Zoosymposia 9: 059–069 (2014)
www.mapress.com/zoosymposia/

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

http://dx.doi.org/10.11646/zoosymposia.9.1.11
http://zoobank.org/urn:lsid:zoobank.org:pub:B9D01EFE-100F-4286-B2DE-93CA264EEE35

Branchiura sowerbyi Beddard, 1892 (Oligochaeta: Naididae) as a test species in ecotoxicology bioassays: a review

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Abstract

Branchiura sowerbyi (Oligochaeta; Naididae; Rhyacodrilinae) is an oligochaete with great potential to be used as a test-species in toxicology bioassays. Since 1950, its life cycle has been studied and nowadays it is well described in the literature. *B. sowerbyi* has a cosmopolitan distribution and can be found in places where *Tubifex tubifex* (normally used in toxicity bioassays) does not occur, especially in tropical regions. Due to its high individual biomass, *B. sowerbyi* is suitable for use in bioaccumulation bioassays. The present study reviews papers that have used this species in toxicology bioassays and were published between 1950 and the first semester of 2012. In the first part, a brief overview is provided of the biology and the life-cycle parameters of *B. sowerbyi.* In the second part, the bioassays are presented and discussed, and in the third and last part, conclusions about research to date and perspectives about future studies are presented. Throughout the investigation, it was possible to find a total of 30 papers that tested approximately 65 different substances (insecticides, metals, industrial chemicals and others). The majority of the bioassays run are 96-h acute water-only tests, only five were chronic bioassays and two involved bioaccumulation. The lack of research and the small number of tested substances in comparison with the standardized *T. tubifex* species (i.e. approximately 9 and 44 tested metals for *B. sowerbyi* and *T. tubifex*, respectively) can be explained by the absence of a bioassay protocol for *B. sowerbyi*, causing researchers to choose other species. It is necessary to undertake more methodological research in order to find a better and common methodology for bioassays.

Key words: bioassay; Oligochaeta; sediment quality; toxicology

Introduction

Aquatic oligochaetes have long been used in bioassays. They were probably collected by Aristotle, who first used them in toxicity tests when rudimentary bioassays were conducted to verify the effects of salt water on such organisms (Chapman 2001; Martin *et al.* 2008). Throughout the last century, their use for testing environmental impact has grown markedly and, over the last decades, with developments in ecotoxicology, these animals have earned a great reputation as potential test-organisms.

According to Warren *et al.* (1998), Chironomidae and Oligochaeta are the most suitable taxa for assessing the effects of contaminants on the environment because their exposure to chemicals occurs not only through water but also sediment intake. Chapman (2001) underscores that oligochaetes are suitable for such assessments because they are key players in water ecosystems; a number of species are cosmopolitan; many have been used in chronic toxicity tests; they are often exposed to contaminants through water and sediments; many species present enough biomass to allow for bioaccumulation tests; they can be easily grown and handled; and they can resist unique physical and chemical conditions.

However, only a few oligochaete species have been studied so far by international agencies for which toxicology-test protocols have been established. This is the case for *Lumbriculus variegatus* (Müller) (Lumbriculidae), *Pristina leidyi* Smith (Naididae) and *Tubifex tubifex* (Müller) (Naididae) (USEPA 2000; ASTM 2005; OECD 2008). The first species is typically found in temperate-climate areas, the second shows a high rate of asexual reproduction—which makes data analysis complicated (i.e. survival), and the last one presents a cosmopolitan distribution despite its low frequency in tropical regions.

Accepted by Adrian Pinder: 12 Dec. 2013; published: 12 Jun. 2014

Other species have been considered as alternative organisms for testing toxicity, such as *Limnodrilus hoffmeisteri* Claparède (Naididae) and *Branchiura sowerbyi* Beddard (Naididae). The latter is an option for tests in tropical areas both because it is a native species and also because of its higher individual biomass in comparison with other oligochaete species (Marchese & Brinkhurst 1996; Raburu *et al.* 2002), which makes it suitable for bioaccumulation assays (Chapman 2001; OECD 2008). However, there is no protocol for testing toxicity with *B. sowerbyi*, which causes researchers to resort to other species.

In light of the current demand, this study aims to provide a review of papers published over the last 50 years on the use of *B. sowerbyi* in ecotoxicological tests, and thus present a compilation of methodological information as well as a discussion about the next steps towards the establishment of a protocol for the species. The review was based on a search of the following platforms: Scielo, SpringerLink and Elsevier.

Life-cycle

B. sowerbyi reproduces by cross-fertilization, during which there is mutual gamete exchange and, a few days after mating, relatively large transparent cocoons are laid (2–4 mm long), which contain between one and eight eggs (Aston 1968). The embryos present early development of gill filaments (one pair per segment) in the rear part of the body, which is a particular taxonomic trait in the species (Bonacina *et al.* 1994). In addition to the respiratory specialization, this species also shows sensorineural specializations that maximize the detection of vibrations in the substrate, water movement and contact, which make them more successful in escaping predators (Drewes & Zoran 1989). This specialization is extremely important because this species, as well as species of the subfamily Tubificinae (Naididae), leave the rear portion of their bodies exposed on the sediment surface and, by moving it, together with the gills, maximize oxygen intake.

Even though it is a tropical-climate species, it is also found in rivers and lakes of some temperate countries, where it is usually used as an indicator of thermal pollution (Aston 1968; Bonacina *et al.* 1994). According to Aston (1968), it is not clear where the species originated, whether in South America or Asia, probably the latter, since there is no record of the species in Amazonia. In Europe, this species was introduced together with water plants from tropical countries, which accounts for its abundance in botanical gardens across the continent. This author has found that *B. sowerbyi* growth and reproduction are affected by temperature, and that no reproductive activity takes place at temperatures lower than 10°C, a finding confirmed by Bonacina *et al.* (1994). This threshold (T_0) is a high temperature in comparison with values for cosmopolitan species such as *L. hoffmeisteri* (T_0 = 4°C) and *T. tubifex* (T_0 = 0°C) (Bonacina *et al.* 1987), which restricts the species' distribution to rivers and lakes in which the average temperature exceeds 10°C.

Aston *et al.* (1982) and Bonacina *et al.* (1994) showed that higher growth and reproduction rates for *B. sowerbyi* are attained at 25°C, at which temperature the population can double in less than two weeks. Marchese & Brinkhurst (1996) found that a higher rate of cocoon production was achieved at 30°C, and a larger number of juveniles per cocoon at 25°C. Among cocoon cultures, at this temperature, full embryonic development and subsequent hatching take nearly three weeks (Nascimento & Alves 2008; Lobo & Alves 2011).

