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Toxic effects of Europium chloride on developing zebrafish (*Danio rerio*) embryos

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Lanthanides, including Europium, show a broad spectrum of industrial applications and their release into environment has been significantly increased during the last decades. However, toxic effects of lanthanides on aquatic biota have not been consistently studied. The aim of the present study was to assess toxicity of Europium chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) on zebrafish embryos under standardised experimental conditions. Embryos were exposed to a range of Europium concentrations (0.05-500 ppm) either for the first 10 or for 72 hrs after fertilisation. The embryonic development was assessed by several biological and physiological endpoints (e.g. mortality rate during the incubation period, hatching rate, standard length at hatch, development of pigmented eyes, body morphometrics, heart contractility as well as heart rate). Exposure of embryos to Europium affected all the parameters of embryonic development resulting in significant dose-dependent mortality, delay of hatching, decreased standard length and heart rate as well as in delayed heart formation. Malformations and significant differences in morphometrics, however, were not observed. The effects of Europium on heart formation and especially on the heart rate, which was manifested starting at concentration as low as 0.05 ppm, suggested that the heart muscle may constitute the target organ for the toxicity of the metal.

Key words: lanthanide, toxicity, zebrafish, development, heart.

INTRODUCTION

Over the past three decades, the release of rare metals into the environment has been significantly increased due to their use in industry and agriculture (Yongxing *et al*., 2000). Europium (Eu) is a rare earth element of the lanthanide series, which is primarily used in nuclear reactor control rods because of its effectiveness in absorbing neutrons. Eu-doped plastics have been used as laser materials, and Eu oxide serves as a phosphor activator, for example in the red phosphors of colour television tubes (Argonne National Laboratory, 2005). Recently, there has been an increasing interest in diagnostic and therapeutic applications of Eu in biomedical nanotechnology due to its fluorescent properties (Kallistratos *et al.*, 1985; Reynaldo *et al.*, 1996; Alptürk *et al.*, 2006).

The biochemistry of lanthanides has been extensively reviewed (Evans, 1990; Cotton, 1991). They can form complexes with many molecules of biochemical interest, since the major ligands for lanthanides are donor groups with negatively charged oxygen atoms [e.g. carboxylic or phosphate groups of amino acids and nucleotides (Gross & Simpkins, 1981; Gersanovski *et al*., 1985; Franklin, 2001)]. Lanthanides enter into reaction with biologically active compounds replacing calcium ions and many others, such as Zn^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Fe^{3+} (Burroughs *et al*., 1992; Sigel & Sigel, 2003).

Toxicity of the lanthanide-containing compounds in vertebrates depends mainly on their chemical form

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and route of administration. It has been shown that oral intake through drinking water or oral administration of $EuCl_3 \cdot 6H_2O$ causes to rats hyperkeratosis of the forestomach and eosinophils infiltration of the stomach submucosa (Ogawa *et al*., 1995). In rabbits, lanthanides provoke conjunctivitis when applied directly on eyes and severe irritation when applied to abraded skin. Intradermal injection of lanthanide chlorides lead to epilation and nodule formation in guinea pigs (Haley *et al*., 1965). It has also been shown that subcutaneous injection of lanthanides causes local calcification with mild fibrosis and accumulation of multinucleated giant cells in mice (Garrett & McClure, 1981). Studies of acute toxicity of Eu in rats revealed that the main symptoms were arched back, writhing, ataxia, lacrymation, stretching of the hind limbs on walking, and laboured and depressed respiration. Death resulted from cardiovascular collapse coupled with respiratory paralysis (Haley *et al*., 1965).

Data about Eu effects on aquatic biota are surprisingly poor, considering the soil enrichment by metals of lanthanide series, caused by humans and occasionally resulting in elevated concentrations in the aquatic environment (Sneller *et al*., 2000). Generally, concentrations of lanthanides increase in the series surface water-pore water-sediments-macrophytes-molluscs; however, aquatic biota tends to show rather high variability in bioaccumulation, depending on the different lanthanide sources and the structure of food chains (Weltje *et al*., 2002). Therefore, adverse effects on certain species, like bottom-dwelling omnivorous and benthivorous cyprinids, should be considered.

