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# Hormetic effect induced by depleted uranium in zebrafish embryos

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ABSTRACT

The present work studied the hormetic effect induced by uranium (U) in embryos of zebrafish (*Danio rerio*) using apoptosis as the biological endpoint. Hormetic effect is characterized by biphasic dose-response relationships showing a low-dose stimulation and a high-dose inhibition. Embryos were dechorionated at 4 h post fertilization (hpf), and were then exposed to 10 or 100 μg/l depleted uranium (DU) in uranyl acetate solutions from 5 to 6 hpf. For exposures to 10 μg/l DU, the amounts of apoptotic signals in the embryos were significantly increased at 20 hpf but were significantly decreased at 24 hpf, which demonstrated the presence of U-induced hormesis. For exposures to 100 μg/l DU, the amounts of apoptotic signals in the embryos were significantly increased at 20, 24 and 30 hpf. Hormetic effect was not shown but its occurrence between 30 and 48 hpf could not be ruled out. In conclusion, hormetic effect could be induced in zebrafish embryos in a concentration- and time-dependent manner.

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## 1. Introduction

Uranium (U) is a metal contaminant widely distributed in our environment (Li et al., 2015; Shao et al., 2016). It reaches the environment from different sources including U production activities (Krishnapriya et al., 2015; Phillips and Watson, 2015), U mine tailings and ore wastes (Lee and Yang, 2010) and depleted uranium (DU) munitions in conflict areas and at test sites (Crean et al., 2013). Exposures to U have aroused public health concerns due to the internal irradiation and/or chemical toxicity (Li et al., 2015; Choy et al., 2006). As a result, immense efforts have been devoted to understanding and modeling the behavior of U in the environment, such as oxidative corrosion of carbide inclusions at the surface of U metal (Scott et al., 2011), precipitation and adsorption of U and its relationship with other contaminants within the geological materials of the surface (Phillips and Watson, 2015) and translocation of U from water to foodstuff while cooking (Krishnapriya et al., 2015), etc. Extensive effort has also been spent on characterization and remediation of U contamination in the environment including chemical extraction (Choy et al., 2006; Crean et al., 2013), enrichment and separation of U from radioactive wastewater (Shao et al., 2016), removal from aqueous solution by nanoparticles and graphene composites (Li et al., 2015), U biomineralization (Choudhary and Sar, 2011), and rhizofiltration to remediate U-contaminated groundwater (Lee and Yang, 2010). A review can also be found in Gavrilescu et al. (2009). In particular, U can be found in aquatic systems at concentrations varying from 0.01 μg/l to 2 mg/l, depending on the geological background (WHO, 2001).

The biological effects of U have also been studied in non-human species but most of these studies are related to acute exposures and bioaccumulation patterns (Bywater et al., 1991; Labrot et al., 1999; Poston, 1982). It was established that U could trigger the production of free radical species (Miller et al., 2002; Yazzie et al., 2003) and could suppress the activities of enzymes related to antioxidant defenses such as catalase and superoxide dismutase in exposures to 20 and 100 μg/l of U on zebrafish (Barillet et al., 2005, 2007). A reduction in the hatching rate and a delay in hatching were also observed on zebrafish embryos after being exposed to 20 and 250 μg/l of DU (Bourrachot et al., 2008). A range of effects from exposures to environmental relevant levels of DU were studied on adult zebrafish by Barillet et al. (2011). The authors reported that U was highly bioconcentrated in fish, according to a time- and concentration-dependent model. Information related to the effects of U exposure in freshwater fish, especially that related to sublethal concentrations of U, is essential for realistic environmental risk assessment. Such information is available in the literature (Barillet

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et al., 2005, 2007; Buet et al., 2005; Cooley et al., 2000; Kelly and Janz, 2009; Labrot et al., 1996; Lerebours et al., 2009; Lourenço et al., 2010).

One important phenomenon for a low-dose environmental stressor is hormesis, which is characterized by biphasic dose-response relationships showing a low-dose stimulation and a high-dose inhibition (Calabrese, 2008; Calabrese and Baldwin, 2002; Calabrese and Linda, 2003). A comprehensive review on the mechanisms underlying the hormetic dose-response which were mediated through receptor and/or cell signaling pathways was given by Calabrese (2013). In particular, it was remarked that the hormetic dose-response was highly generalizable and was independent of the biological model in use, the endpoint measured and the chemical class (Calabrese, 2013). In fact, hormesis has been observed for a broad range of chemicals, metals, herbicides and also physical process like radiation exposure (Azzam et al., 1996; Bond et al., 1991; Calabrese and Baldwin, 1997, 2000, 2001, 2003, 2008; Choi et al., 2012; Cohen, 1995; Damelin et al., 2000; Elmore et al., 2005; Hayes, 2007; Hooker et al., 2004; Lefcort et al., 2008; Luckey, 1982; Mitchel et al., 1999; Ng et al., 2015a; Rithidech and Scott, 2008; Shadley and Wolff, 1987; Shen et al., 2009). Recently, a reduction in the apoptotic signals induced on embryos after exposure to low concentration of U was reported by Ng et al. (2015b). Different processes, including elimination of naturally aberrant cells by early apoptosis, had been proposed to explain the radiation-induced hormetic effect (Vaiserman, 2010).

As such, for realistic risk assessment of U in the environment, it is pertinent to carry out further studies to better understand the hormetic effect induced by U in living organisms. The primary objective of the present study was to study the U-induced hormetic effect in the embryos of zebrafish (*Danio rerio*) using apoptosis as the biological endpoint. In particular, the dependence on the concentration and time of the U-induced hormetic effect was also examined. The effects of a low concentration (10 μg/l) and a high concentration (100 μg/l) DU on zebrafish embryos (with 1 h exposure time) were studied at four different time points, namely, 20, 24, 30 and 48 hpf. As PTU treatment was needed when studying the embryos at 30 and 48 hpf, separate experiments were also carried out to confirm that treating zebrafish embryos with the desired concentration of PTU (i.e., 75 μM) would not affect the amount of apoptotic signals on the embryos. Zebrafish has become a popular model for studying the toxicity and biological effects of different environmental stressors because of the considerable homology between zebrafish and human genomes which include conservation of most DNA repair-related genes (Barbazuk et al., 2000).

## 2. Material and methods

### 2.1. Zebrafish embryos

Adult zebrafish (*D. rerio*) were maintained in fish tanks at 28.5 °C with a 14/10 h light-dark cycle. Spawning was triggered when the 14 h photoperiod began. A specially designed plastic collector was used to collect embryos after the start of the photoperiod (Choi et al., 2010). All embryos were collected within 15–30 min to ensure the synchronization of developmental stages of the embryos. Immediately after collection, the embryos were transferred to an incubator maintained at 28.5 °C until they developed into 4 h post fertilization (hpf). To avoid absorption of DU in the chorions instead of the embryos themselves, the chorion of each embryo was carefully removed with a pair of forceps at 4 hpf.

