Journal of Hazardous Materials 213–214 (2012) 413–420

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxicity assessment of zebrafish following exposure to CdTe QDs

Wei Zhang[a,b,c,*], Kuangfei Lin[a,b,c,*], Youna Miao[a,b,c], Qiaoxiang Dong[d], Changjiang Huang[d], Huili Wang[d], Meijin Guo[e], Xinhong Cui[f]

[a] State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, East China University of Science and Technology, Shanghai 200237, PR China
[b] Shanghai Key Laboratory of Functional Materials Chemistry, East China University of Science and Technology, Shanghai 200237, PR China
[c] School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, PR China
[d] Zhejiang Provincial Key Lab for Technology and Application of Model Organisms, Wenzhou Medical College, Wenzhou 325035, PR China
[e] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China
[f] Shanghai Institute of Landscape Gardening, Shanghai 200233, PR China

ARTICLE INFO

Article history:
Received 20 October 2011
Received in revised form 1 February 2012
Accepted 7 February 2012
Available online 14 February 2012

Keywords:
TGA-CdTe QDs
Zebrafish
Developmental toxicity
Behavioral toxicity

ABSTRACT

CdTe quantum dots (QDs) are nanocrystals of unique composition and properties that have found many new commercial applications; therefore, their potential toxicity to aquatic organisms has become a hot research topic. The lab study was performed to determine the developmental and behavioral toxicities to zebrafish under continuous exposure to low concentrations of CdTe QDs (1–400 nM) coated with thioglycolic acid (TGA). The results show: (1) the 120 h LC_{50} of 185.9 nM, (2) the lower hatch rate and body length, more malformations, and less heart beat and swimming speed of the exposed zebrafish, (3) the brief burst and a higher basal swimming rate of the exposed zebrafish larvae during a rapid transition from light-to-dark, and (4) the vascular hyperplasia, vascular bifurcation, vascular crossing and turbulence of the exposed FLI-1 transgenic zebrafish larvae.

1. Introduction

Quantum dots (QDs) have been employed in many recent applications, such as solar cells, light-emitting devices, biological and medical imaging because of their small size, bright fluorescence, narrow emission spectra, broad absorption spectra, and high photostability [1–5]. The QDs' potential biological toxicity has become a health concern due to their inherent chemical composition and nanoscale properties [6–8]. Literature reports show that QDs may induce cell death and change gene expression [9,10]. Given the many types of QDs have widely different physical and chemical properties; there is a great need for more research to supplement the present knowledge regarding their toxic effects [11–13].

Cadmium–telluride (CdTe) based QDs are semiconductor nanocrystals that hold the greatest commercial potential. Their optical properties make them beneficial for multicolor and long-lived fluorescent probes [14,15]. CdTe QDs are increasingly found in environmental water samples via waste streams from industries that synthesize or use CdTe and through clinical and research uses. Therefore, there is an urgent need to determine the CdTe QDs induced ecotoxicological effects to aquatic organisms and to identify convenient biomarkers for early signaling [16–18].

The ecotoxicological effects of CdTe QDs to freshwater mussel Elliptio complanata have been reported showing immune toxicity and can cause oxidative stress or DNA damage [18]. The toxicity might be due to the leaching of toxic heavy metals from the colloidal form and/or the size and surface chemistry of CdTe QDs [19,20]. The impact of continuous exposure to low level of CdTe QDs remains largely unknown [21,22].

Recent studies employing algae, daphnia, zebrafish, trout, and zooplankton have demonstrated that the nanoparticles (NPs) might cause varying degrees of toxicity to the test organisms [23–29]. Zebrafish has increasingly been employed as a model vertebrate for investigating the development toxicity and neurotoxicity of the potential ecotoxicological impacts of NPs releases to aquatic environments [25–29]; zebrafish embryo is commonly used in the early life-stage tests, especially for investigating the toxicity and teratogenicity of chemicals [30]. There has been no report of employing zebrafish embryos and larvae for CdTe QDs toxicity studies.

Thioglycolic acid (TGA) is a popular short and straight-chain stabilizing agent because its small steric hindrance is more effective for surface passivation of QDs than the long and branched-chain agents to produce highly stable TGA-coated CdTe QDs [31–34]. Zebrafish was employed in this research to study the developmental and behavioral toxicities associated with low doses of TGA-CdTe

* Corresponding authors at: Box 563, 130 Meilong Road, Shanghai 200237, PR China. Tel.: +86 21 64253244; fax: +86 21 64253988.
E-mail addresses: wzhang@ecust.edu.cn (W. Zhang), kflin@ecust.edu.cn (K. Lin).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2012.02.014

Table 1
Experimental program of the toxicity study.

Exposure concentrations	Exposure period	Toxicological endpoints	Results
0, 1, 25, 50, 100, 200, 300, 400 μM or nM	6–120 hpf	Mortality (120 hpf)	Fig. 1
0, 1, 25, 50, 100, 200, 300 nM	6–72 hpf	Hatch rate (72 hpf)	Fig. 2
0, 200 nM	6–96 hpf	Malformation (96 hpf)	Fig. 3
0, 1, 25, 50, 100, 200 nM	6–120 hpf	Body length (120 hpf)	Fig. 4
0, 1, 25, 50, 100, 200, 300 nM	6–48 hpf	Heart beat (10 s, 48 hpf)	Fig. 5
0, 1, 4, 16 nM	6–48 hpf	Swimming speed (144 hpf)	Fig. 6
0, 1, 4, 16 nM	6–48 hpf	Swimming speed (light stimulation test) (144 hpf)	Fig. 7
0, 1, 25, 50, 100 nM	6–96 hpf	Vascular patterns (96 hpf)	Fig. 8

QDs exposure based on select physiological parameters of toxic responses; the results have demonstrated significant effects on overall fitness such as mortality, hatch rate, malformation, body length, heart beat, swimming behavior and vascular patterns.

2. Materials and methods

2.1. Chemicals

Water-soluble TGA-CdTe QDs were purchased from Nanosquare Inc. (Tokyo, Japan). Cadmium chloride ($CdC1_2$) and other chemicals were purchased from Merck (Darmstadt, Germany). All chemicals used in the present study were analytical grade.

2.2. Stock solutions of TGA-CdTe QDs

TGA-CdTe QDs were observed to have an irregular shape with a typical size of approximately 3.5 nm by TEM and excellent spectrum characteristic with narrow and sharp peak by Fluorescence Spectroscopy (FS) after purification to control excess Cd^{2+} and TGA.

The stock solution (9.64 μM) was prepared by dissolving TGA-CdTe QDs in 60 mg L^{-1} ocean salt; TGA-CdTe solution was highly stable in terms of QDs properties (i.e., shape and size) during the entire exposure period.