Besides temperature, the concentration of organic matter in the sediment is a factor that affects reproduction and growth in this species. Aston and Milner (1982) found that activated sludge, which is the result of treated effluents, added to sand at a 33% concentration, yields good conditions for the cultivation of these organisms. On the other hand, high concentrations of activated sludge (equal to or higher than 66%) reduce survival, reproduction and growth among individuals, which, according to these authors, may be associated with lower dissolved oxygen due to the high decomposition rate of the organic matter.

This species is usually more abundant in tropical aquatic environments (Alves & Strixino 2000; Raburu *et al.* 2002; Alves & Strixino, 2003; Pamplin *et al.* 2005; Dornfeld *et al.* 2006; Pamplin *et al.* 2006; Jorcin & Nogueira 2008). Dornfeld *et al.* (2006), for instance, reported different densities throughout sampled parts of the Salto Grande reservoir (Americana, São Paulo, Brazil), ranging between 44 and 8711 ind.m^{-2}.

When cultivated in laboratory conditions (25°C), this species exhibits low hatching rate: 34.4%—Marchese & Brinkhurst (1996), 31.0%—Ducrot *et al.* (2007) and 44.93%—Nascimento & Alves (2008). However, the survival rate among juveniles is high and can reach 96% (Ducrot *et al.*, 2007). Sexual maturity was reached in approximately 35 days, with an average daily growth of 0.58 mg.day^{-1} (Marchese & Brinkhurst 1996). After becoming sexually mature, the organisms lay an average of 0.17 cocoons.day^{-1} (Ducrot *et al.* 2007), during two yearly egg-laying cycles lasting approximately 20 weeks each (Ducrot *et al.*

2007; Lobo & Alves 2011). Life expectancy for this species remains unclear but is estimated to lie between 18 (Aston 1968) and 36 months (Ducrot *et al.* 2007). This uncertainty may be related to the difficulty of monitoring such organisms over a long period of time.

Table 1 shows the biological data available in the literature for *B. sowerbyi*.

TABLE 1. *Branchiura sowerbyi* life-cycle parameters (mean values and 95%-confidence interval or standard deviation) (Adapted from Ducrot et al. 2007).

Stage of development	Life-cycle parameter	Values	Reference
Embryos	Cocoon size	2–4 mm	Aston 1968[a]
	Hatching time	7–8 days	Casellato 1984[b]
		21 days	Marchese & Brinkhurst 1996[c]
		17 ± 7 days	Ducrot *et al.* 2007[d]
		14–16 days	Nascimento & Alves 2008[e]
		12–18 days	Lobo & Alves 2011[f]
	Hatching rate	18%	Aston *et al.* 1982[g]
		50%	Casellato 1984
		21%	Marchese & Brinkhurst 1996
		31%	Ducrot *et al.* 2007
		44.93%	Nascimento & Alves 2008
		33.08%	Lobo & Alves 2011
Juveniles	Initial weight	0.44 mg	Liang 1984[h]
		1.82 mg	Marcherse & Brinkhurst 1996
		0.5 [0.3–0.8] mg	Ducrot *et al.* 2007
		1.04 mg	Lobo & Alves 2011
	Survival rate	96.7 [57–62] %	Ducrot *et al.* 2007
		100%	Lobo & Alves 2011
	Growth rate	0.58 mg·day^{-1}	Marchese & Brinkhurst 1996
		2.4 [1.8–1.7] mg·day^{-1}	Ducrot *et al.* 2007
		0.41 ± 0.09 mg·day^{-1}	Lobo & Alves 2011
Adults	First reproduction	42 days	Aston & Milner 1982[i]
		28–35 days	Marchese & Brinkhurst 1996
		60 [57–62] days	Ducrot *et al.* 2007
		40.83 ± 6.88 days	Lobo & Alves 2011
	Weight at first reproduction	40 mg	Aston & Milner 1982
		±23 mg	Marchese & Brinkhurst 1996
		84.1 [77.5–90.6] mg	Ducrot *et al.* 2007
		17.56 ± 4.57 mg	Lobo & Alves 2011
	Growth rate	1.3 [0.9–1.7] mg·day^{-1}	Ducrot *et al.* 2007
	Number of eggs per cocoon	1.94	Marchese & Brinkhurst 1996
		2.2 [1.8–2.6]	Ducrot *et al.* 2007
		1.21 ± 0.08	Nascimento & Alves 2008
		1.73 ± 0.57	Lobo & Alves 2011
	Mean fertility	0.17 cocoons day^{-1}	Marchese & Brinkhurst 1996
		0.16 cocoons day^{-1}	Ducrot *et al.* 2007
		0.13 cocoons day^{-1}	Nascimento & Alves 2008
		0.12 cocoons day^{-1}	Lobo & Alves 2011
	Life expectancy	> 18 months	Aston 1968
		1100 days	Ducrot *et al.* 2007

Conditions for tests shown in Table 1

[a] – Data collected at 20°C, substrate from the Thames, no food added, 32 days' monitoring.

[b] – Data collected at 27°C, substrate made up of silt, decomposing lettuce as food, 365 days' monitoring.

[c] – Data collected at 25ºC, substrate from Lake Eric, no food added, 56 days' monitoring.

[d] – Data collected at 21°C, sterilized natural substrate, *ad libitum* food (3.2 mg of Tetramin® fish food per oligochaete per day), 365

[e] - Data collected at 25ºC, muddy substrate, *ad libitum* food (fish food), 30 days' monitoring.

[f] – Data collected at 25ºC, sandy substrate, *ad libitum* food (fish food, 20 mg per oligochaete per week), 365 days' monitoring.

[g] – Data collected at 25ºC, activated sludge for substrate, no food added, 12 weeks' monitoring.

[h] – Data collected following field observation.

[i] – Data collected at 25ºC, activated sludge mixed with river mud for substrate, no food added, 14 days' monitoring.

Branchiura sowerbyi as a test-species

Thirty-one papers have been found that report on the use of *B. sowerbyi* as a test-species in ecotoxicology assays (Table 2). Results of assays involving single chemicals are presented in Table 3 and results of assays involving combinations of chemicals are presented in Table 4.