### 2.2. Depleted uranium exposure

In the present experiments, DU contamination was achieved using uranyl acetate $UO_2(CH_3COO)_2{\cdot}2H_2O$ (Electron Microscopy Sciences). The DU stock solution as well as the working solutions were prepared as described by Ng et al. (2015b). Briefly, 0.15–0.30 g/l uranyl acetate stock solutions were prepared by dissolving uranyl acetate with MilliQ water. Since uranyl acetate is light-sensitive and would precipitate when exposed to light, all stock solutions were kept in dark and maintained at 4 °C. To minimize the effects due to potential fluctuation of the DU concentration in different sets of experiments due to precipitation, a new DU stock solution was prepared independently and separately for each set of experiments. The stock solution was prepared 1 day before preforming each set of experiments to allow enough time for all the uranyl acetate to dissolve. On the day of experiment, the stock solution was further diluted to the desired concentration in E3 medium and the pH value of the working solution was maintained at 7.

### 2.3. Effect of DU on zebrafish embryos

#### 2.3.1. Exposure protocol

The number of apoptotic cells within the embryos was chosen as the biological endpoint. In the present study, the effects of DU on zebrafish embryos were studied at four different time points, namely, 20 (Case 1), 24 (Case 2), 30 (Case 3) and 48 (Case 4) hpf. For each time-point study, when the embryos developed into 5 hpf, dechorionated embryos were divided into three groups, each having 10 embryos, and were accommodated in three separate Petri dishes with a layer of biocompatible agarose lining the bottoms. The three groups were referred to as:

(A) ***Control (C) group***: in which the embryos were dechorionated without receiving any further treatment;
(B) ***Low-U-dosed ($U_{10}$) group***: in which the embryos were exposed to 10 μg/l of DU for 1 h (from 5 to 6 hpf);
(C) ***High-U-dosed ($U_{100}$) group***: in which the embryos were exposed to 100 μg/l of DU for 1 h (from 5 to 6 hpf).

Uranium can be found in aquatic systems at concentrations varying from 0.01 μg/l to 2 mg/l, depending on the geological background (WHO, 2001). In the present study, two concentrations of DU (i.e., 10 and 100 μg/l) were employed to investigate the effects of DU on zebrafish embryos since these two concentrations were within the range of environmental concentrations found close to mining sites (Antunes et al., 2007) or in drilled wells (Kurttio et al., 2006). The same concentrations of DU were also employed in previous studies (Ng et al., 2015b, 2016) which were found to have successfully induced different effects on zebrafish embryos. In fact, the accumulated levels of DU in zebrafish embryo were measured using an Inductively Coupled Plasma-Mass Spectrometry (ICP-AES, Detection Limit = 9.3 $\mu g\,l^{-1}$) after being digested in 3 M nitric acid at 105 °C for 5 h. However, the accumulated levels of DU in the zebrafish embryos were all below the detection limit of our ICP-AES. The flows of the experiments involving embryos in these three groups were schematically shown in Fig. 1.A volume of 3 ml of E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, 0.1% methylene blue), 3 ml of DU working solution with a concentration of 10 μg/l (diluted in E3) or 3 ml of DU working solution with a concentration of 100 μg/l (diluted in E3) was used in each Petri dish for the ***C***, ***$U_{10}$*** or ***$U_{100}$*** groups, respectively. The levels of U in the working solutions (10 and 100 μg/l) were measured using an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Optima 2100DV, Perkin-Elmer, Wellesley, MA, USA, detection limit of 9.3 $\mu g\,l^{-1}$) after acidification with 0.3 M nitric acid. Exposures of embryos in the ***$U_{10}$*** and ***$U_{100}$*** groups to DU started at 5 hpf

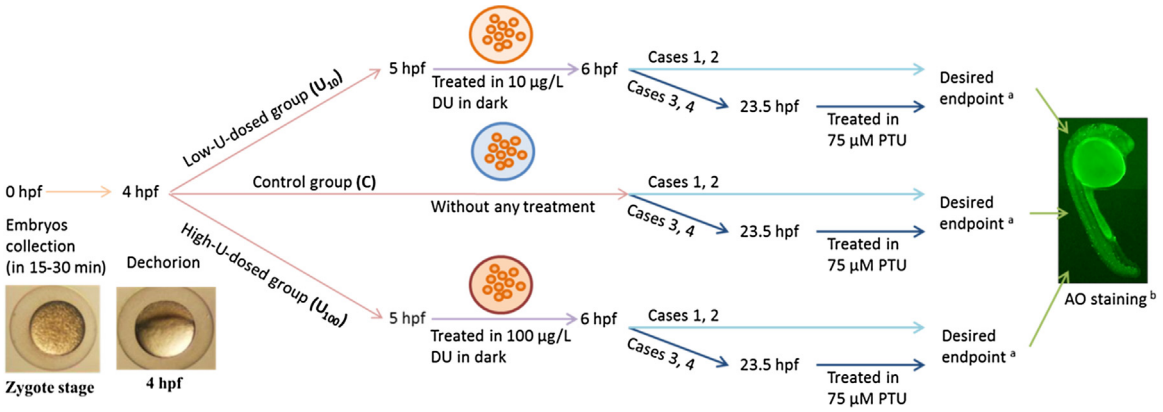


**Fig. 1.** Experimental flow for the embryos in the Control (***C***) group, low-U-dosed (***U*****10**) group and high-U-dosed (***U*****100**) group in the four cases of studies. [a] The desired endpoint refers to 20, 24, 30 and 48 hpf for cases 1–4, respectively. [b] A sample AO-stained embryo in the high-U-dosed (***U*****100**) group which was stained at 24 hpf for 1 h in dark.

when the DNA repair mechanisms had become operative (Miyachi et al., 2003). These embryos were maintained in the DU solution (with concentrations of either 10 or 100 μg/l) for 1 h in dark. Sample working solutions were collected 30 min after the start of the exposure and the DU concentrations were measured again using ICP-AES. All exposure levels were found to have remained constant during the exposures, which was expected due to the short exposure duration (1 h). After that, the embryos were removed from the DU solution and washed with 6 ml of clean E3 medium. Finally, the embryos were transferred to new Petri dishes which contained 3 ml of E3 medium. All the three groups of embryos (***C***, $U_{10}$ and ***U*****100**) were then returned to the 28.5 °C incubator for further development.

It was well established that zebrafish embryos begin to develop pigmentation at around 25 hpf at 28.5 °C (Kimmel et al., 1995; Milos and Dingle, 1978). Since these pigments could obstruct the observation of the stained apoptotic cells, all embryos (in the ***C***, $U_{10}$ and ***U*****100** groups) in Cases 3 and 4 were treated with 75 μM 1-phenyl 2-thiourea (PTU) during embryogenesis at 23.5 hpf (28 somite stage) as suggested by Karlsson et al. (2001) to block the development of pigment cells within embryos. Karlsson et al. (2001) also confirmed that treating zebrafish embryos at such a time point (i.e., 28 somite stage) and such concentration of PTU (i.e. 75 μM) was effective in preserving the transparency of the zebrafish embryos but without interfering with their normal embryonic development. Since all the embryos in Cases 1 and 2 were collected for analysis for apoptosis at 20 and 24 hpf, respectively, which were before the start of pigment development, PTU-treatment was not needed. When the embryos developed to the chosen time points, which were 20 (Case 1: ***C*****_20**, $U_{10\_20}$ and ***U*****100_20**), 24 (Case 2: ***C*****_24**, ***U*****10_24** and ***U*****100_24**), 30 (Case 3: ***C*****_30**, $U_{10\_30}$ and ***U*****100_30**), or 48 hpf (Case 4: ***C*****_48**, $U_{10\_48}$ and ***U*****100_48**), they were collected for staining.

