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Toxicity of Mercury to Growth and Survival of Seabass Larvae, *Lates calcarifer* and the Modifying Effects of Salinity

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ABSTRACT: Short-term chronic toxicity tests were conducted to investigate the adverse effects of mercury (Hg) on growth (measured as dry weight) and survival of seabass larvae, *Lates calcarifer,* and the modifying effects of salinity. Seven-day static-renewal tests were conducted at four salinities: 2, 10, 20 and 30 psu. The experiments were repeated three times for each salinity. Results indicated that seabass larvae were very sensitive to low concentrations of Hg. Based on the actual measured Hg concentrations, the mean NOEC, LOEC and LC_{50} values for survival were 30.8, 52.5 and 46.2 μg L^{-1}, respectively, while the mean NOEC, LOEC, IC_{25} and IC_{50} values for growth were 5.2, 12.6, 8.5 and 19.2 μg L^{-1}, respectively. Salinity did not have any significant effects on the toxicity of Hg on survival and growth of seabass larvae. One possible explanation is that Hg preferentially forms very strong complexes with sulfhydryl groups (-SH) in proteins rather than with chloride.

KEYWORDS: mercury, toxicity, seabass larvae, salinity, growth, survival.

INTRODUCTION

Mercury (Hg) is recognized as one of the most toxic pollutants to living organisms because of its toxicity at very low concentration. Mercury can be bioaccumulated and biomagnified in aquatic food chains[1,2] and can adversely affect human health through seafood consumption. The effects of Hg toxicity are well documented after the Minamata incident in Japan during 1953 to 1961.[3,4]

The toxicity of Hg and other heavy metals is influenced by a number of physico-chemical and biological factors.[5] Variations in physico-chemical factors can modify chemical speciation of metals because the various existing chemical species have a different capacity to bind to and/or to cross biological barriers, and consequently to be delivered to accumulatory sites inside the organism and then exert toxic effects.[6] Among these factors, salinity is an important modifying factor. Salinity (or chloride ion) can cause the complexing of inorganic and organic Hg and influences the chemical speciation of Hg[7] which, in turn, affects its bioavailability and toxicity to living organisms. The toxicity of heavy metals generally increases with decreasing salinity,[8,9] but this remains uncertain with Hg. McLusky et al[10] reported that the effect of salinity on Hg toxicity was not well defined. Although there was a trend of increased toxicity at lower salinities, the differences were not statistically significant; however, no explanations were provided for these findings.

This study was undertaken to investigate the toxic effects of Hg on growth and survival of seabass larvae, *Lates calcarifer,* and the modifying effects of salinity on Hg toxicity. Toxicity tests with the embryo-larval and early juvenile life stages of fish, which are the most or among the most sensitive life stages, can be used to estimate the safe concentrations to fish over their entire life cycle and can also be useful in establishing water quality criteria.[11] *L. calcarifer* was selected as a test organism because of its economic importance in Thailand and in other ASEAN (Association of South-East Asian Nations) countries and because of its tolerance to a wide range of salinities. Moreover, it has shown to be a sensitive fish species for toxicity testing[12] and is often used as test species in Thailand. The results obtained from this study will be useful for Thai national organization such as the Pollution Control Department for setting up or developing coastal water quality standard for aquatic life and human health protection.

Materials and Methods

The toxicity of Hg was evaluated by conducting static-renewal tests with seabass larvae, *L. calcarifer*. The 7-d larval fish survival and growth tests were conducted according to the protocol for sublethal toxicity tests using tropical marine organisms described in CPMS-II[13] and standard test procedures outlined in U.S. EPA.[14] The experiments were conducted at four salinities: 2, 10, 20 and 30 practical salinity units (psu) which represent estuarine and seawater conditions. To assess the relatively sensitivity of the test fish from different batches, the experiments were repeated three times for each salinity.

Experimental Setup

Seabass larvae (approximately 10 days old) obtained from a commercial fish hatchery were acclimated to each test salinity in the laboratory for 48-72 h prior to beginning the tests. The fish were fed with newly-hatched *Artemia* nauplii (<24 h old) during the acclimation period. Batches of water used for acclimation and testing at each salinity were prepared by diluting natural filtered seawater with dechlorinated tap water until the desired salinity (±1 psu) was reached. The batches of water were stored and aerated in 100-L polyethylene tanks.

For each salinity, a series of five concentrations of Hg in geometric interval (3.2, 10, 18, 32 and 56 μg Hg L^{-1}), plus a negative control were tested. Treatments were made from a 1,000 μg Hg L^{-1} stock solution prepared by dissolving 0.1354 g of analytical grade mercuric chloride ($HgCl_2$) in 100 mL of distilled water. Tests were conducted in 1-L glass beakers containing 1 L of test solution. Four replicates were prepared for each treatment. Ten fish were randomly distributed into each test container. The fish were fed with newly-hatched *Artemia* nauplii (<24 h old) twice a day on Days 0-6, increasing the amount of food proportionally as the fish grew. *Artemia* nauplii was provided so that there was only a small quantity of uneaten food left in the test containers. The fish were not fed on the last day of the tests (Day 7). The experiments were conducted at ambient temperature (26-30 °C) with 12:12 h light:dark photoperiod. Test solutions were not aerated during the exposure period and were renewed daily.