2.3. Fish husbandry and embryo collection

Adult zebrafish of wild-type strain (AB) were raised and kept at 28 ± 0.5 °C with a 14:10 dark/light photoperiod (lights on at 8:00 a.m.) in a recirculation system according to standard zebrafish breeding protocols [35]. Water supplied to the system was filtered by reverse osmosis (pH 6.5–7.5), and instant ocean salt was added to the water to raise the conductivity to 450–1000 μs cm^{-1}. Zebrafish were fed twice daily with live Artemia (Jiahong Feed Co., Tianjin, China) and dry flake diet (Zeigler, Aquatic Habitats, Apopka Florida, USA). The development status of zebrafish embryos and larvae were observed with an Inverted Microscope (×8-50, Olympus, Japan).

Zebrafish embryos used for chemical exposure were obtained from spawning adults in tanks overnight with the sex ratio of 1:1. Embryos were collected within 1 h after the light was switched on and rinsed in embryo medium [35]. The fertilized and normal embryos were inspected and staged for the following experiment under a stereomicroscope (Nikon, Japan) according to Kimmel's descriptions [36].

2.4. Zebrafish toxicity test

To explore TGA-CdTe stability and determine total Cd loading on zebrafish, embryos were exposed to TGA-CdTe test solutions (200 and 400 nM) for 6 d and then passed through a filtration membrane (10 kDa cutoff, Amicon® Ultra-15 Centrifugal Filter Devices, Millipore Corporation, Billerica, MA, USA) to determine the amount of Cd released from intact QDs. Cd^{2+} was measured using graphite-furnace atomic-absorption spectrophotometry (novAA400, Analytik Jena AG, Germany) following Creed's descriptions [37]. The exposure concentrations and period, and toxicological endpoint of zebrafish used for each experiment were listed in Table 1. In order to determine the exposure concentrations for each toxicological endpoint, based on previous toxicity information about these compounds, we had carried out a great deal of preliminary experiments. According to the results, we chose the lowest concentration (400 nM) which almost led to all the embryos death at 120 hpf as upper exposure limit for mortality test. As for other toxicological endpoints, selected concentrations (1–300 nM) can result in significant difference or obvious symptom compared to the controls. Exposure period of each experiment were dependent on zebrafish development stage. Such experimental methods had literature support [29,38,39].

To determine the LC_{50}, normal embryos were exposed to the blank control (60 mg L^{-1} ocean salt) and TGA-CdTe solutions (1, 25, 50, 100, 200, 300, 400 nM) from 6 to 120 hpf. Embryos were kept in sterile 96-well plates, with one embryo per well containing 200 μL of the solution. Plates were covered with sealing films to prevent evaporation. For each treatment, 30 embryos were performed in light-controlled incubator. After observing and recording the zebrafish mortality at 120 hpf, the results were plotted for estimating the LC_{50} values. Another series of test runs employing $CdC1_2$ solutions (1, 25, 50, 100, 200, 300, 400 μM) were conducted to evaluate its influence on zebrafish mortality by the same method in order to determine which played a more important role in toxic effect on zebrafish between QDs and released Cd^{2+}.

Normal embryos were exposed to the control and TGA-CdTe (1, 25, 50, 100, 200, 300 nM) from 6 to 72 hpf. For each batch of zebrafish embryo, the hatch rate was measured at 72 hpf. After normal embryos (6 hpf) were exposed to the control and TGA-CdTe (200 nM) for 4 d (96 hpf), the malformation of zebrafish at 96 hpf were observed with an Inverted Microscope (Nikon, Japan).

Normal embryos (6 hpf) were exposed to the control and TGA-CdTe (1, 25, 50, 100, 200 nM) for 5 d (120 hpf), and then the body length of zebrafish were determined at 120 hpf. After normal embryos exposure to the control and TGA-CdTe (1, 25, 50, 100, 200, 300 nM) from 6 to 48 hpf, the 10-s heart beats of zebrafish were determined at 48 hpf.

Normal embryos were exposed to the control and TGA-CdTe (1, 4, 16 nM) from 6 to 48 hpf, and further subjected to behavior assessment at 144 hpf. Two sets of observations were made after a 10 min adaption: larvae locomotion was first observed in visible light for 20 min and then the larvae's swimming speed during the 90-min light-to-dark (20 min for each period) transition stimulation was recorded [38,39]. The test was monitored with the Zebralab Video-Track system (Videotrack, version 3.5) equipped with a Sony one-third inch Monochrome camera (Model DR2-HIBW-CSBOX, 30Fps) and an infrared filter. The entire recording hardware was linked to the computer control program and kept insulated from environmental conditions in a sealed opaque plastic box (Zebrabox, ViewPoint Life Science, France). The data (movement frequency, travelled distance and total movement duration)

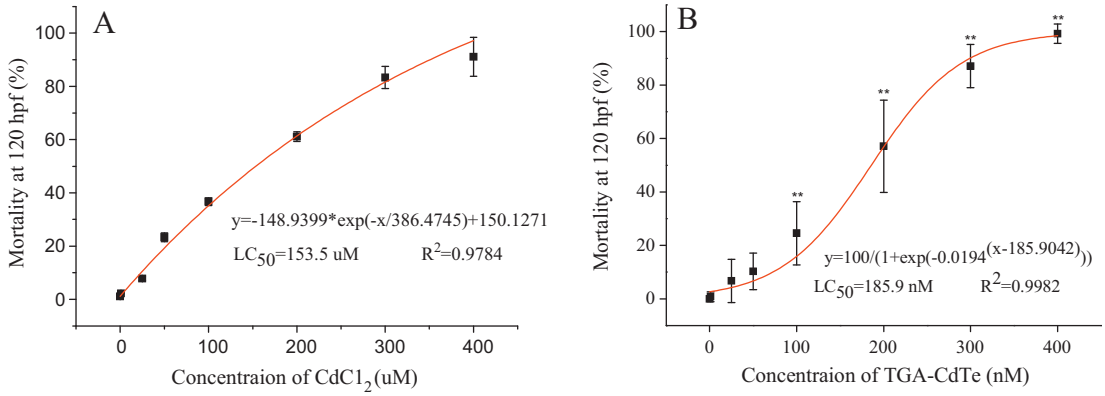

Fig. 1. Effect of exposure concentration on zebrafish mortality at 120 hpf ($n = 30$): (A) $CdCl_2$ and (B) TGA-CdTe. Double asterisks (**) indicate significant difference from control at $P < 0.01$. Values represent the mean ± standard error of three replicates.

were collected every 60 s and further analyzed using custom Open Office. Org 2.4 software.