### 2.3.2. Quantification of apoptosis by vital dye staining

In the present project, apoptosis was chosen as the biological endpoint. Apoptosis has been widely adopted as the biological endpoint to study the effects on zebrafish embryos. When the zebrafish embryos developed to 20 (Case 1), 24 (Case 2), 30 (Case 3) or 48 (Case 4) hpf, the apoptotic signals on the entire embryos were quantified. Among different staining methods, vital dye acridine orange (AO) staining was chosen for the present project to reveal apoptosis within the embryos as recommended by other studies (Yasuda et al., 2008; Mei et al., 2008). Briefly, the embryos were transferred into a medium containing 2 μg/ml of AO (Sigma, St. Louis, MO, U.S.) and stained in dark for 60 min. After that, the embryos were washed twice thoroughly in the culture medium, and were then anaesthetized using 0.0016 M tricaine (Sigma, St. Louis, MO, U.S.) before being counted for the apoptotic signals (i.e., the number of apoptotic cells) under a fluorescent microscope. Three images focusing on different sections of each embryo were captured under the fluorescent microscope with a magnification of 40×. These images were then combined into a single image and the amount of apoptotic signals in each of the embryo was counted using a computer program called "Particle Counting 2.0" (developed by J. Zhang). For each case, four sets of experiments each involving 30 embryos were independently performed on different days.

## 2.4. Effect of PTU on zebrafish embryos

### 2.4.1. Exposure protocol

To confirm that treating zebrafish embryos with the desired concentration of PTU (i.e., 75 μM) would not affect the amount of apoptotic signals, experiments were performed to compare the apoptotic signals on untreated embryos and those treated with 75 μM of PTU. When the dechorionated embryos developed into 5 hpf, they were divided into two groups: (A) ***Control (P*****ctrl**) **group** in which the embryos were maintained in 3 ml of E3 medium, and (B) ***PTU-treated (P*****75 μM**) **group** in which the embryos were maintained in 3 ml of 75 μM of PTU from 23.5 hpf until 24 hpf. Each group had 10 embryos and was accommodated in two separate Petri dishes with a layer of biocompatible agarose lining at the bottom. In addition, the effect of PTU on the apoptotic signals on U-treated embryos was also tested. In this part of the experiment, when the dechorionated embryos developed into 5 hpf, they were divided into a total of six groups each having 12 embryos:

(A) ***Control (P*****ctrl**) **group**: in which the embryos were maintained in 3 ml of E3 medium until 24 hpf;
(B) ***PTU-treated (P*****75 μM**) **group**: in which the embryos were maintained in 3 ml of 75 μM of PTU from 23.5 hpf until 24 hpf;
(C) ***Low-U-dosed and non-PTU-treated (P*****U10_E3**) **group**: in which the embryos were exposed to 10 μg/l of U for 1 h (from 5 to 6 hpf) and were then kept in E3 medium until 24 hpf;
(D) ***Low-U-dosed and PTU-treated (P*****U10_PTU**) **group**: in which the embryos were exposed to 10 μg/l of U for 1 h (from 5 to 6 hpf) and were then treated with 75 μM of PTU from 23.5 hpf until 24 hpf;
(E) ***High-U-dosed and non-PTU-treated (P*****U100_E3**) **group**: in which the embryos were exposed to 100 μg/l of U for 1 h (from 5 to 6 hpf) and were then kept in E3 medium until 24 hpf; and
(F) ***High-U-dosed and PTU-treated (P*****U100_PTU**) **group**: in which the embryos were exposed to 100 μg/l of U for 1 h (from 5 to 6 hpf) and were then treated with 75 μM of PTU from 23.5 hpf until 24 hpf.

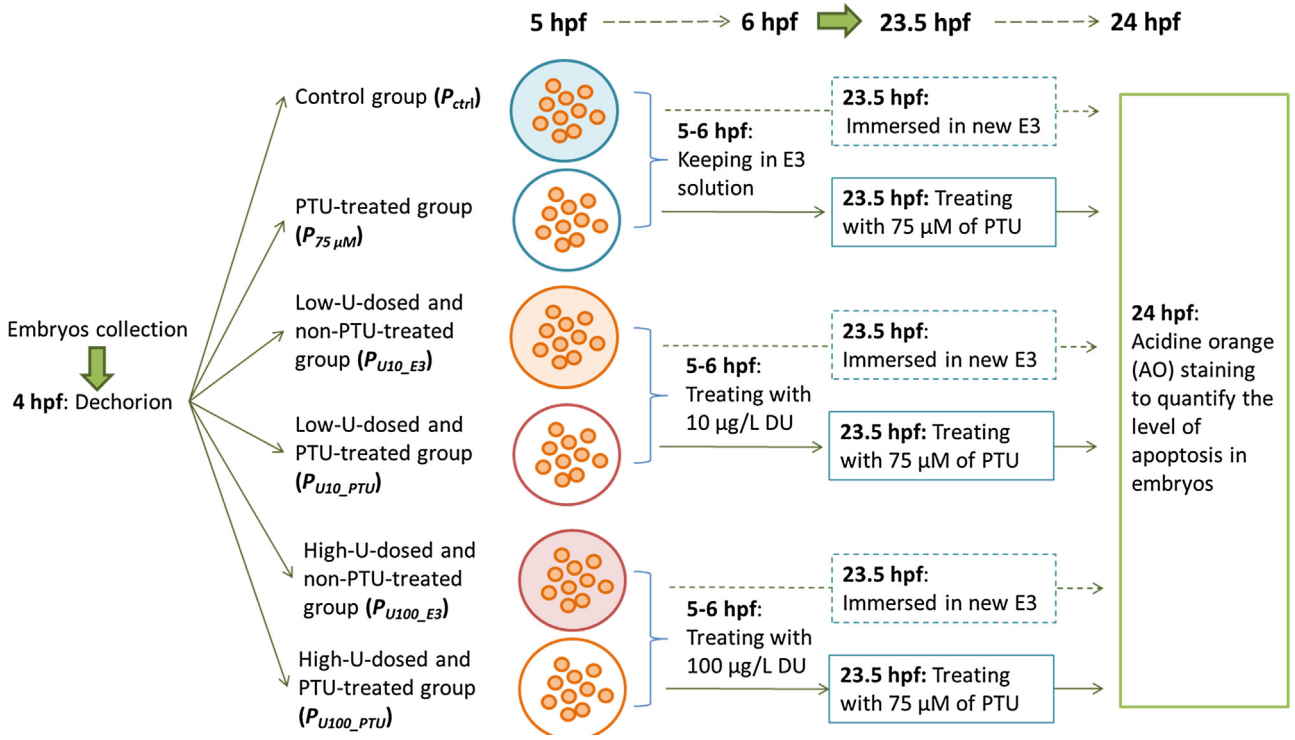


**Fig. 2.** Experimental flow for the embryos in the Control ($P_{ctrl}$) group, PTU-treated ($P_{75\ \mu M}$) group, low-U-dosed and non-PTU-treated ($P_{U10\_E3}$) group, low-U-dosed and PTU-treated ($P_{U10\_PTU}$) group, high-U-dosed and non-PTU-treated ($P_{U100\_E3}$) group and high-U-dosed and PTU-treated ($P_{U100\_PTU}$) group to verify the effect of PTU on zebrafish embryos.

