

2023-06-10

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<https://pearl.plymouth.ac.uk/handle/10026.1/20984>

10.1016/j.scitotenv.2023.162816

Science of the Total Environment

Elsevier

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Tritium: Its relevance, sources and impacts on non-human biota

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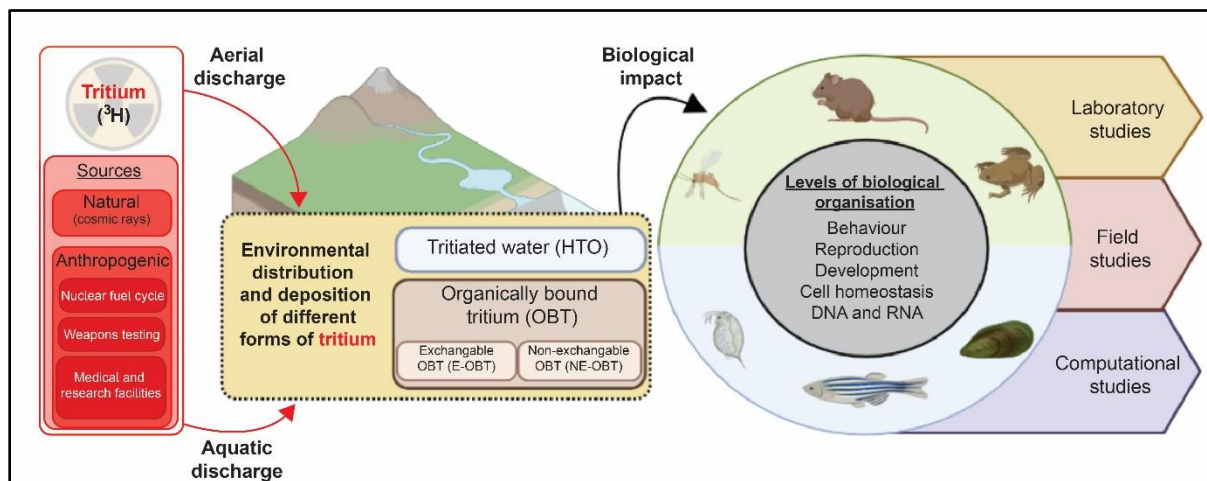
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Graphical Abstract



27 **Highlights:**

28

- 29 • Tritium emissions likely to further increase due to expansion of nuclear processes (85)
- 30 • Tritium (³H) is quickly integrated in the environment and biological systems (78)
- 31 • Sources, properties and effects of ³H in non-human biota critically examined (79)
- 32 • Studies in non-human biota (NHB) are inclined towards bivalves, fish and rodents (82)
- 33 • Integrated approaches required to assess the impact of ³H to fill the knowledge gaps (86)

34

35

1 **Abstract**

2 Tritium (³H) is a radioactive isotope of hydrogen that is abundantly released from the nuclear industries.
3 It is extremely mobile in the environment and in all biological systems, representing an increasing concern
4 for the health of both humans and non-human biota (NHB). The present review examines the sources and
5 characteristics of tritium in the environment, and evaluates available information pertaining to its
6 biological effects at different levels of biological organisation in NHB. Despite an increasing number of
7 publications in the tritium radiobiology field, there exists a significant disparity between data available for
8 the different taxonomic groups and species, and observations are heavily biased towards marine bivalves,
9 fish and mammals (rodents). Further limitations relate to the scarcity of information in the field relative
10 to the laboratory, and lack of studies that employ forms of tritium other than tritiated water (HTO). Within
11 these constraints, different responses to HTO exposure, from molecular to behavioural, have been
12 reported during early life stages, but the potential transgenerational effects are unclear.
13 Transgenerational, epigenetic studies and the application of rapidly developing “omics” techniques could
14 help to fill these knowledge gaps and further elucidate the relationships between molecular and
15 organismal level responses through the development of radiation specific adverse outcome pathways.
16 The use of a greater diversity of keystone species and exposures to multiple stressors, elucidating other
17 novel effects (e.g. by-stander, germ-line, transgenerational and epigenetic effects) offer opportunities to
18 improve environmental risk assessments for the radionuclide. These could be combined with artificial
19 intelligence (AI) including machine learning (ML) and ecosystem-based approaches.

