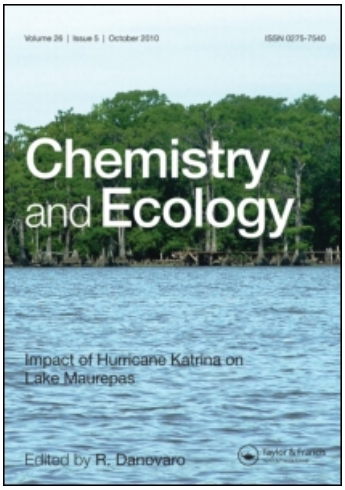


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# Acute toxicity of industrial detergent (Neatex) and corrosion inhibitor (Norust CR486) to early stages of cichlids: *Tilapia guineensis*

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The biological effects of uncontrolled discharge of industrial chemicals into the Niger Delta environment of Nigeria need to be investigated, and the acute toxicity assay represents a useful tool. This study investigated the toxicity of two chemicals (Neatex and Norust CR 486) to early life stages of an economically important fish (*Tilapia guinensis*) from fresh and brackish waters of the Niger Delta environment. The American Society for Testing and Material-recommended semi-static renewal bioassay was adopted. *Tilapia guineensis* (7 d old) were exposed to varying concentrations of the chemicals, and LC50 values were measured at 96 h. Results indicated that exposure durations, concentrations and environmental conditions influenced the effects of the chemicals. The estimated 96-h LC50 values from the fresh and brackish water tests were 7.91 and 15.32 mg $l^{-1}$ for Neatex and 4.03 and 5.30 mg $l^{-1}$ for Norust CR 486, respectively. The results showed that Norust CR 486 was more toxic than Neatex. The estimated 96-h LC50 and mean percentage mortality values for fresh and brackish water tests were statistically significant only for Neatex at $P < 0.05$ ($t$-value = 11.91) and $P < 0.05$ ($t$-value = 3.21), respectively. There were also significant differences between the test and control experiments for both chemicals at $P < 0.05$. The observed sensitivity of the fish to both chemicals provides a rationale for regulatory surveillance of the chemicals in the Niger Delta environment.

*Keywords*: Toxicity; Chemicals; Life-stage; Fish

## 1. Introduction

Discharge of toxic chemicals into the environment causes a wide range of direct and indirect adverse effects on biological systems. These vary from cell distortion to ecosystem degradation. The severity of the effects depends on the type, property, dosage, and exposure duration of the chemical. The major entry points of chemicals into surface waters are usually through point-source industrial discharges and run-offs.

Detergents are widely used in both industrial and domestic premises to wash equipment, installations, heavy-duty machines, vehicles, and oil-soiled materials. They are used in pesticide formulations and dispersal of oil spills at sea. Hazardous effects of cationic, anionic, and

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non-ionic detergents on aquatic organisms have been reported [1–3]. All detergents destroy the external mucus layers that protect the fish from bacteria and parasite infection. They are known to cause severe damage to the gills, and fish deaths have occurred at concentrations of 15 ppm and above [4].

Corrosion inhibitors are used in a wide range of applications, such as oil pipelines, domestic central-heating systems, industrial water-cooling systems, and metal extraction plants. Their toxic effects on aquatic resources such as fish have necessitated the need for regular monitoring of water bodies that receive effluents containing these chemicals.

Reports have shown that linear alkylbenzene sulfonate (LAS), a major component of detergents and corrosion inhibitors, is usually poorly broken down in rivers and may be toxic to aquatic organisms [5]. Previous works on toxicity of industrial detergent and corrosion inhibitors include the works of Nunes *et al.* [6], Kalmanzon *et al.* [7], Ghorpade *et al.* [8], and Pillard *et al.* [9].

The Western Niger Delta ecozone consists of fresh, brackish, and marine environments. The region is one of the largest wetlands of the world [10] and is globally significant because it supports the world's third largest mangrove forest, rich in biodiversity [11]. The discovery of large resources of oil and gas in the region has resulted in massive industrialization within a relatively short time. These industries over the years have generated an array of effluents, which have degraded the aquatic ecosystem.