Embryos in the $P_{U10\_E3}$, $P_{U10\_PTU}$, $P_{U100\_E3}$ and $P_{U100\_PTU}$ groups were exposed to U with concentrations of either 10 or 100 μg/l at 5 hpf for 1 h in dark as described in Section 2.3 above. After that, the embryos were washed with clean medium and then maintained in E3 medium at 28.5 °C. At 23.5 hpf, the embryos in the $P_{75\ \mu M}$, $P_{U10\_PTU}$ and $P_{U100\_PTU}$ groups were immersed into 3 ml of 75 μM of PTU while those in $P_{ctrl}$, $P_{U10\_E3}$ and $P_{U100\_E3}$ groups were immersed into 3 ml of new E3 medium at the same time. All groups of embryos were then returned to the 28.5 °C incubator for further development. The experimental flow involving embryos in this part was schematically shown in Fig. 2.

### 2.4.2. Quantification of apoptosis by vital dye staining

As the pigments developed in the zebrafish embryos after 24 hpf might obstruct the apoptotic signals (Bladen et al., 2005), all embryos in this test were stained with AO for 1 h in dark when they developed into 24 hpf and the number of apoptotic cells within the embryos were counted under the fluorescent microscope using 40× magnification. After anaesthetizing the embryos with 0.0016 M tricaine, three images focusing on different sections of each embryo were captured using the same exposure condition. Again, the computer program "Particle Counting 2.0" was employed to determine the amount of apoptotic cells in each embryo. Two individual sets of experiments were performed.

### 2.5. Statistical analysis

As described above in Sections 2.3 and 2.4, the apoptotic signal on each zebrafish embryo was counted. For the study on the effect of DU on zebrafish embryos, in order to combine the data from different replicates for each experimental condition, the numbers of apoptotic signals for the low-U-dosed ($N_{U10}$) group and for the high-U-dosed ($N_{U100}$) group were first transformed to, respectively, the normalized net number of apoptotic signals $N_{U10}{}^{*} = [(N_{U10} - N_c)/N_c]$ and $N_{U100}{}^{*} = [(N_{U100} - N_c)/N_c]$, taking the average number of apoptotic signals for the control ($N_C$) group as the background apoptotic signals of the corresponding groups of embryos. For the study on the effect of PTU on zebrafish embryos, the average numbers of apoptotic signals for different groups of embryos were obtained and denoted as $N_{pctrl}$, $N_{p75\ \mu M}$, $N_{pU10\_E3}$, $N_{pU10\_PTU}$, $N_{pU100\_E3}$ and $N_{pU100\_PTU}$. The data are presented as the average number of apoptotic signals ± standard error. The Student's *t*-test was used to determine the differences between the compared groups. Cases with *p* values ≤ 0.05 were considered as corresponding to statistically significant differences between the compared groups.

## 3. Results

### 3.1. Effect of DU on zebrafish embryos

In the present study, the effects of DU on zebrafish embryos were studied at four time points, namely, 20 (Case 1: $C_{\_20}$, $U_{10\_20}$ and $U_{100\_20}$), 24 (Case 2: $C_{\_24}$, $U_{10\_24}$ and $U_{100\_24}$), 30 (Case 3: $C_{\_30}$, $U_{10\_30}$ and $U_{100\_30}$) or 48 hpf (Case 4: $C_{\_48}$, $U_{10\_48}$ and $U_{100\_48}$). The results were summarized in Table 1.

The present results demonstrated that the number of apoptotic signals for the $U_{10}$ group of embryos was significantly higher than that of the $C$ group of embryos in all sets of experiments when studied at the earliest studied time point of 20 hpf, and dropped to significantly smaller than that of the $C$ group of embryos when studied at a later time point of 24 hpf. This revealed the presence of U-induced hormesis and suggested elimination of naturally aberrant cells by early apoptosis. Such a hormetic effect vanished when the embryos were examined at 30 and 48 hpf. On the other hand, the amount of apoptotic signals for the $U_{100}$ group of embryos was significantly larger than that of the $C$ group of embryos in all sets of experiments when studied at 20, 24 and 30 hpf. When the embryos

**Table 1**
Average net normalized number of apoptotic signals ($N^*$) ± standard error obtained from embryos in the $U_{10}$ and $U_{100}$ groups in the four sets of experiments for the four cases (Cases 1–4).

| | $U_{10}$ | $U_{100}$ |
|---|---|---|
| Case 1 (20 hpf) | 0.469 ± 0.064<br>[a] $n$ = 37<br>[b] $p = 8.87 \times 10^{-9}$ # | 0.233 ± 0.043<br>[a] $n$ = 40<br>[c] $p = 8.09 \times 10^{-6}$ # |
| Case 2 (24 hpf) | −0.185 ± 0.039<br>[a] $n$ = 35<br>[b] $p$ = 0.00110 # | 0.336 ± 0.075<br>[a] $n$ = 35<br>[c] $p$ = 0.000131 # |
| Case 3 (30 hpf) | −0.026 ± 0.053<br>[a] $n$ = 39<br>[b] $p$ = 0.356 | 0.409 ± 0.073<br>[a] $n$ = 37<br>[c] $p = 6.33 \times 10^{-6}$ # |
| Case 4 (48 hpf) | −0.049 ± 0.030<br>[a] $n$ = 40<br>[b] $p$ = 0.090 | −0.042 ± 0.028<br>[a] $n$ = 38<br>[c] $p$ = 0.116 |

Cases with $p \leq 0.05$ are considered to correspond to statistically significant differences between the compared groups.
[a] $n$: total number of embryos in four sets of replicates in a particular group.
[b] $p$: $p$ values obtained using Student's $t$-test to compare between the $C$ and $U_{10}$ groups of embryos.
[c] $p$: $p$ values obtained using Student's $t$-test to compare between the $C$ and $U_{100}$ groups of embryos.

**Table 2**
Average number of apoptotic signals ($N^*$) ± standard error obtained from embryos in the $P_{ctrl}$, $P_{75\ \mu M}$, $P_{U10\_E3}$, $P_{U10\_PTU}$, $P_{U100\_E3}$ and $P_{U100\_PTU}$ groups in the two sets of experiments.

| | $N_{pctrl}$ | $N_{p75\ \mu M}$ | $N_{pU10\_E3}$ | $N_{pU10\_PTU}$ | $N_{pU100\_E3}$ | $N_{pU100\_PTU}$ |
|---|---|---|---|---|---|---|
| Set 1 | 102 ± 9<br>$n$ = 11<br>[a] $p$ = 0.411 | 105 ± 11<br>$n$ = 9 | 74 ± 4<br>$n$ = 10<br>[b] $p$ = 0.083 | 83 ± 4<br>$n$ = 9 | 123 ± 7<br>$n$ = 12<br>[c] $p$ = 0.176 | 133 ± 8<br>$n$ = 11 |
| Set 2 | 122 ± 12<br>$n$ = 10<br>[a] $p$ = 0.389 | 127 ± 13<br>$n$ = 9 | 93 ± 10<br>$n$ = 12<br>[b] $p$ = 0.382 | 97 ± 9<br>$n$ = 9 | 160 ± 12<br>$n$ = 12<br>[c] $p$ = 0.377 | 155 ± 9<br>$n$ = 11 |

$n$: number of embryos in a particular group.
Cases with $p \leq 0.05$ are considered to correspond to statistically significant differences between the compared groups.
[a] $p$: $p$ values obtained using Student's t-test to compare between the $P_{ctrl}$ and $P_{75\mu M}$ groups of embryos.
[b] $p$: $p$ values obtained using Student's t-test to compare between the $P_{U10\_E3}$ and $P_{U10\_PTU}$ groups of embryos.
[c] $p$: $p$ values obtained using Student's t-test to compare between the $P_{U100\_E3}$ and $P_{U100\_PTU}$ groups of embryos.

were studied at 48 hpf, the amount of apoptotic signals of the $U_{100}$ groups of embryos dropped to the background level.