20

21 **Keywords:** Nuclear Energy, Tritium (³H), Tritiated Water (HTO), Environment, Radiation dose, Toxicity,
22 Risk assessment.

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24

25

26 **1. Introduction**

27 While tritium (^3H), the radioactive isotope of hydrogen (physical half-life: 12.3 years), originates naturally
28 from the action of cosmic-ray-induced nuclear reactions in the atmosphere (Oms et al., 2019; Synzynys et
29 al., 2018), anthropogenic sources contribute a greater proportion to the environment (Kim et al., 2019;
30 Nie et al., 2021; Péron et al., 2016). Anthropogenic sources include nuclear fission reactors and
31 decommissioning (including dismantling and spent fuel reprocessing), past nuclear weapons testing (until
32 the early 1960s), nuclear incidents such as Fukushima, Japan in 2011 (Jha, 2021), tritium production
33 facilities, and medical and research facilities (Fiévet et al., 2013; Happell et al., 2004; Harms et al., 2016).
34 In the near future, the release of tritium is expected to increase with the implementation of new reactors
35 (e.g., European Pressurised Reactor) and the development of the International Thermonuclear
36 Experimental Reactor nuclear fusion facility (Larsen and Babineau, 2020; Rety et al., 2010).

37 Compared to other radionuclides, tritium is released in the environment by nuclear sectors, in huge
38 quantity. For example, an overview of historic trends in liquid discharges of radioactive substances carried
39 out between 1979-1988 showed a 10 fold increase with a peak value of ≈ 820 TBq in 1986-1987 in the
40 discharge of ^3H from different nuclear facilities in the UK (McCubbin et al., 2001).

41 In countries like China with growing importance and use of nuclear power, the nuclear power plants
42 (NPPs) discharged 137 TBq^{-1} of HTO on average between 1993-2009 (Dallas et al., 2016a; Yang et al.,
43 2012). During the period 2005-2008, the two NFRPs discharging into the English Channel/ Irish Sea (i.e.
44 Sellafield, UK and La Hague, France) discharged ≈ 1000 - $10,000 \text{ TBq}^{-1}$ of liquid ^3H . Elevated concentrations
45 of tritium (^3H) have also been reported in sediment and at different trophic levels of biota from the Severn
46 estuary, UK (McCubbin et al., 2001), in common with its accidental release in Cardiff Bay, UK and at
47 Fukushima Daiichi, Japan (Bezhenar et al., 2021; Fiévet et al., 2013; Povinec et al., 2013).

48 Once in the environment, tritium reacts with oxygen and is quickly integrated into numerous cycles of the
49 biosphere as tritiated water (HTO) (Figure 1). Due to its chemical properties, it is extremely mobile in
50 biological systems (Bay et al., 2020; Hanslík et al., 2017; Larsen and Babineau, 2020) and may be found in
51 all hydrogenated molecules and associated water in the biosphere. (Ducros et al., 2018; Eyrolle et al.,
52 2018). Tritium exists in tissues in three forms: (i) Tissue Free Water Tritium (TFWT) and associated with
53 the organic matter (OBT) under two forms (ii) bound to oxygen and nitrogen atoms into the tissue,
54 exchangeable organically bound tritium (E-OBT) (iii) bound to carbon atoms into the tissue, non-
55 exchangeable organically bound tritium (NE-OBT) (Baumgaertner et al., 2009; Jaeschke et al., 2011; Nie
56 et al., 2021).