Zebrafish (*Danio rerio*) belongs to the family Cyprinidae and is a model organism (Lele & Krone, 1996) which offers several compelling experimental advantages, whereas the toxicity profiles for zebrafish and mammals are strikingly similar (McGrath & Li, 2008). One of the advantages of zebrafish is a large number of readily available eggs, which allow multifactorial tests on embryos. Experiments with fish embryos are easy to perform and they may serve as a sound alternative to those using mature fish (Wedekind *et al*., 2007; Lammer *et al*., 2009).

The aim of the present study is to evaluate the potential toxic effects of Eu on developing embryos of the model freshwater organism *D. rerio*.

MATERIALS AND METHODS

Breeding was performed on fish obtained from wild stocks and maintained in the laboratory for at least three generations. Sexually mature zebrafish were kept in 40 l full glass aquaria with a flow-through system (flow rate 10-20 l hr^{-1}, 28.5 ± 1 °C, pH 7.2 ± 0.2 and total hardness 24 °dH). Light and dark periods were 14 and 10 hrs, respectively, and fish were fed with commercially available artificial diet (ZM-400, Tetra Werke, Melle, Germany) twice per day.

Fertilised eggs were obtained from reproducing adult zebrafish according to the procedures described by Westerfield (1995). Eggs collected within two hours after spawning were staged according to standard protocols (Kimmel *et al*., 1995) and exposed to a range of Eu concentrations (0, 0.05, 0.5, 5, 50, 100, 200, 320, 400 and 500 ppm) in triplicate for each one. Europium (III) chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) (CAS 13759-92-7) was obtained from Aldrich Chem.

For each Eu concentration, totally 120 eggs were incubated in a Petri dish at 28.5 ± 1 °C for 72 hrs. Additionally, for some concentrations (200, 320, 400 and 500 ppm), the eggs were exposed to the metal only for the first 10 hpf (hrs post fertilization) and placed afterwards into clean fresh water. The development of embryos and larvae was monitored under a light stereomicroscope. Mortality and hatching were recorded every hour. Dead (non-translucent and dark coloured) eggs were removed. Live as well as dead embryos were examined for malformations under a light microscope. Dead embryos were placed in a NaCl saturated solution, before the examination, until they became clear. Morphometric analysis was carried out on a set of distances among several landmarks defined on the digital photographs of larvae. Distances were determined using an image analysing software (NIKON Digital Sight DS-L2). A total of nine raw distances [eye diameter, mouth to posterior margin of pericardial cavity, posterior margin of pericardial cavity to urogenital opening, urogenital opening to posterior tip of notochord, the highest point of head (dorsally from rhombencephalon) to posterior tip of notochord, posterior tip of notochord to anterior margin of dorsal fin, posterior tip of notochord to posterior margin of dorsal fin, mouth to the highest point of head, posterior margin of pericardial cavity to the highest point of head] along with eight areas (eye, yolk sac, dorsal-caudal fin, ventral fin, notochord, otic capsule, otoliths and body area) were recorded. The effect of body size was removed by a standard regression method (Lleonart *et al*., 2000).

The presence of well-developed heart was assessed at the eye-stage (48 hpf). The heart rate (beats per minute) was measured at 48 and 72 hrs after ferti-

lisation in triplicate for each embryo. Only animals showing a stable rhythm were included in the analysis. Immediately after hatching, the larvae were placed on a graduated (Newbauer) slide and their standard length (StL) was determined under stereomicroscope.

Mortality of embryos was analysed by probit analysis (Finney, 1971) assuming the Eu concentration as the single predictor and the natural response rate to be zero.

The effects of Eu on hatching rate of zebrafish were analysed using the Kaplan-Meier procedure (Hosmer & Lemeshow, 1999). Only successfully hatched larvae were included in the analysis. The overall statistical significance of the presence of Eu (Mantel-Cox test) as well as pairwise differences among hatching curves (log-rank test) were estimated. The median hatching time along with its confidence interval was determined for each concentration of Eu.

Differences in StL at hatching were assessed by ANCOVA (n = 40 per treatment) with exposure (10 or 72 hpf) as fixed factor and log-transformed Eu concentration as covariate. The standardised values of morphometric variables were subjected to multivariate analysis of variance as well as to discriminant analysis in order to assess eventual differences in body form between the groups of larvae exposed to different Eu concentrations.

