the solvent carrier. However, a thin white film remained on the inside of the vial after dissolving in solvent.

The difficulties encountered with 6-CV were most obvious. Several attempts to dissolve 6-CV into the solvent and/or dilution water were unsuccessful. Some of the compound did dissolve, however, the amounts were inconsistent and far below the full sample that was weighed out. Standard techniques (extended stirring and/or heating) to aid dissolving were employed, but were ineffective. It was concluded that the solubility of 6-CV in water and the solvent carriers were lower than expected (based on reported values) and this value had been exceeded. The reported solubility value for 6-CV was 132 ± 3 mg/L in double distilled water at 25^{o}C according to the chemical manufacturer (McKague, pers. comm., 1997). However, the results of the chemical analyses indicated the solubility in dilution water was closer to 20 mg/L, which was exceeded with the higher test concentrations (maximum 125 mg/L). Investigations into the reasons for the observed losses in 3,4,5-TCG and 4,5-DCC indicated that a very fine precipitate had formed on the bottom of the sample bottle. Solubility limits of 3,4,5-TCG may also have been exceeded. The manufacturer provided a solubility level of 313 ± 16 mg/L (McKague, pers. comm., 1997) but Feniak (1993) measured a water solubility for this compound as 2.5 mg/L. The solubility in double distilled water was also confirmed during this study by K.L.E. Kaiser (NWRI) as 309 mg/L. Possibly other ions present in the dilution water reduced the solubility of 3,4,5-TCG.

In addition, the method of preparing the stock solution may have contributed to the solubility problems. Each chemical, except 4,5-DCG, was weighed out, dissolved in a small amount of solvent, then diluted in water to produce the stock solution for a particular test, from which volumes were diluted to produce the concentration series. In contrast, the entire sample of 4,5-DCG was dissolved in solvent to produce a highly concentrated stock solution from which aliquots were taken to produce the concentration series. This difference may have caused the observed precipitation in the other chemicals because of the limited amount of solvent used to dissolve the chemicals. As a result, the verified concentrations are inconsistent and often very different from the nominal values.

The bioassays suggested the animals responded to a series of concentrations that were consistent with a dose-response curve, rather than the inconsistent concentration range suggested by the chemical analyses. The measured values should not necessarily be discounted, however, the results must be interpreted with caution. The measured values were generally much lower than the nominal concentrations, thus, they should be considered as the minimum exposure values. The result of using these values would be to overestimate the toxicity of the chemicals. Use of the nominal values to calculate endpoints would result in underestimating the toxicity. Thus, the measured and nominal values should be considered the range of the actual toxicity values. To simplify the differences, only measured values of stock solutions were considered for calculations and concentrations of ‘old’ solutions at the end of the renewal periods was disregarded. Endpoint values for 4,5-DCG were based on verified concentrations since these values were consistently over 93% of nominal values. To provide a more complete profile of the toxicity of these chemicals, values from other FRAP studies and the literature were compared to the results of the bioassays.

3.2.1 Chlorophenolics

The concentration of chlorophenolic compounds (4,5-DCG, 3,4,5-TCG, 4,5-DCC and 6-CV) that was toxic to organisms varied between one and two orders of magnitude, depending on the chemical. In general, there was good agreement between results obtained from this study and literature values accepted by Guidelines Division for their toxicological database. There was no

distinct difference, in terms of acute toxicity, between sensitivity of invertebrates and fish, however the alga, *Selenastrum capricornum*, appeared to be less sensitive than other species tested (Table 5). While algae were not part of the data requirements, existing data were reviewed during guideline development.

White sturgeon, *Acipenser transmontanus* was included in the data tables and figures for comparison purposes as a ecologically relevant species to the Fraser River. Fry that were 40 to 60 days old were tested with 4,5-DCG, 4,5-DCC and 6-CV by Farrell and Bennett (1998). In comparison with other organisms, sturgeon exhibited similar sensitivity to these chemicals and, except for 6-CV, were not the most sensitive organism (Table 5, Figure 3). Their dose-response curve, based on a 24-h LC_{50} (lethal concentration at which 50% of test organisms die) with fry, appeared to be less steep than the other species (Figure 2), suggesting they may be more susceptible to chronic effects or they may have better toxicological mechanisms to detoxify the chemicals. Sturgeon sensitivity was of interest to FRAP since this is an ecologically relevant species whose populations are declining in the Fraser River.

Of the four chlorophenolics tested, 3,4,5-TCG and 4,5-DCC were generally the most toxic and 6-CV was the least toxic (Table 5, Figure 3). 6-CV had the widest range of LC_{50} values from 0.41 mg/L (*A. transmontanus*) to >100 mg/L (*C. tentans*). No effects were observed with *C. tentans* at any of the test concentrations of 6-CV, based on a 48-h mortality range finder test. These differences in sensitivity may have been due to interspecies differences, or as a result of difficulties encountered with dissolving 6-CV in the stock solutions, potentially resulting in concentration errors. No toxicity information was available for 6-CV from other studies, except Farrell and Bennett (1998), so comparisons to literature values (or other studies) were not possible.

Two chlorophenolics, 4,5-DCG and 6-CV, affected behaviour patterns in *O. mykiss* (Table 6). A loss of equilibrium was observed with 4,5-DCG. At lower concentrations, there appeared to be some recovery, however mortality resulted at higher concentrations. Behavioural effects were also observed with exposure to 6-CV. At concentrations between 2.48 and 9.93 mg/L 6-CV, *O. mykiss* lay bent and immobile at the container bottom. No other species displayed behavioural effects. Kennedy et al. (1996, 1995) also found chlorinated guaiacols produced biochemical

effects such as impaired disease resistance, cortisol depression, and leucocrit elevation in *O. mykiss*.

Sublethal toxicity was similar for *C. dubia* reproduction and *P. promelas* growth; however toxicity of the chemicals varied by two orders of magnitude, based on IC_{50} values (inhibitory concentration at which 50% of test organisms exhibit an adverse effect) (Table 7, Figure 4). The most toxic chlorophenolic was 4,5-DCC (IC_{50} = 0.41 mg/L *P. promelas*; IC_{50} = 0.099 mg/L *C. dubia*) while the least toxic was 6-CV (IC_{50} = 15.7 mg/L for *P. promelas*; IC_{50} = 6.5 mg/L for *C. dubia*). These results suggest that 4,5-DCC and 6-CV affect reproduction in *C. dubia*, while the effects of 3,4,5-TCG, 4,5-DCC and 6-CV are more acute with *P. promelas*.

Leach and Thakore (1975) reported an LC_{50} for 3,4,5-TCG in *O. mykiss* of 0.75 mg/L and Cherr et al. (1987) reported an EC_{50}/NOEL (EC_{50} is the effective concentration at which a response in 50% or test organisms is observed and NOEL is the no-observed effect level) in *Strongylocentrotus purpuratus* (sea urchin) sperm bioassay of 2.0/1.0. Renberg et al. (1980) reported a 96-h LC_{50} in harpacticoid, *Nitocra snipipes* (crustacean), of 5.2 mg/L for 4,5,6-TCG. McLeay (1987) in his literature review reported a *O. mykiss* LC_{50} of 0.7 to 1.0 mg/L for TCG. These results were similar to the *P. promelas* LC_{50} values of 0.33 to 0.65 reported by this study. The 3,4,5-TCG tests with *C. tentans* had a measured value of only 11.2% of the nominal. This test was repeated using a different method to prepare the stock and exposure solutions, however the results were not available in time to be included in this report.

Servizi et al. (1968) reported a 96-h LC_{50} in *Oncorhynchus nerka* of 2.4 to 2.7 mg/L and *O. gorbuscha* of 2.0 mg/L for 4,5-DCC. Feniak (1993) reported a 48-h LC_{50} for 4,5-DCC in *Daphnia magna* of 18 μmol/L. Huttula et al. (1981) reported a 24-h LC_{50} in *D. magna* of 2.9 mg/L for 3,5-DCC and 2.3 mg/L for 4,5-DCC. McLeay (1987) reported the rainbow trout LC_{50} to range from 0.5 to 1.0 for DCC. These values correspond well with the values calculated in this study: 0.3 to 3.2 mg/L.

In general, increased chlorination increases toxicity of phenolics (Salkinoja-Salonen et al., 1991; Oikari et al., 1987). The values reported here appear to be in line with other values reported for these groups of compounds. The pH of the solution is also a factor for the phenolic compounds,

which can vary the LC_{50} by a factor of 10. It is therefore difficult to make direct comparisons to the literature. However, considering the relatively close agreement with the literature values, it seems reasonable to recognise the data generated by this study as valid, with limitations. The measured and nominal values should be considered the range in the actual values.

3.2.2 Abietic Acid

Lethal toxicity of abietic acid was similar for all species tested, and is comparable for this study and literature values (Table 5, Figure 3). The range of 7-day LC_{50} values are 1.9 mg/L (*P. promelas*) to 6.06 mg/L for *Salmo trutta* (brown trout). Abietic acid appears to be generally less lethally toxic than the chlorophenolic compounds, however, the wide range of values from some of the chlorophenolics limits this conclusion. The dose-response curve for *C. tentans* is not steep in comparison to its response with the other chemicals (Figure 2). This is further evidence that abietic acid is relatively less toxic than the chlorophenolics.

Abietic acid also has relatively low sublethal toxicity to *P. promelas* and *C. dubia*, based on 7-day growth and reproduction studies, respectively, since IC_{50} values are very similar to the LC_{50} values from the acute tests (Table 5). In comparison with the other PME chemicals, sub-lethal effects are relatively low for both growth and reproduction. Only 6-CV was less toxic.

In the case of abietic acid, the *P. promelas* LC_{50} calculated using a nominal value is 2.36 mg/L. The literature value reported in the TerraBase database (a toxicity database based on predicted toxicity, rather than bioassays; TerraBase Inc., Burlington, Ontario) is 2.4 mg/L. The values are very close lending support for the use of the nominal values for these exposures. A literature review reported the 96-h LC_{50} of abietic acid in *O. mykiss* as 0.7 to 1.5 mg/L (McLeay, 1987).