Water quality parameters (temperature, pH, salinity and dissolved oxygen [DO]) were measured at the beginning of the test and every 24 h in the freshly prepared (new) test solutions, with the exception of DO which was measured in the new and old test solutions. Approximately 90% of the test solutions were renewed daily. In order to confirm the actual Hg concentrations, subsamples of freshly prepared solutions as well as the old solutions after 24-h exposure period were analyzed for Hg by the cold vapor atomic absorption spectrophotometer technique.

The number of surviving and dead fish in each test container were counted and recorded daily. Dead fish and other debris were removed when the solutions were renewed. The criterion for determining fish mortality was the cessation of movement, even after gentle prodding. At the end of the test, the number of surviving fish was counted in each replicate. To determine growth (dry weight), the fish were rinsed in a distilled water ice bath to remove any salts and debris, transferred to pre-weighed foil pans, and dried at 105 °C for a minimum of 6 h. After cooling in a dessicator, the pans were weighed.

For the tests to be considered valid, mean control survival had to be ≥80% and there must be at least a 2- to 4-fold increase in dry weight during the 7-d test period. In addition, water quality parameters had to be maintained within acceptable ranges.

Data Analysis

The endpoints measured in the 7-d larval fish test were adverse effects on survival and growth (measured as dry weight). The statistical endpoints determined were the NOEC, LOEC, LC_{50}, IC_{25} and IC_{50} values. The NOEC (No Observed Effect Concentration) is the highest concentration tested that does not result in a statistically significant adverse response relative to the control. The LOEC (Lowest Observed Effect Concentration) is the lowest concentration tested that results in a statistically significant adverse response relative to the control. Both the NOEC and LOEC values for survival and growth were calculated using the TOXSTAT software program.[15] An LC_{50} (concentration that was lethal to 50% of the test organisms) value was estimated for survival using the EFFL software program. For growth, IC_{25} and IC_{50} values (concentration that resulted in 25% and 50% inhibition on growth, respectively) were estimated by the linear interpolation method using the ICPIN program.[16]

Results and Discussion

Table 1 shows the results of measured Hg concentrations in freshly prepared and old test solutions. Measured Hg concentrations were generally within 20 to 30% of the nominal concentrations. Analyses of the old test solutions showed that the concentrations of Hg were approximately 40-80% lower than the Hg concentrations in the new solutions. Moreover, this reduction in measured Hg concentration was greater at the higher concentrations

Table 1. Nominal and measured mercury concentrations in new (freshly prepared) and old (after 24-h exposure period) test solutions. Values represent the range of concentrations measured in the three experiments of each salinity.

Salinity (psu)	Nominal Hg Concentration (μg L^{-1})	Hg Concentration in New Solutions (μg L^{-1})	Hg Concentration in Old Solutions (μg L^{-1})
2	0	ND	ND
	3.2	2.6 - 4.1	1.6 - 2.3
	10	9.0 - 13.2	4.2 - 5.9
	18	17.2 - 23.4	4.6 - 7.1
	32	31.6 - 40.7	7.2 - 10.3
	56	54.9 - 69.4	10.7 - 17.1
10	0	ND	ND
	3.2	3.1 - 3.2	1.2 - 1.3
	10	10.0 - 10.3	2.3 - 3.6
	18	18.4 - 18.9	4.5 – 5.0
	32	32.4 - 32.8	6.6 - 7.8
	56	54.5 - 56.1	9.2 - 12.1
20	0	ND	ND
	3.2	2.7 - 4.2	1.1 - 2.3
	10	8.2 - 13.2	3.0 - 4.9
	18	14.9 - 23.5	4.8 - 5.9
	32	26.7 - 40.8	7.0 - 8.4
	56	45.1 - 63.1	7.2 - 10
30	0	ND	ND
	3.2	2.5 - 3.7	1.1 - 1.4
	10	8.6 - 10.6	2.3 - 3.3
	18	15.7 - 19.3	3.1 - 4.6
	32	26.9 - 34.1	5.6 - 7.2
	56	47.2 – 70.0	6.7 - 8.7

ND = Not dectected (<0.3 μg L^{-1} Hg)

compared to lower concentrations. A decrease in Hg concentrations after addition to water has been also reported by Sharp and Neff[17] and Laporte et al[6] The loss of Hg from the test solutions was due to volatilization, adsorption on the container walls[18,19] and uptake by the larval fish and *Artemia* nauplii. Based on these data, daily renewal of test solutions is recommended to ensure continuous exposure to the desired Hg concentrations for the duration of the tests. All results reported in this study are based on measured Hg concentrations of the new test solutions. The results of survival and growth of larval seabass after 7-d exposure to various Hg concentrations and at four salinities (2, 10, 20 and 30 psu) are summarized in Table 2. No mortality was observed in any of the controls. For the growth data, mean initial weight per individual fish in the three experiments ranged from 0.13-0.45 mg dry weight. Mean final weight in the controls ranged from 1.35-2.99 mg dry weight. This represented a 7- to 10-fold increase in the weight of the control fish relative to the initial fish weight. This exceeded the criterion of a 2- to 4-fold increase in weight specified in CPMS-II.[13] The variation in fish size used in this study was due to different batches of fish available from the hatchery. However, the difference in initial fish weight did not appear to affect the toxicity of Hg to seabass larvae as indicated by the similar results obtained. Studies on other aquatic species also indicated that Hg toxicity did not change significantly with varying size. Verma et al[20] reported that the size of a freshwater fish, *Notopterus notopterus* ranging from 40 to 120 mm, had no significant effect on the toxicity of Hg to the fish. Similar results were also reported for the white shrimp, *Penaeus setiferus,* by Green *et al.*[21]

A summary of statistical endpoints for each test (NOEC, LOEC, LC_{50}, IC_{25} and IC_{50}) is presented in Table

Table 2. Survival and growth of larval seabass after 7-d exposure to various mercury concentrations. Three experiments were conducted at different times for each salinity and each experiment consisted of four replicates per concentration.