57 It is generally accepted that E-OBT equilibrates very quickly with TFWT, which is itself at equilibrium with
58 the water molecules in the surrounding environment. The metabolism of the NE-OBT mostly leads to the
59 production of tritiated water at rates dependent on the nature of the tritiated organic molecules and their
60 function in the body. Then, it is expected that tritium as NE-OBT presents an extended biological half-life
61 since it remains bound until the catabolism of the tritiated molecule. The NE-OBT is therefore more stable
62 than E-OBT in tissues and it can provide useful information regarding longer term exposures to tritium
63 (Antonova et al., 2022; Baumgaertner et al., 2009; Eyrolle et al., 2018; Jaeschke et al., 2011; Le Goff et al.,
64 2014). Given sustained and future increased discharges of tritium, its unique behaviour in different
65 compartments of the environment, tritium represents an increasing concern for the health of both
66 humans and non-human biota (NHB). However, compared to human studies, little is known about tritium
67 characteristics in NHB (Beresford et al., 2008; Tornero and Hanke, 2016). In this context, the present work
68 aims to critically analyse the available literature regarding the sources, environmental relevance and
69 biological effects of tritium in NHB. We also aim to highlight knowledge gaps for future tritium research.
70 Literature surveys were carried out through keyword and key-phrase searches in Google scholar,
71 ScienceDirect, and PubMed, including “tritium radiation”, “tritium sources”, “fission energy”, “tritium
72 effects”, “tritium dosimetry”, “tritium in the environment”, “tritium in biota”, “tritiated water”,
73 “organically bound tritium” and any combination thereof. Studies on NHB were grouped according to
74 computational, field or laboratory-based studies. Reported effects were grouped according to exposure
75 (acute versus chronic), taxa, and biological level of organisation.

76 It is not the purpose of the present paper to review the literature regarding the fate of radionuclides
77 including tritium in plant populations. Thorough reviews on impact of ionising radiations are already
78 available (Boyer et al., 2009; Caplin and Willey, 2018; Mousseau and Moller, 2020).

79 **2. Tritium in the environment**

80 Considering that about 99% of the tritium present in the atmosphere is in the form of tritiated water, it is
81 expected that it enters the ocean rapidly through vapour exchange, precipitation and river runoff (Liger
82 et al., 2018; Oms et al., 2019). Levels of tritium in the aquatic environment vary according to latitude,
83 season, and proximity to urbanisation and nuclear facilities (Ansari et al., 2018; Chae and Kim, 2018;
84 Harms et al., 2016). Figure 2 shows the average global concentration of tritium, and its distribution
85 between the northern and southern hemispheres between 1996 and 2016 (Oms et al., 2019; UNSCEAR,
86 2016). More than half of the world’s reactors are located in North America and Western Europe, with less

87 than 10 percent are in developing countries (Adamantiades and Kessides, 2009; Oms et al., 2019), and
88 this is reflected in the higher concentration present in the northern hemisphere.

89 In Canada, three of the largest reactors in the country are located on the shores of the Great Lakes. The
90 maximum concentration of tritium was reported along the northern shores of Lake Ontario with a
91 concentration (8.4 BqL^{-1}) more than twice as high as offshore waters (3.5 BqL^{-1}) (Dove et al., 2021). In
92 France, tritium levels ranging from about 3 to 4 BqL^{-1} were reported in non-nuclearized coastal rivers but
93 in the River Rhône, characterised by a high density of nuclear facilities, concentrations fluctuated between
94 2.50 and 12.85 BqL^{-1} , with a mean of 6.31 BqL^{-1} (Jean-Baptiste et al., 2018). In the United States, Fourmile
95 Branch, an area that received contaminated effluent from nuclear weapons material production facilities,
96 tritium concentration in water from ponds adjacent to the contaminated stream ranged from 1570 to
97 1920 BqL^{-1} , with an average concentration (1790 BqL^{-1}) approximately twenty times higher than the
98 average (70 BqL^{-1}) measured above the stream (Yu et al., 2020).

99 Different regulations exist to try and limit the presence of tritium in the aquatic environment. Table 1
100 shows the highly varied limits for drinking water and Table 2 provides site-specific limits for nuclear
101 facilities for waterborne discharges. Whilst there are some limits for discharges of tritium to protect
102 human health, regulations for the protection of NHB are still poorly defined (Andersson et al., 2009).