3.2.3 Retene

No LC_{50} values could be calculated for retene since the concentration range for acute toxicity exceeded the solubility of the compound in water, which was estimated at 250 μg/L (Kaiser, pers. comm., 1997). As a result, retene is not considered lethal to aquatic organisms based on aquatic bioassays, since no significant mortality occurred with any test organism (Table 5). The lack of

acute toxicity to fish has been demonstrated in previous studies with retene (Billiard and Hodson, 1997).

Although acute toxicity was not significant, retene is currently one of the pulp mill chemicals of most concern because of the potential dioxin-like effects. It has been shown to be a potent MFO inducer, particularly with prolonged exposure (Parrott et al., 1995; Fragoso et al., 1996 in Billiard and Hodson, 1997). In addition, concentrations as low as 32 μg/L resulted in chronic toxicity to developing stages of rainbow trout (Billiard and Hodson, 1997). In this study, retene was toxic to *C. dubia* reproduction at a LOEL of 66 μg/L (LOEL is the lowest observable effect level) and affected growth in larval *P. promelas*, although at higher concentrations (430 μg/L) Results are shown in Table 5 and Figure 4. Although the levels measured in the Fraser River sediments (6 to 450 ng/g) and water (0.41 to 0.85 ng/L) are orders of magnitude lower than those causing observed toxic effects, organisms may potentially bioaccumulate retene to toxic concentrations.

4.0 COMPARISON OF FRASER RIVER PME CHEMICAL LEVELS TO CONCENTRATIONS CAUSING TOXICITY

While these PME chemicals exhibited toxic effects to the test organisms, the range of concentrations that produced these effects was orders of magnitude higher than the maximum levels measured downstream of pulp mills in the Fraser River from 1994-96 (Table 2; Sekela et al., 1995; Brewer et al., 1997). In effect, based on the available toxicity database, these chemicals do not appear to be chemicals of concern in the Fraser basin at the present time. However, this conclusion is based on a chemical-by-chemical approach and may not reflect the behaviour of these compounds in a mixture, as their cumulative effects may be more toxic. In addition, the potential for bioaccumulation has not been addressed.

The toxicity database consists of lethal and sublethal information, however more sensitive responses and complete lifecycle data is still lacking. Biochemical responses were not investigated but are expected to be significant, particularly for retene, which has been identified as a potent MFO inducer in fish (Parrott et al., 1995). Both 4,5-DCG and 6-CV produced atypical behaviour in rainbow trout. Since the endpoint for those tests was mortality, behavioural effects were not

quantified. Effects on behaviour may affect survival by affecting both their foraging ability and their predator avoidance (Wood et al., 1997).

Richardson and Levings (1996) measured the levels of chlorophenolics in benthic organisms from the lower Fraser River, near Agassiz, BC, in March 1993. 3,4,5-TCG was present in most organisms and sediments at levels higher than any other chlorophenolic. Levels in invertebrates ranged from 0.2 to 1.9 ng/g wet weight, while the prickly sculpin (*Cottus asper*), a benthivorous fish, had levels of 3,4,5-TCG higher than any other organisms or those found in the sediment (2.3-5.8 ng/g). This is approximately a 10-fold biomagnification factor between sculpin and their prey (invertebrates), which suggests magnification (or transfer) of chlorophenolics through the food web, potentially affecting birds and other wildlife. Since the input of chlorophenolics probably declined sharply in 1990/91 due to changes in the pulp mill bleaching processes, it is possible that the levels in sculpin reflect, to some degree, bioaccumulation from higher historic levels in benthic organisms. This would be expected to equilibrate to concentrations in water and sediment within a year. No data exists that links tissue residues with toxic effects.

In general, the environmental levels of many of these chemicals were low, relative to concentrations that produce toxic effects. Levels in the environment continue to decline as the use of molecular chlorine is phased out of the pulp and paper process. This is particularly true for the chlorophenolics, however, retene remains a pulp mill chemical of concern due to its dioxin-like effects, chronic toxicity, and potential to bioaccumulate.

5.0 WATER QUALITY GUIDELINE DEVELOPMENT

As a result of difficulties encountered during the recent toxicity testing studies, some scientific uncertainty surrounding the exposure concentrations remains. Consequently, the resulting final data set obtained is not expected to fully meet the requirements for the development of nationally approved Canadian Water Quality Guidelines (WQG) (CCME, 1991). Further testing and data analysis is required to address the data gaps and uncertainty for all the chemicals.

In an effort to make the most prudent and scientifically defensible use of existing data, the current toxicity data was used to develop effects-based reference values for the Fraser Basin based on the CCME WQG derivation procedure. These reference values will be submitted to the CCME process as draft guidelines for full national guideline review and approval pending the final outcome of additional testing and analysis. For the purposes of the FRAP study, the recommended reference values are presented in Table 8. In all cases, the most sensitive endpoint was observed from tests in this study.

The reference value for 4,5-DCG was based on the 96-h LC_{50} with *Chironomus tentans*. A safety factor of 0.05 was used since the endpoint was acute. The reference value was set at 0.11 mg/L. WQGs could not be developed because the dataset did not meet the minimum requirements. This was based on a decision by Guidelines Division to consider the studies with *Acipenser transmontanus* (white sturgeon) (Bennett and Farrell, 1998; Bennett, 1996) as unacceptable. This study was exploratory in nature and not conducted according to a standardised toxicological test method. In addition, some of the data was outside the range of data collected for other fish species. Outlier data estimated using novel approaches are not used for guideline development. An additional test with a species other than rainbow trout is required for guideline development.

The reference value for 3,4,5-TCG was based on a 7-day TEC (threshold effects concentration) for survival with *Pimephales promelas*. The safety factor was 0.1 since the chronic endpoint values were higher. The reference value was set at 0.0151 mg/L. WQGs could not be developed based the toxicological tests conducted in this study because the concerns over actual exposure concentrations compromised the integrity of the data.

The reference value for 4,5-DCC was based on a 7-day TEC value for reproduction in *Ceriodaphnia dubia*. The safety factor was 0.1 since this was a chronic study. The reference value was set at 0.0071 mg/L. WQGs could not be developed based the toxicological tests conducted in this study because the concerns over actual exposure concentrations compromised the integrity of the data. In addition, the sturgeon study from Bennett (1996) (and Farrell and Bennett, 1998) was considered unacceptable for the reasons listed above, thus the minimum data requirements were not met.

The reference value for 6-CV was based on a 96-h LC_{50} with *Oncorhynchus mykiss*. A safety factor of 0.05 was used since the endpoint was acute. The reference value was set at 0.130 mg/L. WQGs could not be developed because the dataset did not meet the minimum requirements. As discussed above, the toxicological tests conducted in this study were not accepted as primary studies and the sturgeon was considered unacceptable.

The reference value for abietic acid was based on a 7-day TEC value for growth with *Pimephales promelas*. The safety factor was 0.1 since this was a chronic study. The reference value was set at 0.05 mg/L. WQGs could not be developed based the toxicological tests conducted in this study because the concerns over actual exposure concentrations compromised the integrity of the data.

The reference value for abietic acid was based on a 7-day TEC value for reproduction with *Ceriodaphnia dubia*. The safety factor was 0.1 since this was a chronic study. The reference value was set at 0.0051 mg/L. WQGs could not be developed based the toxicological tests conducted in this study because the concerns over actual exposure concentrations compromised the integrity of the data.

The reference values relative to the critical data point and other toxicity data are shown in a series of charts for each of the six chemicals in this study (Figures 5 - 10).

The United States Environmental Protection Agency (US-EPA, 1993) has established Ambient Water Quality Concentrations for several of these compounds including 6-CV (acute 2.13 mg/L; chronic 0.1 mg/L), 4,5-DCC (acute 0.89 mg/L; chronic 0.075 mg/L). The US-EPA chronic value for 6-CV is virtually identical to the reference value developed for the Fraser Basin (0.130 mg/L). The chronic value for 4,5-DCC, however is an order of magnitude higher than the Fraser Basin reference value (0.0071 mg/L).

6.0 CONCLUSIONS

3,4,5-TCG, 4,5-DCC, 6-CV and abietic acid were found to exhibit both chronic and acute toxic effects, while retene toxicity was generally chronic. While 4,5-DCG was only tested for acute toxicity, behavioural effects in fish were observed at non-lethal concentrations. The acute toxicity

values for a variety of exposure times and species (expressed as LC_{50}) ranged from 0.33 to 13.9 mg/L, while chronic threshold effects concentrations (TEC) ranged from 0.051 to 6.9 mg/L. Ambient levels of individual PME chemicals in the Fraser Basin were orders of magnitude below these levels. However, these short-term, acute, and single-chemical toxicity tests should be used with caution when assessing effects of PME on aquatic life because PMEs consist of a complex mixture of chemicals and single-chemical toxicity tests do not address possible additive or synergistic effects. The issue of bioaccumulation also remains to be addressed. Although WQGs are generally derived for single chemicals, it is also not possible to identify and test every chemical in the effluent.

Some tests are not long enough to cover all life stages, and potentially sensitive endpoints such as reproduction. These bioassay endpoints are not the most sensitive that could be measured; more sensitive biomarkers such as biochemical (e.g., disease resistance, cortisol depression, leucocrit elevation, or mixed function oxygenase [MFO] induction) and behavioural endpoints may occur at ambient concentrations in the Fraser Basin. Large data gaps regarding more subtle toxic effects of the chemicals remain.

In addition, these chemicals are predominantly associated with sediments and can be at relatively high concentrations in those sediments where their toxicity remains unknown and there is a potential for bioaccumulation or biomagnification. The tests conducted to date do not incorporate sediment or dietary exposures.

While acute toxicity testing is an important first step in environmental assessment, a complete assesment requires investigation of more sensitive endpoints for the chemicals in pulp mill effluents at species and community levels.