In Nigeria and in particular the Niger Delta, existing data on environmental impact assessments by most oil companies are lacking in ecological risk assessments of industrial chemicals [10, 11]. Ecological risk assessments are usually conducted for the purpose of defining the extent of hazardous-waste contamination of the aquatic biota. Bioassays are used to measure the magnitude of existing biological damage to fish, benthic, and other organisms using mortality, impaired physiology, biochemical abnormality, and behavioural aberration as assessment end-points [12].

Even within the regulatory framework, standard toxicity test data on chemicals are lacking in the Niger Delta region. In this regard, lethality tests are particularly useful in predictive assessment of environmental quality of chemicals discharged into the aquatic ecosystem. This is to ensure that substantial safety factors and margins are met. The objective of this study is to access the toxicity of commonly used industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486) to *Tilapia guineensis*. This is with a view of proposing an environmental monitoring programme for these chemicals in the Nigerian costal region where petroleum activities are high.

## 2. Materials and method

### 2.1 *Collection and acclimatization of test organisms*

*Tilapia guineensis* (fish) from fresh and brackish environments of the Nigerian Niger Delta ecological zone were collected on the first day post-hatching in enclosed tanks, and daily observations were made. The test species were collected from cultured fresh and brackish water farms at Kpakiama (fresh) and Abua (brackish) in the Niger Delta area.

Acclimatization to laboratory conditions were carried out in holding tanks with length × height × width dimensions of 100 cm × 100 cm × 100 cm for 7 d prior to commencing the test. The physico-chemical conditions similar to the Niger Delta environment [10] were maintained in the holding tanks with dilution water from the fish habitat.The holding tanks were maintained in the laboratory at room temperature of 26 ± 2°C with a 16:8 h light: darkness

Table 1. Physico-chemical characteristics of the chemicals as contained in the Materials and Safety Data Sheet (MSDS).

| Properties | Neatex | Norust CR 486 |
|---|---|---|
| State or form | Liquid | Liquid |
| Colour | Light brown | Colourless |
| Odour | Pleasant | Pungent |
| Composition | Linear alkyl benzene sulfonate, Sodium hydroxide, sodium carbonate, and ammonium oxalate | Heterocyclic derivatives, cationic surfactant, and alkaline sulfide in ethylene glycol |
| Solubility | Soluble | Soluble |
| Specific gravity | 1.04 | 1.09 |
| pH | 10.62 | 1.97 |

photoperiod. The pH was within the range of 5.5–8, and the dissolved oxygen was greater than 6.00 mg $l^{-1}$.

### 2.2 *Test chemicals*

Two industrial chemicals, Neatex (liquid detergent) and Norust CR 486 (corrosion inhibitor), were used for the 96-h acute toxicity test. The constituents for both chemicals are given in table 1. The chemicals were collected from manufacturers with the trade names Manuex Nig Ltd (Neatex) and Ceca Incorporated (Norust CR 486). Both chemicals are currently used by oil industry operators in the Nigerian Niger Delta area.



### 2.3 *Rationale*

The choice of fish (*Tilapia guineensis*) in this study was based on the recommendation of Beeby [13]. The suitability of *Tilapia guineensis* as a test organism was based on its availability, sensitivity, and ease of maintenance under laboratory conditions.

### 2.4 *Bioassay procedure*

The semi-static renewal bioassays were conducted in amber-coloured wide-mouth glass tanks measuring 40 cm × 25 cm × 25 cm. The bioassay procedure started with a range-finding test [14, 15]. This was used to determine the range of concentrations that would produce the desired LC50 effect in the test organisms. The screening test was carried out with four different concentrations of the test chemicals.

Stock solutions of 40 mg $l^{-1}$ were prepared by dissolving the chemicals in the dilution water from which serial dilutions of 2.5, 5, 10, and 20 mg $l^{-1}$ were made. The concentrations of the chemicals were analytically confirmed. The ecological relevance of these concentrations was based on the observation that the mortality of fish occurred in detergent exposure concentrations above 15 mg $l^{-1}$ [4].