103 In aquatic biota, an equilibrium between HTO and water is achieved in less than a day due to regulation
104 of the water balance by respiration and osmoregulation processes (Calmon and Garnier-Laplace, 2001).
105 As for OBT, its incorporation is mainly believed to occur through ingestion of tritiated food. OBT levels in
106 aquatic biota are influenced by the presence of different physicochemical forms of organic tritium in the
107 ecosystem, such as dissolved organic molecules, detritic or fresh organic particles, and fine technogenic
108 particles. These forms have different origins (autogenic/allogenic) and different uptake pathways and
109 transfer rates and, therefore, OBT concentrations in organisms are expected to vary depending on the
110 surrounding environment (Baburajan et al., 2020; Eyrolle et al., 2018; Nie et al., 2021). However,
111 knowledge relating to the behaviour of the different physicochemical forms of OBT as well as its
112 consequences in NHB is limited and rather outdated (Eyrolle et al., 2018). Previous studies have further
113 highlighted the need for a more evidence-based assessment of the impacts of tritium on natural biota,
114 which could also pose threats to human health via the food chain (Galeriu et al., 2008; McCubbin et al.,
115 2001; Melintescu et al., 2011; Zhao et al., 2021).

116 In terrestrial animals, HTO is up taken from contaminated water in drink and food, and can be produced
117 by the catabolism of organic molecules. Animals can also incorporate OBT into their tissues and fluids by

118 consuming tritiated food. The retention time and incorporation into the dry matter of tissues/organs are
119 generally higher for constitutive and storage molecules than for water (Le Goff et al., 2014; Takeda, 1991).
120 Previous studies have also reported OBT in some reservoirs where organic matter is preserved, such as
121 in soils (Thompson et al., 2015) and aquatic surface sediments (Eyrolle-Boyer et al., 2014; Eyrolle et al.,
122 2019), raising concerns about the transfer of OBT stocks to the water cycle and living organisms (Galeriu
123 et al., 2008; Ota et al., 2017).

124 Evaluation of terrestrial wildlife inhabiting areas with a history of radiation contamination is important to
125 provide information on the bioavailability and dynamics of radionuclides in the environment (Cleary et
126 al., 2021; Kelsey-Wall et al., 2005). However, few data are available in this regard (Beresford et al., 2016;
127 Kelsey-Wall et al., 2005), and laboratory based studies are often employed to fill the gaps (Galeriu et al.,
128 2005). In the past few decades, computational assessments and transfer models have been developed
129 that aim to predict and calculate the movement of tritium through the environment and plant and animal
130 tissues (Dallas et al., 2016b; Galeriu et al., 2005; Keum et al., 2006; Nie et al., 2021; Vives et al., 2022)
131 (Figure 3). Models assessing the exposure of wildlife to radiation would benefit from OBT-HTO data for
132 biota to improve accuracy when studying phylogenetically different organisms (Kim et al., 2019; Vives et
133 al., 2022). This would also allow comparisons to be made of spatial and temporal effects on
134 bioaccumulation or uptake as well as potential species-specific impacts (Beresford et al., 2016; Kim et al.,
135 2013a).

136 **3. Dose rate benchmarks, regulatory values and bioaccumulation for non-human biota.**

137 In common with other radionuclides, biota can be exposed externally and/or internally to tritium
138 (Goodhead et al., 2004; Melintescu and Galeriu, 2011). It is, therefore, critical to accurately determine the
139 absorbed radiation dose that could be linked to observed effects in order to improve environmental risk
140 assessment (ERA) and protection (Adam-Guillermin et al., 2012; Nushtaeva et al., 2020). In contrast to
141 human health, where physical, biological and clinical dosimetry have been well established, there has
142 been very limited progress in estimating radiation doses in NHB, with efforts mainly aiming to filter out
143 situations of minimal risk to the individual or population (Andersson et al., 2009; Beaugelin-Seiller et al.,
144 2020; Beresford et al., 2020; Garnier-Laplace et al., 2010; Mothersill et al., 2020; Real and Garnier-Laplace,
145 2020).