7.0 RECOMMENDATIONS

- Canadian WQGs should be developed for the PME chemicals of concern in the Fraser Basin. However, toxicity testing should focus on more sensitive endpoints, as opposed to the

standard lethal and sub-lethal testing conducted in this study. These alternative studies may also be used to fulfil data requirements.

- Until WQGs are developed, the reference values presented in this report should be used to assess potential impacts in receiving waters and to evaluate pollution control measures at pulp mills.

- The potential for bioaccumulation of PME chemicals should be investigated, particularly for retene and 6-CV, which are known to be more stable in the environment.

- Interaction effects of PME chemicals should be investigated. While the individual chemicals do not appear to be very toxic relative to their levels in the Fraser Basin, mixtures of these chemicals may produce more deleterious effects.

- Since retene accumulates in the bed sediments, it would be more appropriate to develop sediment quality guidelines for this chemical. The potential for long term exposure and bioaccumulation in the benthic community food chains should be investigated for retene.

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TABLES

Table 1. The selected pulp mill effluent chemicals of concern in the Fraser Basin.

Chlorophenolics	Resin acids	Fatty acids	PAHs
4,5-dichloroguaiacol	**abietic acid**	lignoceric	**retene**
3,4,5-trichloroguaiacol	12/14-chlorodehydroabietic acid*		benzo(*e*)pyrene
4,5-dichlorocatechol	dihydroisopimaric acid		
6-chlorovanillin			

* 12-chlorodehydroabietic acid and 14-chlorodehydroabietic acid are distinct compounds that cannot be separated during analysis.

Note: Compounds in bold were chosen for WQG development.

Table 2. Range of levels of selected PME priority chemicals measured in the Fraser Basin from 1992-96 (from Sekela et al., 1995; Brewer et al., 1997).

Chemical Class	Chemicals*	Range of values in Fraser Basin**	Sample type
chlorophenolic	4,5-DCC	0.11-1.6	bed sediment
		<0.59-670	suspended sediment
		<0.1-2.6	clarified water
chlorophenolic	4,5-DCG	0.004-0.78	bed sediment
		<0.1-69	suspended sediment
		<0.01-9	clarified water
chlorophenolic	3,4,5-TCG	<0.14-0.36	bed sediment
		<0.1-55	suspended sediment
		<0.02-5.6	clarified water
chlorophenolic	6-CV	0.23-3.5	bed sediment
		<0.65-1100	suspended sediment
		<0.11-16	clarified water
fatty acid	lignoceric acid	23-530	bed sediment
		70-460	suspended sediment
		7.4-1200	whole water
PAH	B[e]P	0.25-25	bed sediment
		2.3-100	suspended sediment
		<0.1	clarified water
PAH	retene	6-450	bed sediment
		28-190	suspended sediment
		0.41-0.85	clarified water
resin acid	CDAA	<0.58-34.5	bed sediment
		<1.4-900	suspended sediment
		<0.6-46	whole water
resin acid	abietic acid	31-2800	bed sediment
		170-32000	suspended sediment
		<3.5-390	whole water
resin acid	DIPA	0.32-31	bed sediment
		<2.1-1700	suspended sediment
		<1.3-6.7	whole water

* 4,5-DCG = 4,5-dichloroguaiacol
3,4,5-TCG = 3,4,5-trichloroguaiacol
4,5-DCC = 4,5-dichlorocatechol
6-CV = 6-chlorovanillin
B[e]P = benzo[e]pyrene
CDAA = 12/14-chlorodehydroabietic acid
DIPA = dihydroisopimaric acid

**units for bed and suspended sediments are ng/g dry weight
units for clarified/whole water are ng/L

Table 3. Available and required toxicity data for the development of WQGs for the PME chemicals of concern in the Fraser Basin.

Chemical	Available Toxicity Data[a,b]	Required Toxicity Studies
4,5-DCG	<u>FISH</u> 96-h LC_{50} = 4.8 mg/L for *Poecilia reticulata* (guppies) (2)[15]. 96-h LC_{50} = 2.3 mg/L for Oncorhynchus mykiss (rainbow trout) (UN[c])[13]. 96-h LC_{50} = 2.9 mg/L for *Brachydanio rerio* (zebrafish) adults (UN[d])[10]. 96-h LC_{50} = 2.0 mg/L for *B. rerio* larvae (UN[d])[10]. LOEL = 0.5 mg/L for *B. rerio* embryos (UN[d])[10]. 24-h LC_{50} = <0.001-0.01 mg/L for *Acipenser transmontanus* larvae (white sturgeon)[f17] 24-h LC_{50} = 2.5 mg/L for *A. transmontanus* fry[f17] <u>INVERTEBRATES</u> 24-h LC_{50} = 3.1-6.2 mg/L for *Daphnia magna* (2)[15]. 25-min LD_{50} = 100 mg/L for *Tubifex tubifex* (UN[d])[2]. <u>PLANTS/ALGAE</u> 96-h LC_{50} = 29 mg/L (0.015 mM/100mL) for *Selenastrum capricornutum* (2)[6]. 12 to 14-d LOEL = 10 mg/L and 12 to 14-d NOEL = 1 mg/L for growth in *Chlorella pyrenoidosa* (UN[c])[14]. 10 to 14-d LOEL = 5-15 mg/L and 10 to 14-d NOEL = 20 mg/L for growth in *Lemna perpusilla* (UN[c])[14].	<u>FISH</u> One study on one cold-water fish species native to North America. *Oncorhynchus mykiss* (rainbow trout) recommended. <u>INVERTEBRATES</u> One study on one non-planktonic invertebrate species. Freshwater insect recommended. <u>PLANTS/ALGAE</u> No freshwater plant toxicity studies required.
3,4,5-TCG	<u>FISH</u> 96-h LC_{50} = 0.75 mg/L for *O. mykiss* (2)[8].	<u>FISH</u> One study on one fish species, except *O. mykiss*. *Pimephales promelas* (fathead minnow) recommended.

Table 3 continued

Chemical	Available Toxicity Data[a,b]	Required Toxicity Studies
3,4,5-TCG	<u>INVERTEBRATES</u> 48-h LC_{50} = 7.3 mg/L for *D. magna* (2)[11]. 48-h LC_{50} = 4.5 mg/L for *D. magna* in humic water (2)[11]. <u>PLANTS/ALGAE</u> 96-h LC_{50} = 5.6 mg/L (0.0025 mM/100mL) for *S. capricornutum* (2)[6]. 12 to 14-d LOEL = 0.1 mg/L and 12 to 14-d NOEL = 1 mg/L for growth in *C. pyrenoidosa* (UN[c]) [14]. 10 to 14-d LOEL = 5 mg/L and 10 to 14-d NOEL = 1 mg/L for growth in *L. perpusilla* (UN[c]) [14].	<u>INVERTEBRATES</u> One study on one non-planktonic invertebrate species. Freshwater insect recommended. <u>PLANTS/ALGAE</u> No freshwater plant toxicity studies required.
4,5-DCC	<u>FISH</u> 24-h LC_{50} = 2.3 mg/L for *S. trutta* (UN[c])[4]. 96-h LC_{50} < 1.0 mg/L for *O. mykiss* (UN[c])[9]. 24-h LC_{50} = 0.01 mg/L for *A. Transmontanus* larvae[f17] 24-h LC_{50} = 0.51 mg/L for *A. Transmontanus* fry[f17] <u>INVERTEBRATES</u> No freshwater invertebrate toxicity data were available.	<u>FISH</u> One study on one cold-water fish species native to North America. *O. mykiss* recommended. One study on one other fish species. *P. promelas* recommended. <u>INVERTEBRATES</u> One study on one planktonic invertebrate species native to North America. *Daphnia magna* recommended. One study on one non-planktonic invertebrate. Freshwater insect recommended.

Table 3 continued

Chemical	Available Toxicity Data[a,b]	Required Toxicity Studies
4,5-DCC	PLANTS/ALGAE 96-h LC_{50} = 4.5 mg/L (0.0025 mM/100mL) for *S. capricornutum* (2)[6]. 12 to 14-d LOEL = 4 mg/L and 12 to 14-d NOEL = 2 mg/L for growth in *C. pyrenoidosa* (UN[c])[14]. 10 to 14-d LOEL = 5 mg/L and 10 to 14-d NOEL = 1 mg/L for growth in *L. perpusilla* (UN[c])[14]	PLANTS/ALGAE No freshwater plant toxicity studies required.
6-CV	FISH 24-h LC_{50} = 0.41 mg/L for *A. transmontanus* fry[f17] INVERTEBRATES No freshwater invertebrate toxicity data were available. PLANTS/ALGAE No freshwater plant toxicity data were available.	FISH One study on one cold-water fish species native to North America. *O. mykiss* recommended. One study on one other fish species. *P. promelas* recommended. INVERTEBRATES One study on one planktonic invertebrate species native to North America. *D. magna* recommended. One study on one non-planktonic invertebrate. Freshwater insect recommended. PLANTS/ALGAE No freshwater plant toxicity studies required.
Abietic acid	FISH 96-h LC_{50} = 0.7 mg/L for *O. mykiss* (Un[c])[7]. 96-h LC_{50} = 5.45 mg/L for *O. mykiss* (2)[3]. 96-h LC_{50} = 6.06 mg/L for *Salmo trutta* (brown trout) (2)[3]. 24-h LC_{50} = 12 mg/L for *Oryzias latipes* (medaka) (Un[c])[16].	FISH One study on one fish species, except *O. mykiss*. *P. promelas* recommended.