Confidence intervals and differences for the proportions of embryos with well-developed heart at 48 hpf were estimated using binomial test (n = 154 - 331, according to various treatments). Changes in heart rate after exposure to Eu were analysed by ANCOVA (type IV sum of squares) with the exposure time (10 or 72 hpf) and assessment time (48 and 72 hpf) as fixed factors and log-transformed Eu concentration as covariate (n = 40 per treatment).

All analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

The presence of Eu affected significantly the developmental parameters of zebrafish (Table 1). Under the conditions of continuous 72 hpf exposure, mortality started at 100 ppm, whereas exposure to 500 ppm of Eu killed 100% of embryos. The LD_{50} estimated by probit function was equal to 277.1 ppm with 95% CI within 233.9-323.0 ppm. When exposed for 10 hpf, the death of embryos was significantly attenuated (LD_{50} = 448.6; 95% CI: 366.4-574.9 ppm). The ratio

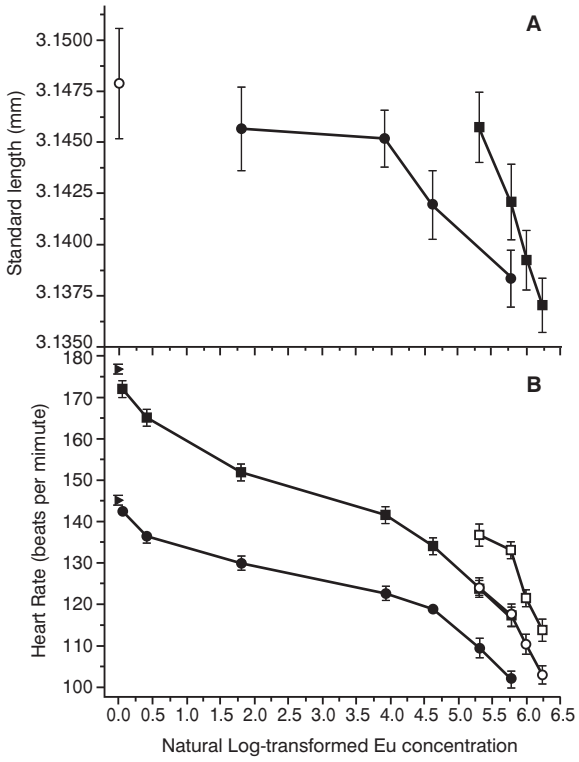

FIG. 1. A. Effects of exposure to Eu on the StL of the newly hatched zebrafish. Open circle: control; Filled circles: 72 hpf exposure; Squares: 10 hpf exposure. B. Dependence of the heart rate of zebrafish embryos (48 and 72 hpf) on the concentration of Eu (0-500 ppm) and duration of exposure (10 or 72 hpf). Triangles: control (lower: 48 hpf, upper: 72 hpf); Circles: exposure to Eu and heart rate at 48 hpf; Squares: exposure to Eu and heart rate at 72 hpf; Shaded symbols: 72 hpf exposure; Open symbols: 10 hpf exposure. Error bars represent 95% CI.

of median potencies was 0.618 with 95% CI within the range of 0.397-0.821.

Because of significant mortality starting at 100 ppm, the hatching rate was analysed only for alive and successfully hatched embryos. Exposure to Eu significantly increased the median hatching time as well as the overall duration of hatching (Mantel-Cox, $p < 0.001$) with 72 hpf exposure resulting in a more profound delay. Under conditions of both 10 and 72 hpf exposure, the median hatching time increased approximately up to 83 hpf, after which extensive mortality of embryos was observed.

The StL of newly hatched larvae (Fig. 1A) was significantly affected at high Eu concentrations ($p < 0.001$). After continuous 72 hpf exposure, a slight but significant decrease of mean StL in comparison to the control group was observed at 100 ppm (ln-transformed = 4.61) of Eu ($p < 0.01$) and became more pronounced at 320 ppm (ln-transformed = 5.77) ($p <$

TABLE 1. Parameters of embryonic development of zebrafish after either 10 or 72 hpf exposure to Eu

