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# SITE-SPECIFIC MARINE WATER-QUALITY CRITERION FOR CYANIDE

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**Abstract**—A site-specific marine water–quality criterion for cyanide was developed for Puget Sound, Washington, USA. The U.S. Environmental Protection Agency (U.S. EPA) national cyanide water-quality criterion is driven by toxicity data for the eastern rock crab, *Cancer irroratus*, a species not resident to the U.S. western coast (West Coast). The reported LC50 for *C. irroratus* is six times lower than any other marine species tested. Cyanide acute toxicity tests were conducted using first stage zoeae of all four species of *Cancer* spp. resident to Puget Sound to develop a site-specific criterion for this water body. Testing with Puget Sound *Cancer* spp. reveals sensitivities 24 times less, on average, than *C. irroratus*. Recalculation of the Puget Sound water-quality criterion for cyanide, by substituting the new *Cancer* spp. data for the *C. irroratus* data, results in water-quality criterion protecting marine life against acute and chronic toxicity of 9.4 and 2.9 μg/L cyanide, compared to the U.S. EPA national value of 1.0 μg/L for both acute and chronic toxicity.

**Keywords**—Cyanide Site-specific criterion *Cancer irroratus* Puget Sound

## INTRODUCTION

Cyanide is commonly found in wastewaters from a variety of industrial practices, such as mining and oil refineries, as well as municipal wastewaters [1]. It exists in these effluents in three basic forms: free cyanide, simple cyanides, and complex cyanides. Free cyanide occurs as two species (hydrogen cyanide and the ionized $CN^-$), depending on pH and temperature [2,3]. Free cyanide is the most toxic form and the one used in laboratory toxicity tests to derive U.S. Environmental Protection Agency (U.S. EPA) water-quality criterion (WQC) [1]. Simple cyanide (e.g., sodium cyanide) normally disassociates into free cyanide and salts in aqueous solutions. Complex cyanides can take a variety of forms, but cyanide–metal complexes (e.g., iron cyanide, nickel cyanide) are the dominant cyanide forms in industrial wastewaters. The latter complexes are normally several orders of magnitude less toxic than free cyanides because their toxicity is largely due to disassociation of free cyanide from these complexes [4].

In petroleum refineries, the primary cyanide sources are conversion units, such as cracking units. Hydrogen cyanide is formed from cracking nitrogen-containing organic compounds in crude oils. Because hydrogen cyanide is corrosive, it forms metal–cyanide complexes in the overhead vapor systems associated with the conversion units. As a result, cyanide discharged in refinery effluent is not in the free form but rather is complexed with several metals (e.g., iron, nickel) [5].

Methods for distinguishing free cyanide from some of the complexed cyanides are not available as routine analytical procedures for wastewaters. The weak acid disassociable method, which is sometimes used for compliance monitoring, will not measure very stable complexes, such as iron and cobaltous cyanide, but will measure cyanide complexes with cadmium, copper, nickel and zinc [3,5]. In compliance monitoring, this can overestimate the free cyanide occurring in an effluent and hence overestimate its potential toxicity.

Considering these issues regarding cyanide chemistry and the deficiencies of approved analytical methods for measuring it, our intent was to develop a site-specific WQC for cyanide in oil refinery effluents discharged to Puget Sound by conducting a water effect ratio study [6]. In conducting water effect ratio studies, it is important to utilize an indicator species that is sensitive to the chemical of concern at or near the existing WQC because sensitive species are more capable of detecting small differences in bioavailability [6]. The identification of a sensitive species for water effect ratio study testing with cyanide was accomplished by conducting a literature search and reviewing data from current U.S. EPA WQC [1].

The literature search revealed no consequential testing of marine species with cyanide since publication of the U.S. EPA WQC in 1984. The marine WQC were derived in accordance with Stephan et al. [7] using data from eight taxa (Table 1). The resulting final acute value (FAV) calculated from Table 1 is 2.030 μg/L. The criterion maximum concentration, which is the FAV divided by 2 to provide an approximation of the LC1, is 1.015 μg/L [7]. The criterion maximum concentration is the concentration protecting 95% of the marine genera from acute toxicity or may be a concentration selected to protect a species of economic or key ecologic importance [7]. Because the acute toxicity data for the eastern rock crab, *Cancer irroratus*, were based on tests with larvae, U.S. EPA [1] judged that they predicted more reliably cyanide's chronic toxicity than would be obtained using the standard U.S. EPA practice of dividing the FAV by the acute–chronic ratio. Therefore, the acute and chronic criteria for cyanide are the same: 1.015 μg/L [1].