Salinity (psu)	Exp. No.	Nominal Concentration ($\mu g\ L^{-1}$)	Actual Concentration ($\mu g\ L^{-1}$)	Mean Survival[1] (%) (N = 4)	Mean Individual Growth[1] (mg dry weight) (N = 4)	Growth (%of Control) (N = 4)
2	1	Control	<0.3	100	1.35	100
		3.2	4.08	100	1.37	101.5
		10	13.19	100	1.02	75.6
		18	23.39	92.5	0.69*	51.1
		32	40.69	92.5	0.46*	34.1
		56	69.34	40*	0.30*	22.2
	2	Control	<0.3	100	2.83	100
		3.2	2.63	100	2.82	99.7
		10	8.98	100	2.07*	73.1
		18	17.22	100	1.43*	50.5
		32	31.58	95	0.97*	34.3
		56	55.58	77.5*	0.42*	14.8
	3	Control	<0.3	100	1.76	100
		3.2	3.2	100	1.56	88.6
		10	10.1	100	1.09	61.9
		18	18.37	97.5	0.79*	44.9
		32	32.18	92.5	0.53*	30.1
		56	54.89	22.5*	0.47*	26.7
10	1	Control	<0.3	100	1.73	100
		3.2	3.09	100	1.52	87.9
		10	10.03	100	0.95*	54.9
		18	18.42	100	0.68*	39.3
		32	32.52	92.5	0.38*	22
		56	55.50	32.5*	0.16*	9.3
	2	Control	<0.3	100	2.79	100
		3.2	3.08	100	2.76	98.9
		10	10.18	100	1.98*	71
		18	18.38	100	1.27*	45.5
		32	32.77	97.5	0.92*	33
		56	56.09	70*	0.40*	14.3
	3	Control	<0.3	100	1.36	100
		3.2	3.17	100	1.23	90.4
		10	10.3	92.5	0.81*	59.6
		18	18.88	100	0.57*	41.9
		32	32.39	82.5*	0.38*	27.9
		56	54.52	22.5*	0.33*	24.3

3. Statistical comparison (ANOVA) revealed that those endpoints were not significantly different between the four salinities tested ($p > 0.05$), suggesting that salinity had no significant influence on the Hg toxicity on the survival and growth of seabass larvae. The mean NOEC, LOEC and LC_{50} values for survival were 30.8, 52.5 and 46.2 $\mu g\ L^{-1}$, respectively, while the mean NOEC, LOEC, IC_{25} and IC_{50} values for growth were 5.2,

Table 2. (Cont.)

Salinity (psu)	Exp. No.	Nominal Concentration (µg L^{-1})	Actual Concentration (µg L^{-1})	Mean Survival[1] (%) (N = 4)	Mean Individual Growth[1] (mg dry weight) (N = 4)	Growth (%of Control) (N = 4)
20	1	Control	<0.3	100	2.99	100
		3.2	4.21	97.5	2.38	79.6
		10	13.17	100	2.05*	68.6
		18	23.46	92.5	1.45*	48.5
		32	40.80	87.5	0.75*	25.1
		56	63.10	5*	0.25*	8.4
	2	Control	<0.3	100	1.32	100
		3.2	3.13	100	1.25	94.7
		10	10.37	100	1.08	81.8
		18	18.64	92.5	0.71*	53.8
		32	33.31	85	0.66*	50
		56	58.75	32.5*	0.42*	31.8
	3	Control	<0.3	100	2.47	100
		3.2	2.65	100	2.29	92.7
		10	8.18	100	1.63*	66
		18	14.91	100	1.18*	47.8
		32	26.66	97.5	1.06*	42.9
		56	45.11	55*	0.63*	25.5
30	1	Control	<0.3	100	1.28	100
		3.2	3.23	100	1.18	92.2
		10	10.57	100	0.80*	62.5
		18	19.30	90	0.60*	46.9
		32	31.26	40*	0.32*	25
		56	59.06	0*	0*	0
	2	Control	<0.3	100	1.43	100
		3.2	3.72	100	1.27	88.8
		10	10.32	100	1.08*	75.5
		18	19.08	90	0.80*	55.9
		32	34.10	82.5	0.58*	40.6
		56	60.97	20*	0.51*	35.7
	3	Control	<0.3	100	1.90	100
		3.2	2.45	100	1.79	94.2
		10	8.64	100	1.34*	70.5
		18	15.71	90	0.96*	50.5
		32	26.85	97.5	0.81*	42.6
		56	47.2 5	2.2*	0.55*	29

[1]Asterisks indicate values where responses were significantly ($P < 0.05$) lower than the control.
Exp. = Experiment

12.6, 8.5 and 19.2 µg L^{-1}, respectively. From these results, survival was significantly reduced at concentrations greater than 30.8 µg L^{-1}, while growth was significantly reduced at concentrations greater than 5.2 µg L^{-1}.