Eu concentration (ppm)	Observed death rate		Estimated median hatching time (hpf) ± 95% CI[a]		Proportion of embryos without heart beat at 48 hpf	
	Exposure time		*Exposure time*		*Exposure time*	
	10 hpf	72 hpf	10 hpf	72 hpf	10 hpf	72 hpf
0	0/125	0/60	n. e.	56 ± 0.7	1/125 (1%)	2/154 (1%)
0.05	n.e.	0/60	n. e.	57 ± 0.9	n. e.	6/163 (4%)
0.5	n.e.	0/60	n. e.	63 ± 1.6	n. e.	18/231 (8%)
5	n.e.	0/80	n. e.	69 ± 3.7	n. e.	41/255 (16%)
50	n.e.	0/80	n. e.	75 ± 4.7	n. e.	69/259 (27%)
100	n.e.	10/200 (5.0%)	n. e.	81 ± 4.9	n. e.	79/274 (29%)
200	11/128 (8.6%)	36/198 (18.2%)	61 ± 0.5	82 ± 6.2	18/98 (18%)	100/331 (30%)
320	27/112 (24.1%)	99/200 (49.5%)	66 ± 3.3	85 ± 7.4	22/85 (26%)	94/290 (32%)
400	46/120 (38.3%)	150/190 (78.9%)	76 ± 3.8	n. e.	27/72 (37%)	n. e.
500	56/103 (54.4%)	200/200 (100%)	84 ± 6.6	n. e.	35/72 (49%)	n. e.

[a] Kaplan-Meier analysis
n. e.: not estimated

0.001). Above this concentration, the differences in StL were not estimated due to the extensive mortality of embryos. A dose-dependent decrease in StL was also observed after 10 hpf exposure. Moreover, there was a significant interaction between the factor of exposure time and Eu concentration covariate ($p < 0.001$), indicating that the high concentrations of the metal, whether for 10 or 72 hpf exposure, tend to produce more similar negative effects on the growth of embryos.

Malformations of body shape, eye and yolk sac were not seen in dead or alive embryos, except for heart aplasia in most dead embryos. When corrected for body size, the morphometric measurements were not significantly different even at highest Eu concentrations used. Very poor discrimination (32% of cases) was also achieved by means of discriminant analysis (data not shown). Therefore, it is concluded that exposure to Eu has no teratogenic effect and, more generally, does not result in significant alterations of the body shape.

Significant effects of Eu on the development and functionality of embryonic heart were detected. Exposure to Eu delayed the differentiation of the heart muscle depending on the concentration of metal (Table 1; binomial test, $p < 0.001$). At concentration of 320 ppm, the proportions of normal embryos were not significantly different between 10 and 72 hpf exposure times. At higher concentrations, most embryos were dead after continuous exposure, whereas for 10 hpf exposure the proportion of animals with normally developed heart continued to decline. Death of embryos was accompanied by complete heart aplasia in most cases.

Furthermore, even in the successfully heart developing embryos, the heart rate was affected by the presence of the metal. The mean values of beats per minute along with 95% CI for different treatments at different time points are shown in Figure 1B. Concentration values (ln-transformed) was the most strong covariate ($p < 0.001$, partial $Eta^2 = 0.440$), causing a time-dependent decrease in the heart rate. The latter was also dependent on the exposure time ($p < 0.001$, partial $Eta^2 = 0.225$) as well as on the time point of measurement (i.e. 48 or 72 hpf; $p < 0.001$, partial $Eta^2 = 0.044$). There were also significant interactions of Eu concentration with the factors of exposure duration ($p < 0.001$, partial $Eta^2 = 0.184$) and the time point of measurement ($p < 0.05$, partial $Eta^2 = 0.006$), indicating that the differences between exposure and measurement time points were attenuated at high concentrations of the metal.

DISCUSSION

The results of our study reveal significant toxic effects of Eu on developing zebrafish eggs. Heart rate and hatching time of embryos may be pointed out as the most sensitive endpoints of the presence of the metal. Significant changes in these parameters were observed at the lowest Eu concentration (0.05 ppm) after 72 hpf exposure. The presence of Eu also resulted

in a significant delay of heart formation starting at Eu concentration of 0.5 ppm. At 100 ppm of Eu and above, a significant decrease in growth and onset of mortality were observed. Exposure to Eu for the first 10 hpf, while resulted in a lesser mortality, exerted strong and lasting effects on the heart function and development of animals. At high concentrations, these effects came close to those of continuous 72 hpf exposure.