The *C. irroratus* data strongly drive the cyanide criterion because this species is six times more sensitive than any other tested. A review of U.S. EPA WQC documents for other chemicals (e.g., arsenic, cadmium, copper, zinc) that include *Cancer* spp. in their data sets reveals that this taxon is in the most

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Table 1. U.S. Environmental Protection Agency national marine water-quality criterion acute toxicity database for cyanide

| Rank | Species | Genus mean acute value (μg/L) |
|---|---|---|
| 8 | Common Atlantic slippershell, *Crepidula fornicata* | >10,000 |
| 7 | Amphipod, *Ampelisca abdita* | 995.9 |
| 6 | Winter flounder, *Pseudopleuronectes americanus* | 372 |
| 5 | Sheepshead minnow, *Cyprinodon variegatus* | 300 |
| 4 | Mysid, *Mysidopsis bahia*/*bigelowi*[a] | 118.4 |
| 3 | Atlantic silverside, *Menidia menidia* | 59 |
| 2 | Copepod, *Acartia clausi* | 30 |
| 1 | Rock crab, *Cancer irroratus* | 4.893 |

[a] Note that both *M. bahia* and *M. bigelowi* have recently been reclassified to the genus *Americamysis.*

sensitive 30% (ranging from 9 to 30%) of taxa tested for any given chemical [8–11]. Arsenic was the only chemical for which *Cancer* spp. was the most sensitive taxon tested. However, even for arsenic, it did not drive the criterion, as several other species were of comparable (within a factor of 2) sensitivity.

Considering *C. irroratus*'s relative sensitivity to other toxicants and its nonresidency in Puget Sound, we decided to test all four *Cancer* spp. resident to Puget Sound. If the latter were comparable in sensitivity to *C. irroratus,* a water effects ratio study would be conducted with one of the resident species to learn whether cyanide's toxicity in Puget Sound waters differed from that observed by testing in standard laboratory water. If resident species were less sensitive, then the Puget Sound data would be substituted for the *C. irroratus* data to derive a site-specific cyanide criterion for Puget Sound. The rationale behind this substitution is that a tested species is supposed to be representing not only itself but also a large number of untested species. Test species should be deleted only when the site lacks the test species and anything closely related to the test species or when some other tested species better represents the close relatives present [6]. By testing all *Cancer* spp. resident to Puget Sound, substitution of *C. irroratus* would be justified.

## MATERIALS AND METHODS

### *Test organisms*

Four species of *Cancer* are resident to Puget Sound: *C. magister, C. productus, C. gracilis,* and *C. oregonensis* [12]. Ovigerous females of all four species release their zoeae from January through April of each year in Puget Sound. Ovigerous *C. magister* were collected in central Puget Sound (Port Gardner) using a beam trawl at a depth of 20 to 60 m. *C. oregonensis* were collected in northern Puget Sound (Lopez Pass, San Juan Islands) using a rock dredge in 15 to 30 m of water. *C. productus* and *C. gracilis* were both collected by SCUBA at 3 to 10 and 3 to 15 m of depth in central Puget Sound (Alki Point and Port Ludlow, respectively). After collection, ovigerous females were held in frequently renewed, 8 to 10°C water and immediately transported to our laboratory, where they were held in recirculating culture facilities until zoeae release. Test organisms were identified by a qualified expert in cancroid crab taxonomy.

Adults were checked daily for zoeal release, and on release adults were placed individually into 28-L aquaria containing fresh, filtered natural seawater. This was done in the evening to facilitate collection of newly released zoeae (<20 h old) the following morning. Zoeae (1,000–2,000) were extracted by dipping a 1,000-ml beaker into the aquarium and slowly filling it with water. To allow for prezoeael molting, zoeae were held for several hours prior to being introduced to the test chambers but were still <24 h old at the onset of testing. This molt requires 1 h in *C. magister* and is presumably similar in the other species, although no information could be found in the literature [13]. Wide-bore, smooth glass tubes (4–8-mm i.d.) with rubber bulbs were used to transfer test organisms.

### *Physical system*

The test chambers used in the acute static renewal toxicity tests were 600-ml borosilicate glass beakers chemically cleaned following American Society of Testing and Materials procedures [14]. Each beaker contained 400 ml of test medium. They were placed in an environmental chamber at 10 ± 1°C and covered with Parafilm® (American National Can, Joplin, MO, USA) to reduce loss of cyanide via volatilization.

