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Toxicity of Cyanide, Iron-Cyanide Complexes, and a Blast-Furnace Effluent to the Banana Prawn, *Penaeus monodon*

F. Pablo,* R. T. Buckney, R. P. Lim

Department of Environmental Biology and Horticulture, University of Technology, Sydney, Westbourne Street, Gore Hill, NSW 2065, Australia

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Toxicity of cyanides to various aquatic organisms has been reviewed (Eisler 1991; USEPA 1984). However, very few data are on estuarine or marine mysid species. Perhaps the most widely used species overseas for toxicity testing was *Mysidopsis* sp. (Lussier *et al.* 1985). Both acute and chronic toxic data are available, and, in general, the species was found to be sensitive.

The present study evaluates the toxicity of cyanides to an Australian prawn, *Penaeus monodon.* The organism is widely distributed in Australian waters and has ecological and commercial importance in being a source of food for both fish and humans. The toxicity of NaCN and the cyanide complexes $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (commonly found in wastes of blast-furnace operations) was evaluated with the objective that data would be useful for reevaluating Australian water quality guidelines with respect to cyanides. The toxicity of an effluent sample from a blast-furnace rundown episode to *P. monodon* was also evaluated. Additional studies were conducted to investigate the toxicity of iron-cyanide complexes at the sublethal concentration range (below estimated 96-hr LC50 value), at pH of 9 and with slightly increased free cyanide content by spiking with NaCN.

MATERIALS AND METHODS

Banana prawns, *Penaeus monodon,* were obtained from Gold Coast Marine Hatcheries, QLD. When delivered to the laboratory, they were 12 ± 2 days past the post-larval stage and approximately 1-cm long. The prawns were acclimated to laboratory conditions in 50-L glass aquaria at 24°C with a recirculating filter system for at least 5 days before they were used for toxicity tests. They were fed daily with 24-hr post-hatch nauplii of *Artemia salina* until 24 hr prior to testing. Mortality during the acclimation period should be less than 10% for the prawns to be acceptable for use in the toxicity tests. The prawns were used within 1 month of delivery to the laboratory.

The seawater, research grade water, reagents and effluent assessed for toxicity to *P. monodon* were as described in Pablo et al. (1997). The methods for chemical analyses and equipment used for water quality parameters were likewise the same.

The toxicity test consisted of three replicates each of a control and 5 to 6 concentrations of the test chemical. At least 12 hr before the commencement of the test, ~850 mL seawater were transferred into each of 2-L beakers. The beakers were

Present address: Centre for Ecotoxicology, NSW EPA, Westbourne Street, Gore Hill. NSW 2065, Australia
Correspondence to: F. Pablo

covered with plastic wrap, and allowed to acclimate in Labec environmental cabinets set at 23±1°C. An hour before the test, each of the seawater beakers was spiked with an accurately measured stock cyanide solution and stirred to make a homogeneous solution. Meanwhile, the prawns were also acclimated to experimental conditions at least 1 hr before the toxicity test. The prawns were each caught in 20-cm glass tubes (diameter 3 cm) with nylon mesh covering the bottom end, taking care to maintain 10-15 mL of water to prevent the prawn from being exposed to air at any time. Incubating each prawn in a glass tube was necessary to prevent cannibalism.

At the start of the test, 10 prawns in individual glass tubes were transferred randomly to each of the test containers, and the solutions were made up to the predetermined 1-L mark by adding seawater. The test organisms in the test solutions were incubated in the environmental cabinets for 96 hr. In the test for NaCN, the test containers were covered with plasticwrap, and the wrap changed whenever necessary for better maintenance of cyanide in solution. To ensure enough dissolved oxygen for the organisms and to maintain correct cyanide concentrations, the solutions were renewed daily by siphoning out old solution down to ~ 1.5-cm level then siphoning or pouring in the new solution. The test organisms were not fed during the test period.

