

# Tritiated Water Exposure in Zebrafish (Danio rerio): Effects on the Early-Life Stages

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#### 20 Abstract

21 Tritium, a radioactive isotope of hydrogen of natural and anthropogenic origin, is ubiquitously 22 present in the environment. Effluents of nuclear center of production are significant 23 anthropogenic sources. With the upcoming project of thermonuclear fusion, tritium releases in 24 the environment may increase. It is therefore important to characterize the ecological risk 25 linked to tritium. The effects of tritiated water (HTO) were therefore studied in zebrafish larvae exposed during 10 days to different dose rates:  $1.1 \times 10^2$ ,  $4.1 \times 10^2$  and  $3.8 \times 10^3$  µGy/h for 26 larvae, corresponding respectively to a water contamination of  $10^4$ ,  $10^5$  and  $10^6$  Bg/mL of 27 28 HTO. Those dose rates were higher than 10  $\mu$ Gy/h which is the threshold recommended to 29 start monitoring ecosystems where radiological contaminants are present. Mortality, embryo-30 larval development, immune-, geno and neuro-toxicity, and alterations of tissues were 31 investigated. Results showed that tritiated water exposure induced DNA damage, ROS 32 production and modulated the expression of genes involved in detoxification processes. 33 Moreover, modifications of the muscular tissues (degradation of myofibrils at 4 dpf and 34 disorganization of mitochondria at later stages) were observed. Results differed with HTO 35 dose rates and with developmental stages. These results will drive future research for the 36 development of new HTO-sensitive biomarkers and will allow us to progress in the 37 characterization of the modes of action of tritium in fish.

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#### 39 Keywords

40 Zebrafish embryo-larvae; tritiated water; biomarkers; DNA damage; ROS production.

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### 42 Highlights

- 43 This study evaluated the effects of tritiated water on zebrafish embryo-larvae
- Larvae were exposed to different dose rates:  $1.1 \times 10^2$ ,  $4.1 \times 10^2$  and  $3.8 \times 10^3 \mu \text{Gy/h}$
- 45 Several endpoints were evaluated: embryo-larval mortality and development,
   46 muscular tissue alteration, geno-, immuno-, neuro-toxicity, reactive oxygen species
   47 production, and gene expression
- The results showed that tritiated water, depending on the dose rate and the stage of
   development considered, induced molecular changes, cellular modifications, as well as
   tissue alterations
- 51

#### 52 1. Introduction

53 The main source of naturally produced tritium is the reaction between cosmic rays and 54 atmospheric gases (Boyer et al. 2009). Anthropogenic sources of tritium, including nuclear 55 reactors, add to this. The nuclear industry releases tritium mostly in the form of tritiated water 56 (HTO) (Boyer et al. 2009, CNSC 2008). The future use of nuclear fusion reactor (ITER 57 project) is expected to meaningfully increase the anthropogenic contribution and aquatic 58 ecosystems are expected to be the main receptors (ASN 2010). Aquatic systems are inhabited 59 by a large variety of species. For these reasons, it seems important to assess the ecological risk of this radionuclide, especially on aquatic ecosystems. All sources combined, worldwide, 60 the annual tritium production reaches about 200 g, corresponding to 7.2x10<sup>16</sup> Bq/year (ASN 61 62 2010).

The approaches of ecological risk assessment used for chemicals are usually also used for ionizing radiation (Garnier-Laplace et al. 2006), using the benchmark of 10  $\mu$ Gy/h for ecosystem protection (Garnier-Laplace et al. 2010). However, ecotoxicity, toxicity mechanism and sub-lethal effects of tritiated waterborne exposure has been poorly studied, particularly for aquatic vertebrates (Adam-Guillermin et al. 2012). It is therefore important to acquire a better knowledge of effects of tritium on fish.

69 Tritium toxicity to fish has been mostly reported on macroscopic endpoints for early life 70 stages (Adam-Guillermin et al. 2012, Sazykina and Kryshev 2003). DNA lesions constitute 71 one of the primary damages from which tritiated water effects can propagate from cell to 72 individual (Mathur-De Vré and Binet 1984). Tritium is known to increase micronucleus 73 frequency (Jaeschke et al. 2011). However, effects other than genotoxicity including effects 74 on hormonal levels and on anti-inflammatory mechanisms were reported (Erickson 1971, 75 Strand et al. 1982). Studies also showed that tritiated thymidine can induce neurotoxic effects 76 (Adam-Guillermin et al. 2013). Neurologic response and immune system response, through 77 the defense from oxidative stress, can therefore be altered by tritium exposure. Effects on 78 these mechanisms can have consequences on survival and/or development of organisms.

In this context, this study aims to augment our knowledge on absorbed dose rates, effects and action mechanisms of tritiated water on fish physiology in the case of chronic contaminations at low and high dose rates of tritium. Fish are known to be relevant sentinels for monitoring the effects of environmental pollution; fish have been used for studies on toxicology and environmental risk assessment for several years (van der Oost et al. 2003). Among them, zebrafish, *Danio rerio*, is commonly used as an ecotoxicological model (Hill et al. 2005). Moreover, the use of early-life stages, considered as the most sensitive part of fish life-cycle,

has been proposed as a relevant bioassay to assess toxic effects of contaminants (Oberemm
2000, OECD 2004a, b, Scholz et al. 2008).

88 In this study, we conducted experiments on zebrafish embryo-larvae in order to assess 89 tritiated water effects at dose rates close to and higher than the environmental protection criteria of 10 µGv/h (Garnier-Laplace et al. 2010). 3 hpf eggs were placed in tritiated water at 90  $10^4$ .  $10^5$  and  $10^6$  Bg/mL for 10 days. These activity concentrations corresponded to theoretical 91 dose rates of  $4x10^1$ ,  $4x10^2$  and  $4x10^3 \mu$ Gy/h (Adam-Guillermin et al. 2013), in order to assess 92 93 battery of biomarkers tritium effects Α were followed, including neurotoxicity 94 (acetylcholinesterase (AChE) activity), defence system (phenoloxidase-like (PO) activity, an 95 enzyme involved in pathogen lysis, as described before (Gagnaire et al. 2013) and reactive 96 oxygen species (ROS) production), and DNA damage (using comet assay). The expression of 97 genes related to these diverse mechanisms (immunotoxicity, neurotoxicity, genotoxicity) was 98 also quantified. These responses measured at low levels of biological organization were 99 related to endpoints measured at the individual level (mortality, hatching success of embryos 100 and developmental parameters). Histopathology of tail muscle was also studied. The total 101 dose rates were calculated for eggs and larvae by considering an external dose rate calculated 102 using HTO water concentration and an internal dose rate calculated using tritium activities 103 measured in individuals, using a protocol developed in a related study (Arcanjo et al. 2019). 104 Results were then compared to observations obtained after exposure of zebrafish larvae to 105 gamma irradiation in previous studies.