146 Table 3 shows screening values proposed by different organisations in order to improve ERA. The United
147 Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 2008) has suggested that

148 chronic dose rates of less than $100 \mu\text{Gyh}^{-1}$ to the most highly exposed organism would be unlikely to incur
149 significant effects on most terrestrial vertebrate communities. Accordingly, the European Union
150 consortium projects, ERICA and PROTECT, have suggested a generic (all species) “no effect” dose rate of
151 $10 \mu\text{Gyh}^{-1}$ (Andersson et al., 2009, 2008; Beresford et al., 2004) . The PROTECT project also proposed a
152 provisional dose rate benchmark of $200 \mu\text{Gyh}^{-1}$ for invertebrates and $2 \mu\text{Gyh}^{-1}$ for vertebrates (Andersson
153 et al., 2008) (See Table 3 for an overview). However, the robustness of this value has been queried because
154 of the small number of data available for different groups of organism (Real and Garnier-Laplace, 2020)
155 and reported differences in accumulation and biotransformation of the different forms of tritium even
156 within the same phylum (Beresford et al., 2016; Jaeschke and Bradshaw, 2013; Kim et al., 2013a). Jaeschke
157 and Bradshaw (2013) observed a disparity in the activity concentration between two phytoplankton
158 species after being exposed to the same concentration of HTO and showed that the ingestion of tritiated
159 phytoplankton resulted in measurable incorporations of tritium into tissues of the bivalve mussels,
160 indicating transfer and concentration up the food chain. Fiévet et al. (2013) studied the kinetics of the
161 turnover of tritium between seawater HTO, biota HTO and OBT. HTO in two algae and a mollusc presented
162 a rapid exchange with seawater HTO but overall tritium turnover between HTO and the whole-organism
163 OBT appeared to be a slow process with a tritium biological half-life on the order of months. In a study
164 with mussels, Yankovich et al., (2011) also suggested the possibility of slow and fast OBT compartments
165 corresponding to differing rates of OBT dynamics. In particular, the model that took account of
166 reproductive processes and tissue compartments produced the best OBT predictions.

167 In common with other types of contaminants, an important concern is tissue-specific accumulation which
168 has implications for radiation dose and hence risk assessments. In rats, for example, a single oral
169 administration of HTO showed that tritium was rapidly and uniformly distributed among tissues (Lee et
170 al., 2019). In this study, OBT and tissue-free HTO concentrations (Bqg^{-1} and both wet and dry samples)
171 showed no significant differences amongst different tissues (e.g. heart, lung, liver, gonads) after 7 and 13
172 days in rats exposed to $3.7 \times 10^4 \text{ Bq HTO}$, and after 17 days exposure to $3.7 \times 10^5 \text{ Bq HTO}$ (Lee et al., 2019).
173 Similar results in tissue specific analyses were obtained for OBT (Bqg^{-1} for both wet and dry sample) on
174 days 1, 3 and 5 after rats had received a single oral administration of HTO ($3.7 \times 10^3 \text{ Bq per gram of body}$
175 weight) (Takeda et al., 1985). However, the distribution of OBT (Bqg^{-1} both wet and dry samples) among
176 tissues was not uniform in rats exposed to tritiated wheat ($0.074 \times 10^3 \text{ Bq per gram of body weight}$) and
177 tritium excreted as urine and faeces was less after the ingestion of tritiated wheat than HTO (Takeda et
178 al., 1985). These observations, along with results of a comparative biokinetic study in rats chronically
179 exposed to tritiated water and tritium-labelled food (Takeda et al., 2001), support the assertion that

180 tritiated organic compounds remain for longer periods of time in the body than tritiated water. Moreover,
181 the distribution pattern of OBT among tissues depends not only on the chemical or biochemical character
182 of each tritiated compound but also on the metabolic activity of each tissue (Kim et al., 2013a; Le Goff et
183 al., 2014; Takeda, 1991; Takeda et al., 1985).