TABLE 2. Use of *B. sowerbyi* as a test-species, tested substances and testing procedure

References	Tested substances	Type of test
Konar & Mullick 1993	Detergent parnol-J, n-hexane, Endosulfan, DDVP, Urea, Phosphate, Zinc, Copper, Iron, Lead (mixed)	Acute 48h (water-only)
Naqvi 1973	Aldrin, Monocrotophos, Dicrotophos, Chlordane, DDT, Dieldrin, Chlorfenethol, Chlorpyrifos, Endrin, Ethion, Azinphosmethyl, Heptachlon, Diofol, Lindane, Melathion, Methoxychlor, Methyl parathion, Parathion, Perthane, Mevinphos, Strobane, Carbaryl, Toxophene	Acute 72h (water-only and with sediment)
Inoue & Kondo 1962	NaPC, Parathion, EPN, Diazinon, DDT, Lindane, Endrin, Dieldrin, Rotenone, Pyrethrins	Acute 96h (water-only)
Ghosh & Konar 1980	Sodium sulfate, Sodium hydroxide, Sodium sulfide, Sodium carbonate, Calcium hypochlorite, Magnesium bisulfite (mixed)	
Kaviraj & Konar 1982	Mercury, Chromium, Cadmium	
Sarkar & Konar 1982	Urea, Amnomium sulphate, Single superphosphate, Muriate of potash, Lime	
Kaviraj & Konar 1983	Cadmium, Mercury, Chromium (mixed)	
Ghosh & Konar 1983	Formalin	
Kaviraj *et al.* 1985	Spent bark of Cinchona	
Ghatak *et al.* 1988	n-heptane, n-hexane	
Ghatak & Konar, 1990	Cadmium, DDVP, Detergent parnol-J, N-heptane (mixed)	
Mullick & Konar 1991	Zinc, Copper, Iron, Lead, n-hexane, detergent parnol-J, endosulfan, DDVP, Urea, Superphosphate (mixed)	
Das & Kaviraj 1994	Cadmium, Potassium Permanganate, Cobalt Chloride (single and mixed)	
Sarkar & Konar 1995	Thiodan, chromium, ekaline FI (mixed)	
Ghosal & Kaviraj 1996	Cadmium (effects of poultry manure on Cd toxicity)	
Dutta & Kaviraj 1996	Cadmium (effects of lime acclimation on Cd toxicity)	
Sarkar & Konar 1997	Thiodan (Endosulfan), Chromium (single and mixed)	
Bhunia *et al.* 2000	Thiocyanate (tested at 3 temperatures: 20, 25 and 28°C)	
Ghosal & Kaviraj 2002	Cadmium, composted manure (mixed)	
Bhunia *et al.* 2003	Aniline (C_6H_7N)	
Kaviraj *et al.* 2004	Methanol	
Das & Das 2005	Copper, CaO (single and mixed)	
Saha *et al.* 2006	Lactic acid, acetic acid, benzoic acid	
Mondal & Kaviraj 2008	Jute-retting water	
Saha & Kaviraj 2008	Cypermethrin	
Mukherjee & Kaviraj 2011	Cobalt	
Chapman *et al.* 1982	Salinity, Temperature, pH, NaPCP, Black Liquor, Mercury, Cadmium, Sewage	Acute 96h (water-only and with sediment)
Kaviraj & Ghosal 1997	Cadmium	Bioaccumulation of Cd after 96h of exposure in contaminated sediments
Casellato & Negrisolo 1989	LAS	Acute 96h (water-only and with sediment), and chronic (140 days)
Casellato *et al.* 1992	LAS (sediment spiked in the laboratory)	Chronic (220 days)
Ducrot *et al.* 2010	Zinc (sediment spiked in the laboratory)	Chronic (partial life cicle and full life cicle tests)

Insecticides were found to be the chemical group with the largest number of tests. Inoue & Kondo (1962) studied the acute effect of a number of insecticides on the mortality of *B. sowerbyi*, and Naqvi (1973) carried out various acute and chronic bioassays to assess toxicity of more than 23 insecticides (Table 2). The acute bioassays were conducted in finger bowls containing only tap water (control) or a solution with toxic substances at 4.4, 21 and 32.2°C. According to this author, temperature had a significant impact on insecticide toxicity: at the extreme values it caused 100% mortality for all tested substances (19 and 18 insecticides, at 4.4 and 32°C respectively), whereas only 8 insecticides (Chlorpyrifos, Ethion, Azinphosmethyl, Dicofol, Parathion, Perthane®, Mevinphos, Toxaphene) led to 100% mortality at 21°C. The author completed bioassays in order to establish sediment influence on the toxicity of pesticides DDT, endrin, toxaphene and chlordane, and it was found that the presence of sediment increases organism resistance to toxic agents and that this species can accumulate large amounts of organochlorine compounds, thus causing toxicity among fish that feed on it. Another interesting finding by this author is their release capacity (detoxification): after a 48h-exposure to 4 ppm Endrin, 500 oligochaetes were transferred to a container containing 5 L of dechlorinated water for 24 h in, and they released enough insecticide to kill 15 mosquitofish, 15 crayfish and 15 shrimps.

Chapman *et al.* (1982) also conducted acute bioassays (96 h), among other substances, with the insecticide/fungicide NaPCP and found different LC50 values from those reported by Inoue & Kondo (1962) (Table 3). The authors also found that the presence of substrate increased the tolerance to contaminants in the water and to changes in environmental variables (pH and salinity).

In addition to insecticides, a number of metals have been subjected to acute bioassays with *B. sowerbyi* as a test-species, and LC50 values can be found in the literature for cadmium (Chapman *et al.* 1982; Kaviraj & Konar 1982; Das & Kaviraj 1994; Ghosal & Kaviraj 1996; Ghosal & Kaviraj 2002), chromium (Kaviraj & Konar 1982; Sarkar & Konar 1997), cobalt (Mukherjee & Kaviraj 2011), copper (Das & Das 2005) and mercury (Chapman *et al.* 1982; Kaviraj & Konar 1982). Kaviraj and Konar (1982) tested acute toxicity (96 h) of mercury, chromium and cadmium and reported LC50 values of 0.007; 10.362 and 4.631 $mg.L^{-1}$ (for Hg, Cr and Cd, respectively). In another study (Kaviraj & Konar 1983), the effects of a mixture of these metals at the ratio of 0.001:0.46:1.0 (Hg, Cr and Cd respectively) was tested using values based on LC50 for metals in isolation (Table 4). Acute tests were conducted in 300 mL beakers with 10 individuals per replication and found LC50 (96 h) of 15 $mg.L^{-1}$. The mixture was more toxic than the metals in isolation. Metal concentration in the mixture was nearly one-fifth of lethal concentrations for the metals tested in isolation.