Dissolved oxygen, pH, salinity and temperature were monitored daily, before and after solution renewal. Twenty-mL subsamples from each of the test solutions were taken daily before and after solution change and analyzed for free and total cyanides. The number of mortalities in each container was noted daily. Death was assumed when the organisms failed to respond to very gentle prodding and there was no movement of legs. The concentration causing mortality to 50% of the test organisms over 96 hr, the 96-hr LC50 value, was determined for each of the test chemicals using the trimmed Spearman-Karber method (Hamilton *et al.* 1977, 1978). Comparison between estimated LC50 values was done by t-test from the SYSTAT package (Kirby, 1993).

A toxicity test of a reference toxicant, dodecyl sulphate sodium salt (DSS), to the same batch of test organisms was conducted concurrently with each cyanide test using a control and 5 concentrations of the DSS. This was conducted to assess the health of the test organisms, laboratory water quality and the experimental conditions for the toxicity test by determining if the LC50 value was within acceptable limits of variability. The acceptable range as determined by the UTS-Centre for Ecotoxicology for DSS to *P. monodon* is 30-60 mg L^{-1}. The procedure for the reference toxicant test followed the standard procedure described above but without replication.

Below are the different tests conducted to investigate the toxicity of NaCN and varying solution compositions of iron-cyanide complexes to *P. monodon.* All tests followed the standard procedure outlined above, involving daily solution renewal with measurement of free and total cyanide concentrations before and after each renewal.

(i) Acute toxicity tests of NaCN, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ to *P. monodon* (appropriate concentrations determined from preliminary range-finding tests).
(ii) Lethal effects of blast furnace effluent on *P. monodon* assessed in 3 replicates each of a seawater control and the following concentrations of effluent: 1.56, 3.12, 6.25, 12.5, 25, 50, 75 and 100%.
(iii) Toxicity of low concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ to *P. monodon,* within a range below their 96-hr LC50 values.
(iv) Toxicity of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ to *P. monodon* at pH of 9. pH of seawater used in tests was adjusted to 9.0 ± 0.1 by dissolving NaOH pellets directly into the water at least 24 hr prior to the test, Concentration ranges used were 2-32 and 20-70 mg L^{-1} as CN for $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, respectively. Seawater controls at natural and elevated pHs were also included in tests.
(v) Toxicity of a mixture of NaCN and $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$ to *P. monodon.*

Test consisted of a control, 2 concentrations of NaCN, 1 concentration of the iron-cyanide complex , and 2 solutions of the mixture of the two compounds.

RESULTS AND DISCUSSION

Results of the preliminary range-finding tests of NaCN, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ against *P. monodon* showed that the following concentration ranges were appropriate for use in definitive tests: 0.025 to 0.15, 2 to 32 and 10 to 105 mg L^{-1} as CN for NaCN, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, respectively. (All concentrations are expressed as total cyanide, as weight of CN per liter solution. It should be noted that for NaCN, the free and total cyanide concentrations are equal; whereas for iron cyanide complexes, the concentration as free cyanide is much less than the total cyanide.)

The results for NaCN showed that prawns exposed to concentrations greater than 150 µg L^{-1} were affected during the first 24 hr of the test. Shortly after exposure to high concentrations of free cyanide, the prawns exhibited slower swimming movements compared to controls. When they eventual] y died, they were lying at the bottom of the tubes and were mostly pink-colored. Hence, free cyanide concentrations > 150 µg L^{-1} were rapidly lethal to *P. monodon.* Prawns exposed to $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ and lower concentrations of NaCN (< 50 µg L^{-1}) were not as rapidly affected by the chemical. Mortality increased with increasing exposure time. The mortality rate was most likely affected by the varying sensitivities of the prawns. Some prawns (mostly those exposed to iron-cyanide complexes) gradually lost their swimming ability and moved to the water surface presumably in an attempt to breathe in air. This was manifested by their jumping movements at the surface with some eventually dying when they were stuck to the wall of the glass tube above the water, It was also observed that there was a large incidence of moulting especially among animals exposed to iron-cyanide complex. The mortality data at low concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ was non-monotonic. This was explored further by conducting additional toxicity tests to *P. monodon* involving concentrations of complexes below their LC50 values (see below).