Water quality parameters recorded during the three experiments ranged from: dissolved oxygen in old test solutions, 2.6-7.0 mg L^{-1}; in new test solutions, 5.9-7.8 mg L^{-1}; temperature, 25.0-29.3 °C; and pH, 6.4-8.4 (Table 4). Salinity ranged within ± 1 psu in each of the

Table 3. Summary of statistical endpoints measured in 7-d larval seabass survival and growth tests. Endpoints were calculated based on actual mercury concentrations

Salinity (psu)	Exp. No.	Survival Effect (µg L^{-1})				Growth Effect (µg L^{-1})					
		NOEC	LOEC	LC_{50}	(95% CI)	NOEC	LOEC	IC_{25}	(95% CI)	IC_{50}	(95% CI)
2	1	40.7	69.3	62.7	(55.1 - 71.2)	13.2	23.4	13.1	(0.2 – 20.1)	24.4	(15.0 - 38.7)
	2	31.6	55.6	> 55.6*		2.6	9.0	8.5	(1.9 – 15.3)	17.8	(10.9 -26.1)
	3	32.2	54.9	44.5	(41.7 - 47.5)	10.1	18.4	6.7	(0.5 – 12.5)	15.9	(5.4 - 24.5)
	Mean	**34.8**	**59.9**	**53.6**		**8.6**	**16.9**	**9.4**		**19.4**	
	SE	**2.9**	**4.7**	**9.1**		**3.1**	**4.2**	**1.9**		**2.6**	
10	1	32.5	55.5	47.5	(43.1 - 52.3)	3.1	10.0	5.8	(0.3 – 8.7)	12.6	(7.0 - 19.9)
	2	32.8	56.1	> 56.1*		3.1	10.2	9.2	(5.9 – 11.4)	16.9	(14.0 - 21.2)
	3	18.9	32.4	42.9	(39.7 - 46.4)	3.2	10.3	6.7	(2.2 – 9.8)	14.9	(7.4 - 19.1)
	Mean	**28.1**	**48.0**	**45.2**		**3.1**	**10.2**	**7.2**		**14.8**	
	SE	**4.6**	**7.8**	**2.3**		**0.03**	**0.1**	**1.0**		**1.3**	
20	1	40.8	63.1	48.2	(44.8 - 51.8)	4.2	13.2	8.0	(0.01 – 20.9)	22.7	(15.9 – 28.0)
	2	33.3	58.8	48.6	(43.4 - 54.5)	10.4	18.6	12.3	(6.1 – 14.9)	32.6	(9.9 - 42.9)
	3	26.7	45.1	> 45.1*		2.7	8.2	6.3	(3.8 – 8.7)	14.0	(10.7 - 22.7)
	Mean	**33.6**	**55.7**	**48.4**		**5.7**	**13.3**	**8.9**		**23.1**	
	SE	**4.1**	**5.4**	**0.2**		**2.4**	**3.0**	**1.8**		**5.4**	
30	1	19.3	31.3	29.1	(26.3 - 32.2)	3.2	10.6	7.4	(4.2 – 9.6)	17.5	(12.8 - 23.7)
	2	34.1	61.0	46.1	(42.5 - 53.0)	3.7	10.3	10.4	(1.9 – 16.6)	24.8	(14.3 - 34.2)
	3	26.9	47.2	> 47.2*		2.5	8.6	7.4	(4.3 – 11.9)	16.7	(10.4 - 26.6)
	Mean	**26.8**	**46.5**	**37.6**		**3.1**	**9.8**	**8.4**		**19.7**	
	SE	**4.3**	**8.6**	**8.5**		**0.4**	**0.6**	**1.0**		**2.6**	
Average four salinities †		**30.8**	**52.5**	**46.2**		**5.2**	**12.6**	**8.5**		**19.2**	

* Excluded from mean LC_{50} calculation (> : greater than)

† Results gave no statistically significant difference among the four salinities tested ($p > 0.05$).

SE = Standard Error; Exp. = Experiment; CI = Confidence Interval

Table 4. Water quality parameters of recorded in 7-d larval seabass survival and growth tests at each salinity

Salinity (psu)	Exp. No.	Temperature (° C)	pH	Dissolved Oxygen (mg L^{-1})	
				Before Renewal (Old Solutions)	After Renewal (New Solutions)
2	1	26.0 - 28.7	7.1 - 7.7	4.0 - 6.8	6.8 - 7.8
	2	25.2 - 28.9	7.2 - 7.8	4.7 - 6.5	7.0 - 7.8
	3	26.0 - 28.4	6.4 - 7.8	5.6 - 7.0	7.3 - 7.8
10	1	27.0 - 28.1	7.1 - 7.8	4.2 - 6.2	7.0 - 7.4
	2	25.6 - 29.3	7.0 - 8.0	4.1 - 5.9	6.8 - 7.5
	3	26.0 - 28.8	6.9 - 7.9	5.1 - 6.7	6.7 - 7.5
20	1	27.5 - 28.5	7.2 - 8.0	2.6 - 6.9	6.1 -7.8
	2	25.0 - 27.6	7.5 - 8.1	4.5 - 6.6	6.8 - 7.2
	3	26.2 - 28.3	7.3 - 8.1	4.5 - 6.1	6.4 - 7.0
30	1	27.2 - 29.2	7.8 - 8.0	4.0 - 5.4	6.0 - 6.2
	2	25.0 - 28.5	7.6 - 8.2	4.1 - 5.9	6.1 - 6.8
	3	26.4 - 28.6	7.7 - 8.4	4.0 - 5.4	5.9 - 6.7

Exp. = Experiment

salinities tested. All water quality parameters were within acceptable ranges and were not likely to have adversely affected test results.