184 Regarding aquatic biota, previous studies have reported that tritium concentration differs between
185 tissues in mussels exposed to HTO and OBT (tritiated amino acid, glycine; T-Gly) (Dallas et al., 2016a;
186 Jaeschke et al., 2011; Jha et al., 2005). Specifically, after HTO exposure, the foot, digestive gland, mantle
187 and adductor muscle had higher OBT activity (Bqg^{-1} dry sample) than the gills and byssus and in all tissues,
188 levels decreased after one day of depuration. Conversely, the OBT (T-Gly) treated group presented the
189 greatest bioaccumulation of tritium in the following order: digestive gland > gills > foot > byssus > mantle,
190 adductor muscle, and all tissue retained activity concentrations significantly above the control values even
191 after 21 days depuration (Jaeschke et al., 2011). A higher concentration (Bqg^{-1} dry sample) in the digestive
192 gland was also reported by Jha et al. (2005) (Jha et al., 2005), while Dallas et al. (2016) (Dallas et al., 2016a)
193 observed that the digestive gland, foot and gill presented higher levels than other tissues, although
194 concentrations varied according to temperature and time. In fish, it was reported that OBT formation rate
195 was significantly higher when fish were exposed to OBT-spiked food compared to HTO (Kim et al., 2013b).
196 This OBT concentration in tissue was higher than the OBT concentration in the food, indicating the
197 bioaccumulation when fish ingest OBT through the food web. Moreover, OBT concentration were higher
198 in viscera than in muscle, suggesting a compartmentalization (Kim et al., 2013b).

199 Considering variations in organisms' metabolisms and experimental exposure scenarios, it is
200 recommended that tissue-specific accumulation should be factored in to dosimetry and ecotoxicological
201 studies for more robust assessments (Dallas et al., 2016b) . While it is not feasible to study tritium transfer
202 in every species, it would be beneficial to improve our understanding of keystone species or ecologically
203 relevant taxonomic groups (Beresford et al., 2016; Dallas et al., 2012), particularly when estimating the
204 potential for transfer through the food web.

205

206 4. Laboratory and field exposure studies

207 As evident in Figures 3 and 4, most of the information on tritium in NHB has been derived from controlled
208 laboratory exposures. Under these exposures, repeated tests can be performed to understand the
209 relationship between dose and effect under controlled, reproducible conditions (Loria et al., 2019). It is
210 particularly important to elucidate the mechanisms involved in biological responses, and potential
211 synergistic, antagonistic or additive effects of factors within the environment that may mask or intensify
212 the detrimental impact of tritium exposure (Dallas et al., 2016a, 2012). That said, however, field studies
213 are more environmentally realistic in determining the biological effects of contaminants, particularly
214 when studying long-term population level effects (Bréchnac, 2017; Loria et al., 2019). Moreover, field
215 studies involving exposure to low radiation levels over a long period of time provide the opportunity to
216 investigate potential adaptation, processes involved in tissue homeostasis and biomagnification (Beaton
217 et al., 2019; McCubbin et al., 2001).