Table 3 continued

Chemical	Available Toxicity Data[a,b]	Required Toxicity Studies
Abietic acid	<u>INVERTEBRATES</u> No freshwater invertebrate toxicity data were available. <u>PLANTS/ALGAE</u> No freshwater plant toxicity data were available.	<u>INVERTEBRATES</u> One study on one planktonic invertebrate species native to North America. *Daphnia magna* recommended. One study on one non-planktonic invertebrate species. Freshwater insect recommended. <u>PLANTS/ALGAE</u> No freshwater plant toxicity studies required.
Retene	<u>FISH</u> LOEL = 100 µg/L for mortality and LOEL << 32 µg/L (lowest concentration tested) for pathology in *O. mykiss* exposed from eyed egg stage until swim-up (30-60 days) (2)[1]. <u>INVERTEBRATES</u> No freshwater invertebrate toxicity data were available. <u>PLANTS/ALGAE</u> No freshwater plant toxicity data were available.	<u>FISH</u> One study on one fish species, except *O. mykiss*. *P. promelas* recommended. <u>INVERTEBRATES</u> One study on one planktonic invertebrate species native to North America. *D. magna* recommended. One study on one non-planktonic invertebrate. Freshwater insect recommended. <u>PLANTS/ALGAE</u> No freshwater plant toxicity studies required.

Footnotes:

[a] Brackets contain the ranking of the available studies according to the CCME protocol (CCME 1991), where 1=primary, 2=secondary, and UN=unacceptable.

[b] Superscripted numbers refer to the references.

[c] Study was ranked unacceptable because the responses and survival of the controls were not reported.

[d] Study was ranked unacceptable because the methods were not described adequately

[e] Study was ranked unacceptable because a single concentration was tested and therefore a dose-response relationship was not demonstrated.

[f] Study was not ranked by Guidelines Division at the time.

References for Table 3:

1. Billiard and Hodson (1997)
2. Cernakova (1994)
3. Flood et al. (1989)
4. Hattula et al. (1981)
5. Kennedy et al. (1995)
6. Kuivasniemi et al. (1985)
7. Leach and Thakore (1976)
8. Leach and Thakore (1979)
9. McKague (1981)
10. Neilson et al. (1990)
11. Oikari et al. (1992)
12. Oris and Giesy (1987)
13. PAPRICAN (1979)
14. Rowe et al. (1982)
15. Salkinoja-Salonen et al. (1981)
16. Sameshima et al. (1986)
17. Bennett (1996)

Table 4. Bioassays conducted to generate data for WQGs development.

Chemical	Organism*	Bioassay
4,5-DCG	• *Oncorhynchus mykiss* • *Chironomus tentans*	• 96-h acute LC_{50} • 96-h acute LC_{50} (water only)**
3,4,5-TCG	• *Pimephales promelas* • *C. tentans*	• 7-d survival and growth (sub-lethal) • 48-h acute LC_{50} (water only)
4,5-DCC	• *P. promelas* • *C. tentans* • *Ceriodaphnia dubia* • *O. mykiss*	• 7-d survival and growth (sub-lethal) • 48-h acute LC_{50} (water only) • 7-d partial lifecycle survival and reproduction (sub-lethal) • 96-h acute LC_{50}
6-CV	• *P. promelas* • *C. tentans*[1] • *C. dubia* • *O. mykiss*	• 7-d survival and growth (sub-lethal) • 48-h acute LC_{50} (water only) • 7-d partial lifecycle survival and reproduction (sub-lethal) • 96-h acute LC_{50}
abietic acid	• *P. promelas* • *C. tentans* • *C. dubia*	• 7-d survival and growth (sub-lethal) • 48-h acute LC_{50} (water only) • 7-d partial lifecycle survival and reproduction (chronic)
retene	• *P. promelas* • *C. tentans*[1] • *C. dubia*	• 7-d survival and growth (sub-lethal) • 48-h acute LC_{50} (water only) • 7-d partial lifecycle survival and reproduction (sub-lethal)

* test details - all bioassays are done in glass containers.
Rainbow trout: 2 replicates with 10 fish per concentration
Fathead minnow: 4 replicates with 10 fish per concentration
Chironomus tentans: 4 replicates with 10 larvae per concentration
Ceriodaphnia dubia: 10 daphnids per concentration (1 per vial)

** 3 replicates with 10 larvae per concentration

[1] definitive tests not conducted due to an absence of observed toxicity in rangefinding tests.

Table 5. **Acute toxicity data for selected Fraser basin chemicals of concern.** Nominal concentration values from this study are shown in brackets.

Chemical	Organism	Endpoint	Value (mg/L)	Confidence Limits (mg/L)	Source
4,5-DCG	*Chironomus tentans*	96-h LC_{50}	***2.2*** (2.1)	1.9-3.1	this study
4,5-DCG	*Oncorhynchus mykiss*	96-h LC_{50}	3.8 (4.0)	3.1-5.2	this study
4,5-DCG	*Acipenser transmontanus*	24-h LC_{50}	2.5	1.0-10.0	Farrell and Bennett (1998)
4,5-DCG	*Daphnia magna*	24-h LC_{50}	3.1-6.2	n/d*	Salkinoha-Salonen et al. (1981)
4,5-DCG	*Poecilia reticulata*	96-h LC_{50}	4.8	n/d	Salkinoha-Salonen, et al. (1981)
4,5-DCG	*Selenastrum capricornutum*	96-h LC_{50}	29	n/d	Kuivasniemi et al. (1985)
3,4,5-TCG	*C. tentans*	48-h LC_{50}	0.60 (5.4)	0.53-0.68	this study
3,4,5-TCG	*O. mykiss*	96-h LC_{50}	0.75	n/d	Kuivasniemi et al. (1985)
3,4,5-TCG	*Pimephales promelas*	7-d LC_{50}	***0.33*** (0.83)	0.29-0.38	this study
3,4,5-TCG	*D. magna*	48-h LC_{50}	7.3	n/d	Oikari et al. (1992)
3,4,5-TCG	*S. capricornutum*	96-h LC_{50}	5.6	n/d	Kuivasniemi et al. (1985)
4,5-DCC	*C. tentans*	48-h LC_{50}	3.2 (3.3)	2.8-3.6	this study
4,5-DCC	*O. mykiss*	96-h LC_{50}	0.45 (0.61)	0.29-0.59	this study
4,5-DCC	*P. promelas*	7-d LC_{50}	***0.36*** (1.5)	0.30-0.60	this study
4,5-DCC	*Ceriodaphnia dubia*	7-d LC_{50}	>0.40 (>1.25)	-	this study
4,5-DCC	*A. transmontanus*	24-h LC_{50}	0.51	0.29-0.87	Farrell and Bennett (1998)
4,5-DCC	*S. capricornutum*	96-h LC_{50}	4.5	n/d	Kuivasniemi et al. (1985)
6-CV	*C. tentans*	48-h NOEL	(≥100)	-	this study
6-CV	*O. mykiss*	96-h LC_{50}	2.6 (13.1)	1.2-5.0	this study
6-CV	*P. promelas*	7-d LC_{50}	13.2 (88.1)	9.8-19.6	this study
6-CV	*C. dubia*	7-d LC_{50}	13.9 (92.8)	9.8-19.6	this study
6-CV	*A. transmontanus*	24-h LC_{50}	***0.41***	0.1-1.0	Farrell and Bennett (1998)
retene	*C. tentans*	48-h NOEL	(≥0.12)	-	this study
	O. mykiss	LOEL (30-60 d)**	***0.1***	n/d	Billiard and Hodson (1997)
retene	*P. promelas*	7-d LC_{50}	>0.86 (>0.5)	-	this study
retene	*C. dubia*	7-d LC_{50}	>0.072 (>0.13)	-	this study

Table 5 continued

Chemical	Organism	Endpoint	Value (mg/L)	Confidence Limits (mg/L)	Source
abietic acid	*C. tentans*	48-h LC_{50}	2.8 (7.2)	2.3-3.3	this study
abietic acid	*O. mykiss*	96-h LC_{50}	5.45	n/d	Flood et al. (1989)
abietic acid	*Salmo trutta*	96-h LC_{50}	6.06	n/d	Flood et al. (1989)
abietic acid	*P. promelas*	7-d LC_{50}	***1.9*** (3.4)	1.4-2.8	this study
abietic acid	*C. dubia*	7-d LC_{50}	3.1 (4.3)	1.8-3.6	this study

* n/d = not determined
** exposed from eyed egg stage to swim-up fry
LC_{50} = lethal concentration producing 50% mortality
LOEL = lowest observed effect level
NOEL = no observed effect level
Note: Values from this study are based on verified concentrations. The most sensitive values are indicated in italicised bold.

Table 6. Atypical and stress behaviour exhibited by *Oncorhynchus mykiss* (rainbow trout) in bioassays.

Chemical	Concentration (mg/L)	Behavioural effect
4,5-DCG	1.8	After 24 hours, loss of equilibrium, swimming on their sides or positioned vertically in the water column. Some recovery seen over the final 72 hrs.
	3.2	Loss of equilibrium after 24 hrs; at 48 hrs ceased swimming and lay motionless on the bottom.
	5.6	Mortality preceded by sluggishness, loss of equilibrium and cessation of movement.
6-CV	2.48 - 9.93	Mortality preceded by lying immobile and bent on the bottom of container. Effects displayed after 24 hrs.

Table 7. **Sub-lethal endpoints from 7-day toxicity tests.** Nominal concentration values are provided in brackets.