#### 108 2. Material & Methods

109 2.1. Chemicals

The chemicals used in this study were purchased from Sigma-Aldrich (St Quentin-Fallavier,
France), as mentioned before (Gagnaire et al. 2015): BSA, Bradford reagent, H<sub>2</sub>DCFDA, LDopa, DMSO, HBSS, DTNB, ATCi, trypan blue, ultrapure water sodium cacodylate,
glutaraldhehyde, osmium tetroxide, toluidine blue. PMA was purchased from Molecular
Probes (Invitrogen, Cergy-Pontoise, France).

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#### 116 2.2 Production of eggs

All experimental procedures performed in this study were previously approved by IRSN
Animal Care Committee and followed French regulations for animal experimentation
(protocols: P2015-13 and P2017-05; registration number of IRSN laboratory: A13-013-07).

120 The eggs were obtained using 20 couples of 6-months old zebrafish (GIS Amagen, Gif sur 121 Yvette, France) as described before in Gagnaire et al. (2015). Genitors were maintained in a 122 Techiplast  $\mathbb{R}$  rearing system with a photoperiod of 12h/12h and a temperature of  $28 \pm 0.5^{\circ}$ C. 123 They were fed three times per day with Tetramin ® fish food (Tetra, Melle, Germany) and 124 Artemias. Embryos were obtained by putting together 2 females and 2 males. All of the eggs 125 spawned by all the genitors were pooled; egg viability was confirmed when the blastula stage 126 was reached at 3 hpf (hours post fertilization) without visible abnormalities. The eggs were 127 considered to be of sufficient quality for the experiment when viability was at least 80% at 24 128 hpf.

129

130 2.3 Exposure of eggs to tritiated water

131 The tritium HTO source is an aqueous solution (97 % purity, 185 MBq, PerkinElmer, 132 Courtaboeuf, France). It was used after being diluted in embryo medium (Westerfield 2007) 133 to the targeted concentrations of  $10^4$ ,  $10^5$  or  $10^6$  Bq/mL.

Egg contamination was done as described before (Arcanjo et al. 2018). Two hundred 3 hpf eggs were randomly distributed in a 25 well-plate or in crystallizing dishes (5 eggs for 2 mL of embryo medium). One 25 well-plate and three crystallizing dishes were used for each dose rate and for the control group. Tritium contamination of embryo-larvae was done in glove box. Exposed eggs (as well as controls) were incubated in the dark at a temperature of  $28 \pm$ 1°C. Eggs were exposed during 10 days to  $10^4$ ,  $10^5$  or  $10^6$  Bq/mL of tritiated water with water renewal every three days.

142 2.4 Calculation of dose rate

143 In order to calculate dose rates, internalization of tritium was assessed using a protocol which 144 aimed to minimize the exchanges between organisms and ambient medium developed in a 145 related study (Arcanjo et al. 2019). Dose rates were calculated using tritium activity 146 concentrations measured in the exposure medium (external dose rate) and in egg or larvae 147 (internal dose rate) applying dose coefficients (DC) calculated with EDEN v2 software 148 (IRSN), according to the hypothesis of an homogeneous tritium distribution in tissues (Table 149 1). A ponderation coefficient of 3 was applied in order to take into account beta rays as 150 recommended in ERICA tool (assessment of impacts of radiation on non-human organisms) 151 (Brown et al. 2008).

- 152
- 153 2.6 Following of embryo-larvae development

Mortality was monitored daily. Starting at 48 hpf, when larvae started to hatch, larvae were examined each day until 10 dpf. Hatching observations were used to calculate hatching time 50% (HT<sub>50</sub>), which represents the time necessary for half of eggs to hatch. This was calculated using REGTOX ® (http://www.normalesup.org/~vindimian/fr index.html).

At 1, 3, 4, 7 and 10 dpf, pictures were acquired in order to measure total length and egg and
yolk bag diameters (Fraysse et al. 2006). Pictures were realized and analysed as described
before (Gagnaire et al. 2015).

161

**162** 2.7 DNA damage

163 The alkaline comet assay was used in order to detect DNA strand breaks (single- and double-164 strand breaks, and alkali-labile sites) as described before (Gagnaire et al. 2015) according to 165 the procedure of Devaux et al. (1998).

166

167 2.8 Measurement of biomarkers of neurotoxicity and immunotoxicity

168 AChE and PO-like activities and ROS production were measured as described before169 (Gagnaire et al. 2015).

170

171 2.9 Following of gene expression

RNA was extracted from three pools of fifteen 4, 7 and 10 dpf larvae per condition and the
methodology described in Gagnaire et al. (2015) was used to obtain the gene expression data.
Briefly, Brillant III Ultra-Fast SYBR® Green QPCR Master Mix was used in addition of
cDNA sample, primers (reverse and forward) and a referent dye on a Mx3000P real-time PCR

176 machine (Stratagene, Agilent). 40 cycles of amplification were used (30 sec at 95°C, 1 min at 177 60°C and 1 min at 72°C) followed by a final step for melting curve analyses. The relative 178 expression ratio of mRNA of every gene normalized by the reference gene was calculated 179 using the threshold cycle Ct on REST-384© version 2 software (Relative Expression 180 Software Tool, http://www.gene-quantification.de/rest-384.html) (Pfaffl et al. 2002). The 181 expression of the housekeeping gene efl (elongation factor 1) was validated between all conditions: Ct means  $\pm$  SE for controls and 4, 7 and 10 dpf larvae contaminated at  $10^4$ ,  $10^5$  or 182  $10^{6}$  Bq/mL were not different (data not shown). 183

184

185 2.10 Microscopical observations

186 Three 4, 7 and 10 dpf larvae were used for microscopical observations as described before187 (Gagnaire et al. 2015).

188

189 2.11 Statistical analyses

190 Results were expressed as means  $\pm$  standard error (se). Normality assumption was verified 191 using Shapiro–Wilk tests. When date were not normal, they were transformed using Boxcox 192 formula. Differences between controls and contaminated larvae were tested with t-tests or 193 ANOVAs followed by an *a posteriori* least significant difference (LSD) post-hoc test in the 194 case of rejection of H<sub>0</sub> when data were normal. Kruskal-Wallis or Mann-Whitney U tests 195 were used when data were not normal. Analyses were performed on STATISTICA Software 196 v12 (StatSoft, Inc., Tulsa, OK, USA). Significance was set at  $p \le 0.05$ .