The bioavailability and toxicity of heavy metals are critically dependent upon their chemical speciation.[22] At low chloride (Cl^-) concentrations, the speciation of inorganic Hg is dominated by three uncharged complexes, $HgCl_2$, HgOHCl and $Hg(OH)_2$, with $Hg(OH)_2$ being the most abundant.[23] At high Cl^- concentrations,the anionic $HgCl_4^{2-}$ is the most predominant form[6,23] and has little or no toxicity.[24] Mercury differs from other toxic heavy metals in that the uncharged $HgCl_2$ is the most bioavailable form instead of the free metal ion.[6, 23,24] The concentration of $HgCl_2$ is more abundant at low Cl^- than at high Cl^- concentrations.[6,23] This should result in an increase in Hg toxicity with decreasing salinity, but the results obtained from this study are not consistent with that assessment.

According to the Hard and Soft Acid-Base (HSAB) principles, Hg is a soft acid and can react faster with soft bases especially N- and S- containing ligands,[25,26] but much more strongly to S- than N- containing ligands.[27] Thus a possible mechanism that reduces the effect of Cl^- on Hg toxicity is that Hg preferentially forms strong complexes with sulfhydryl groups (-SH) in proteins[1,3,28,29] than with Cl^-. The sulfhydryl binding would predominate over Hg(II)-Cl complexes, neutralizing effect of Cl^- concentrations. This result is similar to the case of sediments containing high organic matter contents that salinity had no effect on Hg adsorption, attributed to organic complexation predominating over Cl^- complexation.[30] Because fish contain large amounts of proteins, the amount of sulfhydryl groups contained in fish tissues may determine the amount of Hg that could be adsorbed. Mason et al[23] also speculated that Hg may be sequestered within the cell membrane and react very fast with the sulfhydryl groups.

Growth was found to be a more sensitive measure of adverse effects than survival (Table 3). The inhibitory effect of Hg on growth of seabass larvae was probably due in part to the impact of Hg on prey capture ability. The reduction in feeding may be due to a loss of coordination and earlier satiation of hunger from neurotoxic effect by Hg.[31] Mercury also inhibits the intestinal absorption of nutrients such as amino acids and sugars in fish[32] causing a reduction in growth. In this study seabass larvae were fed with *Artemia* nauplii which can tolerate very high concentrations of Hg[24] and can absorb Hg from the test solutions. Consequently, the seabass larvae could uptake Hg via *Artemia* as well. However, the direct uptake of Hg from water occurs almost entirely across the gills, while the uptake through the skin is minimal.[28] Sublethal concentrations of Hg also affect ion transport and osmoregulatory function in fish by

Table 5. Comparison of the toxicity of mercury on larval fish and other tropical organisms

Metal	Species	Life Stage	Size	Test Duration	Temp. (° C)	Salinity (psu)	Exposure Type	Effect Measure	Statistical Endpoint ($\mu g\ L^{-1}$)	Reference
Hg	Seabass, *Lates calcarifer*	~12-d	0.13 -0.35mg	7-d	25.2 - 28.9	2	Static-renewal	Survival	LC_{50} = 53.56	This study
			0.21- 0.35 mg	7-d	25.6 - 29.3	10		Survival	LC_{50} = 45.22	
			0.22- 0.45 mg	7-d	25.0 - 28.5	20		Survival	LC_{50} = 48.40	
			0.17- 0.38 mg	7-d	25.0 - 29.2	30		Survival	LC_{50} = 37.62	
Hg	Seabass, *Lates calcarifer*	~12-d	0.13 -0.35 mg	7-d	25.2 - 28.9	2	Static-renewal	Growth	IC_{25} = 9.44; IC_{50} = 19.37	This study
			0.21- 0.35 mg	7-d	25.6 - 29.3	10		Growth	IC_{25} = 7.21; IC_{50} = 14.82	
			0.22- 0.45 mg	7-d	25.0 - 28.5	20		Growth	IC_{25} = 8.87; IC_{50} = 23.10	
			0.17- 0.38 mg	7-d	25.0 - 29.2	30		Growth	IC_{25} = 8.42; IC_{50} = 19.68	
Hg	Seabass, *Lates calcarifer*	Juvenile	1.5 cm	96-h		30	Static	Survival	LC_{50} = 260	37
Hg	Seabass, *Lates calcarifer*	Juvenile	1.9 - 2.3 cm	96-h	25 - 27	31 - 32	Static	Survival	LC_{50} = 112.8	38
Hg	Seabass, *Lates calcarifer*	Juvenile	2.5 cm	96-h	28 - 29	29	Static	Survival	LC_{50} = 86	39
Hg	Milkfish, *Chanos chanos*	Juvenile	2.7 cm, 0.2 g	96-h	28 - 30	15 - 16	Static	Survival	LC_{50} = 380	40
Hg	Cresent grunter, *Therapon jarbua*	Juvenile	3 - 4 cm	96-h	24 -33	10 - 30	Static	Survival	LC_{50} = 170 - 1000	41
Hg	Estuarine prawn *Penaeus indicus*	Post Larvae	-	48-h 96-h	19 - 24	-	Flow-through	Survival Survival	LC_{50} = 16 lC_{50} = 15	42
Hg	White shrimp, *Penaeus setiferus*	Post Larvae	7 - 35 mm	96-h	21 - 24	25	Static	Survival	LC_{50} = 17	21
Hg	Giant prawn, *Macrobrachium rosenbergi*	Various larval stages	-	96-h	25.5-27.0	12	Static	Survival	LC_{50} = 50 - 340	43