The order of toxicity as 96-hr LC50 values based on total cyanides was NaCN > $K_3Fe(CN)_6$ > $K_4Fe(CN)_6$ (Table 1 (column 2)). The toxicity of the iron-cyanide complexes was negligible on the basis of total cyanide compared with the toxicity of NaCN, consistent with earlier reports on very low toxicity of metallocyanide complex solutions to fish (Doudoroff *et al.* 1966; Doudoroff 1956). Obviously, the solutions were less toxic than NaCN, as they were made up predominantly of less toxic $[Fe^{III}(CN)_6]^{3-}$ and $[Fe^{II}(CN)_6]^{4-}$ ions. The LC50 value obtained for NaCN to *P. monodon* was within the range of acute values (93-124 µg L^{-1}) estimated for *Mysidopsis* sp. (USEPA 1984). This indicates that *P. monodon* has comparable sensitivity to free cyanide as that of other saltwater mysids.

In the NaCN test solutions, cyanide was exclusively in the free form, mainly as volatile molecular HCN and partly as ionic CN^-. Hence, monitoring of free cyanide was important to ensure that the test organisms were being exposed to accurate cyanide concentrations. It was observed that the measured free cyanide levels were

Table 1. Estimated 96-hr LC50 values from toxicity tests of NaCN, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ at normal and pH 9 to *Penaeus monodon.*

	96-hr LC50 (95% confidence limits) (mg CN L^{-1})	
	at normal pH (~8)	at pH ~9
NaCN	0.110 (0.092 - 0.132)	-
$K_3Fe(CN)_6$	9.1 (6.2 - 13.2)	2.70 (2.50 - 2.92)
$K_4Fe(CN)_6$	60.8 (34.8 - 106.2)	2.41 (1.53 - 3.80)

within 10% of nominal values. The deviation of the measured from nominal concentrations ranged from 0.15 to 27% (average of 8.7%), which was acceptable considering the variability in the analysis of free cyanide and expected losses of HCN. Lost HCN was replenished by daily renewal of test solutions.

In solutions of $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$, the main component was the undissociated complex anion ($[Fe^{III}(CN)_6]^{3-}$ or $[Fe^{II}(CN)_6]^{4-}$), while the concentration of free cyanide was very low. These complexes are subject to dissociation on exposure to UV. The HCN component could be lost to the atmosphere (USEPA 1984). Hence, both free and total cyanide concentrations were monitored during the course of the tests. The measured 96-hr average total cyanides in various concentrations of iron-cyanide solutions were close to nominal concentrations, with average deviations of 1.9 and 3.1% for $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, respectively; while the daily average deviation of measured 96-hr free cyanide concentrations was only 13.5%. This indicated that the complexes were stable and losses of HCN were negligible.

At the LC50 value for $K_3Fe(CN)_6$ of 9.1 mg L^{-1}, the measured free cyanide was greater than 60 µg L^{-1}. This amount of cyanide in the free form may account for most of the toxicity of $K_3Fe(CN)_6$ to *P. monodon,* Figure l(a) shows a good correlation between toxicity of $K_4Fe(CN)_6$ and NaCN to *P. monodon* based on measured free cyanides, indicating that, as in $K_3Fe(CN)_6$, the toxicit of $K_4Fe(CN)_6$ was due mainly to the free cyanide. The complex anions $[Fe^{III}(CN)_6]^{3-}$ and $[Fe^{II}(CN)_6]^{4-}$ had insignificant toxicity. Possibly, the slight toxicity of the anions may have been due to their dissociation to ferric or ferrous and CN- ions (and, consequently, HCN) when HCN penetrates the cells or when the organisms absorb iron.

The blast-furnace effluent examined for toxicity to *P. monodon* was a complex mixture of waste products containing free and complex cyanides. The measured pH and salinity of the effluent were 7.97 and 33 ‰, respectively, which matched the dilution seawater used in the test. The measured free and total cyanide concentrations were 473.9 and 708.1 ppb, respectively. In the toxicity test of effluent to *P. mono don*, the measured free and total cyanide concentrations in various diluted effluent solutions were reasonably maintained during the duration of the test.