218 Although Figure 3 indicates that data are available for different organisms from field studies, most of
219 these have investigated tritium accumulation and very few have been published on the effects of tritium
220 on NHB (Audette-Stuart et al., 2011; Beaton et al., 2019; Gagnaire et al., 2017). Near an operating nuclear
221 site in Canada, Audette-Stuart et al. (2011) reported that frogs collected in areas with above-background
222 levels of tritium (Duke Swamp, 2800 BqL⁻¹) presented lower levels of DNA damage in liver cells compared
223 to frogs inhabiting areas with background levels (214 BqL⁻¹) after an *in vivo* exposure to a challenging dose
224 of 4 Gy ionising radiation. Decreased sensitivity to radiation damage was also observed in cultured liver
225 cells from frogs collected in Duke Swamp when the radiation dose was delivered *in vitro*. The authors
226 suggested that the stress present in the area with a higher concentration of tritium could induce a
227 protection of DNA or a cellular defence mechanism as an adaptive response to radiation. However, further
228 studies are required as these results were based on a small number of samples. In the same region,
229 another study revealed that tritium exposure induced genotoxicity, DNA repair activity, changes in fatty
230 acid composition, and immune, neural and antioxidant responses in fathead minnows fish (Gagnaire et
231 al., 2017). In a subsequent controlled laboratory study in which fish were chronically exposed to a
232 gradient of HTO (12 x10³, 25 x10³ , and 180 x10³ BqL⁻¹) and OBT (tritiated amino acids; 27 x10³ BqL⁻¹),
233 similar observations were reported to those found at the field sites (Gagnaire et al., 2018). Beaton et al.
234 (2019) found that DNA damage in fish exposed in the laboratory was higher than fish exposed in field,
235 whereas enzymatic activities (e.g., SOD and catalase) in the liver were lower in fish exposed under
236 laboratory conditions. A positive monotonic relationship between DNA damage and internalised tritium

237 was observed in both experiments, but no correlation was found between tritium internal concentration
238 and enzymatic activities. This is not surprising considering that organisms in the environment are
239 exposed to a mixture of contaminants and a range of variables (e.g., temperature, pH, salinity), and
240 oxidative stress biomarkers are known to be non-specific (Lourenço et al., 2016; Van der Oost et al.,
241 2003a). An integrative approach, where investigations in the field are followed by controlled laboratory
242 experiments, provides a better understanding of tritium behaviour and is able to identify suitable
243 biomarkers for tritium exposure in order to assess and predict the impact of current and future radiation
244 exposure (Dallas et al., 2016b; Parisot et al., 2015).

245 **4.1. Chronic and acute exposures**

246 As with other radionuclides, an important factor to consider when assessing the biological effects of
247 tritium is the length or duration of exposure. A review of laboratory studies shows that data of sub-chronic
248 and chronic exposures to tritium are predominant for terrestrial biota (Table 4) but acute exposures are
249 prevalent for aquatic biota (Table 5). Acute exposures are useful in demonstrating immediate stress
250 response and for applying in models that study the metabolic behaviour of contaminants inside the body
251 (Giussani et al., 2020; Jaeschke and Bradshaw, 2013). However, chronic and low dose exposures are more
252 relevant for biomonitoring purposes and environmental risk assessments, as well as for studies related to
253 adaptive responses in organisms (Audette-Stuart et al., 2011; Mothersill et al., 2020; Real et al., 2004).

254 In mice, both HTO and OBT were found to induce increased levels of chromosomal aberrations in
255 peripheral blood lymphocytes at concentrations of 1 and 20 MBqL⁻¹ following a one-month chronic
256 exposure. However, excess damage was not observed for HTO when the exposure was protracted to eight
257 months, suggesting that a longer exposure could trigger some compensatory repair mechanism (Roch-
258 Lefèvre et al., 2018). By contrast, Bannister et al. (2016) found no evidence for cytotoxicity or genotoxicity
259 in mouse spleen following chronic exposures (one and eight months) to HTO up to 20 MBqL⁻¹, while Saito
260 (2002) observed that DNA-bound tritium in cells from mouse spleen was lower than in nucleus from liver
261 and brain cells. This highlights the importance of tritium accumulation being tissue- and time-specific
262 (Dallas et al., 2016a; Jha et al., 2005; Pearson et al., 2018).

263 In studies of aquatic biota, effects reported after acute tritium exposure include alteration in gene
264 expression levels, and development, genotoxic and cytotoxic effects. Conversely, sub-chronic and chronic
265 exposures have been performed to assess the impact of tritium in physiological, behavioural responses
266 and genotoxic effects (Table 5). Responses at different level of organisation may be better understood

267 over different timescales of exposure, as acute and chronic exposures may target different metabolic
268 routes, physiological processes or different life stages.