197 For comet assay statistical analysis, raw data of the individual cell tail moment were used. 198 The analyses were performed using R software (R Development Core Team 2004). Tail 199 moments equal to zero were considered as artefact of the image analysis. However, they 200 represent "true" zeros, i.e. cells with no or few DNA damage, so, if any, they were replaced 201 by the smallest value of the slide considered. To deal with the nested design of the comet 202 model was assay, а linear mixed-effects constructed using the *nlme* package 203 (https://CRAN.R-project.org/package=nlme) with treatment (control or HTO) as fixed factor 204 and replicates (slides) as random factor. Then random permutation tests were applied using 205 the *pgirmess* packages (https://CRAN.R-project.org/package=pgirmess) and the number of 206 permutations was fixed to 2000.

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# 209 **3. Results**

- 210 3.1 Tritium dose rate in eggs and larvae
- After exposure to  $10^4$  Bq/mL of HTO, tritium dose rate was lower in 7 and 10 dpf larvae when compared to 4 dpf larvae. After exposure to  $10^5$  Bq/mL, tritium dose rate was lower in 7 and 10 dpf larvae when compared to 24 hpf eggs. After exposure to  $10^6$  Bq/mL, tritium dose rate was lower in 4 and 10 dpf larvae compared to 7 dpf larvae. For the three concentrations, the mean tritium activity was significantly higher in eggs compared to larvae (1.12 and 1.15 times higher for  $10^5$  and  $10^6$  Bq/mL, respectively) (Table 2).
- 217 Despite these slight differences, the mean pondered dose rate of exposure all stages included 218 was calculated as  $1.1 \times 10^2$ ,  $4.1 \times 10^2$  and  $3.8 \times 10^3 \mu \text{Gy/h}$  for nominal exposure HTO 219 concentration of  $10^4$ ,  $10^5$  and  $10^6$  Bq/mL, respectively (Table 2).
- 220
- 221 3.2 Effects on mortality, hatching and embryo-larvae development
- No significant differences in mortality were observed between control and exposed groups for
  all HTO concentrations on all experiments (data not shown; mean cumulated mortality for all
  experiments at 10 dpf of 14.7% for controls and 16.4% for HTO).
- 225 No differences in  $HT_{50}$  between controls and contaminated groups were shown for all of the 226 HTO concentrations tested (Figure 1).
- 227 Morphological analyses performed on eggs and larvae showed differences in several stages depending on the tritium level. At 10<sup>4</sup> Bq/mL, 24 hpf egg diameter and 3 and 7 dpf yolk bag 228 diameter were significantly higher and 4 dpf larvae length was significantly lower in exposed 229 animals when compared to controls. At  $10^5$  Bg/mL, 3 and 7 dpf yolk bag diameter and 3 dpf 230 231 larvae length were significantly lower and 10 dpf yolk bag diameter was significantly higher 232 in exposed animals compared to controls. At  $10^6$  Bg/mL, 24 and 48 hpf egg diameters and 48 233 hpf and 7 dpf volk bag diameters were significantly higher in exposed animals when 234 compared to controls (Table 3).
- 235
- 236 3.3 Effects on DNA damage measured by the comet assay
- After an exposure to  $10^4$  Bq/mL, only 4 dpf exposed larvae presented significantly less DNA damage than controls. After an exposure to  $10^5$  or  $10^6$  Bq/mL, only 4 dpf exposed larvae presented significantly higher DNA damage than controls (Table 4).
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243 3.4 Effects on biomarkers of immunotoxicity and neurotoxicity

244 No effect of HTO was observed on PO-like and AChE activities after 4 days of exposure,245 whatever the dose rate used (Table 5).

246 At 10<sup>4</sup> Bq/mL of HTO, ROS stimulation index showed no significant differences whatever 247 the stage studied (Table 6). ROS basal and stimulated levels were higher in exposed animals 248 compared to controls only for 7 dpf larvae (data not shown). At 10<sup>5</sup> Bg/mL of HTO, ROS stimulation index was higher in exposed 4 dpf larvae compared to controls. ROS stimulated 249 250 levels were higher in contaminated animals compared to controls for 4, 7 and 10 dpf larvae, 251 while ROS basal level was higher in contaminated animals compared to controls only for 7 dpf larvae (data not shown). At 10<sup>6</sup> Bq/mL of HTO, ROS stimulation index was higher in 252 exposed 4 dpf larvae compared to controls. ROS basal level was lower in 7 dpf contaminated 253 254 larvae compared to controls (data not shown).

255

**256** 3.5 Effects on gene expression

- 257 The genes for which the expression was modified in presence of HTO depended on the 258 concentration studied.
- At  $10^4$  Bq/mL of HTO, *mt2* (metallothionin) was 2.72 times more expressed in 4 dpf 259 contaminated larvae compared to controls. At 10<sup>5</sup> Bq/mL, only mpx (myeloid specific 260 261 peroxidase) was 2.7 times less expressed in 4 dpf contaminated larvae compared to controls. 262 After exposure at 10<sup>6</sup> Bg/mL of HTO, *cvp1a* (cvtochrome P450 CYP1A) was 42.74 times 263 more expressed and gstpl (glutathione-S-transferase) was 50 times less expressed in 4 dpf 264 contaminated animals compared to controls; at the same activity concentration, lyz (lysozyme 265 C), mpx and mt2 were 1.45, 2.45 and 1.57 times more expressed in 7 dpf contaminated 266 animals compared to controls, respectively, and *ache* (acetylcholinesterase) was 1.63 times 267 more expressed in 10 dpf contaminated larvae compared to controls (Table 7).
- 268

**269** 3.6 Histological observations

TEM observations on the tail muscular part of 4 dpf larvae showed a similar pattern of responses for all concentration studied. A global degradation of myofibrils with an alteration of actin and myosin filaments on A and I disks was observed (Figure 2). Z stria was still observable, but the connections seemed to be broken (Figure 2). These alterations were rarely observed at 10<sup>4</sup> (Figure 2B) and 10<sup>5</sup> Bq/mL of HTO (Figure 2C) compared to controls (Figure 2A). However, at 10<sup>6</sup> Bq/mL of HTO, the alterations were observed with a higher frequency compared to lower concentrations (Figure 2D). 277 For 4 and 7 dpf larvae exposed to all dose rates, mitochondria seemed to be more numerous 278 compared to controls, indicating a high energetic activity in cells. However, no structural 279 differences appeared in mitochondria between contaminated 4 dpf larvae and controls (data 280 not shown). On the contrary, for 7 dpf larvae, important alterations were observed on muscle 281 mitochondria (Figure 3). For the two highest dose rates, mitochondrium cristae were 282 disorganized or missing; only external membranes of mitochondria were present. Some 283 mitochondria seemed to have disappeared from endomysium, leaving numerous free spaces in 284 muscular tissue (Figure 3C, D). For the lowest dose rate, mitochondria seemed normal but 285 numerous free spaces in the muscular tissue were present compared to controls (Figure 3B).