Due to their chemistry and ionic radius, lanthanides are effective blockers of calcium movement and inhibitors of physiological processes depending on Ca^{2+} transport. In this regard, nerve impulse transmission (Miledi, 1971), skeletal muscles contraction (Morris, 1980), hormonal responses (Borowitz, 1972) and especially cardiac function (Kitzes & Berns, 1979; Durret & Adams, 1980; Fawzi & McNeill, 1985) may be considered as the most important. The inhibition of cardiac muscle contraction by lanthanides may be attributed to either Ca displacement (Ravens, 1975; Durret & Adams, 1980; Fawzi & McNeill, 1985) or binding of Eu at calcium specific sites of Ca^{2+}-Mg^{2+} ATP-ase (Joshi & Shamoo, 1987).

Heart contractility may thus constitute the primary target of Eu toxic effects, taking into account that, in our study, the heart rate was the most sensitive to Eu parameter. At high Eu concentrations, most death events occurred at the second day post fertilisation and were accompanied by aplastic or hypoplastic heart muscle. Our results thus support the idea that an early disruption of Ca-mediated functions exerts detrimental effect on subsequent heart morphogenesis and remodelling in zebrafish (Glickman & Yelon, 2002). In small fish embryos, including zebrafish, the heart function, primarily, does not ensure the oxygen delivery but rather the distribution of nutrients and electrolytes through embryonic tissues (Pelster & Burggren, 1996). Thus, decreased heart function would result in a rather uniform depletion of energy resources and decrease in metabolic rate, which might well explain the delayed hatching, decreased growth and mortality observed. This pattern of toxicity is also consistent with the absence of malformations and body form alterations in our study.

In conclusion, this study demonstrates for the first time the toxic effects of Eu on developing zebrafish embryos. Although decrease in growth and viability was not observed up to 100 ppm of the trace element, a slight decline of cardiac function and delayed hatching were already present in zebrafish embryos exposed to the lowest experimental Eu concentration (0.05 ppm for 72 hpf). It may be further hypothesised that chronic exposure to low Eu concentrations could permanently and negatively affect cardiac function and energy distribution, rising thus cumulative effects and decreased viability of fish offspring at later developmental stages.

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REFERENCES

Alptürk O, Rusin O, Fakayode SO, Wang W, Escobedo JO, Warner IM, Crowe WE, Král V, Pruet JM, Strongin RM, 2006. Lanthanide complexes as fluorescent indicators for neutral sugars and cancer biomarkers. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 9756-9760.

Argonne National Laboratory, 2005. *Europium*. Chicago, USA.

Borowitz JL, 1972. Effect of lanthanum on catecholamine release from adrenal medulla. *Life Sciences*, 11: 959-964.

Burroughs SE, Eisenman G, Horrocks WD Jr, 1992. Characterization of the five-fold Ca^{2+} binding site of satellite tobacco necrosis virus using Eu^{3+} luminescence spectroscopy: a marked size-selectivity among rare earth ions. *Biophysical Chemistry*, 42: 249-256.

Cotton SA, 1991. *Lanthanides and Actinides*. Macmillan, London.

Durret LR, Adams HR, 1980. A comparison of the influence of La^{3+}, D600 and gentamiscin on frequency-force relationships in isolated myocardium. *European Journal of Pharmacology*, 66: 315-325.

Evans CH, 1990. *Biochemistry of the Lanthanides*. Plenum Press, New York.

Fawzi AB, McNeill JH, 1985. Effect of lanthanum on the inotropic response of isoproterenol: role of the superficially bound calcium. *Canadian Journal of Physiology and Pharmacology*, 63: 1106-1112.

Finney DJ, 1971. *Probit analysis*. Cambridge University, New York Press.

Franklin SJ, 2001. Lanthanide-mediated DNA hydrolysis. *Current Opinion in Chemical Biology*, 5: 201-208.

Garrett JIR, McClure J, 1981. Lanthanide-induced calcergy. *Journal of Pathology*, 135: 267-275.

Gersanovski D, Colson P, Houssier C, Fredericq E, 1985. Terbium^{3+} as a probe of nucleic acids structure. Does it alter the DNA conformation in solution? *Biochimica et Biophysica Acta*, 824: 313-323.

Glickman NS, Yelon D, 2002. Cardiac development in zebrafish: coordination of form and function. *Seminars in Cell & Developmental Biology*, 13: 507-513.

Gross DS, Simpkins H, 1981. Evidence for two site binding in the terbium (III) – nucleic acid interaction. *Journal of Biological Chemistry*, 256: 9593-9598.