### 3.2. Effect of PTU on zebrafish embryos

In this part of study, the main objective was to test whether treating zebrafish embryos with 75 μM PTU would affect the amount of apoptotic signals. The study could help to confirm that the PTU-treatment was not the reason causing the changes in the apoptotic signals on embryos in the previous part (Cases 3 and 4) of the experiment. The embryos were divided into six groups, namely $P_{75\ \mu M}$, $P_{U10\_PTU}$ and $P_{U100\_PTU}$ groups where the embryos were treated with 75 μM PTU for 30 min and $P_{ctrl}$, $P_{U10\_E3}$ and $P_{U100\_E3}$ groups where the embryos were kept in E3 medium instead of PTU solution. Two sets of experiments were performed independently and the results were summarized in Table 2.

Two replicates of experiments were performed. For each set of experiment, Student's $t$-test was performed between (i) $P_{75\ \mu M}$ and $P_{pctrl}$ groups; (ii) $P_{U10\_PTU}$ and $P_{U10\_E3}$ groups, and (iii) $P_{U100\_PTU}$ and $P_{U100\_E3}$ groups. The obtained data demonstrate that treating zebrafish embryos with 75 μM PTU did not affect the amount of apoptotic signals on the whole embryos in any group, i.e. low-U-exposed group, high-U-exposed group and non-U-exposed embryos. In addition, when comparing the $P_{U10\_PTU}$ and $P_{U100\_PTU}$ groups with the $P_{75\ \mu M}$ group, significant differences were observed with $p$ values of 0.0053 and 0.0046, respectively. Significant differences were also observed when comparing the $P_{U10\_E3}$ and $P_{U100\_E3}$ groups with the $P_{pctrl}$ group with $p$ values of 0.0013 and 0.0026, respectively. These results agreed with those in Section 3.1 regarding the effect of 10 and 100 μg/l of DU on zebrafish embryos at 24 hpf (Case 1). Such agreement further confirmed that treating zebrafish embryos with 75 μM PTU did not affect the results.

## 4. Discussion

Apoptosis is controlled by the regulated cellular program and can be triggered by exposures to environmental pathogens or toxins (Vaux et al., 1994; Cole and Ross, 2001). In the present study, the DNA damages induced by depleted uranium were revealed through apoptotic events. It is understood that apoptosis occurs during normal zebrafish development (e.g., Cole and Ross, 2001) and can also be caused by exposures to environmental stressors (Vaux et al., 1994; Cole and Ross, 2001). In relation to the apoptosis arising from normal zebrafish development, Cole and Ross (2001) studied the spatial and temporal patterns of apoptosis in zebrafish embryos from 12 to 96 hpf under normal development using the *in situ* terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay, and revealed that the dynamics of apoptosis in zebrafish embryos in general followed the patterns in other organisms (Jacobson et al., 1997; Hensey and Gautier, 1999; Cellerino et al., 2000). As regards the apoptotic events caused by environmental stressors, Bladen et al. (2007) found a correlation between the apoptotic signals and the induction of DNA double strand breaks caused by 6 MeV photons. As background environmental stressors are ubiquitous, there are always damaged cells in the zebrafish embryos set to undergo apoptosis in addition to the normal development, which contributes to the naturally aberrant cells. As such, apoptosis was also employed in the present study to observe the different biological effects in the zebrafish embryos using a low concentration (10 μg/l) and a high concentration (100 μg/l) of U. As shown in Table 1, the apoptotic signals for the $U_{10}$ groups of embryos were significantly fewer than those of the $C$ groups of embryos in all sets of experiments when studied at 24 hpf. The observation agrees with our previous results (Ng et al., 2016). In general, the cells within the zebrafish embryos which were not subjected to any DU exposure could be divided into two groups, namely, (1) the naturally aberrant cells; and (2) healthy cells (Ng et al., 2016). Without any DU exposure (the $C$ group), the naturally aberrant cells would undergo apoptosis from time to time, for instance, at 20, 24, 30 and 48 hpf (Cole and Ross, 2001). Such an amount of apoptosis was regarded as the background apoptotic signal ($N_C$) for the corresponding set of experiment at the corresponding time point. The reduction in the apoptotic signals on embryos (with reference to $N_C$) after treatment with a low concentration of U suggested the presence of U-induced hormesis.

The chemical toxicity of U could arise from toxicant-target interactions or through changing enzymatic activities (Barillet et al., 2005, 2007). In particular, Barillet et al. (2007) reported that U reduced the activities of several enzymes which were involved in the antioxidant defense system in adult male zebrafish. On the other hand, DNA damage was also identified as a main effect of DU in fish (Barillet et al., 2011; Lerebours et al., 2009, 2013; Lourenço et al., 2010). DU could directly affect the normal functions of DNA through covalently bonding to the DNA molecule and thus forming U-DNA adducts (Wilson et al., 2014; Stearns et al., 2005). DU could also indirectly generate free radicals and reactive oxygen species

(ROS) that damaged DNA molecules (Miller et al., 2002; Yazzie et al., 2003; Miura, 2004). Lerebours et al. (2009) studied the expression of DNA repair genes such as *gadd45g* and *rad51* in zebrafish after DU exposures. For a comparison, DNA damages were also considered a major effect of ionizing radiations (Olsvik et al., 2010), although direct damages on DNA molecules resulted from breaking of the hydrogen bonds and oxygen-phosphate bonds (Rak et al., 2011; Razskazovskiy et al., 2000). The *gadd45g* gene was proposed as an indicator for DNA damages induced by both DU (Song et al., 2012) and ionizing radiations (Grace et al., 2002). In particular, sufficient evidence showed that gadd45 proteins were pro-apoptotic (Azam et al., 2001; Bulavin et al., 2003; Kojima et al., 1999; Takekawa and Saito, 1998; Vairapandi et al., 2000; Zhang et al., 2001). Considering the similar downstream events of DNA damages for both exposures to DU and ionizing radiations, it was feasible to explain the DU-induced hormetic effect in terms of elimination of naturally aberrant cells by early apoptosis, which was previously proposed by Vaiserman (2010) as a mechanism underlying radiation-induced hormetic effect.