286 Only 10 dpf larvae exposed to  $10^4$  Bq/mL of HTO showed the same alterations in a more 287 important way compared to 7 dpf larvae, while for 10 dpf larvae exposed to  $10^5$  Bq/mL of 288 HTO, the degradations seemed less present compared to 7 dpf larvae (data not shown). Not 289 enough 10 dpf larvae were available at  $10^6$  Bq/mL of HTO to perform TEM analyses.

290

All of the results were summed up in Table 8.

292

### 294 4. Discussion

**295** 4.1 Dose rates calculated for tritiated water

Our results showed that tritium accumulates in eggs and larvae, as it was already shown for tritiated thymidine (Adam-Guillermin et al. 2013). Our results also showed differences in dose rates calculated in all stages, values being globally higher in eggs or young larvae (4 dpf). These differences could indicate that the HTO transfer behavior mechanisms can vary depending on the life stage considered and the activity concentration of tritium in the medium.

302 Exchanges of HTO between water and organisms are rapid, as maximal dose rates were 303 obtained for 24h eggs. Several studies showed that tritium accumulated in adult organisms 304 equilibrates with HTO concentration of the water and that OBT fraction (tritium bounded to 305 organic matter) equilibrates with the concentration of HTO internalized (Gagnaire et al. 2017, 306 Gagnaire et al. in press, Galeriu and Melintescu 2011, Kim et al. 2019, Kim and Korolevych 307 2013, Kim et al. 2015, Melintescu et al. 2015). A companion paper study presented in details 308 the discussion about internalization results obtained after tritium contamination for two stages (24h eggs and 4 dpf larvae) and two HTO concentrations ( $10^5$  and  $10^6$  Bq/mL) (Arcanjo et al. 309 310 2019).

311

312 4.2 Macroscopic effects of tritiated water (hatching, morphological measurements)

313 No differences of mortality and hatching were observed between conditions. In zebrafish, tritiated thymidine induced a delay in hatching at  $1.91 \times 10^3$  and  $1.05 \times 10^4$  µGy/h (Adam-314 Guillermin et al. 2013). Moreover, we showed no abnormalities in larvae exposed to HTO at 315 316 the same dose rates, while abnormalities were observed in zebrafish larvae after exposure to tritiated thymidine (Adam-Guillermin et al. 2013). Exposure of medaka embryos for 30 days 317 to  $1.2 \times 10^3$  µGy/h of HTO led to vertebral malformations and effects on fecundity of adults 318 319 (Hyodo-Taguchi and Etoh 1993). Moreover, the hatchability of common flounder, *Paralichtys olivaceus*, was modified after 4 days of exposure to  $1.21 \times 10^5 \mu \text{Gy/h}$  of tritium 320 321 (Ichikawa and Suyama 1974). Thus, tritium effects on hatching process seem to be related to 322 the form used, the total dose received and the biological stage.

Tritiated water induced some effects on macroscopical endpoints depending on the dose rate studied. At  $10^4$  Bq/mL (i.e.  $1.1x10^2 \mu$ Gy/h), exposed animals presented differences with the controls for early stages (<4 dpf). At  $10^5$  Bq/mL (i.e.  $4.1x10^2 \mu$ Gy/h), differences between controls and exposed animals were seen only at older stages (3-10 dpf). At  $10^6$  Bq/mL (i.e.  $3.8x10^3 \mu$ Gy/h), exposed animals presented lower egg and/or yolk-bag diameters at early and 328 old stages. Effects on developmental parameters including egg diameter has also been shown 329 on three-spined stickleback, *Gasterosteus aculatus* (Walden 1971), and in puffer, *Fugu* 330 *niphobles* (Ichikawa and Suyama 1974), eggs exposed to  $1.21 \times 10^5$  and  $1.21 \times 10^6 \mu$ Gy/h of 331 HTO, respectively. Indeed, HTO induced some effects on zebrafish larvae development, 332 indicating an effect on metabolism. It would be interesting to study the responses of older 333 larvae to HTO in order to see if the effects persist after 10 dpf.

334

335 4.3 Sub-individual responses to HTO

336 Tritiated water induced effects on DNA damage depending on the studied dose rate. At  $10^4$ 337 Bq/mL (i.e.  $1.1 \times 10^2 \mu$ Gy/h), a decrease of DNA damage was observed in 4 dpf contaminated larvae. An explanation of this result at the low dose rate could be an hormesis effect of 338 339 ionizing radiation, as it was already shown in fish (Mothersill and Seymour 2009). No effect 340 was shown for all dose rates in 7 and 10 dpf larvae. This result could indicate that repair 341 mechanisms are induced after 4 dpf in response to HTO. Other studies have shown that 342 tritium can induce genotoxicity, with an increase of chromosome aberrations in medaka eggs exposed to  $2.08 \times 10^1 \,\mu$ Gy/h (Suyama et al. 1981) and of DNA damage and MN frequency at 343 1.25x10<sup>1</sup> µGy/h in mussels (Jha et al. 2005). Tritiated thymidine also induced an increase of 344 DNA damage in 4 dpf larvae exposed at  $1.05 \times 10^4 \mu Gy/h$  (Adam-Guillermin et al. 2012). 345 DNA damage were also induced in vitro in fish lymphocytes exposed to  $10^{-1}$ - $10^2$  Bg/mL of 346 347 HTO (Stuart et al. 2016) and in fathead minnow, Pimephales promelas, after and in vivo and in situ exposure to tritium (Gagnaire et al. 2017, Gagnaire et al. in press). However, no DNA 348 damage was induced in rainbow trout, Onchorvnchus mvkiss, after exposure to  $7.10^3$  Bg/L 349 (Festarini et al. 2019). These results make us hypothesize that zebrafish could be more 350 351 resistant to HTO effects on DNA damage compared to other species.