FLI-1: EGFP transgenic zebrafish larvae, purchased from Molecular Toxicology Research Center of Oregon State University (USA) which exhibited green fluorescence under fluorescence spectroscopy, were exposed to ocean salt control (60 mg L^{-1}) and TGA-CdTe (1, 25, 50, 100 nM) from 6 to 96 hpf; and their vascular patterns at 96 hpf were further observed by Fluorescence Inverted Microscope (Nikon TE2000-U, Japan) [38,39].

2.5. Data statistical analysis

Each treatment was replicated three times; the results were reported as the average of three parallel determinations of the mixture of three replicated samples. The concentration–response curves which were required to determine LC_{50} values were completed using origin 8.0 (OriginLab, Northampton, MA, USA). One-way ANOVA was performed to calculate statistical significance followed by Dunnett's test to independently compare each exposure group to the control group. All statistical analyses were conducted separately using SPSS 16.0 software (SPSS, Chicago, USA); the P value of less than 0.05 was considered to be statistically significant. The data are presented as mean ± standard error (SE).

3. Results

3.1. $CdCl_2$ and TGA-CdTe affected zebrafish mortality at 120 hpf

For the TGA-CdTe test runs, Cd^{2+} concentrations of 15.5 and 33.8 nM were detected in the filtered samples after the incubating of 200 and 400 nM solutions for 6 d, indicating minimal Cd release (about 8% of total Cd) from TGA-CdTe core under the test conditions. The 120 h LC_{50} of $CdCl_2$ was calculated to be 153.5 μM (Fig. 1A). In the previous study, TGA did not cause obvious toxic effect on zebrafish.

The mortality of zebrafish after exposure to different doses of TGA-CdTe was determined at 120 hpf. Fig. 1B data show that the control and 1 nM TGA-CdTe had no detectable toxicity to zebrafish and that the higher concentrations of TGA-CdTe were increasingly more toxic. The embryo survival rate decreased from 90% of the 25 nM group to 0.88% of the 400 nM group, demonstrating the higher zebrafish developmental toxicity of the higher TGA-CdTe dose. When the concentration of TGA-CdTe was 100 nM, the mortality even exhibited highly significant difference ($P < 0.01$) compared to the controls. The 120 h LC_{50} of TGA-CdTe was 185.9 nM.

3.2. TGA-CdTe affected the hatch rate of zebrafish at 72 hpf

The hatch rate of zebrafish embryos decreased with increasing TGA-CdTe concentration at 72 hpf (Fig. 2). Compared to the controls, 1, 25, 50 and 100 nM exhibited no significant differences during 72 h exposure period. In contrast, the hatch rates of 200 and 300 nM groups (31.66% and 5.98%, respectively) displayed embryonic developmental delay and highly significant ($P < 0.01$) toxicity.

3.3. TGA-CdTe induced zebrafish malformation at 96 hpf

Zebrafish were exposed to 200 nM TGA-CdTe from 6 to 96 hpf and the malformation were observed at 96 hpf (Fig. 3). The control solution (60 mg L^{-1} ocean salt) was not toxic to zebrafish (data not shown), while the treated groups had significantly higher malformation rates relative to the control. Several malformation patterns (i.e., eyespots and melanin developmental inhibition, pericardial edema, vitelline cyst, bent tail, bent spine, and somites decrease) were observed in all the exposure groups. Such observations have suggested that pericardial edema would often occur and that the toxic effect would result in several malformation patterns at the same time.

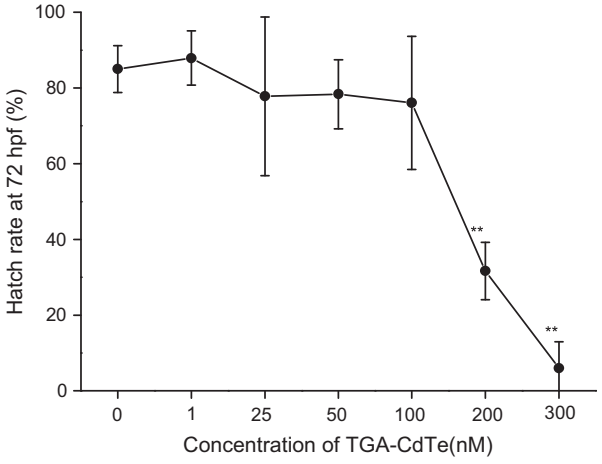

Fig. 2. Effect of TGA-CdTe concentration on hatch rate of zebrafish embryos at 72 hpf ($n = 30$). Double asterisks (**) indicate significant difference from control at $P < 0.01$. Values represent the mean ± standard error of three replicates.