The estimated 96-hr LC50 value of the effluent was 28.2% (95% confidence limits 23.5 and 33.9%). Figure 1(b) shows a comparison of cumulative % mortalities of *P. monodon* in NaCN and different concentrations of effluent based on measured free cyanide. The figure clearly shows good correlation of the toxicity of effluent with free cyanide concentration. Interpolation of average free cyanide concentration at the estimated 96-hr LC50 value (using the two closest effluent concentrations) gives 119.6 µg L^{-1}. This free cyanide is within the 95% confidence limits of the 96-hr LC50 value for NaCN alone (Table 1). The results clearly indicate that cyanide is the significant cause for toxicity in the blast-furnace effluent.

As mentioned earlier, the mortality data at low concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ showed non-monotonic behavior. This has been explored by conducting additional toxicity tests on *P. monodon* involving concentrations of iron-cyanide complexes below their estimated 96-hr LC50 values (Table 1). It was thought that another LC50 value, at a lower concentration than what had already been found, could be derived. Four replicates each of five low concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ were set up (Table 2). The measured total cyanide concentrations closely agreed with those of nominal values, and 96-hr average free and total cyanide concentrations had low variabilities. The mortality data, also given in Table 2, show high variability across 4 replicates and non-monotonic behavior. The total number of mortalities for each concentration was less than 50%. With the results obtained, another LC50 value could not be determined apart from what had already been estimated.

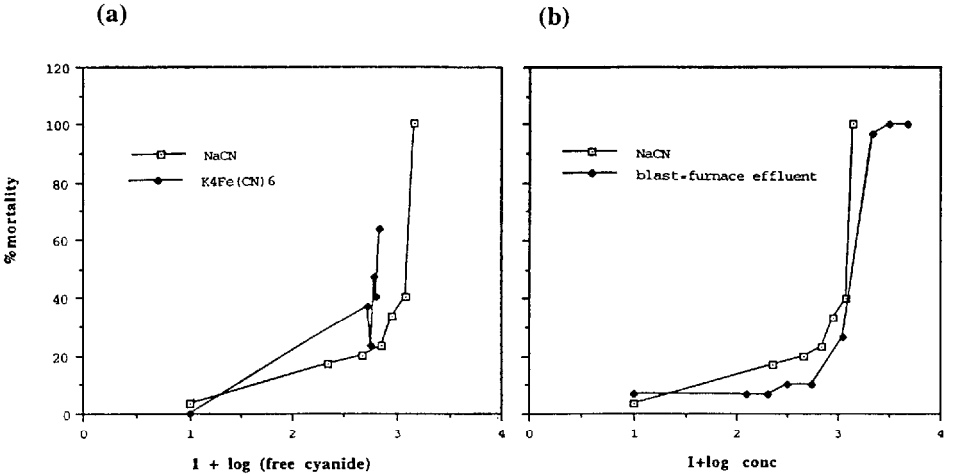

Figure 1. Comparison of mortalities of *Penaeus monodon* in tests with NaCN and (a) $K_4Fe(CN)_6$; (b) blast-furnace effluent on the basis of free cyanide concentrations.

Table 2. Measured 96-hr average free and total cyanide concentrations and number of mortalities of *Penaeus monodon* in the toxicity tests involving low, narrow concentration ranges of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$.

Nominal cone (mg CN L^{-1})	Measured ave 96-hr cone ± s.d		% mortality			
	free cyanide (µg L^{-1})	total cyanide (mg L^{-1})	Set 1	Set 2	Set 3	Set 4
$K_3Fe(CN)_6$						
0	<dl[a]	<dl	0	0	10	0
1	56.0 ± 3.3	1.14±0.19	20	20	20	20
2	62.0±0.4	2.12±0.21	0	30	20	10
4	b	3.99±0.32	0	10	30	20
6	b	5.91±0.34	10	20	50	30
8	b	7.67±0.28	10	30	10	0
$K_3Fe(CN)_6$						
0	<dl[a]	<dl	0	0	0	10
10	51.6±2.6	10.6±1.4	20	10	20	30
20	62.9±7.2	19.9±2.7	40	20	10	20
30	67.3±9.2	29.5±2.1	30	30	40	10
40	76.2±11.2	39.1±2.8	60	60	30	20
50	77.0±10.0	49.5±2.8	20	30	30	50

[a]detection limit ~10 µg L^{-1}

[b]Measurement of free-cyanide by potentiometry in ≤ 3 mg L^{-1} (as CN) $K_3Fe(CN)_6$ was not reliable (at temperature ~25°C).