Cole and Ross (2001) showed that apoptosis eliminated excess cells during zebrafish development. The authors revealed large numbers of apoptotic cells at earlier stages of brain development and attributed most of the scattered apoptosis in the developing brain to ongoing morphogenesis or elimination of damaged or defective cells. Coincidently, high apoptotic levels were observed at 20 hpf upon exposures to low doses of DU. The observation of high apoptotic levels before 24 hpf in the present study supported the proposed explanation of the low-dose-U induced hormetic effect in terms of removal of naturally aberrant cells through early apoptosis. When the embryos were exposed to a low concentration of DU, a relatively small number of healthy cells were damaged, while also a relatively small number of naturally aberrant cells were "further" damaged (in addition to their naturally occurring damages). Some of the damaged cells were then eliminated by early apoptosis before 24 hpf (Cole and Ross, 2001). If the eliminated naturally aberrant cells initially destined to undergo apoptosis at 24 hpf outnumbered the residual damaged healthy cells destined to undergo apoptosis at 24 hpf, the apoptotic signals observed at 24 hpf would be reduced when compared with the background levels observed in the control group of embryos. This was indeed observed when the embryos were treated with 10 μg/l of DU. However, it was interesting to note that such hormetic effect vanished when the embryos were examined at 30 and 48 hpf as the amounts of apoptotic signals on the embryos had returned to the background level at 30 hpf. Such observations suggested that most of the damaged cells were wiped out before 30 hpf, which was 24 h after the embryos were exposed to DU. Such a result also implied that the naturally aberrant cells lasted less than 30 h.

When the embryos were exposed to a higher concentration of DU (i.e., 100 μg/l), the number of apoptotic signals for the $\boldsymbol{U_{100}}$ group of embryos was significantly higher than that for the ***C*** group of embryos in all sets of experiments when studied at 20 hpf. The high level of apoptotic signals remained for a further 10 h, i.e., until 30 hpf. The results agree with those in previous studies (Barillet et al., 2011; Ng et al., 2016; Pereira et al., 2012) where zebrafish embryos were exposed to 100 μg/l of DU. The level of apoptotic signals then returned to background level at 48 hpf. Again, the cells within the zebrafish embryos which were not subjected to any DU exposure could be generally divided into two groups, namely, (1) the naturally aberrant cells; and (2) healthy cells (Ng et al., 2016). However, the outcomes from exposures to a higher DU concentration were expected to be mainly contributed by the damaged healthy cells since there were many more healthy cells than the naturally aberrant cells. The present data therefore demonstrated that U-induced apoptosis took place until a time point between 30 and 48 hpf, which was in sharp contrast to the apoptosis of naturally aberrant cells which lasted less than 30 h. Hormetic effect was not demonstrated in the data, but its occurrence between 30 and 48 hpf could not be ruled out.

In conclusion, the present study assessed the DU-induced apoptosis in zebrafish embryos and its associated dependence on the dose and the time. Our data demonstrated that at a chosen time point, viz., 24 hpf, different DU concentrations played an important role in the biological effects on the zebrafish embryos, leading to a hormetic effect (as given by a reduction in the apoptotic signals) at a low DU concentration, but an inhibitory effect on the increase in the number of normal cells (as given by an increase in the apoptotic signals) at a high DU concentration. The patterns of the U-induced apoptosis for the low and high DU concentrations were also very different. These results provide valuable information on realistic risk assessment of U in the environment.

## Author contributions

Conceived and designed the experiments: CYPN, SHC, KNY. Performed the experiments: CYPN. Analyzed the data: CYPN, KNY. Wrote the paper: CYPN, KNY.

## References


Antunes, S.C., Pereira, R., Gonçalves, F., 2007. Acute and chronic toxicity of effluent water from an abandoned uranium mine. Arch. Environ. Contam. Toxicol. 53, 207–213.

Azam, N., Vairapandi, M., Zhang, W., Hoffman, B., Liebermann, D.A., 2001. Interaction of CR6 (GADD45 g) with proliferating cell nuclear antigen impedes negative growth control. J. Biol. Chem. 276, 2766–2774.

Azzam, E.I., de Toledo, S.M., Raaphorst, G.P., Mitchel, R.E., 1996. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat. Res. 146, 369–373.

Barbazuk, W.B., Korf, I., Kadavi, C., Heyen, J., Tate, S., Wun, E., Bedell, J.A., McPherson, J.D., Johnson, S.L., 2000. The syntenic relationship of the zebrafish and human genomes. Genome Res. 10, 1351–1358.

Barillet, S., Buet, A., Adam, C., Devaux, A., 2005. Dose uranium exposure induce genotoxicity in the teleostean *Danio rerio*? First experimental results. Radioprotection 40, S175–S180.

Barillet, S., Adam, C., Palluel, O., Devaux, A., 2007. Bioaccumulation, oxidative stress and neurotoxicity in *Danio rerio* exposed to different isotopic compositions of uranium. Environ. Toxicol. Chem. 26, 497–505.

Barillet, S., Adam-Guillermin, C., Palluel, O., Porcher, J.M., Devaux, A., 2011. Uranium bioaccumulation and biological disorders induced in zebrafish (*Danio rerio*) after a depleted uranium waterborne exposure. Environ. Pollut. 159, 495–502.

Bladen, C.L., Lam, W.K., Dynan, W.S., Kozlowski, D.J., 2005. DNA damage response and Ku80 function in the vertebrate embryo. Nucleic Acids Res. 33, 3002–3010.

Bladen, C.L., Flowers, M.A., Miyake, K., Podolsky, R.H., Barrett, J.T., Kozlowski, D.J., Dynan, W.S., 2007. Quantification of ionizing radiation induced cell death in situ in a vertebrate embryo. Radiat. Res. 168, 149–157.

Bond, V.P., Benary, V., Sondhaus, C.A., 1991. A different perception of the linear, nonthreshold hypothesis for low-dose irradiation. Proc. Natl. Acad. Sci. U. S. A. 88, 8666–8670.

Bourrachot, S., Simon, O., Gilbin, R., 2008. The effects of waterborne uranium on the hatching success, development, and survival of early life stages of zebrafish (*Danio rerio*). Aquat. Toxicol. 90, 29–36.

Buet, A., Barillet, S., Camilleri, V., Garnier-Laplace, J., 2005. Alterations of oxidative stress parameters in fish as response to direct uranium exposure. Radioprotection 40, S151–S155.

Bulavin, D.V., Kovalsky, O., Hollander, M.C., Fornace Jr., A.J., 2003. Loss of oncogenic H-ras-induced cell cycle arrest and p38 mitogen-activated protein kinase activation by disruption of Gadd45a. Mol. Cell. Biol. 23, 3859–3871.

Bywater, J.F., Banaczkowski, R., Bailey, M., 1991. Sensitivity to uranium of six species of tropical freshwater fishes and four species of cladocerans from Northern Australia. Environ. Toxicol. Chem. 10, 1449–1458.

Calabrese, E.J., 2008. Hormesis: why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27, 1451–1474.

Calabrese, E.J., 2013. Hormetic mechanisms. Crit. Rev. Toxicol. 43, 580–606.

Calabrese, E.J., Baldwin, L.A., 1997. The dose determines the stimulation (and poison): development of a chemical hormesis database. Int. J. Toxicol. 16, 545–560.

Calabrese, E.J., Baldwin, L.A., 2000. Radiation hormesis: the demise of a legitimate hypothesis. Hum. Exp. Toxicol. 19, 76–84.

Calabrese, E.J., Baldwin, L.A., 2001. Hormesis: U-shaped dose response and their centrality in toxicology. Trends Pharmacol. Sci. 22, 285–291.

Calabrese, E.J., Baldwin, L.A., 2002. Defining hormesis. Hum. Exp. Toxicol. 21, 91–97.

Calabrese, E.J., Baldwin, L.A., 2003. Hormesis: the dose–response revolution. Annu. Rev. Pharmacol. Toxicol. 43, 175–197.