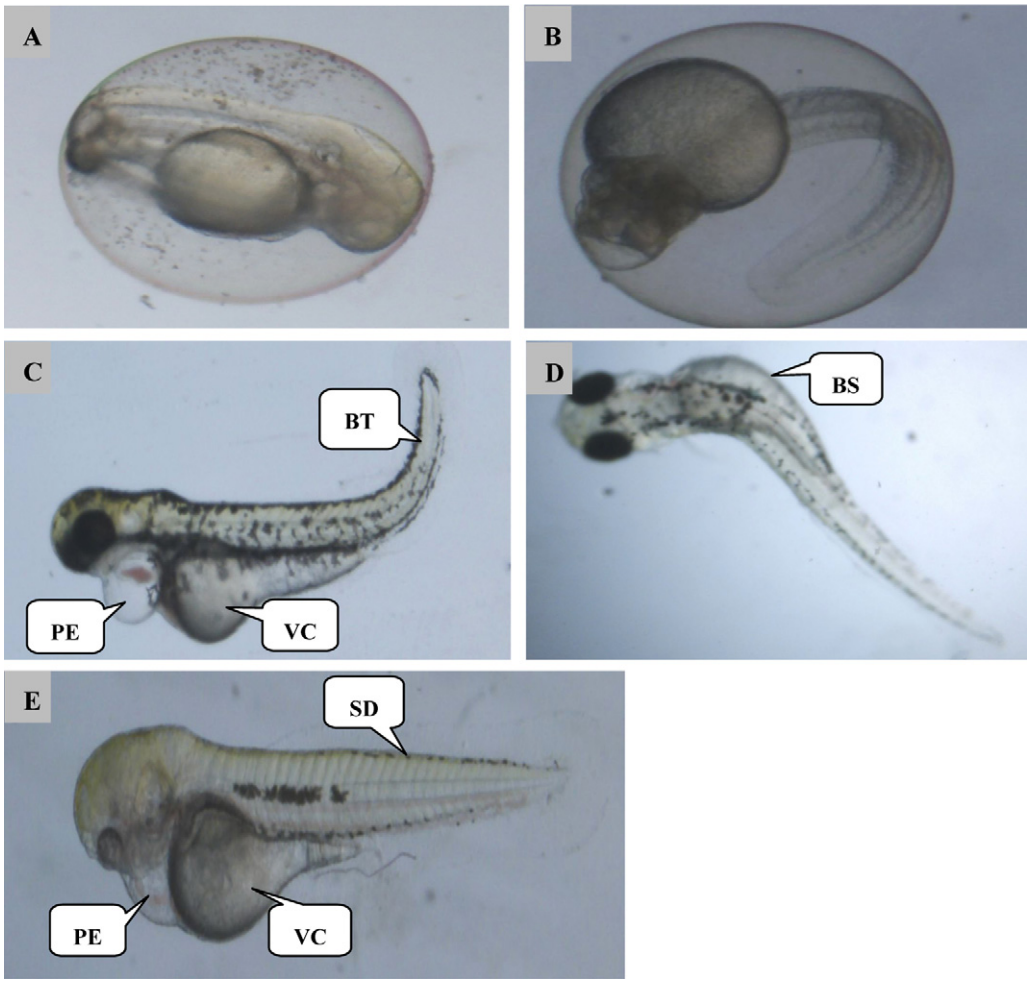

Fig. 3. Malformation patterns of zebrafish after exposure to 200 nM TGA-CdTe: (A) eyespots developmental inhibition, (B) melanin developmental inhibition, (C) pericardial edema (PE), vitelline cyst (VC), bent tail (BT), (D) bent spine (BS), and (E) pericardial edema (PE), vitelline cyst (VC), somites decrease (SD). Scale bar = 0.5 mm.

3.4. TGA-CdTe affected the body length of zebrafish at 120 hpf

The body length of zebrafish reduced with increasing TGA-CdTe concentration at 120 hpf (Fig. 4). Compared to the controls, 1 nM TGA-CdTe did not significantly show the toxicity; whereas 25 nM displayed statistical significance ($P < 0.05$); moreover, 50, 100, and 200 nM treated groups showed the toxicity highly significant ($P < 0.01$), and the body length of zebrafish were only 3139.56, 3157.30, and 3018.67 μm, respectively.

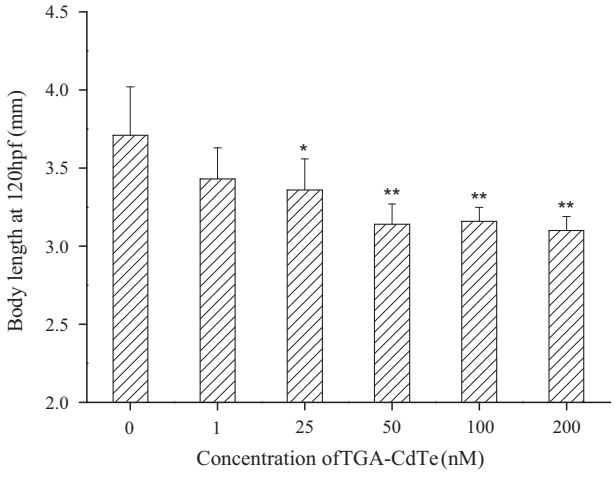

Fig. 4. Effect of TGA-CdTe concentrations on body length of zebrafish at 120 hpf ($n = 30$). Single asterisk (*) and double asterisks (**) indicate significant differences from control at $P < 0.05$ and $P < 0.01$, respectively. Values represent the mean ± standard error of three replicates.

3.5. TGA-CdTe affected the heart beats of zebrafish at 48 hpf

The number of heart beats during a 10 s period for zebrafish embryos was taken at 48 hpf after exposure to TGA-CdTe at various concentrations. Fig. 5 data show that, compared to the control and 1 nM treatment, 25 and 50 nM treatments resulted in the significantly less heart beats ($P < 0.05$); the notably less lower heart beats of the 100, 200 and 300 nM treatments (27.7, 27.3 and 25.8, respectively) than the control (31.5) are evidence of the highly significant embryonic toxicity ($P < 0.01$) of those TGA-CdTe treatments.

3.6. TGA-CdTe affected the swimming speed of zebrafish at 144 hpf

Fig. 6 illustrates the effect of TGA-CdTe concentration on the average swimming speed of zebrafish larvae was measured at 144 hpf. While the average swimming speed after the 1 nM treatment was slightly higher than the control (1.915 mm s^{-1} vs 1.871 mm s^{-1}), the 4 nM treatment had a significant effect ($P < 0.05$) of reducing the swimming speed by 34.9% to 1.217 mm s^{-1} and that the 16 nM treatment had a more significant effect ($P < 0.01$)

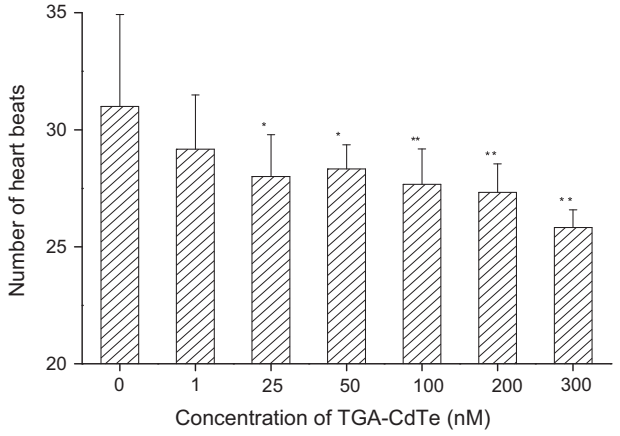

Fig. 5. Effect of TGA-CdTe concentrations on 10 s heart beats of zebrafish embryos at 48 hpf ($n = 30$). Single asterisk (*) and double asterisks (**) indicate significant differences from control at $P < 0.05$ and $P < 0.01$, respectively. Values represent the mean ± standard error of three replicates.

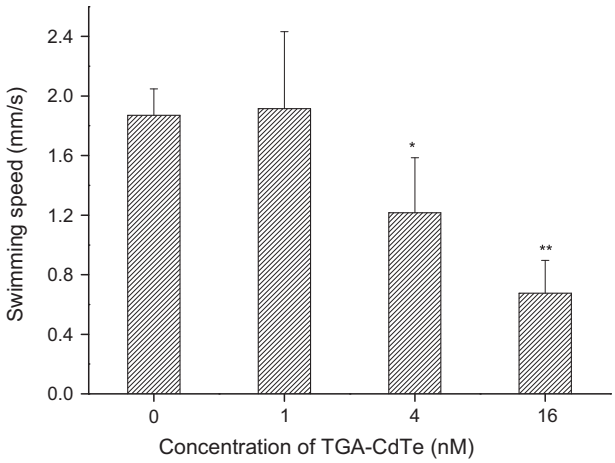

Fig. 6. Effect of TGA-CdTe concentrations on swimming speed of zebrafish larvae at 144 hpf ($n = 30$). Single asterisk (*) and double asterisks (**) indicate significant differences from control at $P < 0.05$ and $P < 0.01$, respectively. Values represent the mean ± standard error of three replicates.

of reducing the speed by 63.9% to 0.675 mm s^{-1}. Such results show that TGA-CdTe can elicit neurobehavior alternations in zebrafish larvae.