Culture and dilution water consisted of aged, filtered (10 μm) natural seawater collected from the National Marine Fishery Service laboratory located in central Puget Sound (Mukilteo), Washington, USA. The water was transported to an outdoor storage tank, located at our laboratory, where it was circulated continuously through a 5-μm carbon core filter and ultraviolet sterilizer for 2 to 5 d prior to use.

### *Test material*

The test material was reagent-grade sodium cyanide (NaCN) purchased from Mallinckrodt Inc. (St. Louis, MO, USA; Lot 7616KMAC). To evaluate the possible loss of cyanide over time, all test concentrations were analytically verified at 0, 48, and 96 h. Two samples were collected at 48 h, one before and one after test solution renewal, so that a total of four samples per test concentration were analyzed for cyanide. This sampling scheme was followed for all tests except the first test with *C. magister,* in which test concentrations were measured only at test initiation and termination.

### *Test procedures*

Toxicity tests were 96-h static renewal tests with test solution renewal at 48 h just after test organisms were fed in accordance with U.S. EPA test methods [15]. During test solution renewal at 48 h, beaker water level was reduced so that 15 to 20% of the test solution remained. New test solution was then added slowly to minimize disturbance of the zoeae and reduce cyanide volatilization. For each test, six test concentrations and a control were replicated five times. Test concentrations for studies presented in this paper were based on preliminary range-finding tests. Several tests (not presented) did not adequately bracket the LC50, were considered unacceptable, and were repeated. Test beakers were positioned randomly and labeled by replicate and concentration. Four replicates were used for collection of biological data; the fifth was a monitoring replicate in which daily water-quality measurements were taken. Zoeae were added randomly to all beakers (including monitoring replicates) until each contained 10 organisms.

Zoeae were fed *Artemia* sp. nauplii ad libitum prior to test initiation and just before solution renewal at 48 h. Preliminary testing demonstrated poor survival (<50%) in 96-h tests with

Table 2. Summary of biological and analytical results. ND = nondetect (<4 μg/L)

| Test | Test concentrations μg/L CN | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | A | B | C | D | E | F | LC50 (95% CI) |
| *Cancer gracilis* #1 | | | | | | | | |
| Measured CN[a] | ND | 102 | 125 | 169 | 207 | 274 | 354 | |
| % Survival[b] | 90 ± 0 | 70 ± 26 | 63 ± 25 | 50 ± 8 | 0 | 0 | 0 | 153 (133–174) |
| *Cancer gracilis* #2 | | | | | | | | |
| Measured CN | ND | 96 | 129 | 140 | 199 | 242 | 317 | |
| % Survival | 95 ± 6 | 88 ± 10 | 73 ± 15 | 33 ± 10 | 3 ± 5 | 0 | 0 | 135 (128–143) |
| *Cancer magister* #1 | | | | | | | | |
| Measured CN | ND | 4 | 7 | 9 | 25 | 40 | 60 | |
| % Survival | 90 ± 8 | 93 ± 5 | 98 ± 5 | 93 ± 5 | 90 ± 8 | 98 ± 5 | 23 ± 10 | 51 (49–54) |
| *Cancer magister* #2 | | | | | | | | |
| Measured CN | ND | 42 | 54 | 66 | 82 | 113 | 151 | |
| % Survival | 90 ± 8 | 95 ± 6 | 83 ± 5 | 85 ± 6 | 45 ± 13 | 28 ± 10 | 10 ± 8 | 92 (82–101) |
| *Cancer oregonensis* #1 | | | | | | | | |
| Measured CN | ND | 37 | 46 | 61 | 78 | 97 | 108 | |
| % Survival | 100 | 100 | 100 | 100 | 95 ± 10 | 88 ± 5 | 50 ± 32 | 111 (105–126) |
| *Cancer oregonensis* #2 | | | | | | | | |
| Measured CN | ND | 74 | 109 | 131 | 141 | 206 | 294 | |
| % Survival | 98 ± 5 | 90 ± 8 | 90 ± 14 | 88 ± 10 | 55 ± 10 | 5 ± 6 | 0 | 154 (146–164) |
| *Cancer productus* #1 | | | | | | | | |
| Measured CN | ND | 104 | 140 | 177 | 225 | 266 | 332 | |
| % Survival | 98 ± 5 | 95 ± 6 | 88 ± 25 | 78 ± 10 | 50 ± 8 | 15 ± 13 | 0 | 219 (204–231) |
| *Cancer productus* #2 | | | | | | | | |
| Measured CN | ND | 105 | 136 | 173 | 213 | 281 | 345 | |
| % Survival | 93 ± 10 | 53 ± 15 | 15 ± 10 | 8 ± 10 | 0 | 0 | 0 | 107 (96–118) |