Ten (10) test organisms were used for each of the tests, including the control for both fresh and brackish experiments. They were conducted in three replicates. The fish were starved for 24 h before the test and during the 96 h test. Aeration was done throughout the test duration [16]. The test solutions were renewed daily, and their physico-chemical constituents were measured throughout the duration of the experiment.

During the 96-h mortality tests, the mean temperature and dissolved oxygen were 26 ± 2°C and 6 ± 0.3 mg $l^{-1}$, respectively. The pH values had a mean of 5.5 ± 0.2 in the freshwater

experiments and $7.2 \pm 0.8$ in the brackish water tests. The mean salinity in the fresh tests was $59.72 \pm 3.4\,\mathrm{mg\,l^{-1}}$ and $3758 \pm 207\,\mathrm{mg\,l^{-1}}$ in the brackish water experiments. The mean TDS and conductivity levels in the freshwater test were $86.81 \pm 2.5\,\mathrm{mg\,l^{-1}}$ and $176.23 \pm 6.8\,\mu\mathrm{S\,cm^{-1}}$, while in the brackish water experiments $6903 \pm 46.6\,\mathrm{mg\,l^{-1}}$ and $13814 \pm 88.6\,\mu\mathrm{S\,cm^{-1}}$ were recorded, respectively.

### 2.5 *Mortality*

Fish mortality was recorded at 24−, 48−, 72−, and 96-h exposure durations. The dead organisms were removed immediately on detection. Fish were considered dead when they failed to show any evidence of opercular activity and did not respond to gentle prodding [17].

### 2.6 *Statistical analysis*

The susceptibility of fish to both chemicals was determined using the probit method of analysis by Finney [18] for a median LC50 at 96 h. Computations of confidence intervals of mortality rates were also obtained from the probit analyses.



## 3. Results

The results of acute toxicity of Neatex and Norust CR 486 to *Tilapia guineensis* are presented below (tables 2 and 3).

Table 2. Mean mortality of *Tilapia guineensis* exposed to different concentrations of Neatex in fresh and brackish water.

| Concentration ($\mathrm{mg\,l^{-1}}$) | Log of dose | Not tested | Neatex fresh | | | | Neatex brackish | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.5 | 0.4 | 10 | 0 | 10 | 20 | 30 | 0 | 0 | 0 | 10 |
| 5 | 0.7 | 10 | 0 | 10 | 23 | 40 | 0 | 0 | 10 | 30 |
| 10 | 1 | 10 | 10 | 23 | 40 | 57 | 7 | 10 | 27 | 47 |
| 20 | 1.3 | 10 | 10 | 40 | 57 | 80 | 7 | 13 | 43 | 60 |

Table 3. Mean mortality of *Tilapia guineensis* exposed to different concentrations of Norust CR 486 in fresh and brackish water.

| Concentration ($\mathrm{mg\,l^{-1}}$) | Log of dose | Not tested | Norust CR 486 fresh | | | | Norust CR 486 brackish | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 0 | 0 | 10 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 |
| 2.5 | 0.4 | 10 | 7 | 20 | 37 | 53 | 0 | 10 | 27 | 47 |
| 5 | 0.7 | 10 | 20 | 33 | 50 | 63 | 17 | 27 | 40 | 53 |
| 10 | 1 | 10 | 23 | 47 | 57 | 67 | 23 | 40 | 57 | 67 |
| 20 | 1.3 | 10 | 37 | 60 | 70 | 80 | 30 | 53 | 60 | 73 |