In another study of mixed substances, Konar & Mullick (1993) conducted bioassays using *B. sowerbyi*, among other organisms, in order to study the influence of detergent endosulfan, organophosphate pesticide DDVP and fertilizers (nitrogen and phosphate) on the toxic behavior and safe elimination of mixtures of zinc, copper, iron and lead. A solution was prepared containing 10 times the calculated LC50 value for zinc, copper and DDPV, and the LC50 value for the other components (LC50 values were based on tests performed with zooplankton) (Table 4). LC50 values were calculated for 10 different admixtures (Table 4). The authors found that the tested compounds present very different toxicity levels depending on the mixture in which they are found and concluded that, in order for a mixture of substances to be safely eliminated in the environment, it is not only the toxicity of the compounds in isolation that needs to be taken into account but, in fact, the toxicity they present when mixed at specific concentrations.

Other studies have also shown the effect of substances on metal toxicity. Ghosal & Kaviraj (1996) studied the influence of turkey manure (not toxic at the maximum tested concentration; 250 $mg.L^{-1}$) on the toxicity of cadmium and found that large amounts of manure reduce the toxicity for the species. Dutta & Kaviraj (1996) investigated the effect of lime acclimatization on the susceptibility of organisms to Cd. In a first test, organisms were acclimatized in lime solutions at 50, 75, 100, 125, 150, 175, 200 and 125 ppm for 96 h before bioassays were carried out with Cd and found that, when they had been acclimatized in concentrations 100 and 150 ppm, their tolerance to Cd increased markedly. In a second test, the authors found a reduced susceptibility to the metal when the organisms had been acclimatized for a period of eight days in a constant 75 ppm solution in comparison to a four-day period (different acclimatization periods were tested: 4, 6, 8 and 12 days). Findings suggest that the Ca^{+2} ion fills the binding sites in the cells of the organism, thus preventing Cd^{+2} from binding and then causing toxicity. However, excess calcium might also prevent oxygen (O_2) attachment, then causing anoxia in the organisms and their death. The authors recommend eight-day acclimatization in a 75-ppm lime solution for 96 h in a solution of 100 to 150 ppm as being ideal to increase resistance to Cd.

TABLE 3. Chemicals and respective LC50 values for species *B. sowerbyi*

Chemicals	LC50 ($mg \cdot L^{-1}$)		Temp.	References
	Water	Sediment	°C	
Metals				
Cadmium (Cd)	0.24	5.7	10	Chapman *et al.* 1982
	4.63		?	Kaviraj & Konar 1982
	36.98 (27.58 – 48.47)		?	Das & Kaviraj 1994
	58.02 (55.78 – 60.34)		25	Ghosal & Kaviraj 1996
	58.02 (55.78 – 60.34)		25	Ghosal & Kaviraj 2002
Chromium (Cr)	10.36		?	Kaviraj & Konar 1982
	207.5		28	Sarkar & Konar 1997
Cobalt (Co)	179.00 (140.00 – 228.00)		34	Mukherjee & Kaviraj 2011
Cooper (Cu)	0.08 (0.07 – 0.10)		20-21	Das & Das 2005
Mercury (Hg)	0.08	3.2	10	Chapman *et al.* 1982
	0.007		?	Kaviraj & Konar 1982
Insecticides				
NaPCP	2.2		25	Inoue & Kondo 1962
	0.28	0.56	10	Chapman *et al.* 1982
Parathion	3.5		25	Inoue & Kondo 1962
EPN	2.06		25	Inoue & Kondo 1962
Diazinon	4.95		25	Inoue & Kondo 1962
DDT	19.91		25	Inoue & Kondo 1962
Lindane	11.6		25	Inoue & Kondo 1962
Endrin	7.66		25	Inoue & Kondo 1962
Dieldrin	4.12		25	Inoue & Kondo 1962
Rotenone	0.25		25	Inoue & Kondo 1962
Pyrethrins	0.56		25	Inoue & Kondo 1962
Thiodan (Endosulfan)	0.88		28	Sarkar & Konar 1997
Cypermethrin	71.12 (64.00 – 78.00)		20	Saha & Kaviraj 2008
Detergents				
LAS	4.38 (3.75 – 5.13)	10.82 (9.27 – 12.64)	10	Casellato & Negrisolo 1989
	4.82 (3.75 – 6.19)		20	Casellato & Negrisolo 1989
Effluents				
Black liquor (%)	0.79	1.4	10	Chapman *et al.* 1982
Sewage (%)	2.5	7.6	10	Chapman *et al.* 1982
Others				
Low pH	3.7	2.6	10	Chapman *et al.* 1982
High pH	10.5	11.3	10	Chapman *et al.* 1982
Salinity (‰)	7.5	12	10	Chapman *et al.* 1982
Temperature (°C)	35	35	10	Chapman *et al.* 1982
Spent Bark of Cinchona	13800		27	Kaviraj *et al.* 1985
n-heptane	2500		27.8	Ghatak *et al.* 1988
n-hexane	3286.5		27.8	Ghatak *et al.* 1988
Cobalt Chloride ($CoCl_2$)	132.62 (115.16 – 152.73)		?	Das & Kaviraj 1994
Potassium Permanganate	0.03 (0.23 – 0.38)		?	Das & Kaviraj 1994
Thiocyanate	217.79 (194.46 – 243.92)		20	Bhunia *et al.* 2000
	186.45 (161.82 – 214.82)		25	Bhunia *et al.* 2000
	166.88 (136.03 – 204.72)		28	Bhunia *et al.* 2000
Aniline	586.50 (498.78 – 691.56)		?	Bhunia *et al.* 2003
Methanol	54890 (53200 – 56630)		?	Kaviraj *et al.* 2004
Quicklime (CaO)	83.00 (72.10 – 95.52)		20-21	Das & Das 2005
Lactic acid	50.82 (48.40 – 53.24)		?	Saha *et al.* 2006
Acetic acid	14.90 (14.48 – 15.43)		?	Saha *et al.* 2006
Benzoic acid	39.47 (38.92 – 40.03)		?	Saha *et al.* 2006