[a] Geometric mean of measured test concentrations.
[b] Mean ± standard deviation of four replicates of 10 organisms each.

unfed zoeae. The number of live zoeae in each test chamber was recorded daily, and the dead were removed. Mortality was defined as lack of movement on gentle agitation. Each test was deemed acceptable if laboratory control mortality did not exceed 10%. Two tests with zoeae from separate females were conducted with each species.

### *Water quality*

At test initiation and every 24 h, water temperature, pH, salinity, and dissolved oxygen were recorded from each treatment's monitoring replicate. Dilution water was aerated prior to testing to ensure that dissolved oxygen exceeded 90% of saturation at test initiation. Water temperatures were maintained at 10 ± 1°C in an environmental chamber. Lighting (10 h light, 14 h dark) was provided with fluorescent bulbs at 50 to 100 foot candles.

### *Analytical chemistry*

Cyanide concentrations were verified using the weak acid disassociable method 4500-CN method I [16]. In this method, hydrogen cyanide is evolved and collected from the water sample using reflux distillation for 1 h with the sample buffered at pH 4.5 using an acetate buffer. The hydrogen cyanide is then quantified using a colorimetric technique with a method detection limit of 4 μg/L. Duplicate samples, matrix spikes, and a standard reference material were analyzed with each batch of samples analyzed.

### *Data analysis*

Mortality data were used to statistically estimate the 96-h median lethal concentration (LC50) and its 95% confidence interval. The LC50s were based on the geometric mean of cyanide concentrations measured over the test duration. The geometric mean of measured test concentrations was considered an appropriately conservative estimate of exposure because of the slight loss of cyanide observed over the course of each test. These statistics were estimated using either probit analysis or binomial probability, depending on the quality of the concentration–percentage response (e.g., presence or absence of 100% response, number of partial responses). Statistical analyses were performed using the TOXIS software package [17].

## RESULTS AND DISCUSSION

### *Water quality*

Water quality was adequately maintained for all tests. Test temperatures were maintained at 10 ± 1°C. Salinity for all tests was maintained at 28 ± 1 g/L. Test solution pH was maintained at 7.7 ± 0.2 standard units and dissolved oxygen at 9.0 ± 0.3 mg/L, precluding the need for aeration during testing.

### *Analytical chemistry*

Measured test concentrations and LC50s calculated on the basis of these concentrations are summarized in Table 2. On average, measured cyanide concentrations were 73% of nominal and relatively stable over each test duration. Coefficients of variation within a test concentration over time averaged 28 ± 19%. Relatively high (>50%) coefficients of variation were seen only in the first *C. magister* test, in which test concentrations were measured only at test initiation and termination.

Coefficients of variation for two test concentrations were high (95% and 67%), but the absolute variation in cyanide concentration was low, with standard deviations of 3.5 and 6.4 μg/L CN, respectively. The relative percentage difference for duplicate samples ($n = 11$) ranged from 0 to 12.5% for all testing. Percentage recovery of matrix spikes ($n = 14$) ranged from 88 to 112%, while recoveries for a standard reference material ($n = 10$) ranged from 87 to 112%.

*Biological results*

All tests reported here exhibited < 10% mortality in the control. Ninety-six-hour LC50s ranged from 51 to 219 μg/L cyanide for all four species. *C. magister* appeared significantly more sensitive than the other three species; the geometric mean of the two LC50s (species mean acute value) of 68 μg/L was lower than those (131, 153, and 144 μg/L cyanide) calculated for *C. oregonensis*, *C. productus*, and *C. gracilis*, respectively (Table 2).

These tests reveal West Coast species of *Cancer* resident to Puget Sound to be less sensitive to cyanide than the U.S. eastern coast (East Coast) yellow rock crab, *C. irroratus*. On average, West Coast species were 24 times less sensitive than *C. irroratus*. The reasons for the significant differences in sensitivity between East Coast and Puget Sound *Cancer* spp. are unclear. Species within a genus are expected to be similar in sensitivity, normally differing in LC50s by less than one order of magnitude [18,19]. Comparative data between East Coast and West Coast *Cancer* spp. are available only for one other chemical: cadmium. Acute toxicity tests resulted in LC50s of 250 and 247 μg/L cadmium for *C. irroratus* and *C. magister*, respectively, suggesting comparable sensitivity [8].