Perhaps the non-monotonic behavior of the mortality data with iron-cyanide complexes was due to individual differences in the organisms' health and/or physiology. and, hence, differences in the way they responded to the different chemical species constituting the iron-complex solutions. After all, the solutions of iron-cyanide complexes consisted (among many possible equilibria existing in

seawater) of competing equilibria of dissociation of ferri- or ferro-cyanide complex and HCN, and reduction/oxidation of Fe^{III}/Fe^{II} depending on available dissolved 02 and other natural oxidizing agents. Such a complicated equilibria system did not exist in NaCN solutions, where response behavior by *P. monodon* was monotonic.

The toxicity of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ to *P. monodon* at pH 9 was conducted to determine whether a higher pH (within tolerable limits of the organisms) would provide protection to the organisms from toxicity of free cyanide. Simple calculations utilizing K_{HCN} and K_f for the iron-cyanide complexes show that the HCN content of solutions of iron-cyanide complexes are lower at pH 9 than at pH 8.

The 96-hr total cyanide concentrations of different test solutions showed close agreement with nominal concentrations, as well as a low variability over time (Table 3 column 3). On the other hand, free cyanide concentrations of the iron-cyanide complex solutions at pH 9 (Table 3 column 2) were not as low as that predicted by calculations. These observations indicate that either the various equilibria in the solution take relatively longer to establish or there were far more competing equilibria to be considered for making better predictions on the amounts of free cyanide.

The slightly higher pH of 9 caused changes in the positions of the H^+/OH^--sensitive equilibria existing in the system. Among these were the dissociation of HCN and consequently that of ferri- or ferro-cyanide complex, and the volubility of ferric and for ferrous hydroxide. In fact, it was observed that the presence of either $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in seawater whose pH was pre-adjusted (at least 24 hr prior to use) to -9 caused the final pH of the solution to drift by approximately 0.3 units overnight. (The measured pH of test solutions decreased overnight from an average of 9.06 ± 0.03 to 8.68 ± 0.06.) This indicated that some OH^- had been removed from solution after standing overnight and involved in displacing some equilibria. Possibly, ferric or ferrous hydroxides and their complexes were formed

Table 3. Measured 96-hr average free and total cyanide concentrations and cumulative % mortalities over 96 hr of *Penaeus monodon* in different concentrations of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ at pH 9.

Nominal cone (mg CN L^{-1})	Mean 96-hr cone ± s.d		Cumulative % mortality			
	free cyanide (µg L^{-1})	total cyanide (mg L-1)	24 hr	48 hr	72 hr	96 hr
$K_3Fe(CN)_6$						
0 (at normal pH)	<dl[a]	<dl	0	0	0	0
0	<dl	<dl	0	0	0	0
2	54.2 ± 8.6	1.98±0.11	0	0	0	17
4	64.0±15.3	3.84±0.27	37	57	90	93
8	b	7.82±0.27	50	63	93	100
16	b	15.5±0.78	53	73	100	100
32	b	31.0±1.4	47	73	100	100
$K_4Fe(CN)_6$						
0 (at normal pH)	<dl[a]	<dl	0	0	0	0
0	<dl	<dl	0	3	3	7
20	55.1±4.0	20.4±2.7	53	80	83	83
50	63.8±5.9	48.1±3.6	73	97	97	97
70	70.4±6.4	68.6±3.8	87	93	93	97

[a]detection limit ~10 µg L^{-1}

[b]Measurement of free cyanide by potentiometry in > 4 mg L^{-1} (as CN) $K_3Fe(CN)_6$ were not reliable (at temperature ~20°C).

while CN^- concentrations increased (with corresponding decrease in HCN) from the reaction of OH^- with HCN and the Fe^{III} or FeII of the iron-cyanide complexes.