Calabrese, E.J., Baldwin, L.A., 2008. Inorganics and hormesis. Crit. Rev. Toxicol. 33, 3–4.
Calabrese, E.J., Linda, A.B., 2003. Toxicology rethinks its central belief. Nature 421, 691–692.
Cellerino, A., Galli-Resta, L., Colombaioni, L., 2000. The dynamics of neuronal death: a time-lapse study in the retina. J. Neurosci. 20, RC92 1–5.
Choi, V.W.Y., Lam, R.K.K., Chong, E.Y.W., Cheng, S.H., Yu, K.N., 2010. Designing experimental setup and procedures for studying alpha-particle-induced adaptive response in zebrafish embryos *in vivo*. Nucl. Instrum. Meth. B 268, 651–656.
Choi, V.W.Y., Cheung, A.L.Y., Cheng, S.H., Yu, K.N., 2012. Hormetic effect induced by alpha-particle-induced stress communicated *in vivo* between zebrafish embryos. Environ. Sci. Technol. 46, 11678–11683.
Choudhary, S., Sar, P., 2011. Uranium biomineralization by a metal resistant *Pseudomonas aeruginosa* strain isolated from contaminated mine waste. J. Hazard. Mater. 186, 336–343.
Choy, C.C., Korfiatis, G.P., Meng, X., 2006. Removal of depleted uranium from contaminated soils. J. Hazard. Mater. 136, 53–60.
Cohen, B.L., 1995. Test of the linear-no threshold theory of radiation carcinogenesis for inhaled radon decay products. Health Phys. 68, 157–174.
Cole, L.K., Ross, L.S., 2001. Apoptosis in the developing zebrafish embryos. Dev. Biol. 210, 123–142.
Cooley, H.M., Evans, R.E., Klaverkamp, J.F., 2000. Toxicology of dietary uranium in lake whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 48, 495–515.
Crean, D.E., Livens, F.R., Sajih, M., Stennett, M.C., Grolimund, D., Borca, C.N., Hyatt, N.C., 2013. Remediation of soils contaminated with particulate depleted uranium by multi stage chemical extraction. J. Hazard. Mater. 263, 382–390.
Damelin, L.H., Vokes, S., Damelin, J.M., Alexander, J.J., 2000. Hormesis: a stress response in cells exposed to low levels of heavy metals. Hum. Exp. Toxicol. 19, 420–430.
Elmore, E., Lao, X.Y., Ko, M., Rightnar, S., Nelson, G., Redpath, J., 2005. Neoplastic transformation *in vitro* induced by low doses of 232 MeV protons. Int. J. Radiat. Biol. 81, 291–297.
Gavrilescu, M., Pavel, L.V., Cretescu, I., 2009. Review: characterization and remediation of soils contaminated with uranium. J. Hazard. Mater. 163, 475–510.
Grace, M.B., McLeland, C.B., Blakely, W.F., 2002. Real-time quantitative RT-PCR assay of GADD45 gene expression changes as a biomarker for radiation biodosimetry. Int. J. Radiat. Biol. 78, 1011–1021.
Hayes, D.P., 2007. Review nutritional hormesis. Eur. J. Clin. Nutr. 61, 147–159.
Hensey, C., Gautier, J., 1999. Developmental regulation of induced and programmed cell death in Xenopus embryos. Ann. N.Y. Acad. Sci. 887, 105–119.
Hooker, A.M., Bhat, M., Day, T.K., Lane, J.M., Swinburne, S.J., Morley, A.A., Sykes, P.J., 2004. The linear nothreshold model does not hold for low-dose ionizing radiation. Radiat. Res. 162, 447–452.
Jacobson, M.D., Weil, M., Raff, M.C., 1997. Programmed cell death in animal development. Cell 88, 347–354.
Karlsson, J., von Hofsten, J., Olsson, P.E., 2001. Generating transparent zebrafish: a refined method to improve detection of gene expression during embryonic development. Mar. Biotechnol. 10, 522–527.
Kelly, J.M., Janz, D.M., 2009. Assessment of oxidative stress and histopathology in juvenile northern pike (*Esox lucius*) inhabiting lakes downstream of a uranium mill. Aquat. Toxicol. 92, 240–249.
Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dynam. 203, 253–310.
Kojima, S., Mayumi-Matsuda, K., Suzuki, H., Sakata, T., 1999. Molecular cloning of rat GADD45g, gene induction and its role during neuronal cell death. FEBS Lett. 446, 313–317.
Krishnapriya, K.C., Baksi, A., Chaudhari, S., Gupta, S.S., Pradeep, T., 2015. Translocation of uranium from water to foodstuff while cooking. J. Hazard. Mater. 297, 183–190.
Kurttio, P., Harmoinen, A., Saha, H., Salonen, L., Karpas, Z., Komulainen, H., Auvinen, A., 2006. Kidney toxicity of ingested uranium from drinking water. Am. J. Kidney Dis. 47, 972–982.
Labrot, F., Ribera, D., Saint-Denis, M., Narbonne, J.F., 1996. In vitro and *in vivo* studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinestérase, catalase and glutathione peroxidase activities in three non-mammalian species. Biomarkers 1, 21–28.
Labrot, F., Narbonne, J.F., Ville, P., Saint Denis, M., Ribera, D., 1999. Acute toxicity, toxicokinetics, and tissue target of lead and uranium in the clean *Corbicula fluminea* and the worm *Eisenia fetida*: comparison with the fish *Brachydanio rerio*. Arch. Environ. Contam. Toxicol. 28, 682–690.
Lee, M., Yang, M., 2010. Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. *vulgaris*) to remediate uranium contaminated groundwater. J. Hazard. Mater. 173, 589–596.
Lefcort, H., Freedman, Z., House, S., Pendleton, M., 2008. Hormetic effects of heavy metals in aquatic snails: is a little bit of pollution good? EcoHealth 5, 10–17.
Lerebours, A., Gonzalez, P., Adam, C., Camilleri, V., Bourdineaud, J.P., Garnier-Laplace, J., 2009. Comparative analysis of gene expression in brain, liver, skeletal muscles, and gills of zebrafish (*Danio rerio*) exposed to environmentally relevant waterborne uranium concentrations. Environ. Toxicol. Chem. 28, 1271–1278.
Lerebours, A., Cambier, S., Hislop, L., Adam-Guillermin, C., Bourdineaud, J.P., 2013. Genotoxic effects of exposure to waterborne uranium, dietary methylmercury and hyperoxia in zebrafish assessed by the quantitative RAPD-PCR method. Mutat. Res. 755, 55–60.
Li, Z.J., Wang, L., Yuan, L.Y., Xiao, C.L., Mei, L., Zheng, L.R., Zhang, J., Yang, J.H., Zhao, Y.L., Zhu, Z.T., Chai, Z.F., Shi, W.Q., 2015. Efficient removal of uranium from aqueous solution by zero-valent iron nanoparticle and its graphene composite. J. Hazard. Mater. 29, 26–33.
Lourenço, J., Castro, B.B., Machado, R., Nunes, B., Mendo, S., Gonçalves, F., Pereira, R., 2010. Genetic, biochemical, and individual responses of the teleost fish *Carassius auratus* to uranium. Arch. Environ. Contam. Toxicol. 58, 1023–1031.