Haley T, Komesu N, Colvin G, Koste L, Upham H, 1965. Pharmacology and toxicology of europium chloride. In: Evans CH, ed. *Biochemistry of the Lanthanides*. Plenum Press, New York: 344-346.

Hosmer DW, Lemeshow S, 1999. *Applied survival analysis*. John Wiley & Sons, New York.

Joshi NB, Shamoo AE, 1987. Binding of Eu^{3+} to cardiac sarcoplasmic reticulum ($Ca^{2+}+Mg^{2+}$)-ATPase-laser excited Eu^{3+} spectroscopic studies. *Biophysical Journal*, 51: 185-191.

Kallistratos G, Vezyraki P, Seferiadis K, Evangelou A, Kalfakakou V, 1985. Fluorescent determination of steroids possessing a semi-quinonoid structure. *Proceedings of the 16th FEBS Congress, Part C*: 377-382.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF, 1995. Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203: 253-310.

Kitzes MC, Berns MW, 1979. Electrical activity of rat myocardial cells in cultures: La^{3+}-induced alterations. *American Journal of Physiology*, 237: C87-C95.

Lammer E, Carr GJ, Wendler K, Rawlings JM, Belanger SE, Braunbeck T, 2009. Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? *Comparative Biochemistry and Physiology – Part C*, 149: 196-209.

Lele Z, Krone PH, 1996. The zebrafish as a model system in developmental, toxicological and transgenic research. *Biotechnology Advances*, 14: 57-72.

Lleonart J, Salat J, Torres GJ, 2000. Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology*, 205: 85-93.

McGrath P, Li C-Q, 2008. Zebrafish: a predictive model for assessing drug-induced toxicity. *Drug Discovery Today*, 13: 394-401.

Miledi R, 1971. Lanthanum ions abolish the "calcium response" of nerve terminals. *Nature*, 229: 410-411.

Morris GE, 1980. The use of lanthanum and cytochalasin B to study calcium effects on skeletal muscle differentiation *in vitro*. *Journal of Cellular Physiology*, 105: 431-438.

Ogawa Y, Suzuki S, Naito K, Saito M, Kamata E, Hirose A, Ono A, Kaneko T, Chiba M, Inaba Y, 1995. Toxicity study of europium chloride in rats. *Journal of Environmental Pathology, Toxicology and Oncology*, 14: 1-9.

Pelster B, Burggren WW, 1996. Disruption of hemoglobin oxygen transport does not impact oxygen-dependent physiological processes in developing embryos of zebrafish (*Danio rerio*). *Circulation Research*, 79: 358-362.

Ravens U, 1975. The effects of lanthanum on electrical and mechanical events in mammalian cardiac muscle. *Naunyn-Schmiedeberg's Archive of Pharmacology*, 288: 133-146.

Reynaldo LP, Villafranca JJ, Horrocks WdeW Jr, 1996. Investigating the effects of posttranslational adenylylation on the metal binding sites of *Escherichia coli* glutamine synthetase using lanthanide luminescence spectroscopy. *Protein Science*, 5: 2532-2544.

Sigel A, Sigel H, 2003. *Metal ions in biological systems (the lanthanides and their interrelations with biosystems)*. Fontis Media S.A. and Marcel Dekker Inc., Switzerland.

Sneller F, Kalf D, Weltje L, Wezel A, 2000. *Maximum permissible concentrations and negligible concentrations for rare earth elements (REEs)*. Bilthoven, The Netherlands.

Wedekind C, von Siebenthal B, Gingold R, 2007. The weaker points of fish acute toxicity tests and how tests on embryos can solve some issues. *Environmental Pollution*, 148: 385-389.

Weltje L, Heidenreich H, Zhu W, Wolterbeek HTh, Korhammer S, de Goeij JJM, Markert B, 2002. Lanthanide concentrations in freshwater plants and molluscs, related to those in surface water, pore water and sediment. A case study in The Netherlands. *The Science of the Total Environment*, 286: 191-214.

Westerfield M, 1995. *The zebrafish book. Guide for the laboratory use of zebrafish (Danio rerio)*. Institution of Neuroscience, University of Oregon Press, Oregon.

Yongxing W, Xiaorong W, Zichun H, 2000. Genotoxicity of lanthanum (III) and gadolinium (Ill) in human peripheral blood lymphocytes. *Bulletin of Environmental Contamination and Toxicology*, 64: 611-616.