? – Not provided

Das & Das (2005) also tested lime effect (CaO) on the toxicity of another metal for water organisms including *B. sowerbyi*. Acute tests were conducted for 96 h, in two test batteries, the first to determine LC50 for copper and lime separately, and the second to find the effect of variations of lime concentrations on Cu

LC50. The findings revealed that Cu is toxic at low concentrations for *B. sowerbyi* (0.08 $mg.L^{-1}$), which is also sensitive to CaO (LC50 = 83.00 $mg.L^{-1}$) when compared to the fish species *Cyprinius carpo* L., which in turn did not present mortality for any CaO concentration lower than 500 $mg.L^{-1}$. The authors have also noted that the presence of Cu reduces lime toxicity; LC50 for lime in the absence of the metal was 83 $mg.L^{-1}$; however, with the addition of 0.08 $mg.L^{-1}$ Cu (LC50 value), no organism mortality was found even when they were exposed to 450 $mg.L^{-1}$ of CaO, which points to an antagonistic relation between both substances. According to the authors, the reduced toxicity may be caused by the same factor reported by Dutta & Kaviraj (1996): the competition between Ca^{+2} and Cu^{+2} ions for binding sites in the cells of the organisms.

Ghosal & Kaviraj (2002) studied the effects of cadmium combined with manure made up of *Pistia stratiotes* leaves, mixed with bovine manure at the ratio of 1:1, decomposed anaerobically for 85 days. The authors found that LC50 for cadmium increases with an increment in the amount of decomposed manure added to the solution. They also found that the oligochaetes accumulated more metal in the presence of the manure.

Also with regard to mixtures with metals, Sarkar & Konar (1997) studied the effect of insecticide Thiodan (Endosulfan) interacting with chromium in 96-h acute bioassays. The mixture was obtained based on the LC50 calculated for the individual substances. The authors found that when they are mixed, LC50 is much lower in comparison with LC50 for the components in isolation (mixture LC50: 166.50 $mg.L^{-1}$ – 166.4 mg Cr + 0.00209 $mg.L^{-1}$ Thiodan; LC50 Cr: 207.5 $mg.L^{-1}$; LC50 Thiodan: 0.882 $mg.L^{-1}$).

Kaviraj & Ghosal (1997) performed bioassays with natural sediments from brackish water lagoons (Sundarban, India), contaminated with Cd. Twelve samples were collected at different spots in the lagoons for laboratory bioassays carried out at 25°C for a period of 96 h. The organisms were placed in 3 L flasks (2.5 g of wet weight) containing 0.125 L of sediment and 2.5 L of Cd-free water (sediment-water ratio: 1:20). *B. sowerbyi* accumulates large amounts of Cd, and the highest reading was 10.50 ± 2.51 $\mu g.g^{-1}$ (organisms kept in sediment containing 5.76 ± 3.23 $mg.kg^{-1}$ cadmium).

Another substance that has been studied well is Linear Alkylbenzene Sulphonate (LAS), an anionic surfactant used in detergents. Casellato & Negrisolo (1989) carried out acute (96 h) and chronic (140 days) tests to assess its effects and have found, like Naqvi (1973) and Chapman *et al.* (1982), that, in acute tests, sediment presence significantly increases this species' tolerance to the tested compound (10.8 $mg.L^{-1}$ and 4.38 $mg.L^{-1}$, LC50 with and without sediment, respectively). The chronic tests, however, showed that low concentrations of LAS dissolved in water (0.5 and 2.5 $mg.L^{-1}$) influenced the species' reproductive behavior. At such concentrations, the organisms presented a discontinuous reproductive period which was clearly altered by the presence of LAS. Fewer cocoons were found within concentrations 0.5 and 2.5 $mg.L^{-1}$ in comparison to the control. The 5 $mg.L^{-1}$ concentration was very similar to the control for all aspects under observation; however, the authors were not able to account for these unexpected results. Still on the study into the effects of long exposure to LAS, Casellato *et al.* (1992) conducted an experiment with contaminated sediment in the laboratory and exposed the species for 220 days. No differences were found between the treatment (25.87 $mg.kg^{-1}$) and the control for the cocoon degeneration rate, hatching rate, number of eggs per cocoon and embryo development time; only the total numbers of cocoons and eggs were slightly larger in the treatment than the control. The authors concluded that, at that concentration, when LAS is present in the sediment, it has a weaker effect on the studied species than when it is dissolved in water. This was the first long-term toxicity experiment using *B. sowerbyi*.

Ducrot *et al.* (2010) also performed chronic bioassays to assess long-term Zn effects on the partial (28 days) and full (179 days) life-cycles of *B. sowerbyi*. In order to assess the effects of the metal on the partial life-cycle (PLC), the authors ran five bioassays: one with young organisms (14 days old), one with young-adults (40 days old), two with adults (60 days old) and one with cocoons. Two kinds of PLC test were performed, one to check the effects on survival and growth (28 days long) and another to check reproduction/ hatching (42 days long). As for the effect on the full life-cycle (FLC), a single bioassay was conducted with young, 14-day-old organisms. In the PLC, the authors found that, for young and adult organisms, there was a significant difference for survival only for the higher concentration tested (3317 $mg.kg^{-1}$, a value 25 times lower than the average value for French water bodies, which is nearly 130 $mg.kg^{-1}$). For young organisms, on the other hand, lower survival was found among organisms exposed to Zn concentrations higher than 1819 $mg.kg^{-1}$. As far as growth is concerned, the PLC test for all concentrations showed that young organisms grow

less when they are exposed to the metal. No significant differences were found in reproduction or hatching tests across the tested concentrations (419–1651 $mg.kg^{-1}$) and the control. The authors found that 67 ± 34% of the organisms born in sediments with Zn concentration higher than 551 $mg.kg^{-1}$ presented deformed gills; however, they were unable to confirm whether the metal was to blame for such deformities due to lack of replications in the experimental design. Following the FLC assays, it was found that the organisms exposed to concentrations between 409 and 1050 $mg.kg^{-1}$ showed lower body weight in comparison to the control. No significant differences have been reported for the other parameters (mortality, maturation time and reproduction). According to the findings, the authors conclude that the species is sensitive to Zn only in the first month of life.

The range of other substances (pure or mixed) that have already been used in bioassays with *B. sowerbyi* can be found in table 2, and calculated LC50 values are shown in tables 3 and 4.

TABLE 4. Mixtures and respective LC50 values for species *B. sowerbyi*.