### 3.1 *Mean percentage mortality*

The mean percentage mortality was observed to be concentration-dependent. The influence of exposure duration and environmental conditions was also observed. The mean percentage mortality for the fish increased as concentrations increased and were higher in the freshwater experiments (figures 1–4). The mean mortalities for Norust CR 486 at 96 h were 53, 63, 67, and 80% in the freshwater tests and 47, 53, 67, and 73% in the brackish water tests for 2.5, 5, 10, and 20 mg $l^{-1}$ concentrations, respectively. Neatex freshwater experiments for the same concentrations had a mean percentage mortality of 30, 40, 57, and 80%, while the brackish water tests had 10, 30, 47, and 60% at 96 h. In all control experiments, no mortality

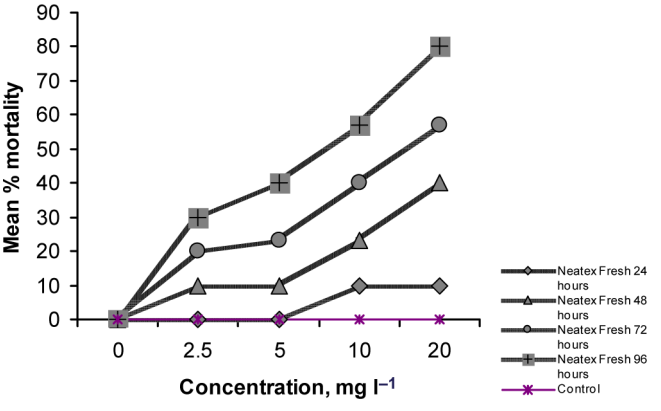


Figure 1. Mean percentage mortality at different hours in freshwater fish (7 d old) exposed to Neatex.

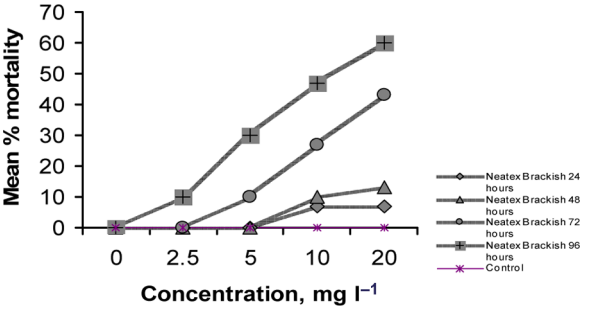


Figure 2. Mean percentage mortality at different hours in brackish water fish (7 d old) exposed to Neatex.

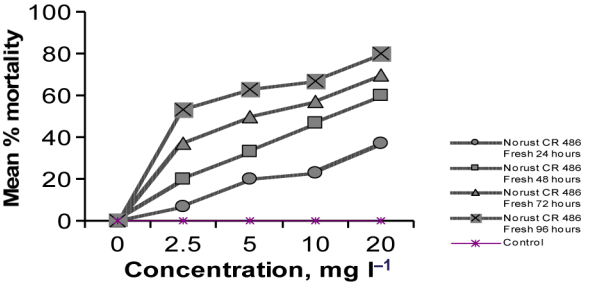


Figure 3. Mean percentage mortality at different hours in freshwater fish (7 d old) exposed to Norust CR 486.

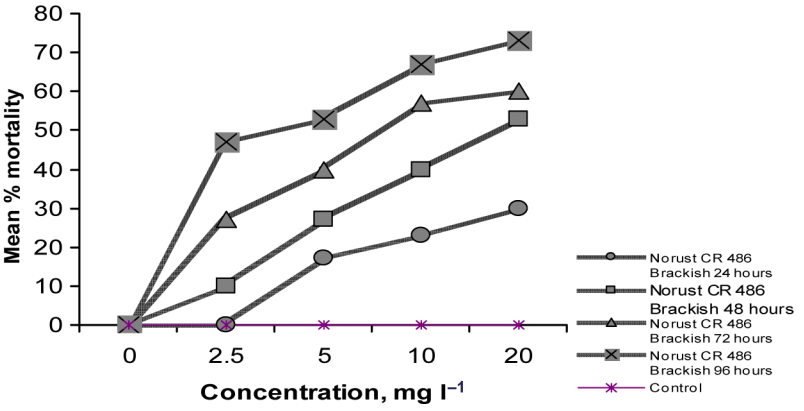


Figure 4. Mean percentage mortality at different hours in brackish water fish (7 d old) exposed to Norust CR 486.

was recorded. The mean percentage mortality for fish at 96 h in the freshwater and brackish water tests was significantly different at $P < 0.05$ for both chemicals. The mean percentage mortality in the control and test experiments was also significantly different at $P < 0.05$. The $t$-values were 3.10, 2.64 (Neatex) and 3.80, 3.73 (Norust CR 486) in the fresh and brackish experiments, respectively.