3.7. TGA-CdTe affected the swimming speed of zebrafish larvae at 144 hpf when subject to light-to-dark photoperiod stimulation

Zebrafish larvae at 144 hpf of the control and 1, 4, 16 nM TGA-CdTe exposure groups were subjected to the light stimulation motor behavior test; the photoperiod stimulation began in light (20 min), followed by two cycles of darkness (20 min) and light (20 min). Fig. 7 data show that the rapid transition from light-to-dark resulted in a similar, brief burst of swimming in all groups (both exposure and control) and that subsequently larvae of 1 and 4 nM TGA-CdTe groups had a higher basal swim rate than the control. The results suggest that zebrafish larvae were sensitive to sudden darkness and exposure stress.

3.8. TGA-CdTe affected the vascular patterns of FLI-1 transgenic zebrafish larvae at 96 hpf

The vascular patterns of FLI-1: EGFP transgenic zebrafish larvae in control and TGA-CdTe exposure groups (1, 25, 50, 100 nM) were observed and recorded by Fluorescence Inverted Microscope at 96 hpf. Fig. 8 shows that the vascular in the 1 nM treatment group

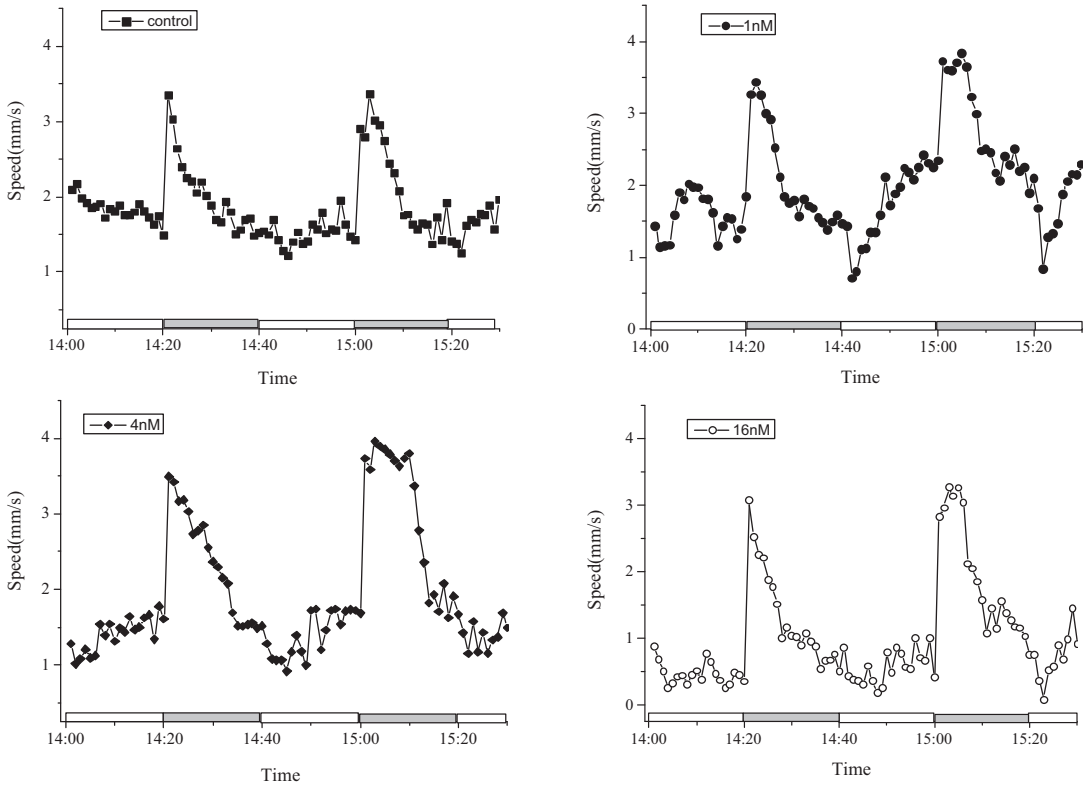

Fig. 7. Effect of TGA-CdTe concentration on swimming speed of zebrafish larvae after subjecting to the 90-min light-to-dark photoperiod stimulation at 144 hpf ($n = 30$).

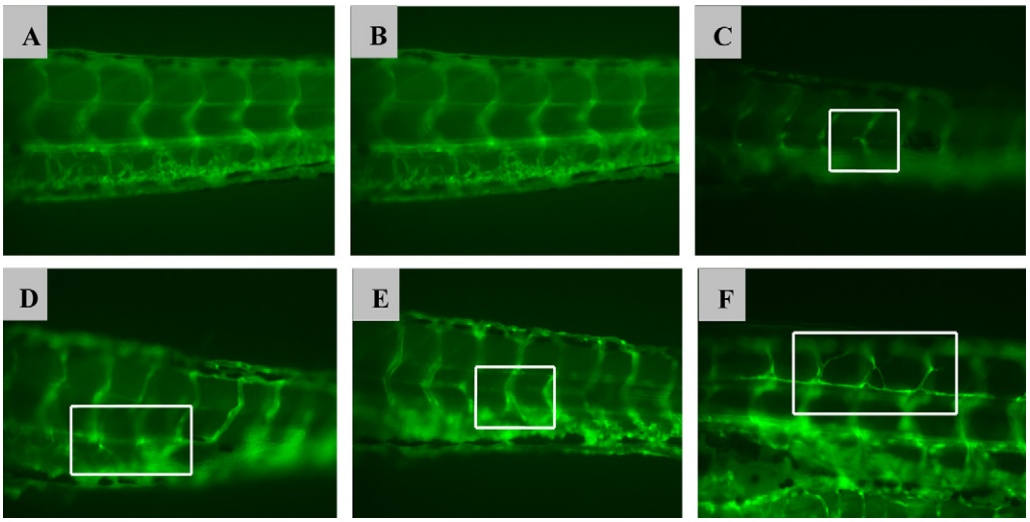

Fig. 8. Effect of TGA-CdTe concentration on vascular pattern of FLI-1 transgenic zebrafish larvae at 96 hpf ($n = 30$) by fluorescence inverted microscope (×200): (A) Control; (B) 1 nM; (C) 25 nM (vascular hyperplasia), (D) 50 nM (vascular crossing and turbulence), (E) and (F) 100 nM (vascular bifurcation). The vascular defects are shown in the white box.