Mixtures	LC50 ($mg \cdot L^{-1}$)	References
Hg + Cr + Cd	15.0 (0.80 – 21.00)	Kaviraj & Konar 1983
Zn + Cu + Fe + Pb	17.07 (1.00 – 35.84)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane	63.69 (12.45 – 114.93)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + PJ	14.58 (1.00 – 41.29)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ	44.57 (7.85 – 81.29)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + Endosulfan	49.80 (18.26 – 81.34)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + DDVP	50.16 (1.00 – 50.16)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + Endosulfan + DDPV	32.37 (1 – 32.37)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + Endosulfan + DDPV + Urea	36.13 (19.91 – 61.35)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + Endosulfan + DDPV + SSP	65.68 (31.11 – 100.25)	Konar & Mullick 1993
Zn + Cu + Fe + Pb + *n*-hexane + PJ + Endosulfan + DDPV + Urea + SSP	31.89 (1 – 31.89)	Konar & Mullick 1993
Cd + $KMnO_4$	41.17 (44.90 – 51.67)	Das & Kaviraj 1994
Cd + $CoCl_2$	65.01 (51.63 – 58.38)	Das & Kaviraj 1994
Cd + 0.25g·L composted manure	70.80 (65.92 – 76.03)	Ghosal & Kaviraj 2002
Cd + 0.50 g·L composted manure	77.47 (71.43 – 84.26)	Ghosal & Kaviraj 2002
Cd + 1.00 g·L composted manure	78.75 (72.98 – 85.00)	Ghosal & Kaviraj 2002
Cd + 6.70 g·L composted manure	85.33 (79.60 – 91.46)	Ghosal & Kaviraj 2002
Cd + 30 mg·L poultry litter	48.55 (45.71 – 51.56)	Ghosal & Kaviraj 1996
Cd + 65 mg·L poultry litter	68.73 (61.86 – 76.36)	Ghosal & Kaviraj 1996
Cd + 125 mg·L poultry litter	77.56 (67.67 – 88.9)	Ghosal & Kaviraj 1996
Cd + 250 mg·L poultry litter	85.76 (76.57 – 96.12)	Ghosal & Kaviraj 1996
Thiodan + Cr	166.5 (122.5 – 212.5)	Sharkar & Konar 1997

Review of bioassay methods

It has been noted that the vast majority of studies included acute bioassays in which only water was used. Some tests were run in Petri dishes and the significant majority in beakers between 250 to 600 mL, in which 200 to 300 mL solutions were assessed, by exposing between 3 and 10 organisms per replication.

Another significant variation in methodology that has been found across the studies concerns test temperatures. Some experiments tested species sensitivity at 10°C (Chapman *et al.* 1982) and others as high as 34°C (Mukherjee & Kaviraj 2011). Temperature is known to influence substance toxicity (Naqvi 1973;

Casellato & Negrissolo 1989; Bhunia *et al.* 2000) and to play a key role in bioassays, in addition to its impact on the biology of reproduction and species growth (Aston 1968; Aston *et al.* 1982; Bonacina *et al.* 1987; Bonacina *et al.* 1994). Chapman *et al.* (1982) have found that the LC50 for temperature is 35°C, which is very close to the temperature (34°C) used by Mukherjee & Kaviraj (2011) in their acute bioassays with cobalt, which may cause uncertainty as to whether the actual cause of mortality among the organisms is due to temperature, to the chemical or to a combination of both.

In chronic bioassays, the most noticeable difference concerns length of exposure, which ranged between 28 (Ducrot *et al.* 2010) and 220 days (Casellato *et al.* 1992), and other variations noted included container size, sediment and water volume, and organism density. In most cases, such methodological discrepancies make the comparison of results impossible. The design of a protocol for bioassays with *B. sowerbyi* should promote methodological standardization which, in turn, may allow for the comparison of results across different laboratories.

Concluding remarks

As we have shown, a number of papers have already been published on ecotoxicity assays with *B. sowerbyi*; however, the lack of a specific protocol for the species makes the comparison of results difficult and causes researchers to choose other species for which there is already a protocol, such as *T. tubifex* (ASTM 2005; OECD 2008) and *L. variegatus* (USEPA 2000; OECD 2008).

According to ASTM (2005), in order for a species to be used in ecotoxicological tests with substrates, it must *(1)* possess an ecotoxicity database that shows sensitivity to a number of chemicals concerning the sediment, *(2)* possess a database for comparison across different laboratories, *(3)* be in physical contact with the sediment, *(4)* always be available for testing, either in cultures or field samples, *(5)* be easily kept in the laboratory, *(6)* be easily identified, *(7)* be ecologically or economically important, *(8)* have broad geographic distribution, be native to the site to be assessed or be of a similar niche as that of native organisms *(9)* be tolerant to physical and chemical variations in the sediment, *(10)* be compatible with the exposure methods and selected endpoints, *(11)* be reviewed and *(12)* be confirmed by answers from natural populations of benthic organisms.

B. sowerbyi has been known to have great potential for use as a test-species; however, a bioassay protocol is required in order for this species to become another tool in programs to assess and monitor the health of tropical water environments.

For chronic tests, we recommend using the methods described in protocol E1706–05 (ASTM, 2005), described for *T. tubifex*: 250 mL flasks with 100 mL of sediment and 100 mL of dechlorinated water, temperature adjusted to 25 or 30°C, instead of 21°C, and maybe, in order to allow for a larger number of juveniles born in the control, a 7-day extension to the final duration of the chronic test if the temperature is 25°C. Test duration would then become 35 days, as recommended by Marchese and Brinkhurst (1996). For acute tests with no sediments (water-only), we recommend using 200 mL flasks with 100 mL of water/solution and 4 or 5 individuals per replication, at 25°C. However, further studies are required in order for a specific protocol for *B. sowerbyi* to be produced.

References

Alves, R.G. & Strixino, G. (2000) Distribuição espacial de Oligochaeta em uma lagoa marginal do Rio Mogi-Guaçu, São Paulo, Brasil. *Iheringia*, Série Zoológica, 88, 173–180. 2000.

Alves, R.G. & Strixino, G. (2003) The sampling of benthic macroinvertebrates using two different methods: waiting trays and an Ekman collector. *Acta Limnologica Brasiliensia*, 15, 3, 1–6.

ASTM – American Society for Testing and Materials (2005) *E1706-05: Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates*, 117 pp.