Luckey, T.D., 1982. Physiological benefits from low levels of ionizing radiation. Health Phys. 43, 771–789.
Mei, J., Zhang, Q.Y., Li, Z., Lin, S., Gui, J.F., 2008. C1q-like inhibits p53-mediated apoptosis and controls normal hematopoiesis during zebrafish embryogenesis. Dev. Biol. 319, 273–284.
Miller, A.C., Stewart, M., Brooks, K., Shi, L., Page, N., 2002. Depleted catalyzed oxidative DNA damage: absence of significant alpha particle decay. J. Inorg. Biochem. 91, 246–252.
Milos, N., Dingle, A.D., 1978. Dynamics of pigment pattern formation in the zebrafish, *Brachydanio rerio*. I. Establishment and regulation of the lateral line melanophore stripe during the first eight days of development. J. Exp. Zool. 205, 205–216.
Mitchel, R.E., Jackson, J.S., McCann, R.A., Boreham, D.R., 1999. The adaptive response modifies latency for radiation-induced myeloid leukemia in CBA/H mice. Radiat. Res. 152, 273–279.
Miura, Y., 2004. Oxidative stress, radiation-adaptive responses, and aging. J. Radiat. Res. 45, 357–372.
Miyachi, Y., Kanao, T., Okamoto, T., 2003. Marked depression of time interval between fertilization period and hatching period following exposure to low dose X-rays in zebrafish. Environ. Res. 93, 216–219.
Ng, C.Y.P., Kong, E.Y., Konishi, T., Kobayashi, A., Suya, N., Cheng, S.H., Yu, K.N., 2015a. Low-dose neutron dose response of zebrafish embryos obtained from the neutron exposure accelerator system for biological effect experiments (NASBEE) facility. Radiat. Phys. Chem. 114, 12–17.
Ng, C.Y.P., Pereira, S., Cheng, S.H., Adam-Guillermin, C., Garnier-Laplace, J., Yu, K.N., 2015b. Combined effects of depleted uranium and ionising radiation on zebrafish embryos. Radiat. Prot. Dosim. 167, 311–315.
Ng, C.Y.P., Pereira, S., Cheng, S.H., Adam-Guillermin, C., Garnier-Laplace, J., Yu, K.N., 2016. Combined effects of alpha particles) and depleted uranium on zebrafish (*Danio rerio*) embryos. J. Radiat. Res., http://dx.doi.org/10.1093/jrr/rrv104 (in press).
Olsvik, P.A., Heier, L.S., Rosseland, B.O., Teien, H.C., Salbu, B., 2010. Effects of combined gamma-irradiation and metal (Al + Cd) exposures in Atlantic salmon (*Salmo salar* L.). J. Environ. Radioact. 101, 230–236.
Pereira, S., Camilleri, V., Floriani, M., Cavalie, I., Garnier-Laplace, J., Adam-Guillermin, C., 2012. Genotoxicity of uranium contamination in embryonic zebrafish cells. Aquat. Toxicol. 109, 11–16.
Phillips, D.H., Watson, D.B., 2015. Distribution of uranium and thorium in dolomitic gravel fill and shale saprolite. J. Hazard. Mater. 285, 474–482.
Poston, T.M., 1982. Observations on the bioaccumulation potential of thorium and uranium in rainbow trout (*Salmo gairdneri*). Bull. Environm. Contam. Toxicol. 28, 682–690.
Rak, J., Kobylecka, M., Storoniak, P., 2011. Single strand break in DNA coupled to the OP bond cleavage. A computational study. J. Phys. Chem. B 115, 1911–1917.
Razskazovskiy, Y., Debije, M.G., Bernhard, W.A., 2000. Direct radiation damage to crystalline DNA: what is the source of unaltered base release? Radiat. Res. 153, 436–441.
Rithidech, K.N., Scott, B.R., 2008. Evidence for radiation hormesis after *in vitro* exposure of human lymphocytes to low doses of ionizing radiation. Dose Response 6, 252–271.
Scott, T.B., Petherbridge, J.R., Harker, N.J., Ball, R.J., Heard, P.J., Glascott, J., Allen, G.C., 2011. The oxidative corrosion of carbide inclusions at the surface of uranium metal during exposure to water vapour. J. Hazard. Mater. 195, 115–123.
Shadley, J.D., Wolff, S., 1987. Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. Mutagenesis 2, 95–96.
Shao, D., Ren, X., Wen, J., Hu, S., Xiong, J., Jiang, T., Wang, X., Wang, X., 2016. Immobilization of uranium by biomaterial stabilized FeS nanoparticles: effects of stabilizer and enrichment mechanism. J. Hazard. Mater. 302, 1–9.
Shen, K., Shen, C., Lu, Y., Tang, X.J., Zhang, C.K., Chen, Z.C., Shi, J.Y., Lin, W., Chen, Y.X., 2009. Hormesis response of marine and freshwater luminescent bacteria to metal exposure. Biol. Res. 42, 183–187.
Song, Y., Salbu, B., Heier, L.S., Teien, H.C., Lind, O.C., Oughton, D., et al., 2012. Early stress responses in Atlantic salmon (*Salmo salar*) exposed to environmentally relevant concentrations of uranium. Aquat. Toxicol. 112, 62–71.
Stearns, D.M., Yazzie, M., Bradley, A.S., Coryell, V.H., Shelley, J.T., Ashby, A., Asplund, C.S., Lantz, R.C., 2005. Uranyl acetate induces hprt mutations and uranium-DNA adducts in Chinese hamster ovary EM9 cells. Mutagenesis 20, 417–423.
Takekawa, M., Saito, H., 1998. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4/MAPKKK. Cell 95, 521–530.
Vairapandi, M., Azam, N., Balliet, A.G., Hoffman, B., Liebermann, D.A., 2000. Characterization of MyD118, Gadd45, PCNA interacting domains: PCNA impedes MyD/Gadd mediated negative growth control. J. Biol. Chem. 275, 16810–16819.
Vaiserman, A.M., 2010. Hormesis, adaptive epigenetic reorganization, and implications for human health and longevity. Dose Response 8, 16–21.
Vaux, D.L., Haecker, G., Strasser, A., 1994. An evolutionary perspective on apoptosis. Cell 76, 777–779.

World Health Organization (WHO), 2001. Depleted uranium: Sources, exposure and health effects, WHO/SDE/SPE/0.1.1. Geneva, Switzerland.
Wilson, J., Young, A., Civitello, E.R., Stearns, D.M., 2014. Analysis of heat-labile sites generated by reactions of depleted uranium and ascorbate in plasmid DNA. J. Biol. Inorg. Chem. 19, 45–57.
Yasuda, T., Yoshimoto, M., Maeda, K., Matsumoto, A., Maruyama, K., Ishikawa, Y., 2008. Rapid and simple method for quantitative evaluation of neurocytotoxic effects of radiation on developing Medaka brain. J. Radiat. Res. 49, 533–540.
Yazzie, M., Gamble, S.L., Civitello, E.R., Stearns, D.M., 2003. Uranyl acetate causes DNA single strand breaks in vitro in the presence of ascorbate (vitamin C). Chem Res. Toxicol. 16, 524–530.
Zhang, W., Hoffman, B., Liebermann, D.A., 2001. Ectopic expression of MyD118/Gadd45/CR6 (Gadd45b/α/g) sensitizes neoplastic cells to genotoxic stress-induced apoptosis. Int. J. Oncol. 18, 749–757.