Data summarized in Table 3 show a mortality of 7% in the controls at pH 9 of the $K_4Fe(CN)_6$ test after 96 hr and none in other controls at normal pH. The remaining organisms at the end of each test in the high pH solutions did not appear to be well or actively swimming as those in the normal pH solutions. The presence of more OH^- and less H^+ in the water may have had a gradual effect on the organisms. However, the presence of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in seawater at pH ~9 caused greater % mortality (but slowly lethal) in *P. monodon* compared with complementary solutions at normal pH (see Table 1). The greater toxicity was reflected in the much lower estimated 96-hr LC50 values obtained at pH 9 compared with those obtained at normal pH (Table 1). This observation is in agreement with the trend (10- to 13-fold increase in toxicity) reported by Doudoroff (1976) for the toxicity of tetracyanonickelate complex to fish at higher pH values of 7.4 to 7.8 (freshwater). The greater number of mortalities observed here for the iron-cyanide complexes at pH 9 could not be mainly due to cyanides since, as mentioned earlier, lower free cyanide levels were actually measured. Perhaps, the greater toxicity could have been due to absorption of both OH^- and HCN (and perhaps iron) by the organisms which together had greater adverse effects internally. Hence, instead of providing protection to *P. monodon,* $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ solutions at the higher pH of 9 were more harmful.

Table 4. Measured 96-hr average free and total cyanide concentrations and cumulative % mortalities over 96 hours of *Penaeus monodon* in toxicity tests of NaCN, (a) $K_3Fe(CN)_6$ or (b) $K_4Fe(CN)_6$, and their combinations.

(a) $K_3Fe(CN)_6$

Test solution		Mean 96-hr cone ± s.d		Cumulative % mortality			
$K_3Fe(CN)_6$ (mg CN L^{-1})	NaCN (µg L^{-1})	free cyanide (µg L^{-1})	total cyanide (mg L^{-1})	24 hr	48 hr	72 hr	96 hr
Control		<dl[a]	<dl[b]	0	0	3	3
-	50	49.4±5.0	c	3	3	7	7
-	100	94.6±8.0	c	10	13	20	27
4	-	53.4±7.2	3.87±0.20	17	33	47	47
4	50	65.2±5.5	3.91±0.18	27	47	57	67
4	100	81.4±9.9	3.97±0.22	63	80	90	90

(b) $K_4Fe(CN)_6$

Test solution		Mean 96-hr cone ± s.d		Cumulative % mortality			
$K_4Fe(CN)_6$ (mg CN L^{-1})	NaCN (µg L^{-1})	free cyanide (µg L^{-1})	total cyanide (mg L^{-1})	24 hr	48 hr	72 hr	96 hr
Control		<dl[a]	<dl[b]	3	3	3	3
-	50	48.1±4.9	c	7	17	17	17
-	120	105.8±14.2	c	0	7	13	27
30	-	56.8±8.2	29.2±3.7	7	23	30	37
30	50	76.5±11.5	26.9±2.9	3	20	27	43
30	120	108.7±6.8	29.0±3.5	13	37	57	60

[a]detection limit ~2 µg L^{-1}

[b]lowest detectable concentration ~10 µg L^{-1}

[c]total cyanide = free cyanide

In experiments where iron-cyanide complex solutions were spiked with NaCN, the measured free cyanide concentrations were higher than those where NaCN was not added (Table 4). Total cyanide concentrations measured for the solutions were close to nominal concentrations, ensuring accurate preparation of solutions (Table 4). Comparison of the mortality data obtained for NaCN alone, either iron-cyanide complex alone, and iron-cyanide complexes spiked with NaCN showed that greater mortalities of *P. monodon* were recorded where CN^- was added to either complex (Table 4). The greater the amount of NaCN added (and consequently greater free cyanide), the greater the increase in % mortality. The results show that *P. monodon* was very sensitive to increases in free cyanide in solutions predominantly made up of iron-cyanide complex. These observations are consistent with the earlier point made and published findings (Doudoroff *et al.* 1966; Doudoroff 1956), that in solutions of iron-cyanide complexes, the toxicity is mainly due to free cyanide (HCN + CN-).

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