### 3.2 *Estimated 96-h LC50*

The acute toxicity of both chemicals was also evaluated using estimated 96-h LC50 values in varying concentrations. The estimated 96-h LC50 values for Neatex and Norust varied in the fresh and brackish water tests (tables 4 and 5). In Neatex, the estimated 96-h LC50 ranged between 7.91 mg $l^{-1}$ (freshwater) and 15.32 mg $l^{-1}$ (brackish water), while in Norust, it ranged between 4.03 mg $l^{-1}$ (freshwater) and 5.30 mg $l^{-1}$ (brackish water). The estimated 96-h LC50 values obtained showed that Norust was more toxic than Neatex in both freshwater and brackish water fish. Although the freshwater fish were more sensitive, this was only statistically significant in Neatex at $P < 0.05$ ($t$-value = 11.91). The probit analysis also

Table 4. Acute toxicity profile of fresh and brackish water fish to Neatex exposure.

| | Days | LC50 | Confidence limit | Probit equations | Slope |
|---|---|---|---|---|---|
| Freshwater | 7 | 7.91 ± 073 | 3.51–14.69 | $y = 3.57 + 1.59 \log$ (concentration) | 4.36 ± 0.91 |
| Brackish water | 7 | 15.32 ± 0.17 | 8.89–34.94 | $y = 2.99 + 1.70 \log$ (concentration) | 3.83 ± 0.05 |

Table 5. Acute toxicity profile of fresh and brackish water fish to Norust exposure.

| | Days | LC50 | Confidence limit | Probit equations | Slope |
|---|---|---|---|---|---|
| Freshwater | 7 | 4.03 ± 0.76 | 0.45–15.74 | $y = 4.40 + 1.00 \log$ (concentration) | 9.89 ± 1.13 |
| Brackish water | 7 | 5.30 ± 1.60 | 1.19–14.76 | $y = 4.38 + 0.87 \log$ (concentration) | 16.91 ± 0.3 |

showed that the 96-h LC50 values decreased with increasing concentrations, indicative of an increase in toxicity with increasing concentrations and exposure duration.

## 4. Discussion

Indiscriminate discharges of industrial chemicals into the aquatic environment are bound to expose organisms living and breeding there, to multiple stressors of varying sources and intensity. In this study, fish fingerlings were exposed to two commonly used chemicals (industrial detergents and corrosion inhibitors), which are normally discharged into the fresh and brackish water environment of the Niger Delta region of Nigeria, an ecological zone where industrial activities are on the increase. *Tilapia guineensis* is an economically important cichlid, found in these fresh and brackish waters exposed to chemical contaminations.

This study exposed the vulnerability of early life stages of *Tilapia guineensis* fingerlings to chemical contaminants. The mortality of fish fingerlings exposed to varying concentrations of industrial detergent (Neatex) and corrosion inhibitor (Norust CR 486) were influenced by toxicity-modifying factors such as exposure duration, concentrations, type of chemicals, life stages, and environmental conditions. In all concentrations, organisms showed varying degrees of stress to Neatex and Norust exposure. The toxicity of Neatex and Norust CR 486 was first manifested as retarded swimming activity and erratic movement in the test organisms.