Table 5. (Cont.)

Metal	Species	Life Stage	Size	Test Duration	Temp. (° C)	Salinity (psu)	Exposure Type	Effect Measure	Statistical Endpoint ($\mu g\ L^{-1}$)	Reference
Hg	Giant prawn, *Macrobrachium rosenbergi*	Post larvae (P_{4-5})	-	96-h	28	12	Static	Survival	LC_{50} = 31	44
Hg	Pacific oyster, *Crassostrea gigas*	Embryo	-	48-h	20 ± 1	33.79 ± 0.07	Static	Development	EC_{50} = 6.7	45
Hg	Mussel, *Mytilus edulis*	Embryo	-	48-h	17 ± 1	33.79 ± 0.07	Static	Development	EC_{50} = 5.8	45
Hg	Dungeness crab, *Cancer magister*	Zoeae	-	96-h	15 ± 1	33.79 ± 0.07	Static	Survival	LC_{50} = 8.2	45
Hg	Clam, *Donax faba*		1.0 - 1.5 cm	96-h	27 - 32	-	Static	Survival	LC_{50} = 160	46
Hg	Phytoplankton, *Tetraselmis* sp.	5-d	-	96-h	23 -26	34	Static	Cell densities	IC_{25} = 25; IC_{50} = 34	47
Cu	Seabass, *Lates calcarifer*	12-d	0.63 - 1.67 mg	7-d	27.0 - 30.0	23 -29	Static -renewal	Survival Growth	LC_{50} = > 1400 IC_{25} = > 1400	35
Cd	Seabass, *Lates calcarifer*	12-d	0.63 - 1.67 mg	7-d	27.0 - 30.0	23 -29	Static -renewal	Survival Growth	LC_{50} = 6360 IC_{25} = 3010	35
Zn	Seabass, *Lates calcarifer*	12-d	0.63 - 1.67 mg	7-d	27.0 - 30.0	23 -29	Static -renewal	Survival Growth	LC_{50} = >7330 IC_{25} = > 7330	35

inhibition of Na^+/K^+ -ATPase activity.[33,34]

The toxicity values of Hg on larval seabass compared to other tropical aquatic organisms (fish, shrimp, prawn, bivalve, crab and phytoplankton) are shown in Table 5. Mercury was found to be more highly toxic to larval invertebrates than larval fish. The seabass larvae were more sensitive to Hg toxicity than the juvenile stage of seabass, milkfish *Chanos chanos* and cresent grunter *Therapon jarbua*. This is because earlier life stages of aquatic organisms are generally more sensitive to metal toxicity than older stage or adults.[8,11] Comparison to other tropical fish larvae could not be made due to a lack of the data. It would be expected that phytoplankton species are more sensitive to metal toxicity than other animals. However, the opposite result showed that *Tetraselmis* sp. was less sensitive to Hg than larval seabass. This is possibly due to the use of a static test resulting in a large reduction of Hg in the test solutions in which the algae could tolerate over 96-h exposure. In comparison to other metals copper (Cu), cadmium (Cd) and zinc (Zn) tested by Thongra-ar and Musika,[35] Hg was found to be more toxic to larval seabass than the other three metals with the toxicity ranking of Hg > Cu > Cd > Zn. The toxicity of Hg on growth of seabass larvae is greater than that of Cu, Cd and Zn by approximately 200-, 300- and 1000-fold, respectively.

The lethal concentration (LC_{50}) and inhibition concentration of growth (IC_{25} and IC_{50}) for Hg to the seabass larvae tested over a 7-d period are much higher than the concentrations found along the entire coastline of Thailand which ranged from <0.01 to 0.54 µg L^{-1} during 1997 to 1998.[36] With few exceptions, generally the level of Hg in Thai coastal waters does not exceed the National Coastal Water Quality Standard of 0.1 µg L^{-1}.[36] For the protection of aquatic life, the recommended interim ASEAN marine water quality criteria for total concentration of Hg in seawater is 0.16 µg L^{-1}. [4] This value is approximately 1/280 and 1/120 of the values of the 7-d LC_{50} and 7-d IC_{50} values, respectively, obtained in this study. This suggests that if Hg concentrations in the aquatic environment meet the recommended criteria, seabass larvae would not be adversely affected.