Chemical	Organism	Endpoint	Value (mg/L)	Confidence Limits (mg/L)
3,4,5-TCG	*Pimephales promelas*	IC_{50}	>0.51 (>0.99)	-
3,4,5-TCG	*P. promelas*	LOEL	0.51 (0.99)	-
3,4,5-TCG	*P. promelas*	TEC	***0.36**** (0.70)	-
3,4,5-TCG	*P. promelas*	NOEL	0.26 (5.1)	-
4,5-DCC	*P. promelas*	IC_{50}	0.41 (1.7)	0.31-0.52
4,5-DCC	*P. promelas*	LOEL	0.30 (1.3)	-
4,5-DCC	*P. promelas*	TEC	0.21 (0.88)	-
4,5-DCC	*P. promelas*	NOEL	0.15 (0.63)	-
4,5-DCC	*Ceriodaphnia dubia*	IC_{50}	0.099 (0.31)	0.077-0.16
4,5-DCC	*C. dubia*	LOEL	0.10 (0.31)	-
4,5-DCC	*C. dubia*	TEC	***0.071**** (0.22)	-
4,5-DCC	*C. dubia*	NOEL	0.050 (0.15)	-
6-CV	*P. promelas*	IC_{50}	15.7 (104)	-
6-CV	*P. promelas*	LOEL	9.8 (65)	-
6-CV	*P. promelas*	TEC	6.9 (46)	-
6-CV	*P. promelas*	NOEL	4.9 (33)	-
6-CV	*C. dubia*	IC_{50}	6.5 (43)	4.5-7.6
6-CV	*C. dubia*	LOEL	4.9 (33)	-
6-CV	*C. dubia*	TEC	***3.5**** (23)	-
6-CV	*C. dubia*	NOEL	2.5 (17)	-
retene	*P. promelas*	IC_{50}	>0.86 (>0.50)	-
retene	*P. promelas*	LOEL	0.43 (0.25)	-
retene	*P. promelas*	TEC	0.30 (0.17)	-
retene	*P. promelas*	NOEL	0.21 (0.12)	-
retene	*C. dubia*	IC_{50}	0.066 (0.12)	-
retene	*C. dubia*	LOEL	0.072 (0.13)	-
retene	*C. dubia*	TEC	***0.051**** (0.095)	-
retene	*C. dubia*	NOEL	0.036 (0.067)	-
abietic acid	*P. promelas*	IC_{50}	1.9 (3.4)	1.6-2.2
abietic acid	*P. promelas*	LOEL	0.71 (1.26)	-
abietic acid	*P. promelas*	TEC	***0.50**** (0.88)	-
abietic acid	*P. promelas*	NOEL	0.35 (0.62)	-
abietic acid	*C. dubia*	IC_{50}	2.6 (3.6)	2.3-2.9
abietic acid	*C. dubia*	LOEL	3.6 (5.0)	-
abietic acid	*C. dubia*	TEC	2.5 (3.5)	-
abietic acid	*C. dubia*	NOEL	1.8 (2.5)	-

IC_{50} = inhibitory concentration at which 50% of test organisms are adversely affected
LOEL = lowest observed effect level
NOEL = no observed effect level
TEC = threshold effects concentration
* most sensitive endpoint used for reference value calculations highlighted in italicised bold.

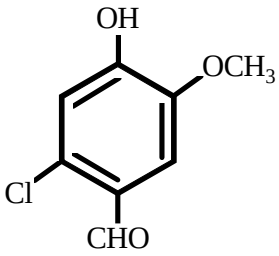
Table 8. Recommended effects-based reference values for pulp mill effluent chemicals of concern in the Fraser Basin.

Parameter	Reference Value (mg/L)	Critical Value (mg/L)	Safety Factor	Test	Organism
Chlorophenolics					
4,5-dichloroguaiacol	0.11	2.2	0.05	96-h LC_{50}	*Chironomus tentans*[1]
3,4,5-trichloroguaiacol	0.0151	0.181	0.1	7-d TEC (survival)	*Pimephales promelas*[1]
4,5-dichlorocatechol	0.0071	0.071	0.1	7-d TEC (reproduction)	*Ceriodaphnia dubia*[1]
6-chlorovanillin	0.130	2.6	0.05	96-h LC_{50}	*Oncorhynchus mykiss*[1]
Resin Acids					
abietic acid	0.05	0.5	0.1	7-d TEC (growth)	*Pimephales promelas*[1]
PAHs					
retene	0.0051	0.051	0.1	7-d TEC (reproduction)	*Ceriodaphnia dubia*[1]

[1] This study

LC_{50} = lethal concentration at which 50% of organisms die
TEC = threshold effects concentration

FIGURES



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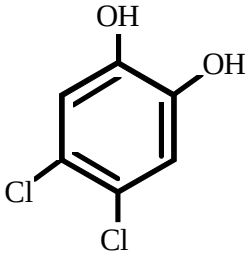
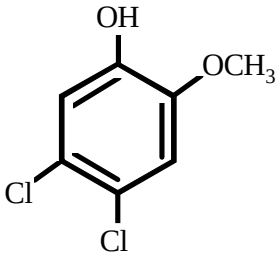


4,5-chlorocatechol



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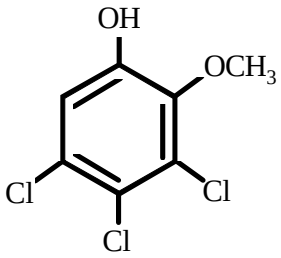

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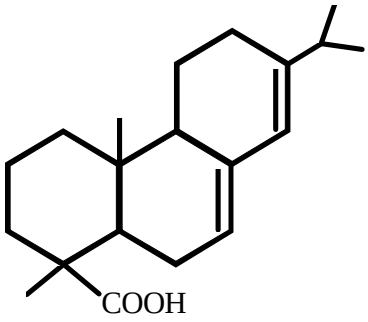
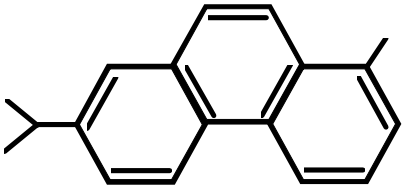


abietic acid



retene

Figure 1. Structures of PME chemicals selected for WQGs development.

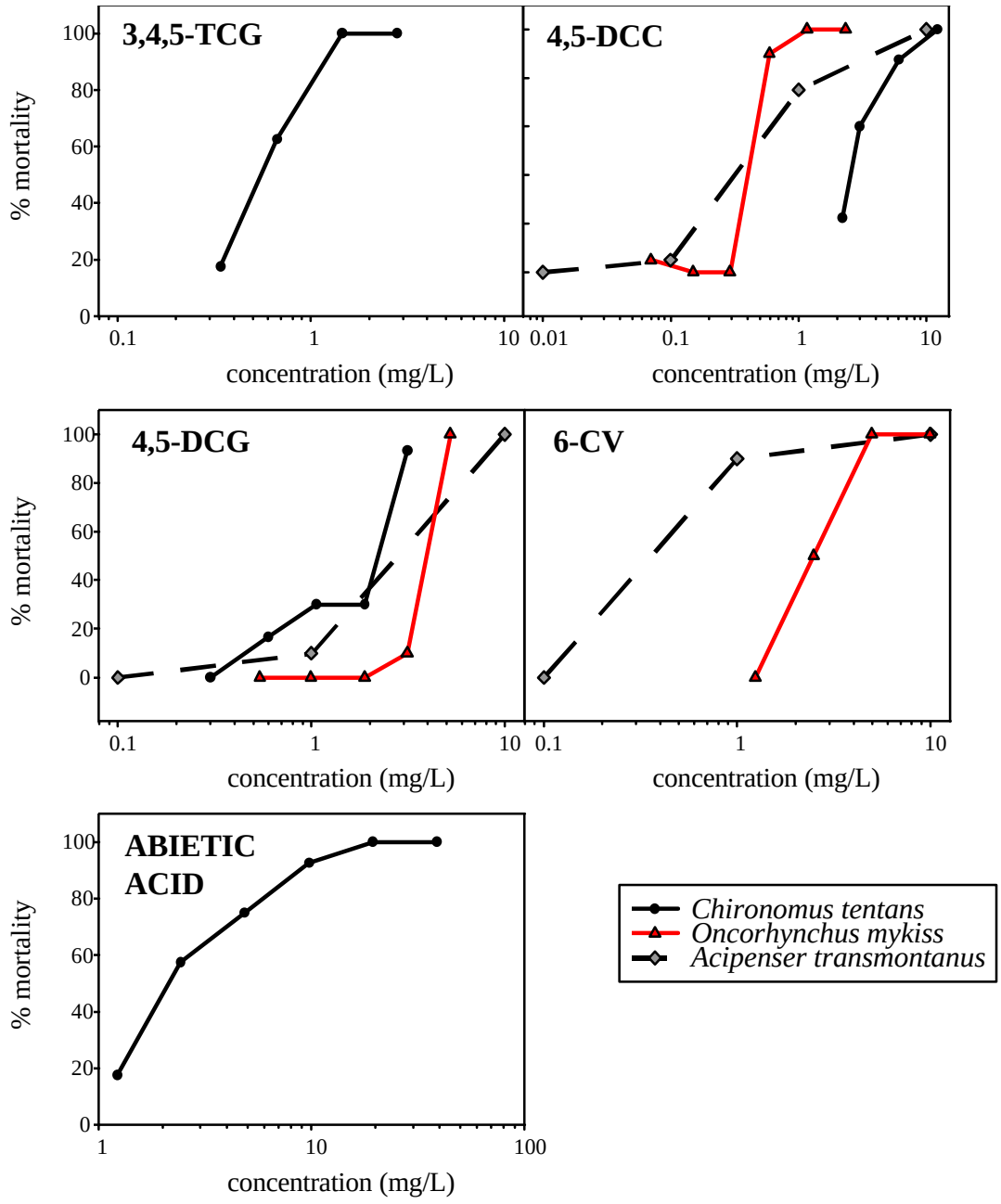


Figure 2. **Dose-response curves for PME chemicals of concern in the Fraser Basin.**