(B) was very similar to that in control (A) and that the 25, 50 and 100 nM treatments resulted in abnormal vascular patterns such as vascular hyperplasia (C, 25 nM), vascular crossing and turbulence (D, 50 nM), and vascular bifurcation (E and F, 100 nM).

4. Discussions

QD core metals (e.g., cadmium, lead, zinc, and mercury) could be released under oxidative and photolytic conditions, and many core materials are known to be toxic to vertebrate systems at relatively low concentrations [11]. Therefore, the assessment of nanoparticle stability under exposure conditions is necessary to interpret biological effects. Cd, a CdTe QDs core material, is an important factor in determining TGA-CdTe toxicity. In addition to the release of free Cd^{2+}, CdTe QDs may affect due to its size at the colloidal scale [40]. King-Heiden showed that Cd^{2+} was toxic to zebrafish larvae and that the QD's nanoscale properties were even more potent and produced toxicological endpoints distinct from that of Cd^{2+} [41]. Although the results of this study have confirmed the toxic effect of Cd^{2+} on zebrafish, they also strongly suggest other contributors to the observed zebrafish toxicity given the low concentrations of Cd^{2+} releases. As shown in Fig. 1, the released amount of free Cd^{2+} (15.5 or 33.8 nM) in the TGA-CdTe treatment groups (200 or 400 nM) for 120 h was not far enough to explain approximately 57.1% (200 nM) or 99.2% (400 nM) zebrafish mortality, because of considering that 15.5 or 33.8 nM Cd^{2+} just resulted in zebrafish mortality less than 2% (the 120 h LC_{50} of $CdCl_2$ 153.5 μM). Therefore, we can conclude that nanoscale effect of TGA-CdTe QDs should play a more important role in the observed mortality as King-Heiden's descriptions [41].

In the present study, TGA-CdTe induced various malformations including eyespots and melanin developmental inhibition, pericardial edema, vitelline cyst, bent tail, bent spine and somites decrease due to one or more possible mechanisms. Cheng et al. [42] attributed the spinal deformities to reduced formation of myosin and/or myotome necessary for the healthy musculo-skeletal system development. We hypothesize that the bent spine was related to the muscle or skeleton based on the broken, disorganized, and loosen array muscle fibers observed in the previous research. Moreover, to gain insight into potential mechanisms of malformations, we observed toxic effect following exposure to a single concentration of $CdCl_2$ (25 μM Cd), and the results indicated that Cd exposure can result in pericardial edema, vitelline cyst, bent spine, while unrelate to malformed tail, so nanoscale properties of TGA-CdTe QDs might be unique contributor to bent tail. Blechinger et al. [43] also reported similar observation.

Behavioral analysis often serves as a sensitive tool for detecting sublethal chemical effects [44]. In this study, we found TGA-CdTe can also disturb the neurobehavior of zebrafish larvae. For example, its swimming behavior was adversely affected by TGA-CdTe exposure. The much reduced larval swimming speed suggests the significant effect of low dose TGA-CdTe on larval behavior. Zebrafish larvae at 144 hpf of the 4 and 16 nM TGA-CdTe groups swam at a much slower speed at an inactive stage (1.217 and 0.675 mm s^{-1}) in the 20 min light period. All treatment groups elicited a burst of swimming responding to light-to-dark photoperiod stimulation, and 1 or 4 nM TGA-CdTe exposure resulted in a higher basal rate of swimming than the control. Zebrafish displayed a biorhythm showing that larvae became active after exposure to sudden darkness and then slow down consistent with literature reports [45,46].

The typical behavior of low activity under normal conditions and hyperactivity under stress suggests that TGA-CdTe exposure altered neurobehavior of zebrafish larvae. The effects of TGA-CdTe on the neural circuits and the physiological or biochemical mechanisms of the locomotive behaviors responding to the photoperiod stimulation are still unclear. Recent published studies suggest a significant involvement of motor neurons and muscle fiber in the overall locomotive behavior [47–49]. Zebrafish larvae possess two types of skeletal muscle fibers, slow (red) fibers and fast (white) fibers, which could contribute to their behavioral responses [50,51]. Functionally, the red fibers are de-recruited during fast burst swimming, while the white fibers are inactive during slow swimming [52,53]; therefore, the white fibers might have played an important role for burst swimming during photoperiod stimulation. Future studies are necessary to reveal the underlying mechanism for TGA-CdTe induced physiological or neurochemical changes and explore further the stress-related behavioral responses in zebrafish larvae, in particular their relationship with whole-body cortisol level, because it is the main mediator of physiological response to stress in fish [54].

After exposure to TGA-CdTe from 6 to 96 hpf, the vascular patterns of FLI-1: EGFP transgenic zebrafish larvae showed abnormalities such as vascular hyperplasia, vascular crossing and turbulence, and vascular bifurcation. TGA-CdTe was previously found to aggregate in back and abdominal vascular of zebrafish

resulting in vascular obstruction which might have contributed to the observed abnormalities. That malformation of pericardial edema was observed at the same time suggest the two results might be related, which is needed in future studies.

Results obtained from a study of TGA-CdTe removal have indicated that the clearance was negligible; a more thorough analytical approach to TGA-CdTe pharmacokinetics, such as the determination of body burden, metabolism and elimination kinetics, environmentally relevant exposures, will be conducted in the near future.

5. Conclusion

To simulate the actual environmental exposures of aquatic animals dwelling in a TGA-CdTe contaminated environment, the zebrafish of the treated samples were exposed to low doses of TGA-CdTe from embryonic stage to adulthood. The following can be concluded from the results of this study.