These results corroborate those described by other authors, showing a non-linear genotoxic response to HTO (Bannister et al. 2016, Stuart et al. 2016). This kind of responses could be due to reparation and/or decrease of damages due to efficient cell elimination by apoptosis. This hypothesis is realistic as a transcriptomic analysis performed in a companion study of 24 hpf and 4 dpf larvae exposed to HTO showed that some genes involved in DNA damage repair and anti-apoptotic response (e.g. *bcl2l1*, *xpc*, *gadd45bb* et *xrcc1*) were over-expressed in exposed animals (Arcanjo et al. 2018).

As a whole, these results indicated an increase of DNA damage in presence of tritium, which is coherent with the known mechanisms of action of ionizing radiations (Adam-Guillermin et al. 2012).

363 In our study, ROS production was modified by HTO with again a response depending on the dose rate tested. Four dpf larvae exposed to 10<sup>5</sup> Bq/mL (i.e. 4.1x10<sup>2</sup> µGy/h) presented an 364 increase in ROS stimulation index as a result of an increase of both basal and stimulated ROS 365 366 levels. At this dose rate, the expression of mpx (myeloid specific peroxidase) gene, which is 367 involved in reduction of H<sub>2</sub>O<sub>2</sub> during the oxidative burst, was lower in contaminated larvae, indicating that HTO induced effects on elements playing a role in oxidative stress. Indirect 368 369 effects of HTO, by a modulation of ROS levels, could therefore be responsible of the DNA 370 damage observed. Moreover, ROS stimulated levels were decreased in 7 dpf animals exposed 371 to  $10^6$  Bg/mL (i.e.  $3.8 \times 10^3$  µGy/h). The decrease of ROS PMA-stimulated levels by HTO 372 could indicate that fish may not be able to stimulate defence capacities to an infectious 373 disease.

- For other HTO dose rates tested, mpx expression was increased only in 7 dpf larvae exposed to  $10^6$  Bq/mL (i.e.  $4.8 \times 10^3$  µGy/h). No effect was shown on ROS stimulation index at  $10^4$ Bq/mL (i.e.  $1.1 \times 10^2$  µGy/h). Even though there is no direct link between ROS production levels and DNA damage, our results showed that the study of ROS production allows to better understand the mechanisms deployed in response to HTO exposure in zebrafish larvae.
- 379 Interestingly, the expression of several genes related to detoxication process (*mt2*, *cyp1a* and 380 *gstp1*) were modified after 4 and 7 days of exposure to  $10^4$  Bq/mL (i.e.  $1.1x10^2 \mu$ Gy/h) and 381  $10^6$  Bq/mL (i.e.  $4.8x10^3 \mu$ Gy/h). MT are known to play a role in cell protection towards 382 oxidative stress (Sato and Kondoh 2002). Detoxication could therefore be a mechanism 383 relevant in the study of effects of HTO in aquatic organisms.
- The expression of *lyz* gene was slightly increased only in 7 dpf larvae contaminated to  $10^5$ Bq/mL (i.e.  $4.1x10^2 \mu$ Gy/h), possibly indicating a stimulation of the innate immune system. However, this response was transient as it was observed neither in older larvae nor at other dose rates.
- 388
- 389 No effect of HTO was observed on PO-like activity after 4 days of exposure for all dose rates.390 This biomarker does not seem relevant for the study of HTO effects on fish.
- 391 No effect was observed on AChE activity after 4 days of exposure to all dose rates of HTO. A
- 392 slight increase in *ache* expression was shown only in 10 dpf larvae exposed to  $10^6$  Bq/mL (i.e.
- **393**  $4.8 \times 10^3 \,\mu$ Gy/h). Therefore, the nervous system does not seem to be affected by tritium.
- 394

395 Histological observations of 4 dpf larvae showed global degradation of myofibrils of tail 396 muscle for all dose rates, with an increase of observations of these alterations with the dose 397 rate. The affected part being the contractile part of the muscle, an increase in alterations could 398 eventually have consequences on larvae motility. In 7 and 10 dpf larvae, the alterations to 399 myofibrils observed at 4 dpf seemed to be repaired, probably due to the important cell 400 replacement in this organism in embryo-larval development. However, the intensity of the 401 alterations observed on mitochondria for 7 and 10 dpf larvae suggests an important functional 402 alteration or accelerated aging, possibly leading to the end of the energetic process of 403 muscular tissue and alteration in cell respiration (Delbart, 2000). This phenomenon could be 404 due to a progressive muscular dystrophia. The increase of mitochondria alterations could also 405 be linked to the increase of ROS basal level in exposed larvae, which could be toxic for the 406 organelle when excessively present. However, for 10<sup>5</sup> Bg/mL of HTO, the alterations seemed to be less important on 10 dpf larvae compared to 7 dpf larvae, while for 10<sup>4</sup> Bq/mL of HTO 407 408 alterations seemed more important at 10 dpf compared to 7 dpf. As for DNA damage, we can 409 hypothetize that this result could be due to an increase of mitochondria destruction by cell 410 autophagy and apoptosis in 10 dpf larvae as a compensatory response to HTO induced-stress. 411 It would be interesting to study older larvae to demonstrate if the alterations are still present 412 or repaired by the organism.

413

## 414 4.4 Comparison to gamma irradiation

415 Results of previous studies on zebrafish larvae exposed to gamma irradiation showed that 416 gamma irradiation induced either a delay in hatching after acute exposure (0.3-2 Gy during 1 417 min) (Pereira et al. 2011), or an acceleration of hatching and abnormalities after 96h exposure at  $3.33 \times 10^1$  and  $2.37 \times 10^4$  µGy/h (Gagnaire et al. 2015, Simon et al. 2011). Gamma irradiation 418 419 at  $2.37 \times 10^4$  µGy/h also induced effects on 4 dpf zebrafish larvae, with a decrease of volk bag 420 diameter in exposed animals, indicating an increase in energetic reserve consumption 421 (Gagnaire et al. 2015). We saw here that HTO induced no effects on hatching and slight 422 effects on developmental parameters (increase of yolk-bag diameter, decrease of length) 423 rather indicating a disruption of energy consummation in these larvae. On the contrary, 424 tritiated thymidine induced drastic effects (malformations, hatching delay) at dose rates to 425 DNA of 1-7 mGy/h, indicating an important toxicity of this form for these high dose rates 426 (Adam-Guillermin et al. 2013). Therefore, different types of exposure to radionuclides have 427 different consequences on hatching process and development.