Both chemicals caused a sharp increase in mortality over a large concentration range which were slightly more in Norust CR 486 (brackish water test). This might be due to the presence of heterocyclic derivatives and surfactants components in Norust CR 486. This result is consistent with the observations of other related studies [19–22]. The varying degrees of mortality reported in this study are corroborated by Sparling [23], who reported that differences in an organism's biological adjustment, behavioural response to changes in water chemistry, and osmotic conditions depend on the stage of development. The implication of this observation is that early larval stages of fish are not only vulnerable to chemical contaminants but usually adversely affected. Survival is dependent on the degree of fecundity of the parent organisms.

Differential acute toxicity levels observed for both chemicals at different concentrations and exposure durations were a reflection of the effect of toxicity-modifying factors. Test organisms showed better tolerance at lower concentrations, which did not necessarily mean complete compensation for the chemicals. Resistance may have at least added metabolic cost and negatively influenced energy budget [24]. Estimated 96 h LC50 values obtained in this study compared with GESAMP rating [25] showed that both chemicals were slightly toxic to the early life stages of the fish.

It has also been reported that the toxicity of chemicals can be altered by variations in water chemistry, which affects the bioavailability of the chemicals to fish [26–28]. Relative differences observed in the mean percentage mortality and 96-h LC50 values between the fresh and brackish water test may not be unconnected with the varying osmoregulatory demands of the different environments. It has been reported that in freshwater environments, any physical damage in external tissues allows more water to enter the body of the organism and salt to escape. This places an additional burden on the kidneys, which ultimately results in death [26]. This probably accounts for the higher mortality in the freshwater test.

The significant difference observed in the mortality between the control and the test concentrations showed that the forensic threshold was exceeded. This is an indication that the chemicals may have induced the death of the fish. The result of this study raises an environmental concern, which calls for constant monitoring of chemicals discharged into the waters of the fragile Niger Delta ecological zone.

## References


[1] T. Madsen, H.B. Boyd, D. Nylén, A.R. Pedersen, G.I. Petersen, F. Simonsen. *Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products*. Environmental Project No. 615 (2001).

[2] S.E. Belanger, D.H. Davidson, J.L. Farris, D. Reed, D.S. Cherry. Effects of cationic surfactant exposure to a bivalve mollusc in stream mesocosms. *Environ. Toxicol. Chem.*, **12**, 1989–1802 (1993).

[3] R.S. Boethling, D.G. Lynch. Quaternary ammonium surfactants. In *Detergents, The Handbook of Environmental Chemistry, Volume 3. Part F. Anthropogenic Compounds*, N.T. de Oude (Ed.), pp. 145–177, Springer, Berlin (review).

[4] D.R.M. Passino, S.B. Smith. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. *Environ. Toxicol. Chem.*, **6**, 901–907 (1987).

[5] P. Lightowlers. *Still Dirty: a Review of Action Against Toxic Products in Europe*. A report for WWF – UK (2004).

[6] B. Nunes, F. Carvalho, L. Guilhermino. Acute toxicity of widely used pharmaceuticals in aquatic species: *Gambusia holbrooki, Artemia partheonetica* and *Tetrasemis chui. Ecotoxicol. Environ. Saf.*, **61**, 413–419 (2005).

[7] E. Kalmanzon, Y. Rahamim, Y. Barenholz, S. Carmeli, E. Zlotkin. Receptor-mediated toxicity of pahutoxin, a marine trunkfish surfactant. *Toxicon.*, **42**, 63–71 (2003).

[8] N. Ghorpade, V. Mehta, M. Khare, P. Sinkar, S. Krishnan, C.V. Rao. Toxicity study of diethyl phthalate on freshwater fish *Cirrhina mrigala. Ecotoxicol. Environ. Saf.*, **53**, 255–258 (2002).

[9] D.A. Pillard, J.S. Cornell, D.L. DuFresne, M.T. Hernandez. Toxicity of benzotriazole and benzotriazole derivative to three aquatic species. *Wat. Res.*, **35**, 557–560 (2001).

[10] Shell Petroleum Development Company (SPDC). *Environmental Impact Assessment (EIA) of the Jones Creek Field Development Plan* (1998).