This study suggests that, in nature, seasonal fluctuations in salinity in an estuarine environment is not likely be an important factor influencing the toxicity of Hg on seabass larvae. However, additional testing with other estuarine organisms is recommended to further evaluate effects of salinity on Hg toxicity to other living organisms. In addition, studies on other environmental factors influencing Hg toxicity, such as temperature and pH, should be investigated to fully characterize the adverse effects of Hg on aquatic organisms.

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References

1. Hodson P (1988) The effect of metal metabolism on uptake, disposition and toxicity in fish. *Aquat Toxicol* **11**, 3-18.
2. Wren CD and Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. *Environ Pollut* **71,** 205-41.
3. Manahan SE (1992) *Toxicological Chemistry*, *2nd edition*. Lewis Publishers, Boca Raton.
4. Deocadiz ES, Diaz VR and Otico PFJ (1999) ASEAN marine water quality criteria for mercury. In: *ASEAN Marine Water Quality Criteria: Contextual Framework, Principle, Methodology and Criteria for 18 Parameter* (Edited by McPherson CA, Chapman PM, Viger GA and Ong KS), pp XIII-1 - XIII-34. EVS Environment Consultants, North Vancouver and Department of Fisheries, Malaysia.
5. Bryan GW (1976) Heavy metal contamination in the sea. In: *Marine Pollution* (Edited by Johnston R), pp 185-302. Academic Press, New York.
6. Laporte JM, Truchot JP, Ribeyre F and Boudou A (1997) Combined effects of water pH and salinity on the bioaccumulation of inorganic mercury and methylmercury in the shore crab (*Carcinus maenas*). *Mar Pollut Bull* **34**, 880-93.
7. Hahne HCH and Kroontje W (1973) Significant of pH and chloride concentration on behavior of heavy metal pollutions: mercury(II), cadmium(II), zinc(II), and lead(II). *J Environ Qual* **2**, 444-50.
8. Wang W (1987) Factors affecting metal toxicity to (and accumulation by) aquatic organisms - overview. *Environ Internat* **13**, 437-57.
9. Wright DA (1995) Trace metal and major ion interactions in aquatic animals. *Mar Pollut Bull* **31,** 8-18.
10. McLusky DS, Bryant V and Campbell R (1986) The effect of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanogr Mar Biol Ann Rev* **24**, 481-520.
11. McKim JM (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J Fish Res Board Can* **34**, 1148-54.
12. Hutomo M, Darmayati Y and Hindari D (1995) Development of toxicity testing protocols for ASEAN species. In: *ASEAN-Canada Marine Environmental Quality Technical Papers and Mission Report* (Edited by Watson D and Ong KS), pp 30-9. EVS Environment Consultants, Vancouver and Department of Fisheries, Malaysia.
13. CPMS-II (ASEAN-Canada Cooperative Programme on Marine Science- Phase II) (1995) *Draft protocol for sublethal toxicity tests using tropical marine organisms*. Technical Study: 5/555-01.11 (112-15). CIDA Project No. 149/15461.