Gamma irradiation at 3.33x10<sup>1</sup> µGy/h decreased ROS stimulation index due to an increase of 429 430 ROS basal levels in irradiated larvae (Gagnaire et al. 2015). It should be noted that this dose rate is close to the benchmark value of  $10^1 \mu Gy/h$  recommended for ecosystem protection 431 432 towards ionizing radiations (Garnier-Laplace et al. 2010). Moreover, 24 hpf eggs exposed to 2.37x10<sup>4</sup> µGy/h showed an increase in DNA damage (Gagnaire et al. 2015). Taken together, 433 434 the present results confirmed the existence of an oxidative stress conducting to DNA damage 435 after fish exposure to radionuclides like tritium. The induction of an oxidative stress can 436 therefore be considered as a common mode of action to both kind of exposure: external 437 irradiation and HTO contamination.

438

439 Neither HTO nor gamma irradiation induced any effect on phenoloxidase activity. HTO 440 modulated the expression of genes involved in detoxication, while gamma irradiation at 441  $3.33 \times 10^1 \mu$ Gy/h decreased EROD activity in 4 dpf larvae (Gagnaire et al. 2015). Detoxication 442 could therefore be a mechanism common to several types of radionuclides and relevant in the 443 study of effects of such contaminants in aquatic organisms.

444

445 At the tissular level, HTO induced alterations in myofibrils after 4 days of exposure. It is 446 interesting to point out that the same alteration was already observed in a more pronounced 447 way with gamma irradiation, conducting to a dilatation of endomysium (Gagnaire et al. 2015), 448 not observed with HTO. The alterations to muscle seem to be a common mode of action of 449 HTO and gamma irradiation. However, HTO induced no effect on neurotransmission. On the contrary, gamma irradiation induced disruptions of this mechanism at the biochemical and 450 451 molecular levels (Gagnaire et al. 2015). Moreover, HTO modulated the expression of lvz 452 gene, while gamma irradiation did not (Gagnaire et al. 2015). These results showed again that 453 tritium and gamma irradiation have different mechanisms of action in zebrafish.

454

455 It is important to consider that energy deposition following an exposure to tritiated thymidine 456 directly impacts the DNA, contrary to HTO and gamma irradiation for whom the dose is 457 distributed in a more homogenous way in the cell, therefore more diluted (Adam-Guillermin 458 et al. 2012). The doses calculated at the scale of an egg or a larvae for HTO, tritiated 459 thymid in and gamma irradiation are therefore not directly comparable.

460

461 5. Conclusion

462 The present study investigated the effects of a contamination to HTO at different dose rates 463 on biomarkers and macroscopical parameters of zebrafish larvae. The results showed that 464 tritium was internalised in eggs and in larvae at all dose rates tested. They also showed that 465 differences in the sensitivity of biomarkers measured can exist. Some effects were observed 466 on the parameters of development. We showed that HTO could modify zebrafish parameters 467 including ROS production and induced muscle alterations and DNA damage. These parameters could therefore be relevant parameters for assessing tritium-related stress. 468 469 However, the responses to HTO were not linear with the increasing dose rate, probably due to 470 apoptosis. On the contrary, PO-like and AChE activities did not seem relevant to study of 471 tritium effects in larvae. However, effects differed considering the dose rate of HTO used.

472 HTO effects on zebrafish larvae were observed at several levels at dose rates ranging from  $1.1 \times 10^2$  to  $3.8 \times 10^3$  µGy/h, which is in the range or even higher than the derived consideration 473 reference levels (DCRLs) for fish of 4.1x101-4.1x102 µGy/h (ICRP 2008). These DCRLs 474 475 correspond to a "band of dose rate within which there is likely to be some chance of 476 deleterious effects of ionizing radiation occurring to individuals" (ICRP 2008). Our results 477 seem to validate the accuracy of this DCRL. However, the dose rates we tested were greatly 478 higher than the benchmark value of 10 µGy/h for the protection of all ecosystems towards 479 ionizing radiations (Garnier-Laplace et al. 2010). As at the lowest dose rate of HTO tested, we 480 saw few effects, hence our results do not seem to question the relevance of this protection 481 threshold.

When comparing results of HTO to gamma irradiation, some mechanisms seem common for both types of exposure (muscle alterations, DNA damage, oxidative stress), but gamma irradiation seem more deleterious to zebrafish larvae at lower or higher dose rates than the ones tested on HTO on the present study. Some more experiments are planned to acquire the whole results presented here on similar dose rate of irradiation gamma and of tritiated thymidine in order to refine a value for the Relative Biological Effectivness (RBE) of tritium (Adam-Guillermin et al. 2012).

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491 Figure captions

492

**493** Figure 1: Hatching Time (HT<sub>50</sub>) in control and contaminated zebrafish larvae for  $10^4$  (A),  $10^5$ **494** (B) and  $10^6$  (C) Bq/mL of HTO. Error bars correspond to 95% confidence intervals of the **495** HT<sub>50</sub> values.

- 496
- **497** Figure 2: muscular tissue of 4 dpf zebrafish larvae observed by TEM in control (A) and 498 contaminated at  $10^4$  (B),  $10^5$  (C) and  $10^6$  (D) Bq/mL of HTO. A: A disk, E: endomysium, I: I 499 disk, M: M stria, My: myofibrils, S: sarcomere, Z: Z stria, Mi: mitochondria.
- 500

501 Figure 3: muscular tissue of 4 dpf zebrafish larvae observed by TEM in control (A) and 502 contaminated at  $10^4$  (B),  $10^5$  (C) and  $10^6$  (D) Bq/mL of HTO. My: myofibrils, S: sarcomere, 503 Z: Z stria, Mi: mitochondria.

504

Table 1: external and internal dose coefficients (DC) calculated with EDEN v2 software (IRSN) for zebrafish egg and larvae.