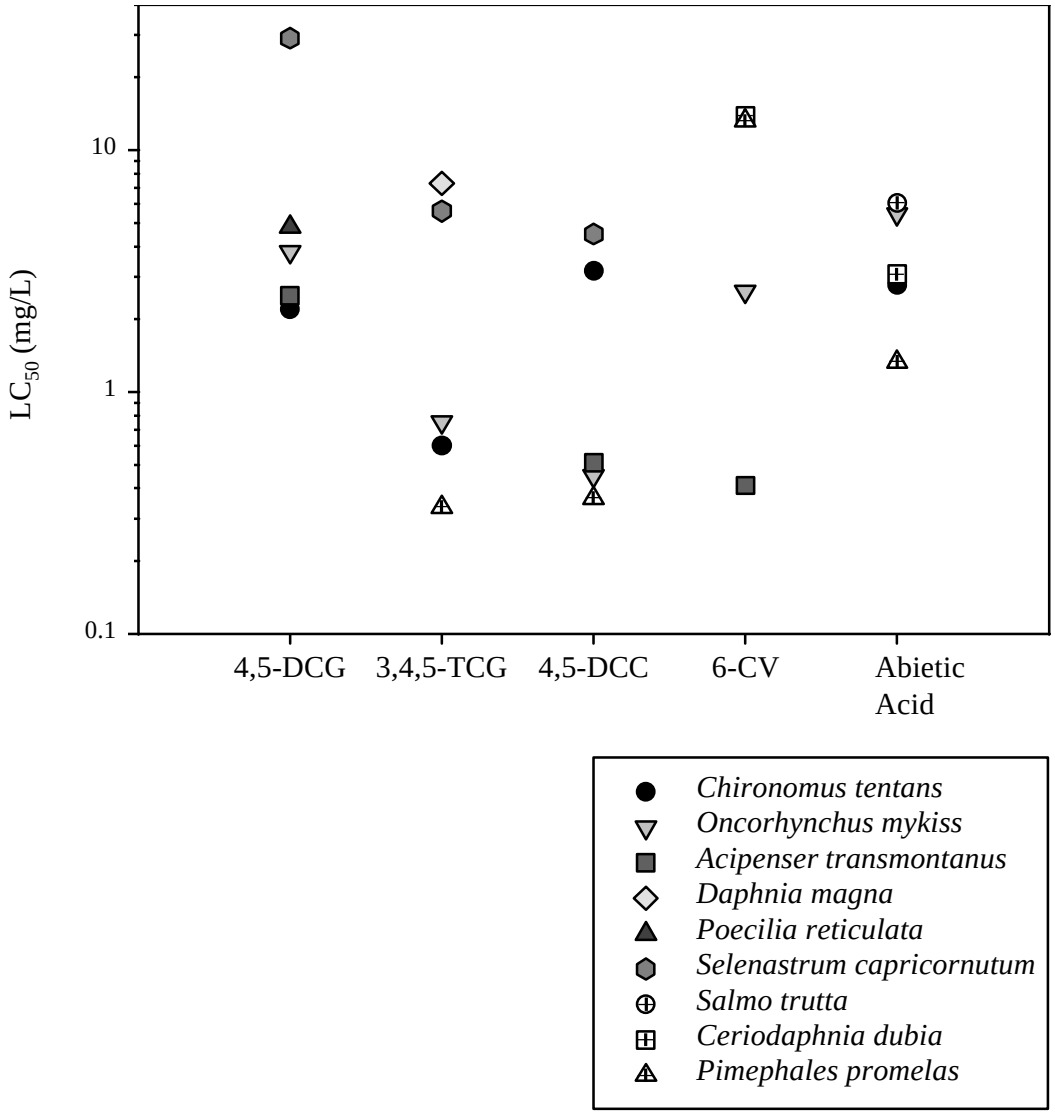


Figure 3. **Comparison of LC_{50} values for PME chemicals.** No LC_{50} value was obtained for retene. No acute toxicity was observed with *C. tentans* for 6-CV. Test durations are species dependent and described in Table 3. Data presented here include this study and literature values, as cited in Table 3.

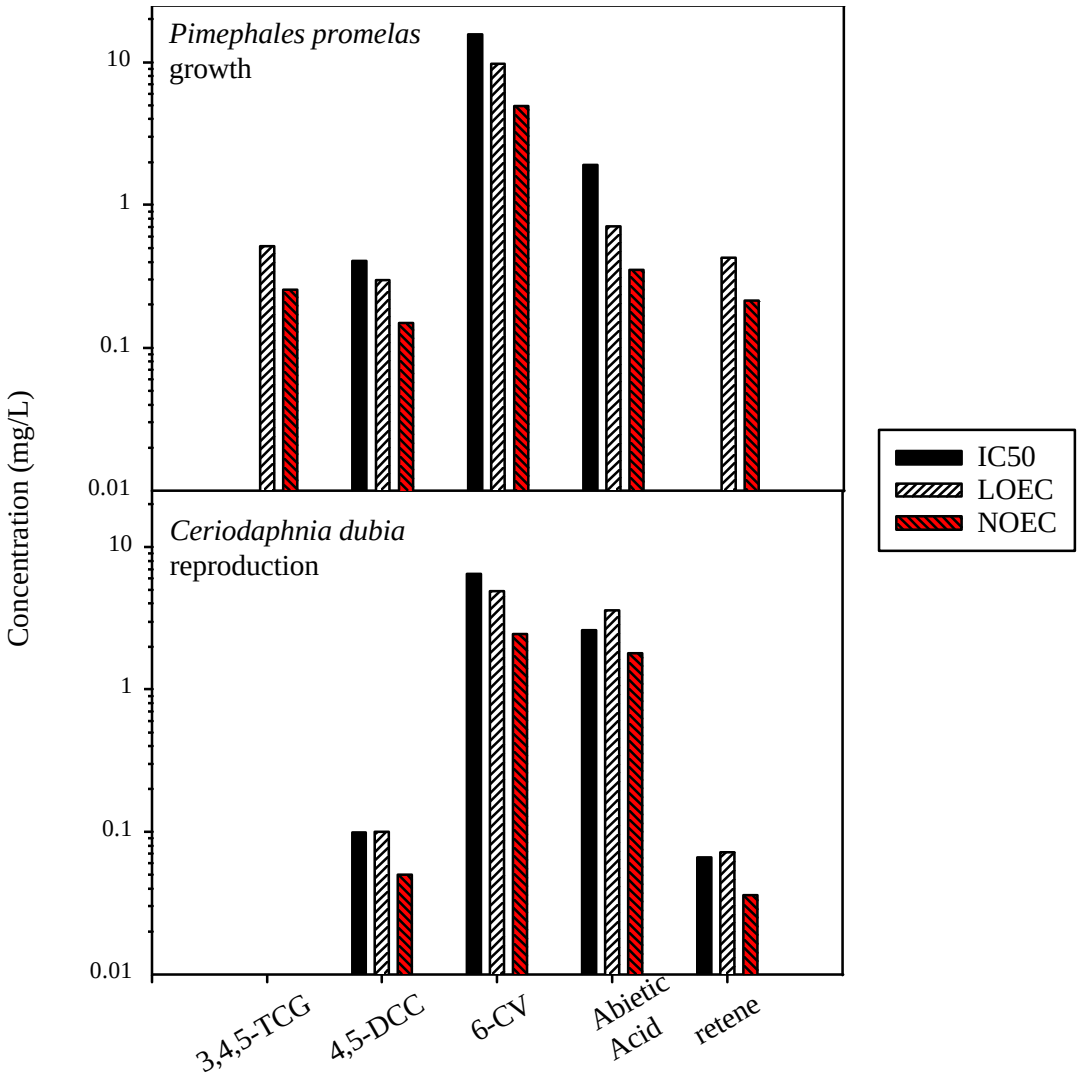


Figure 4. **Comparison of sublethal endpoints of *Pimephales promelas* (fathead minnow) growth bioassay and *Ceriodaphnia dubia* 7-d reproduction bioassay for PME chemicals.**

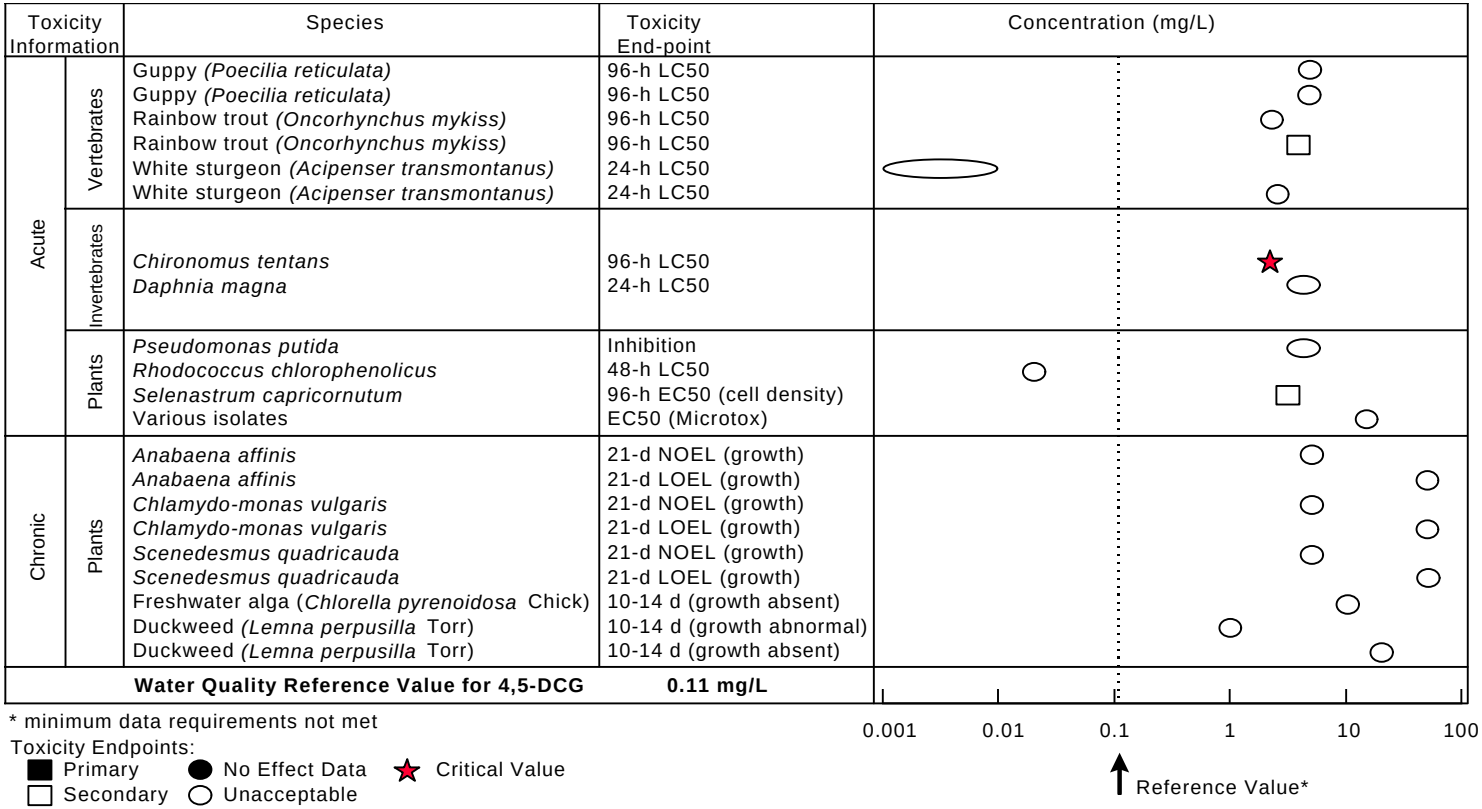


Figure 5. **Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for 4,5-dichloroguaiacol (4,5-DCG).**