Differences in cyanide speciation and, therefore, toxicity between the studies is unlikely, as hydrogen cyanide is the dominant (>94%) form of free cyanide in waters below pH 8.3 and a temperature of 25°C [3]. The only apparent difference between our study and the *C. irroratus* study was a higher test temperature (20°C) for the latter. Several attempts were made to conduct tests at 20°C with *C. productus*, but the zoeae did not tolerate the higher temperature, and excessive (>70%) control mortality was observed.

We propose replacing the *C. irroratus* data in the cyanide toxicological database [1] with that for Puget Sound *Cancer* spp. as a site-specific criterion. This proposal appears justified because *C. irroratus* is not resident to Puget Sound and because data for all resident species of this genus have been generated, ensuring protection of the local *Cancer* species. According to Stephan et al. [7], when data are available for multiple species within a genus and one datum differs by a factor of 10 or more from other data, then these data should be considered an outlier and excluded from the data set. In this case, the West Coast *Cancer* data are consistent (within a factor of 4), while the *C. irroratus* data are different by greater than a factor of 10. Using only the West Coast *Cancer* data, species mean acute values and genus mean acute values were calculated according to Stephan et al. [7]. The genus mean acute value was then incorporated into the U.S. EPA [1] data set (Table 3). In the United States, genus mean acute values are usually used to derive WQC [7].

Based on these recalculations, cyanide's revised FAV is 18.8 μg/L, and its criterion maximum concentration is 9.4 μg/L. Because *Cancer* spp. no longer drives cyanide's WQC, the final chronic value would be obtained by multiplying the geometric mean of the acute–chronic ratios (ACRs) available for marine species by the FAV. However, because there are currently only two ACRs available for marine species and at least three are required by the guidelines, it was necessary to consider freshwater data in developing a final ACR [7]. Chronic toxicity tests conducted in freshwater are used to supplement the marine chronic database on the thesis that freshwater and saltwater organisms differ insignificantly in sensitivity to chemicals whose toxicity is unaffected by salinity [18,19]. Cyanide satisfies this criterion. The geometric mean for the two marine cyanide ACRs of 1.62 and 8.31 and the four freshwater cyanide ACRs of 9.11, 10.59, 7.63, and 7.32 was calculated to derive the final ACR of 6.45. The freshwater ACR of 68.29 for the isopod *Asellus communis* was not included because it was an order of magnitude higher than the other ACRs and because the species is acutely resistant to cyanide. The ACR for *A. communis* was excluded by U.S. EPA for the same reasons when it derived the final freshwater ACR for cyanide [1]. Applying the final ACR to the FAV results in a final chronic value of 2.9 μg/L cyanide. The latter is an estimate of the chronic threshold concentration for marine species exposed to bioavailable forms (free) of cyanide.

Table 3. Revised cyanide water-quality criterion database using West Coast, USA, *Cancer* toxicity data. Final acute value = 18.8 μg/L; criterion maximum concentration = (18.8 μg/L)/2 = 9.4 μg/L; acute: chronic ratio = 6.45; final chronic value = 2.9 μg/L

| Rank | Species | Genus mean acute value (μg/L) |
|---|---|---|
| 8 | Common Atlantic slippershell, *Crepidula fornicata* | >10,000 |
| 7 | Amphipod, *Ampelisca abdita* | 995.9 |
| 6 | Winter flounder, *Pseudopleuronectes americanus* | 372 |
| 5 | Sheepshead minnow, *Cyprinodon variegatus* | 300 |
| 4 | Cancroid crabs, *Cancer magister, orgegonensis, productus* and *gracilis* | 118.5 |
| 3 | Mysid Shrimp, *Mysidopsis bahia*/*bigelowi* | 118.4 |
| 2 | Atlantic silverside, *Menidia menidia* | 59 |
| 1 | Copepod, *Acartia clausi* | 30 |

In summary, addition of acute cyanide toxicity data for four species of West Coast cancroid crabs showed that, on average, they were 24 times less sensitive than the only East Coast cancroid crab previously tested. When these new data are substituted for the East Coast crab data in the U.S. EPA WQC database to derive site-specific criteria for Puget Sound, the acute criterion increases from 1.0 to 9.4 μg/L and the chronic criterion from 1.0 to 2.9 μg/L cyanide.

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