1) Zebrafish is a convenient model organism for assessing potential risks of TGA-CdTe exposure.
2) Since the amounts of Cd^{2+} released from TGA-CdTe QDs of the treated samples were too small to have caused the observed zebrafish toxicities, the QDs scale effect might have been a more important factor.
3) The low dose TGA-CdTe exposure (1–400 nM) affected overall fitness of zebrafish; the LC_{50} of TGA-CdTe at 120 hpf was 185.9 nM. Effects on mortality (120 hpf), hatch rate (72 hpf), body length (120 hpf) and heart beat (10 s, 48 hpf) were dose dependent of greater effect at increasing dose.
4) The TGA-CdTe exposure induced zebrafish malformation (96 hpf), and altered the swimming speed (144 hpf) and vascular patterns (96 hpf).
5) The unique original investigation of developmental toxicity produced important and valuable information for assessment of TGA-CdTe because toxicants that affect embryonic development exert their effects at lower concentrations than those required to affect adults or to cause general cytotoxicity. The developmental toxicity data will help to establish water-quality standards to protect aquatic life.
6) Further studies are needed to investigate the mechanisms underlying the developmental and behavioral changes.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (40901148, 40871223), the Major State Basic Research Development Program of China (2011CB200904), the Scientific Project on Treatment and Control of Water Pollution (2012ZX07115), the National Environmental Protection Public Welfare Science and Technology Research Program of China (200909089), the National Forestry Public Welfare Science and Technology Research Program of China (201104088), the Fundamental Research Funds for the Central Universities (WB0911011), the Natural Science Foundation of Shanghai (11ZR1409400), and Zhejiang Provincial Science Foundation of China (Y5100333). We also would like to thank the anonymous referees for their helpful comments on this paper.

References

[1] L.L. Wang, H.Z. Zheng, Y.J. Long, M. Gaoa, J.Y. Hao, J. Du, X.J. Mao, D.B. Zhou, Rapid determination of the toxicity of quantum dots with luminous bacteria, J. Hazard. Mater. 177 (2010) 1134–1137.
[2] W.C.W. Chan, S. Nie, Quantum dot bioconjugates for ultrasensitive nonisotopic detection, Science 281 (1998) 2016–2018.
[3] M.M.B. Moronne Jr., P. Gin, S. Weiss, A.P. Alivisatos, Semiconductor nanocrystals as fluorescent biological labels, Science 281 (1998) 2013–2016.
[4] D.S. Wang, J.B. He, N. Rosenzweig, Z. Rosenzweig, Superparamagnetic Fe_2O_3 beads-CdSe/ZnS quantum dots core–shell nanocomposite particles for cell separation, Nano Lett. 4 (2004) 409–413.
[5] M. Dahan, S. Levi, C. Luccardini, P. Rostaing, B. Riveau, A. Triller, Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking, Science 302 (2003) 442–445.
[6] V.L. Colvin, The potential environmental impact of engineered nanomaterials, Nat. Biotechnol. 21 (2003) 1166–1170.
[7] E. Casals, S. Vázquez-Campos, N.G. Bastús, V. Puntes, Distribution and potential toxicity of engineered inorganic nanoparticles and carbon nanostructures in biological systems, Trends Anal. Chem. 27 (2008) 672–683.
[8] B. Dubertret, P. Skourides, D.J. Norris, V. Noireaux, A.H. Brivanlou, A. Libchaber, In vivo imaging of quantum dots encapsulated in phospholipid micelles, Science 298 (2002) 1759–1762.
[9] A.O. Choi, S.E. Brown, M. Szyf, D. Maysinger, Quantumdot-induced epigenetic and genotoxic changes in human breast cancer cells, J. Mol. Med. 86 (2008) 291–302.
[10] A.M. Derfus, W.C.W. Chan, S.N. Bhatia, Probing the cytotoxicity of semiconductor quantum dots, Nano Lett. 4 (2004) 11–18.
[11] R. Hardman, A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors, Environ. Health Perspect. 114 (2006) 165–172.
[12] M.N. Moore, Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ. Int. 32 (2006) 967–976.
[13] M.J. Kim, C. Park, K. Choi, T.H. Yoon, Implications of trap emission observed from quantum dot nanoparticles accumulated in toxicity test organism, *Daphnia magna*, Bull. Korean Chem. Soc. 29 (2008) 1101–1102.
[14] C.B. Murray, D.J. Norris, M.G. Bawendi, Synthesis and characterization of nearly monodisperse CdE (E = S, Se, Te) semiconductor nanocrystallites, J. Am. Chem. Soc. 115 (1993) 8706–8715.
[15] C. Park, K.H. Lim, D. Kwon, T.H. Yoon, Biocompatible quantum dot nanocolloids stabilized by gum Arabic, Bull. Korean Chem. Soc. 29 (2008) 1277–1279.
[16] L.Z. Zhao, R.T. Liu, X.C. Zhao, B.J. Yang, C.Z. Gao, X.P. Hao, Y.Z. Wu, New strategy for the evaluation of CdTe quantum dot toxicity targeted to bovine serum albumin, Sci. Total Environ. 407 (2009) 5019–5023.
[17] M. Yan, Y. Zhang, K.D. Xu, T. Fu, H.Y. Qin, X.X. Zheng, An in vitro study of vascular endothelial toxicity of CdTe quantum dots, Toxicology 282 (2011) 94–103.
[18] F. Gagné, J. Auclair, P. Turcotte, M. Fournier, C. Gagnon, S. Sauvé, C. Blaise, Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity, Aquat. Toxicol. 86 (2008) 333–340.
[19] A.R. Clapp, I.L. Medintz, J.M. Mauro, B.R. Fisher, M.G. Bawendi, H. Mattoussi, Fluorescence resonance energy transfer between quantum dot donors and dye-labeled protein acceptors, J. Am. Chem. Soc. 126 (2004) 301–310.
[20] V.M. Fthenakis, Life cycle impact analysis of cadmium in CdTe PV production, Renew. Sust. Energy Rev. 8 (2004) 303–334.
[21] P.C. Naha, A. Casey, T. Tenuta, I. Lynch, K.A. Dawson, H.J. Byrne, M. Davoren, Preparation, characterization of NIPAM and NIPAM/BAM copolymer nanoparticles and their acute toxicity testing using an aquatic test battery, Aquat. Toxicol. 92 (2009) 146–154.
[22] K.L. Aillon, Y.M. Xie, N. El-Gendy, C.J. Berkland, M.L. Forrest, Effects of nanomaterial physicochemical properties on in vivo toxicity, Adv. Drug Deliv. Rev. 61 (2009) 457–466.
[23] K. Hund-Rinke, M. Simon, Ecotoxic effect of photocatalytic active nanoparticles TiO_2 on algae and daphnids, Environ. Sci. Pollut. Res. 13 (2006) 225–232.
[24] S.B. Lovern, R. Klaper, *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C_{60}) nanoparticles, Environ. Toxicol. Chem. 25 (2006) 1132–1137.
[25] K.J. Lee, P.D. Nallathamby, L.M. Browning, C.J. Osgood, X.H. Xu, In vivo imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos, ACS Nano 1 (2007) 133–143.
[26] R.J. Griffitt, R. Weil, K.A. Hyndman, N.D. Denslow, K. Powers, D. Taylor, D.S. Barber, Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*), Environ. Sci. Technol. 41 (2007) 8178–8186.
[27] T.B. Henry, F.M. Menn, J.T. Fleming, J. Wilgus, R.N. Compton, G.S. Sayler, Attributing effects of aqueous C_{60} nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression, Environ. Health Perspect. 115 (2007) 1059–1065.
[28] C.Y. Usenko, S.L. Harper, R.L. Tanguay, In vivo evaluation of carbon fullerene toxicity using embryonic zebrafish, Carbon 45 (2007) 1891–1898.
[29] X.S. Zhu, L. Zhu, Y. Li, Z.H. Duan, W. Chen, P.J.J. Alvarez, Developmental toxicity in zebrafish embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC_{60}) and fullerol, Environ. Toxicol. Chem. 26 (2007) 976–979.
[30] C. Schulte, R. Nagel, Test acute toxicity in the embryo of zebrafish, *Brachydanio rerio* as alternative to the acute fish test-preliminary results, ATLA 22 (1994) 12–19.
[31] M.A. Jhonsi, R. Renganathan, Investigations on the photoinduced interaction of water soluble thioglycolic acid (TGA) capped CdTe quantum dots with certain porphyrins, J. Colloid Interface Sci. 344 (2010) 596–602.
[32] J.N. Tian, R.J. Liu, Y.C. Zhao, Q. Xu, S.L. Zhao, Controllable synthesis and cell-imaging studies on CdTe quantum dots together capped by glutathione and thioglycolic acid, J. Colloid Interface Sci. 336 (2009) 504–509.