Aston, R.J. (1968) The effect of temperature on the life cycle, growth and fecundity of *Branchiura sowerbyi* (Oligochaeta: Tubificidae). *Journal of Zoology*, 154, 29–40.
http://dx.doi.org/10.1111/j.1469-7998.1968.tb05038.x

Aston, R.J. & Milner, A.G.P. (1982) Conditions required for the culture of *Branchiura sowerbyi* (Oligochaeta: Tubificidae) in activated sludge. *Aquaculture*, 26, 155–166.
http://dx.doi.org/10.1016/0044-8486(81)90118-6

Aston, R.J., Sadler, K. & Milner, A.G.P. (1982) The effects of temperature and the culture of *Branchiura sowerbyi* (Oligochaeta, Tubificidae) on activated sludge. *Aquaculture*, 29, 137–145.
http://dx.doi.org/10.1016/0044-8486(82)90040-0

Bhunia, F., Saha, N.C. & Kaviraj, A. (2000) Toxicity of thiocyanate to fish, plankton, worm and aquatic ecosystem. *Bulletin of Environmental Contamination and Toxicology,* 64, 197–204.

Bhunia, F., Saha, N.C. & Kaviraj, A. (2003) Effects of Aniline – an aromatic amine to some freshwater organisms. *Ecotoxicology*, 12, 397–404.

Bonacina, C., Bonomi, G. & Monti, C. (1987) Progress in cohort cultures of aquatic Oligochaeta. *Hydrobiologia,* 155, 163–169.

Bonacina, C., Bonomi, G. & Marzuoli, D. (1994) Quantitative observations on the population ecology of *Branchiura sowerbyi* (Oligochaeta, Tubificidae). *Hydrobiologia*, 278, 267–274.
http://dx.doi.org/10.1007/bf00142334

Casellato, S. (1984) Life-cycle and karyology of *Branchiura sowerbyi* Beddard (Oligochaeta, Tubificidae). *Hydrobiologia*, 115, 65–69.
http://dx.doi.org/10.1007/bf00027895

Casellato, S. & Negrissolo, P. (1989) Acute and chronic effects of a surfactant on some freshwater tubificid species. *Hydrobiologia*, 180, 243–252.

Casellato, S., Aiello, R., Negrisolo, P. & Seno, M. (1992) Long-term experiment on *Branchiura sowerbyi* Beddard (Oligochaeta, Tubificidae) using sediment treated with LAS (Linear Alkylbenzene Sulphonate). *Hydrobiologia*, 232, 169–173.
http://dx.doi.org/10.1007/bf00017476

Chapman , P. M., Farrell, M.A. & Brinkhurst, R.O. (1982) Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. *Aquatic Toxicology*, 2, 47–67.
http://dx.doi.org/10.1016/0166-445x(82)90005-4

Chapman, P.M. (2001) Utility and relevance of aquatic oligochaetes in ecological risk assessment. *Hydrobiologia*, 463, 149–169.

Das, B.K. & Kaviraj, A. (1994) Individual and interactive lethal toxicity of cadmium, potassium permanganate and cobalt chloride to fish, worm and plankton. *Geobios*, 21, 223–227.

Das, B.K. & Das, N. (2005) Impacts of quicklime (CaO) on the toxicity of copper ($CuSO_4.\ 5H_2O$) to fish and fish food organisms. *Chemosphere*, 61, 186–191.
http://dx.doi.org/10.1016/j.chemosphere.2005.02.064

Dornfeld, C.B., Alves, R. G., Leite, M.A. & Espíndola, E.L.G. (2006) Oligochaeta in eutrophic reservoir: the case of Salto Grande reservoir and their main affluent (Americana, Sao Paulo, Brazil). *Acta Limnologica Brasiliensia*, 18**,** 2, 189–197.

Drews, C.D. & Zoran, M.J. (1989) Neurobehavioral specializations for respiratory movements and rapid escape from predators in posterior segments of the tubificid *Branchiura sowerbyi*. *Hydrobiologia*, 180, 65–71.
http://dx.doi.org/10.1007/bf00027538

Ducrot, V., Péry, A.R.R., Quéau, H., Mons, R., Lafont, M. & Garric, J. (2007) Rearing and estimation on life-cycle parameters of the tubificid worm *Branchiura sowerbyi*: Application to ecotoxicity testing. *Science of the Total Environment*, 384, 252–263.

Ducrot, V., Billoir, E., Péry, A.R.R., Garric, J. & Charles, S. (2010) From individual to population level effects of toxicants in the tubificid *Branchiura sowerbyi* using threshold effect models in a bayesian framework. *Environmental Science and Technology*, 44, 3566–3571.

Dutta, T.K. & Kaviraj, A. (1996) Effects of lime acclimation on the susceptibility of two freshwater teleosts and one oligochaete worm to metallic pollutant cadmium. *Folia Biologica*, 44, 143–148.

Ghatak, D.B., Hossain, M.M. & Konar, S.K. (1988) Acute toxicity of n-heptane and n-hexane on worm and fish. *Environmental and Ecology*, 6, 943–947.

Ghatak, D.B. & Konar, S.K. (1990) Acute toxicity of mixture of heavy metal cadmium, pesticide DDVP, detergent parnol J and petroleum product n-heptante on fish, plankton and worm. *Environmental and Ecology*, 8, 1239–1248.

Ghosal, T.K. & Kaviraj, A. (1996) Influence of poultry litter on the toxicity of cadmium to aquatic organisms. *Bulletin of Environmental Contamination and Toxicology*, 57, 1009–1015.
http://dx.doi.org/10.1016/s0045-6535(01)00162-x

Ghosal, T.K. & Kaviraj, A. (2002) Combined effects of cadmium and composted manure to aquatic organisms. *Chemosphere*, 46, 1099–1105.

Ghosh, T.K. & Konar, S.K. (1980) Toxicity of chemicals and wastewater of paper and pulp mills to worms, plankton and molluscs. *Indian Journal of Environmental Health*, 22, 278–285.

Ghosh, T.K. & Konar, S.K. (1983) Effects of formalin on aquatic ecosystem. *Environment and Ecology*, 1, 273–276.

Inoue, T. & Kondo, K. (1962) Susceptibility of *Branchiura sowerbyi*, *Limnodrilus socialis* and *Limnodrilus willeyi* for several agricultural chemicals. *Botyu Kagaku*, 27, 97–99.