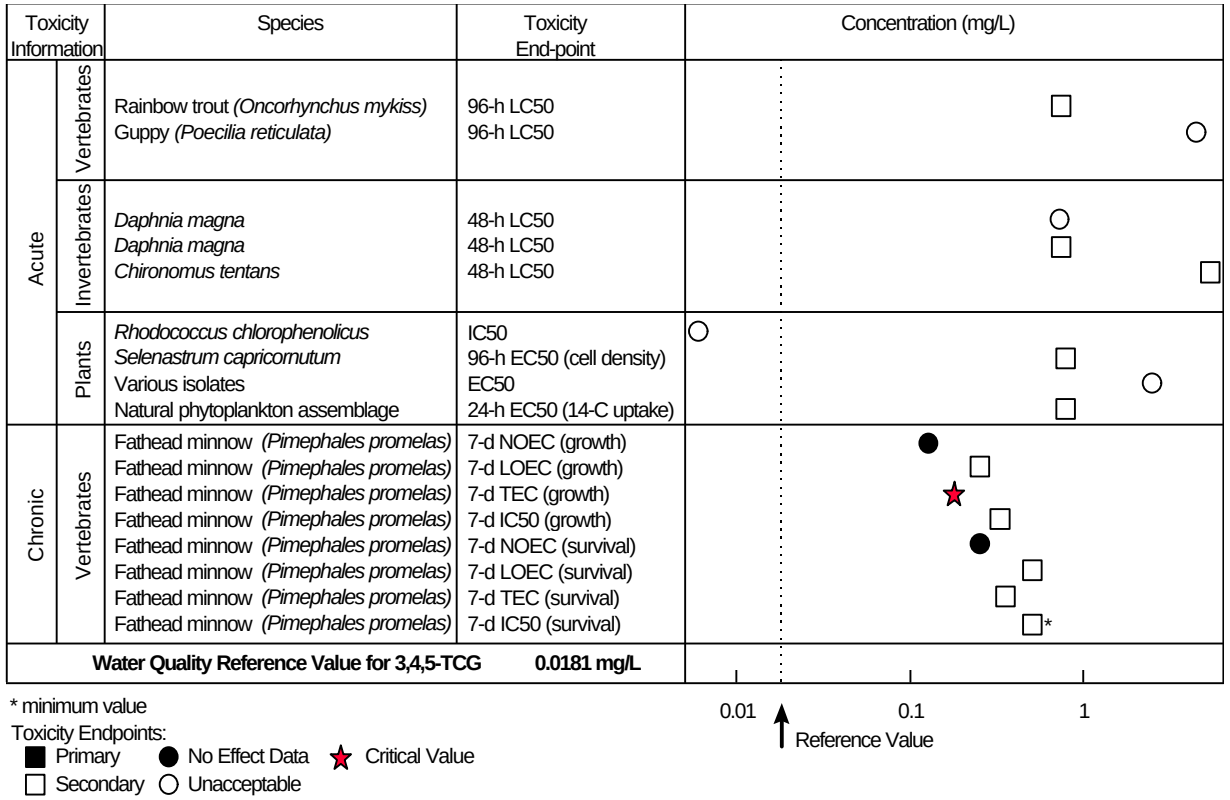


Figure 6. Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for 3,4,5-trichloroguaiacol (3,4,5-TCG).

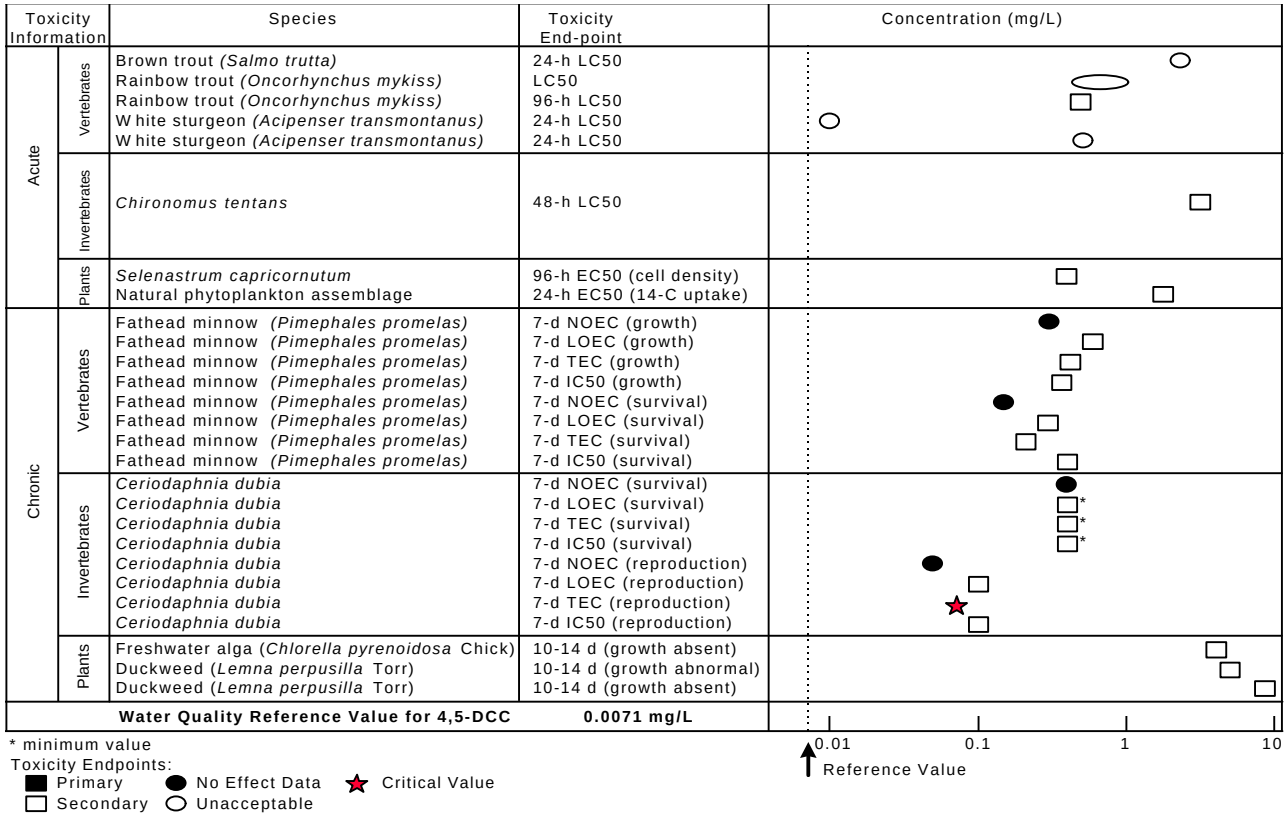


Figure 7. Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for 4,5-dichlorocatechol (4,5-DCC).

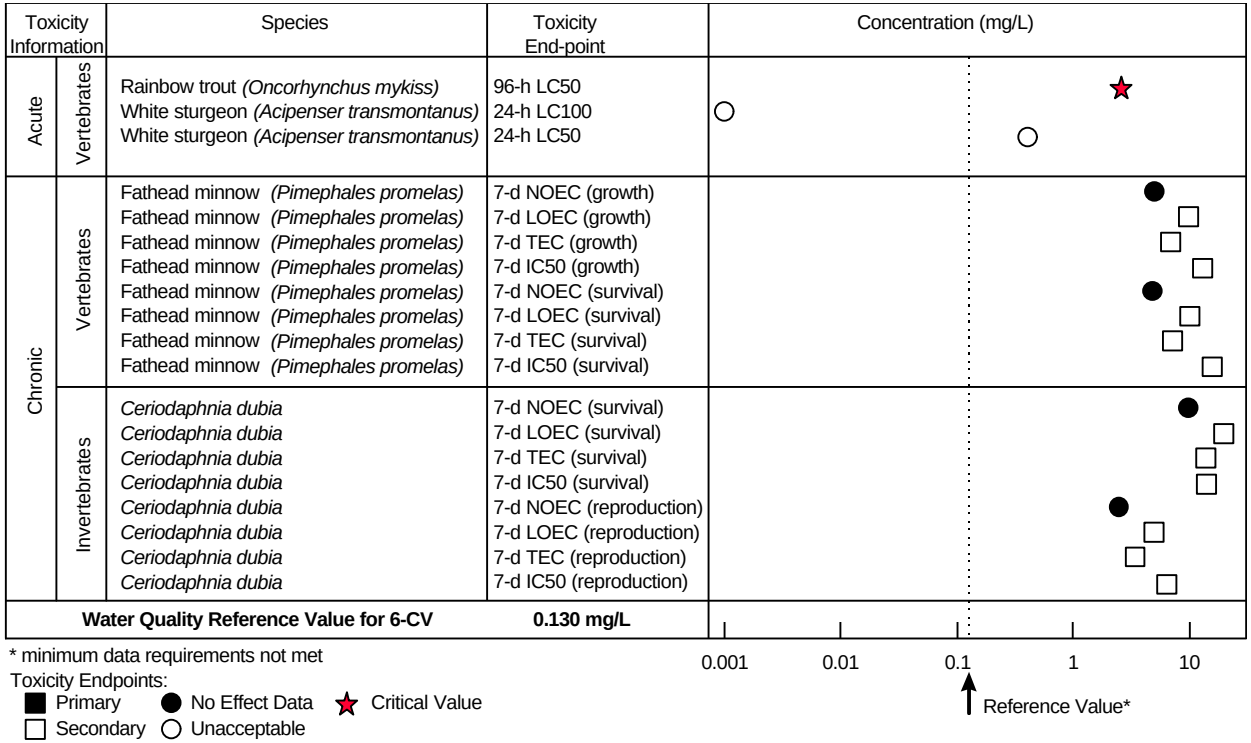


Figure 8. Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for 6-chlorovanillin (6-CV).