[33] X.Q. Fan, J.J. Peng, S.G. Yan, L. Wang, Y.Q. He, Study on the interaction of CdTe quantum dots with ferulic acid and protocatechuic aldehyde by optical spectroscopy, J. Lumin. 131 (2011) 2230–2236.
[34] S.D. Souza, E. Antunes, T. Nyokong, Synthesis and photophysical studies of CdTe quantum dot-monosubstituted zinc phthalocyanine conjugates, Inorg. Chim. Acta 367 (2011) 173–181.
[35] M. Westerfield, The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Brachydanio rerio*), University of Oregon Press, Eugene, 1995, pp. 267–272.
[36] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic development of the zebrafish, Dev. Dyn. 203 (1995) 253–310.
[37] J.T. Creed, T.D. Martin, J.W. O'Dell, Determination of Trace Elements by Stabilized Temperature Graphite Furnace Atomic Absorption, US Environmental Protection Agency, 1994.
[38] M.Y. Wang, J.F. Chen, K.F. Lin, Y.H. Chen, W. Hu, R.L. Tanguay, C.J. Huang, Q.X. Dong, Chronic zebrafish PFOS exposure alters sex ratio and maternal related effects in F1 offspring, Environ. Toxicol. Chem. 9999 (2011) 1–8.
[39] H.H. Huang, C.J. Huang, L.J. Wang, X.W. Ye, C.L. Bai, Q.X. Dong, Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctane sulphonicacid (PFOS), Aquat. Toxicol. 98 (2010) 139–147.
[40] Y. Zhang, W. Chen, J. Zhang, J. Liu, G. Chen, C. Pope, In vitro and in vivo toxicity of CdTe nanoparticles, J. Nanosci. Nanotechnol. 7 (2007) 497–503.
[41] T.C. King-Heiden, P.N. Wiecinski, A.N. Mangham, K.M. Metz, D. Nesbit, J.A. Pedersen, R.J. Hamers, W. Heideman, R.E. Peterson, Quantum dot nanotoxicity assessment using the zebrafish embryo, Environ. Sci. Technol. 43 (2009) 1605–1611.
[42] S.H. Cheng, A.W.K. Wai, C.H. So, R.S.S. Wu, Cellular and molecular basis of cadmium-induced deformities in zebrafish embryos, Environ. Toxicol. Chem. 19 (2000) 3024–3031.
[43] S.R. Blechinger, J.T. Warren Jr., J.Y. Kuwada, P.H. Krone, Developmental toxicology of cadmium in living embryos of a stable transgenic zebrafish line, Environ. Health Perspect. 110 (2002) 1041–1046.
[44] A.S. Kane, J.D. Salierno, G.T. Gipson, T.C. Molteno, C. Hunter, A video-based movement analysis system to quantify behavioral stress responses of fish, Water Res. 38 (2004) 3993–4001.
[45] R.C. MacPhail, J. Brooks, D.L. Hunter, B. Padnos, T.D. Irons, S. Padilla, Locomotion in larval zebrafish: influence of time of day, lighting and ethanol, Neurotoxicology 30 (2009) 52–58.
[46] D.A. Prober, J. Rihel, A.A. Onah, R.J. Sung, A.F. Schier, Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish, J. Neurosci. 26 (2006) 13400–13410.
[47] P. Drapeau, D.W. Ali, R.R. Buss, L. Saint-Amant, In vivo recording from identifiable neurons of the locomotor network in the developing zebrafish, J. Neurosci. Methods 88 (1999) 1–13.
[48] H. Flanagan-Steet, M.A. Fox, D. Meyer, J.R. Sanes, Neuromuscular synapses can form in vivo by incorporation of initially aneural postsynaptic specializations, Development 132 (2005) 4471–4481.
[49] E. Levin, M. Aschner, U. Heberlein, D. Ruden, K. Welsh-Bohmer, S. Bartlett, K. Berger, L. Chen, A. Corl, D. Eddins, R. French, K. Hayden, K. Helmcke, H. Hirsch, E. Linney, G. Lnenicka, G. Page, D. Possidente, B. Possidente, A. Kirshner, Genetic aspects of behavioral neurotoxicology, NeuroToxicol. 30 (2009) 741–753.
[50] M. Greek-Walker, G.A. Pull, A survey of red and white muscle in marine fish, J. Fish Physiol. 7 (1975) 295–300.
[51] S.D. Buckingham, D.W. Ali, Sodium and potassium currents of larval zebrafish muscle fibers, J. Exp. Biol. 207 (2004) 841–852.
[52] R.R. Buss, P. Drapeau, Activation of embryonic red and white muscle fibers during fictive swimming in the developing zebrafish, J. Neurophysiol. 87 (2002) 1244–1251.
[53] N. Sylvain, D. Brewster, D. Ali, Zebrafish embryos exposed to alcohol undergo abnormal development of motor neurons and muscle fibers, Neurotoxicol. Teratol. 32 (2010) 472–480.
[54] R.J. Egan, C.L. Bergner, P.C. Hart, J.M. Cachat, P.R. Canavello, M.F. Elegante, S.I. Elkhayat, B.K. Bartels, A.K. Tien, D.H. Tien, S. Mohnot, E. Beeson, E. Glasgow, H. Amri, Z. Zukowska, A.V. Kalueff, Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish, Behav. Brain Res. 205 (2009) 38–44.