Jorcin, A. & Nogueira, M.G. (1998) Benthic macroinvertebrates in the Paranapanema reservoir cascade (southeast Brazil). *Brazilian Journal of Biology*, 68, 4, 1013–1024.
http://dx.doi.org/10.1590/s1519-69842008000500009

Kaviraj, A. & Konar, S.K. (1982) Acute toxicity of mercury, chromium and cadmium to fish, plankton and worm. *Geobios*, 9,

97–100.
Kaviraj, A. & Konar, S.K. (1983) Lethal effects of mixture of mercury, chromium and cadmium on fish. *Environment and Ecology*, 1, 153–158.
Kaviraj, A. & Ghosal, T.K. (1997) Partitioning and bioaccumulation of cadmium from the sediment of brackishwater ponds in Sundarban, India. *Asian Fisheries Science*, 9, 269–274.
Kaviraj, A., Ghosh, T., Roy, L.P.K. & Som, U. (1985) Acute toxicity of spent bark of Cinchona to fish, plankton and worm. *Environment and Ecology*, 3, 345–347.
Kaviraj, A., Bhunia, F. & Saha, N.C. (2004) Toxicity of methanol to fish, crustacean, oligochaete worm and aquatic ecosystem. *International Journal of Toxicology*, 23, 55–63.
Konar, S.K. & Mullick, S. (1993) Problems of safe disposal of petroleum products, detergents, heavy metals and pesticides to protect aquatic life. *The Science of the Total Environment*, Supplement, 989–1000.
Liang, Y. (1984) Annual production of *Branchiura sowerbyi* (Oligochaeta: Tubificidae) in the Donghu Lake, Wuhan, China. *Chinese Journal of Oceanology and Limnology*, 2, 1, 102–108.
Lobo, H. & Alves, R.G. (2011) Reproductive cycle of *Branchiura sowerbyi* (Oligochaeta: Naididae: Tubificinae) cultivated under laboratory conditions. *Zoologia*, 28, 427–431.
http://dx.doi.org/10.1590/s1984-46702011000400003
Marchese, M.R. & Brinkhurst, R.O. (1996) A comparison of two tubificid oligochaete species as candidates for sublethal bioassay tests relevant to subtropical and tropical regions. *Hydrobiologia*, 334, 163–168.
http://dx.doi.org/10.1007/bf00017366
Martin, P., Martinez-Ansemil, E., Pinder, A., Timm, T. & Wetzel, M.J. (2008) Global diversity of oligochaetous clitellates ("Oligochaeta"; Clitellata) in freshwater. *Hydrobiologia*, 595, 117–127.
http://dx.doi.org/10.1007/s10750-007-9009-1
Mondal, D.K. & Kaviraj, A. (2008) Ecotoxicological effects of jute retting on the survival of two freshwater fish and two invertebrates. *Ecotoxicology*, 17, 207–211.
http://dx.doi.org/10.1007/s10646-007-0185-6
Mukherjee, S. & Kaviraj, A. (2011) Ecotoxicological assessment of cobalt used as supplement in diet of common carp *Cyprinus carpio*. *Bulletin of Environmental Contamination and Toxicology*, 87, 527–530.
Mullick, S. & Konar, S.K. (1991) Combined influence of heavy metals, petroleum products, detergents, pesticides and fertilizers on the worm *Branchiura sowerbyi*. *Environment and Ecology*, 9, 1032–1037.
Naqvi, S.M.Z. (1973) Toxicity of twenty-three insecticides to a tubificidae worm *Branchiura sowerbyi* from the Mississippi Delta. *Journal of Economic Entomology*, 66, 70–74.
Nascimento, H.L.S. & Alves, R.G. (2008) Cocoon production and hatching rates of *Branchiura sowerbyi* Beddard (Oligochaeta, Tubificidae). *Revista Brasileira de Zoologia,* 25, 1, 16–19.
OECD – Organization for Economic Cooperation and Development (2008). *315*: *Guidelines for the Testing of Chemicals: Bioaccumulation in Sediment-dwelling Benthic Oligochaetes*. 32 pp.
Pamplin, P.A.Z., Rocha, O. & Marchese, M. (2005) Riqueza de espécies de Oligochaeta (Annelida, Clitellata) em duas represas do rio Tietê (São Paulo). *Biota Neotropica*, 5, 1, 1–8.
http://dx.doi.org/10.1590/s1676-06032005000100007
Pamplin, P.A.Z., Almeida, T.C.M. & Rocha, O. (2006) Composition and distribution of benthic macroinvertebrates in Americana Reservoir (SP, Brazil). *Acta Limnologica Brasiliensia*, 18, 2, 121–132.
Raburu, P., Mavuti, K.M., Harper, D.M. & Clark, F.L. (2002) Population structure and secondary productivity of *Limnodrilus hoffmeisteri* (Claparede) and *Branchiura sowerbyi* Beddard in the profundal zone of Lake Naivasha, Kenya. *Hydrobiologia*, 488, 153–161.
http://dx.doi.org/10.1007/978-94-017-2031-1_15
Saha, S. & Kaviraj, A. (2008) Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. *Bulletin of Environmental Contamination and Toxicology*, 80, 1, 49–52.
Saha, N.C., Bhunia, F. & Kaviraj, A. (2006) Comparative toxicity of three organic acids to freshwater organisms and their impact on aquatic life. *Human and Ecological Risk Assessment*, 12, 192–292.
Sarkar, S.K. & Konar, S.K. (1982) Acute toxicity of agricultural fertilizers to aquatic organisms. *Geobios*, 9, 225–228.
Sarkar, U.K. & Konar, S.K. (1995) The combined effects of pesticide thiodan, heavy metal chromium and detergent ekaline FI on fish and fish food organisms. *Journal of Environmental Biology*, 16, 19–26.
Sarkar, U.K. & Konar, S.K. (1997) Interactions of insecticide thiodan (Endosulfan) and heavy metal chromium and impact on fish and aquatic ecosystem. *Chemistry and Ecology*, 13, 271–278.
USEPA – United States Environmental Protection Agency. (2000) *EPA/600/R-99/064: Methods for measuring the toxicity and bioaccumulation of sediment associated contaminant with freshwater invertebrates.* 2ª ed., Washington D.C., 192 pp.
Warren, L.A., Tessier, A. & Hare, L. Modeling cadmium accumulation by benthic invertebrates in situ: The relative contributions of sediment and overlying water reservoirs to organism cadmium concentrations. *Limnology and Oceanography*, 43, 7, 1442–1454.
http://dx.doi.org/10.4319/lo.1998.43.7.1442