	DC (µGy/h per Bq/g)	24 hpf	4 dpf	7 dpf	10 dpf
	Internal DC	6.71x10 <sup>-3</sup>	6.71x10 <sup>-3</sup>	6.71x10 <sup>-3</sup>	6.71x10 <sup>-3</sup>
_	External DC	3.77x10 <sup>-5</sup>	5.21x10 <sup>-5</sup>	$1.03 \times 10^{-4}$	1.15x10 <sup>-4</sup>

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**524 Table 2**: tritium dose rates ( $\mu$ Gy/h) based on internal concentrations in 24 hpf eggs and 4, 7 and 10 dpf larvae exposed to HTO. Values are expressed **525** as means  $\pm$  se, n=7-15 per condition. Bold values indicate a significant difference between conditions. Letters indicate significant difference between **526** stages for a given HTO water concentration (Kruskal-Wallis test, p≤0.05): a<b<c.

527

Nominal HTO concentration (Bq/mL)	24 hpf	24 hpf 4 dpf		10 dpf	Mean
<b>10<sup>4</sup></b>	$9.1 x 10^1 \pm 1.2 x 10^1$ ab	$1.7 \text{x} 10^2 \pm 1.3 \text{x} 10^1 \text{ b}$	$9.5 \times 10^1 \pm 1.3 \times 10^1$ a	$8.5 \times 10^1 \pm 1.1 \times 10^1$ a	$1.1 \times 10^2 \pm 5.2 \times 10^1$
10 <sup>5</sup>	$5.2x10^2 \pm 1.3x10^1 c$	$4.6x10^2 \pm 1.3x10^1$ bc	$2.8 \times 10^2 \pm 9.8$ a	$3.7 x 10^2 \pm 1.6 x 10^1$ ab	$4.1x10^2 \pm 1.1x10^1$
10 <sup>6</sup>	$3.8 \times 10^3 \pm 1.5 \times 10^2$ ab	$3.3x10^3 \pm 1.5x10^2$ a	$4.7x10^3 \pm 2.4x10^2 \text{ b}$	$3.4 \times 10^3 \pm 1.5 \times 10^2$ a	$3.8 \times 10^3 \pm 8.5 \times 10^2$

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**Table 3**: measurements in mm of egg diameter, total length and yolk bag (YB) diameter in zebrafish egg and larvae control and exposed (at  $10^4$ ,  $10^5$ and  $10^6$  Bq/mL of HTO) groups. Values are expressed as means of at least 10 replicates. Bold values indicate a significant difference between conditions. T test, p $\leq 0.05$ ; a, b: significantly different, a $\leq b$  n.d.: not determined.

533

	24 1	npf	48	hpf	3 dj	of 534	
	Egg diameter (µm)	YB diameter (µm)	Egg diameter (µm)	YB diameter (µm)	Length (µm)	YB diameter (µm)	
Control	1177.5 ± 11.4 a	489.6 ± 12.4	465.8 ± 6.2	$1173.0 \pm 11.0$	$2943.5 \pm 126.7$	$347.6 \pm 21.1$ a	
HTO 10 <sup>4</sup> Bq/mL	$1213.4 \pm 10.5 $ b	$478.3 \pm 5.3$	$474.2 \pm 6.0$	$1201.8 \pm 13.5$	$3127.5 \pm 46.0$	$399.1 \pm 12.5 \text{ b}$	
Control	$1175.3 \pm 12.4$	$502.8 \pm 5.8$	1164.1 ± 8.9	494.0 ± 8.7	$3208.9 \pm 39.3$ b	$410.7 \pm 9.9$ b	
HTO 10 <sup>5</sup> Bq/mL	$1127.5 \pm 21.2$	$508.6 \pm 12.3$	$1152.3 \pm 21.2$	498.1 ± 13.6	$3017.5 \pm 57.1$ a	358.7 ± 10.9 a	
Control	1156.4 ± 11.3 a	509.4 ± 5.2	1155.1 ± 16.1 a	456.7 ± 6.6 a	n.d.	n.d.	
HTO 10 <sup>6</sup> Bq/mL	$1226.1 \pm 10.3$ b	$506.0 \pm 8.2$	$1213.2 \pm 14.9 $ b	$503.9 \pm 11.2$ b	n.d.	n.d.	

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	4 d	pf	7 d	pf	10 dpf				
	Length (µm)	YB diameter (µm)	Length (µm)	YB diameter (µm)	Length (µm)	YB diameter (µm)			
Control	$3480.0 \pm 30.8 b$	351.1 ± 8.8	$3950.5 \pm 52.7$	$325.2 \pm 6.4 a$	$3688.6 \pm 78.9$	$312.5 \pm 11.6$			
HTO 10 <sup>4</sup> Bq/mL	$3335.1 \pm 63.4 a$	$329.5 \pm 15.0$	$4027.5 \pm 35.8$	$361.9 \pm 12.5 \text{ b}$	$3585.2 \pm 112.2$	$294.7 \pm 11.7$			
Control	3406.6 ± 35.1	325.6 ± 10.2	3995.6 ± 37.1	$387.2 \pm 9.7$ b	4029.2 ± 70.4	$291.6 \pm 7.8$ a			
HTO 10 <sup>5</sup> Bq/mL	3351.9 ± 39.2	339.1 ± 8.5	3915.2 ± 57.7	$322.0 \pm 8.3$ a	3876.6 ± 76.3	317.1 ± 8.5 b			
Control	$3300.8 \pm 47.4$	$325.7 \pm 10.4$	3779.4 ± 63.9	282.4 ± 8.8 a	n.d.	n.d.			
HTO 10 <sup>6</sup> Bq/mL	3379.1 ± 57.6	$322.1 \pm 6.3$	$3625.1 \pm 60.6$	$339.6 \pm 8.5$ b	n.d.	n.d.			

**Table 4:** mean tail moments (comet assay) obtained for zebrafish 24 hpf embryos (n=3 pools of 10) and 4, 7 and 10 dpf larvae (n=10) control and exposed (at  $10^4$ ,  $10^5$  and  $10^6$  Bq/mL of HTO) groups. Mix model (R), p≤0.001; Bold values indicate a significant difference between conditions. a, b: significantly different, a<b. n.d.: not determined.