14. U.S. EPA (1992) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms*. EPA/600/4-91/021. United States Environmental Protection Agency, Cincinnata, Ohio.
15. Gulley DD, Boelter AM and Bergman HL (1990) *TOXSTAT Release 3.2*. Department of Zoology and Physiology, University of Wyoming, Laramie, WY.
16. Norberg-King TJ (1993) *A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach (Version 2.0)*. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. Tech. Report 03-93 of the National Effluent Toxicity Assessment Center.
17. Sharp JR and Neff JM (1980) Effects of the duration of exposure to mercuric chloride on the embryogenesis of the estuarine teleost, *Fundulus heteroclitus*. *Marine Environ Res* **2**, 195-213.
18. Carr RA and Wilkniss PE (1973) Mercury: short-term storage of natural waters. *Environ Sci Tech* **7**, 62-3.
19. Lo JM and Wai CM (1975) Mercury loss from water during storage: mechanisms and prevention. *Anal Chem* **47**, 1869-70.
20. Verma SR, Chand R and Tonk IP (1985) Effects of environmental and biological variables on the toxicity of mercuric chloride. *Water Air Soil Pollut* **25**, 243-8.
21. Green FAJr, Anderson JW, Petrocelli SR, Presley BJ and Sims R (1976) Effect of mercury on the survival, respiration and growth of postlarval white shrimp, *Penaeus setiferus*. *Mar Biol* **37**, 75-81.
22. Luoma SN (1983) Bioavailability of trace metals to aquatic organisms- a review. *Sci Total Environ* **28**, 1-22.
23. Mason RP, Reinfelder JR and Morel FMM (1996) Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ Sci Technol* **30**, 1835-45.
24. Okasako J and Siegel S (1980) Mercury antagonists: effects of sodium chloride and sulfer group (VIa) compounds on excystment of the brine shrimp artemia. *Water Air Soil Pollut* **14,** 235-40.
25. Sparks DL (1995) *Environmental soil chemistry*. Academic Press, San Diego.
26. Stucki JW, Bailey GW and Gan H (1995) Redox reactions in phyllosilicates and their effects on metal transport. In: *Metal Speciation and Contamination of Soil* (Edited by Allen HE, Huang CP, Bailey GW, Bowers AR), pp.113-181. CRC Press, Inc, Boca Raton.
27. Skyllberg U, Xia K, Bloom PR, Nater EA and Bleam WF (2000) Binding of mercury(II) to reduced sulfur in soil organic matter along upland-peat soil transects. *J Environ Qual* **29**, 855-65.
28. Olson KR, Bergman HL and Fromm PO (1973) Uptake of methyl mercuric chloride and mercuric chloride by trout: a study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. *J Fish Res Board Can* **30**, 1293-9.
29. Spry DJ and Wiener JG (1991) Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. *Environ Pollut* **71**, 243-304.
30. Parkpoin P, Thongra-ar W, DeLaune RD and Jugsujinda A (2001) Adsorption and desorption of mercury by Bangpakong river sediments as influenced by salinities. *J Environ. Sci. Health A* **36**, 623-40.
31. Weis JS and Khan AA (1990) Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus* from a polluted habitat. *Marine Environ Res* **30,** 243-9.
32. Farmanfarmaian A, Pugliese KA and Sun LZ (1989) Mercury inhibits the transport of D-glucose by the intestinal brush border membrane vesicles of fish. *Marine Environ Res* **28**, 247-51.
33. Stagg RM, Rusin J and Brown F (1992) Na^+, K^+-ATPase activity in the gills of the flounder (*Platichthys flesus*) in relation to mercury contamination in the Firth of Forth. *Marine Environ Res* **33**, 255-66.
34. Wright DA and Welbourn PM (1993) Effects of mercury exposure on ionic regulation in the crayfish *Orconectes propinquus Environ Pollut* **82**, 139-42.
35. Thongra-ar W and Musika C (1997) Short-term chronic toxicity of cadmium, zinc and copper on larval seabass, *Lates calcarifer*. In: *ASEAN Environmental Management: Quality Criteria and Monitoring for Aquatic Life and Human Health Protection* (Edited by Vigers G, Ong KS, McPherson C, Millson N, Watson I and Tang A), pp IV-27 - IV- 33. Proceedings of the ASEAN-Canada Technical Conference on Marine Science (24-28 June 1996), Penang, Malaysia.
36. Chongprasith P and Wilairatanadilok W (1999) Are Thai waters really contaminated with mercury?. In: *ASEAN Marine Environmental Management: Towards Sustainable Development and Integrated Management of the Marine Environment in ASEAN* (Edited by Watson I, Vigers G, Ong KS, McPherson C, Millson N, Tang A and Gass D), pp11-26. Proceedings of the Fourth ASEAN-Canada Technical Conference on Marine Science (26-30 October 1998), Langkawi, Malaysia.
37. Wainiya W and Tharnbubpa C (1981) *Acute toxicity of mercury and copper to white sea bass, Lates calcarifer (Bloch)*.Technical paper No. MFL/24/4. Department of Fisheries.
38. Chayarat C (1985) *Acute toxicity of mercury, lead and their mixtures to pla ka-pong khao, Lates calcarifer (Bloch)*. M.Sc. Thesis, Kasetsart University, Bangkok.
39. Tamiyavanich S (1984) Bioassay studies on the impacts of heavy metals on certain marine animals. In: *Proceedings of the Third Seminar on the Water Quality and the Quality of Living Resources in Thai Waters*, 26-28 March 1984. pp 490-3. National Research Council of Thailand.
40. Diaz VR (1995) Preliminary results of acute toxicity tests for mercury and cadmium on milkfish (*Chanos chanos*) juvenile. In: *ASEAN Criteria and Monitoring: Advances in Marine Environmental Management and Human Health Protection* (Edited by Watson D, Ong KS and Vigers G), pp 112-5. Proceedings of the ASEAN-Canada Midterm Technical Review Conference on Marine Science (24-28 October 1994), Singapore.
41. Navanarasest N and Menasveta P (1986a) Acute toxicity of some toxicants to brackishwater fishes. In: *Proceedings of the Third National Marine Science Seminar*. 6-8 August 1986, National Research Council of Thailand. Volume 3.
42. McClurg TP (1984) Effects of fluoride, cadmium and mercury on the estuarine prawn *Penaeus indicus*. *Water SA* **10**, 40-5.
43. Piyan BT, Law AT and Cheah SH (1985) Toxic levels of mercury for sequential larval stages of *Macrobrachium rosenbergii* (de Man). *Aquaculture* **46**, 353-9.
44. Navanarasest N and Menasveta P (1986b) Acute toxicity of different toxicants to giant prawn (*Macrobrachium rosenbergii*) larvae. In: *Proceedings of the Third National Marine Science Seminar*. 6-8 August 1986, National Research Council of Thailand. Volume 3.
45. Martin M, Osborn KE, Billig P and Glicksteins N (1981) Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvea. *Mar Pollut Bull* **12**, 305-8.
46. Sommanee P (1980) Toxicity of heavy metals on *Donax faba* Chemniz. *Thai Fish Gazette* **32**, 391-402.
47. Gonzales DCQ (1997) Chronic toxicity of cadmium, copper and mercury to unicellular marine algae, *Tetraselmis* sp./ *Tetraselmis tetrahele*. In: *ASEAN Environmental Management: Quality Criteria and Monitoring for Aquatic Life and Human Health Protection* (Edited by Vigers G, Ong KS, McPherson C, Millson N, Watson I and Tang A), pp X-9 - X-12. Proceedings of the ASEAN-Canada Technical Conference on Marine Science (24-28 June 1996), Penang, Malaysia.