[11] A.B.M. Egborge. Pollution of Warri River at Opete. *Oil Pollut. Nigeria*, **2**, 238 (2000).

[12] C. Fuller, J. Bonner, C. Page, A. Ernest, T. McDonald, S. McDonald. Comparative toxicity of oil dispersant and oil plus dispersant to several marine species. *Environ. Toxicol. Chem.*, **23**, 12, 2941–2949 (2004).

[13] A. Beeby. What do sentinels stand for? *Environ. Pollut.*, **112**, 285–298 (2001).

[14] American Society for Testing and Materials Standards (ASTM). Standard practice for conducting acute toxicity tests with fishes, macro invertebrates and amphibians. In *Annual Book of American Society for Testing and Materials Standards*, **11**, 4, 378–397, E 729–88a, ASTM, Philadelphia, PA. (1989).

[15] J.A. Hall, L.A. Golding. *Acute Toxicity Test Protocol; Standard Method for Whole Effluent Toxicity Testing: Development and Application. Report No. MFE80205*. NIWA Report for the Ministry of the Environment, Wellington, New Zealand (1998).

[16] D.J. Reish, P.S. Oshida. Manual of method in aquatic environment research Part 10 short-term static bioassays. *FAO Fish Tech. Pap.*, **247**, 62 (1986).

[17] D.J. Spurgeon, S.P. Hopkins. The effects of metal contaminated soils on the growth, sexual development and early cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. *Ecotoxicol. Environ. Saf.*, **35**, 86–95 (1996).

[18] D.J. Finney. *Probit Analysis*, 3rd edition Cambridge University Press, Cambridge (1971).

[19] A.A. George, J.R. Clark. Acute toxicity of two Corexit dispersants. *Chemosphere*, **40**, 897–906 (2000).

[20] A. Scarlett, T.C. Galloway, M. Canty, E.L. Smith, J. Nilsson. Comparative toxicity of two oil dispersants, superdispersants – 25 and Corexit 9527, to a range of coastal species. *Environ. Toxicol. Chem.*, **15**, 1219–227 (2005).

[21] L.I.N. Ezemonye, A. Enuneku. Evaluation of acute toxicity of cadmium and lead to amphibian tadpole (toad: *Bufo maculatus* and frog: *Ptychadena bibroni*). *J. Aquat. Sci.*, **20**, 33–36 (2005).

[22] T.A. Johnson, L.M. Miller, D.M. Whittle, S.B. Brown, D.M. Wieg, A.R. Kapuscinnsk, W.C. Leggett. Effects of materially transferred organochlorine contaminants of early life survival in a fresh-water fish. *Environ. Toxicol. Chem.*, **24**, 2594–2602 (2005).

[23] D.W. Sparling, G. Linder, C.A. Bishop. *Ecotoxicology of Amphibians and Reptiles*, Society of Environmental Toxicology and Chemistry, Pensacola, FL (2002).

[24] W.J. Langston, S.K. Spence. Biological factors involved in metal concentrations observed in aquatic organisms. In *Metal Speciation and Bioavailability in Aquatic Systems*, A. Tressier, D.R. Turner (Eds), pp. 407–478, Wiley, Chichester, UK (1995).

[25] Group of Experts on Scientific Aspects on Marine Pollution (GESAMP). *The Health of the Seas. UNEP Regional Seas Reports and Studies No. 16* (1982)

[26] N.R. Bury, J.C. McGeer, C.M. Wood. Effects of altering freshwater chemistry on physiological responses of rainbow trout to silver exposure. *Environ. Toxicol. Chem.*, **18**, 49–55 (1999).

[27] J.G. Richards, R.C. Playle. Protective effects of calcium against the physiological effects of exposures to a combination of cadmium and copper in rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.*, **77**, 1035–1047 (1999).

[28] R.C. Playle, R.W. Gensemer, D.G. Dixon. Copper accumulation on gills of fathead minnows: Influence of water hardness, complexation and pH of the gill microenvironment. *Environ. Toxicol. Chem.*, **11**, 38–391 (1992).