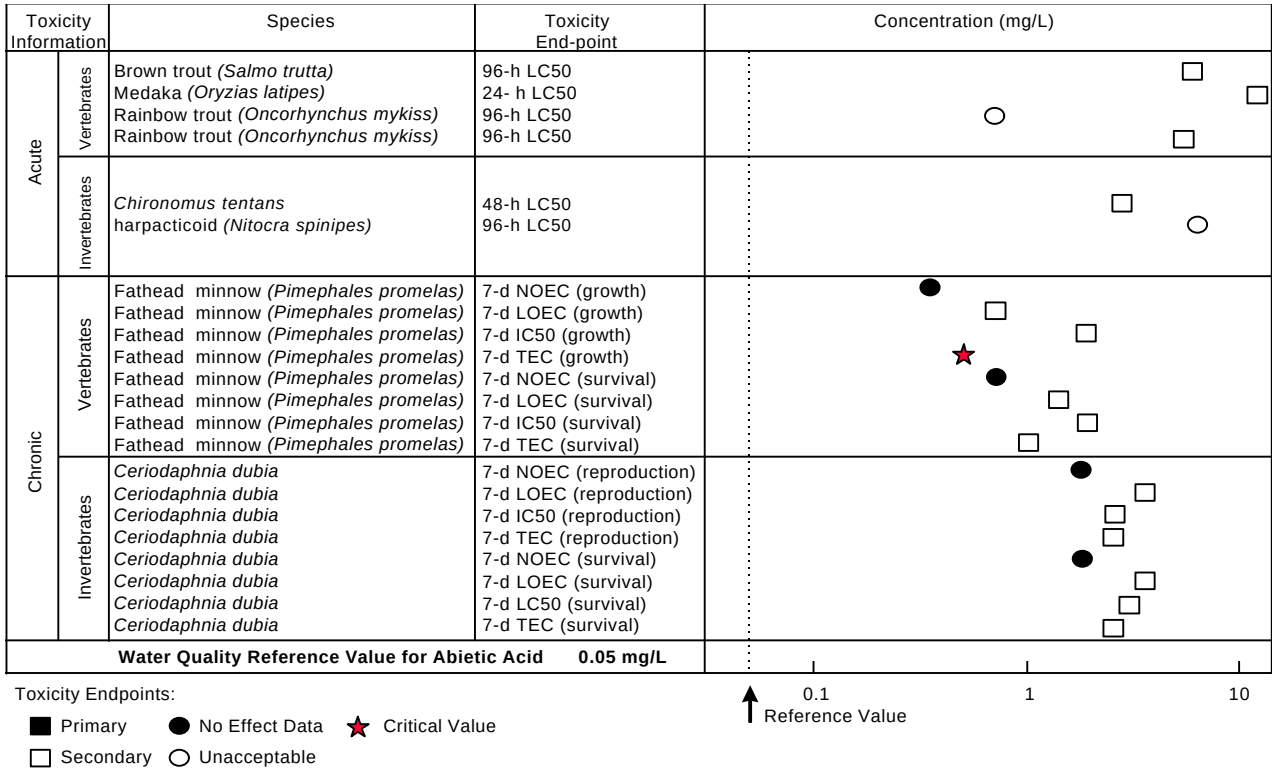


Figure 9. Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for abietic acid.

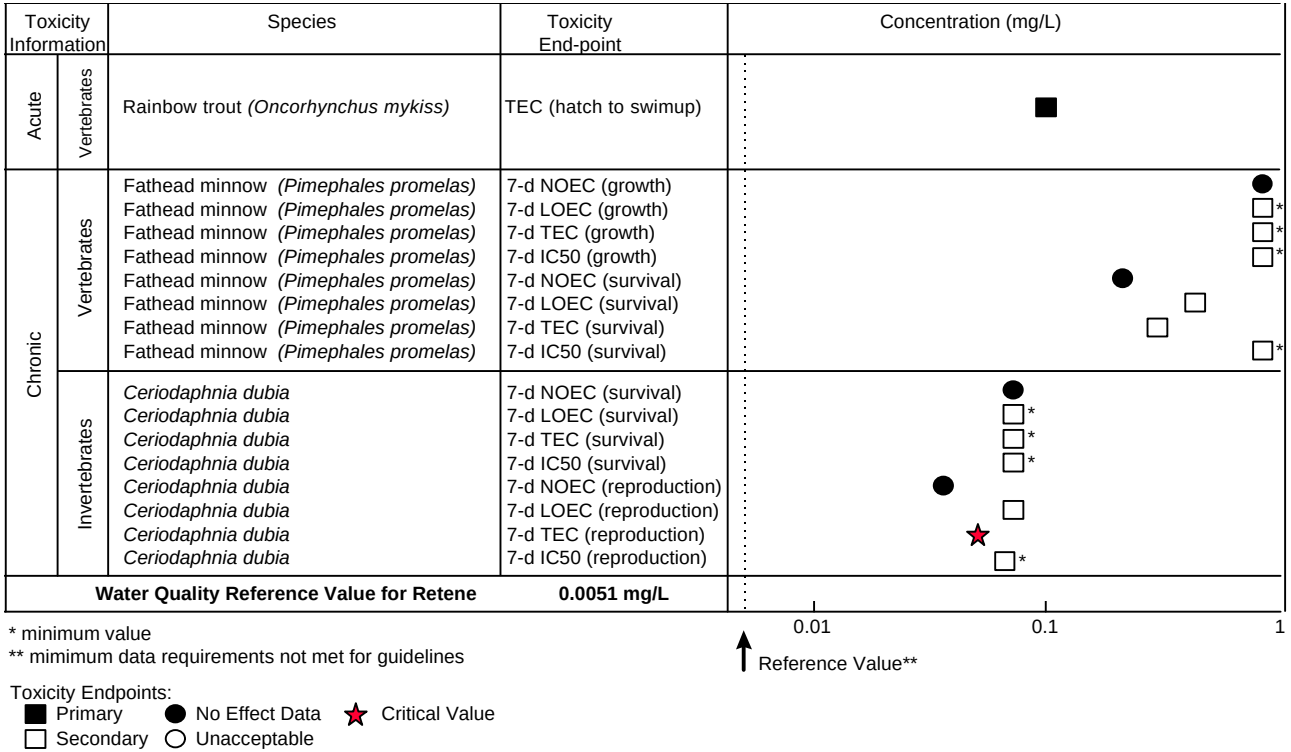


Figure 10. Water quality reference value in relation to the critical toxicity endpoint value and toxicity data for retene.

APPENDIX 1

Table A 1. Chemical identifications of standard compounds.

Common name	IUPAC name	CAS Registry #	CA Index name	Formula
4,5-dichlorocatechol	4,5-dichloro-1,2-benzenediol	3428-24-8	1,2-benzenediol, 4,5-dichloro-	$C_6H_4Cl_2O_2$
4,5-dichloroguaiacol	4,5-dichloro-2-methoxyphenol	2460-49-3	phenol, 4,5-dichloro-2-methoxy-	$C_7H_6Cl_2O_2$
3,4,5-trichloroguaiacol	3,4,5-trichloro-2-methoxyphenol	57057-83-7	phenol, 3,4,5-trichloro-2-methoxy-	$C_7H_5Cl_3O_2$
6-chlorovanillin	2-chloro-4-hydroxy-5-methoxybenzaldehyde	18268-76-3	benzaldehyde,2-chloro-4-hydroxy-5-methoxy-	$C_8H_7ClO_3$
abietic acid	7,13-abietadien-18-oic acid or 1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1R-(1a,4ab,4ba,10aa)]-1-phenanthrenecarboxylic acid	514-10-3	1-phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1R-(1a,4ab,4ba,10aa)]-	$C_{20}H_{30}O_2$
retene	7-isopropyl-1-methylphenanthrene or 1-methyl-7-(10methylethyl)phenanthrene	483-65-8	phenanthrene, 1-methyl-7-(10methylethyl)-	$C_{18}H_{18}$

APPENDIX 2

Table A 2. Quantitative analyses of standards for chlorophenolics, retene and abietic acid used for calibration.

Chemical	Stated purity	Stated concentration (mg/L)	Observed concentration (mg/L)
4,5-DCG	99%	-	-
3,4,5-TCG (std 728)	99%	1	1.0
3,4,5-TCG (std 814)	99%	1	1.1
4,5-DCC (std 723*)	99%	1	1.0
4,5-DCC (std 723)	99%	1	0.9
6-CV (std 81*)	99%	1	1.0
6-CV (std 820)	99%	1	1.0
abietic acid (std 821*)	90-95%	1	1.0
abietic acid (std 813)	90-95%	100	96.9
retene (std*)	98%	10	10.0
retene (std 88)	98%	100	136.6
retene (std 813)	98%	10	13.2

* standard used for calibration