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	24 hpf	4 dpf	7 dpf	10 dpf
Control	$4.80 \pm 0.31$	$3.68 \pm 0.14$ b	$0.87 \pm 0.06$	$0.42 \pm 0.03$
HTO 10 <sup>4</sup> Bq/mL	$4.93 \hspace{0.1 in} \pm \hspace{0.1 in} 0.21$	$1.39 \pm 0.06$ a	$1.47 \pm 0.10$	$0.39 \pm 0.03$
Control	$1.82 \pm 0.09$	$0.84 \pm 0.04$ a	$2.20 \pm 0.11$	n.d.
HTO 10 <sup>5</sup> Bq/mL	$1.87 \pm 0.09$	$1.30 \pm 0.07$ b	$2.47 \pm 0.13$	n.d.
Control	$1.46 \pm 0.06$	$0.86 \pm 0.04$ a	$1.15 \pm 0.08$	$0.78 \pm 0.06$
HTO 10 <sup>6</sup> Bq/mL	$1.78 \pm 0.13$	$2.09 \pm 0.13$ b	$1.47 \pm 0.09$	$0.83 \pm 0.07$

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**Table 5**: PO-like (U/larva) and AChE activities (nmol ATCi/min/mg proteins) in zebrafish larvae contaminated during 4 days at  $10^4$ ,  $10^5$  and  $10^6$  Bq/mL of HTO. Values are means of 10 replicates for each condition; standard error is presented. T-test or U Mann Witney test, p<0.05.

	PO-like	activity	AChE activity				
	(U/la	rva)	(nmol / pi	mg/mg s)			
Control	1.11 ±	0.09	167.9	±	21.3		
HTO 10 <sup>4</sup> Bq/mL	1.03 ±	0.07	195.7	±	38.0		
Control	1.29 ±	0.12	68.1	±	10.8		
HTO 10 <sup>5</sup> Bq/mL	1.08 ±	0.04	63.9	±	10.4		
Control	1.63 ±	0.35	173.7	±	16.0		
HTO 10 <sup>6</sup> Bq/mL	1.94 ±	0.28	146.7	±	10.4		

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**Table 6**: ROS stimulation index in *D. rerio* larvae contaminated during 10 days at  $10^4$ ,  $10^5$ and  $10^6$  Bq/mL of HTO. Values are expressed as means of 20 replicates (10 for stimulated levels and 10 for basal levels); standard error is presented. T test, p0.05; Bold values indicate a significant difference between conditions. a, b: significantly different, a<br/>b.

559

ROS stimulation index		4	dpf			7 dj	of	10 dpf			
Control	1.17	±	0.06		1.18	±	0.03	1.22	±	0.02	
HTO 10 <sup>4</sup> Bq/mL	1.24	±	0.10		1.20	±	0.03	1.22	±	0.03	
Control	1.08	±	0.08	a	1.21	±	0.06	1.15	±	0.07	
HTO 10 <sup>5</sup> Bq/mL	1.22	±	0.02	b	1.28	±	0.05	1.19	±	0.09	
Control	1.14	±	0.10	a	1.26	±	0.17	0.87	±	0.08	
HTO 10 <sup>6</sup> Bq/mL	1.58	±	0.12	b	1.12	±	0.16	0.78	±	0.09	
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03											
24											
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**Table 7**: relative expression of genes (normalized to reference gene *ef1*) compared to control in larvae contaminated during 10 days at  $10^4$ ,  $10^5$  and  $10^6$  Bq/mL of HTO (n=9). Analysis with REST-384©, p≤0.05; bold values indicate a significant difference compared to control.

		4 dpf			7 dpf		10 dpf			
	10 <sup>4</sup> Bq/mL	10 <sup>5</sup> Bq/mL	10 <sup>6</sup> Bq/mL	10 <sup>4</sup> Bq/mL	10 <sup>5</sup> Bq/mL	10 <sup>6</sup> Bq/mL	10 <sup>4</sup> Bq/mL	10 <sup>5</sup> Bq/mL	10 <sup>6</sup> Bq/mL	
ache	1.38	1.74	0.99	1.39	1.19	1.57	1.28	1.31	1.63	
bax	1.61	1.28	0.74	1.29	0.77	1.27	1.12	0.75	1.48	
chat	1.29	1.40	0.79	-1.40	1.29	1.29	2.13	1.07	1.43	
cypla	1.63	1.55	42.74	1.52	0.83	0.86	-1.17	0.78	1.30	
gstp l	0.93	1.34	0.02	1.36	1.27	0.95	1.02	0.74	0.69	
lyz	1.13	0.76	1.34	1.79	0.84	1.45	-1.76	0.92	1.29	
mpx	1.20	0.37	1.02	1.34	0.59	2.45	1.50	0.83	1.52	
mt2	2.72	0.98	0.87	1.04	1.39	1.57	1.50	1.37	1.48	

		10*	' Bq/mL			10	° Bq/mL		10° Bq/mL			
Stage	24 hpt	4 dpt	/ dpt	10 dpt	24 hpt	4 dpt	/ dpt	10 dpt	24 hpt	4 dpt	7 dpt	10 dpt
Dose rate (µGy/h)	9.1x10'	1.7x10 <sup>2</sup>	9.5x10'	8.5x10'	5.2x10 <sup>2</sup>	4.6x10 <sup>2</sup>	2.8x10 <sup>2</sup>	3.7x10 <sup>2</sup>	3.8x10 <sup>3</sup>	3.3x10 <sup>3</sup>	4.7x10°	3.4x10 <sup>3</sup>
Total dose (µGy)	2.2x10 <sup>3</sup>	1.6x10*	1.6x10*	2.0x10*	1.2x10*	4.5x10*	4.7x10*	8.8x10*	9.1x10*	3.2x10°	7.8x10°	8.2x10 <sup>-</sup>
Develop- ment	Egg diam > C	Length < C	RV diam > C	Ø	Ø	Ø	RV diam < C	RV diam > C	Egg diam > C	Ø	RV diam > C	ND
DNA damage	Ø	< C	Ø	Ø	Ø	> C	Ø	ND	Ø	>T	Ø	Ø
Gene expression	-	Mt2 > C	Ø	Ø	-	Mpx < C	Ø	Ø	-	Cyp1a>T GSTp1 <t< td=""><td>Mpx &gt; C Lyz &gt; C MT2 &gt; C</td><td>Ache &gt; C</td></t<>	Mpx > C Lyz > C MT2 > C	Ache > C
ROS production	-	Ø	Basal and stimulated activities > C	Ø	-	Stimulated activity > C Index > C	Stimulated and basal activities > C	Stimulated activity > C	-	Index > C	Stimulated activity < C	Ø
Histology of muscle	-	Rare alterations of myofibrils	Mitochondria alterations	Mitochondria alterations	-	Rare alterations of myofibrils	Mitochondria alterations	Mitochondria alterations	-	Alterations of myofibrils	Mitochondria alterations	ND

Table 8: synthesis of results obtained for exposition of zebrafish to three dose rates of HTO. C: control; Ø: no significant effect; -: not tested;
ND: not determined. Diam: Diameter.

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