269 **4.2. Studies on different animal groups**

270 An overview of studies aiming to assess potential impact of tritium on different animal groups has been
271 summarised in Table 4. Rodents, such as mice and rats, have long served as laboratory research models
272 due to the anatomical, physiological and genetic similarity to humans. They have been considered
273 preferred models also because of their short gestation period, short life cycle, small size and ease of
274 maintenance. Accordingly, Figure 4 and Table 4 show that mammals, and mainly mice and rats, are
275 predominant in laboratory studies involving tritium. Although these studies have been performed using
276 high concentrations and with the aim to address potential toxicity to human health, they provide valuable
277 data and generate mechanistic information that could help understanding tritium effects in mammals and
278 other biota. Molecular to behavioural effects have been reported under laboratory settings after acute (Li
279 et al., 2021) and chronic (including transgenerational) exposure scenarios (Bannister et al., 2016; Gao et
280 al., 1999; Roch-Lefèvre et al., 2018) (Table 4). Observed effects include a decrease in offspring survival
281 (Cahill et al., 1975; Clerici et al., 1984), a reduction in weight of reproductive organs (Cahill and Yuile,
282 1971; Laskey et al., 1973) and sterile F1 (Cahill et al., 1975). Alterations in the nervous system at molecular
283 (Laskey and Bursian, 1976) and behavioural levels (Gao et al., 1999; Wang and Zhou, 1995) have also been
284 reported. The availability of the complete genome sequence for rats and mice also opens up possibilities
285 to implement genetic techniques and develop transgenic mice, and use them in toxicology studies
286 (Ankley et al., 2010; Kratchman et al., 2018), and, regarding tritium research, monitor DNA inversion
287 frequency and assess genotoxicity (Bannister et al., 2016).

288 The majority of nuclear facilities are connected to rivers and lakes and, directly or indirectly, to the marine
289 environment (Adam-guillermin et al., 2012; Landrigan et al., 2020). Despite being a popular freshwater
290 model species, to our knowledge only one study has employed daphnids as a biological model to assess
291 the impacts of tritium (Gudkov and Kipnis, 1996). Thus, a long-term exposure to HTO was performed for
292 five generations in *Daphnia magna*, and alterations in reproductive performance, increasing
293 abnormalities during embryogenesis, decrease in survival rate and differences in cytological in terms of
294 increased number of nucleoli per cell were reported (Table 4). This species might not be ecologically
295 representative of aquatic invertebrates (Dallas et al., 2012) but it allows relevant multigenerational
296 exposure scenarios and potential perturbations in population dynamics to be understood (Atienzar and
297 Jha, 2004).

298 Tritium studies in aquatic mammals are scarce, and fish represents the main source of data available for
299 vertebrates (Adam-Guillermin et al., 2012; Beresford et al., 2016) (Figure 2, Table 5). Due to the well-
300 known physiology and life cycle of certain fish species, different approaches, from molecular (e.g., Arcanjo
301 et al., 2018; Festarini et al., 2019) to behavioural studies (e.g., Arcanjo et al., 2020; Festarini et al., 2016),
302 have been conducted in the laboratory (e.g., Arcanjo et al., 2018; Festarini et al., 2019, 2016; Gagnaire et
303 al., 2020) or field setting (Gagnaire et al., 2017). These models share some advantages with rodents, such
304 as known and short life cycles, but, to our knowledge, no study has assessed transgenerational effects of
305 tritium exposure in fish.

306 Most data relating to tritium effects are available for the early life stages of zebrafish and medaka, two of
307 the most popular fish models (Table 5). In zebrafish, observations following acute and chronic exposures
308 above $0.4 \times 10^3 \mu\text{Gy h}^{-1}$ include decreases in swimming activity, thyroid hormone and hatching rate, as well
309 as DNA damage and altered expression of genes involved in detoxification processes and muscle
310 contraction. Studies using lower tritium concentrations and longer exposure periods are required to
311 determine if these biomarkers are sensitive to environmentally-realistic radiation doses and whether any
312 effects can be overcome after chronic exposure.

313 Relationships between ambient (water) and tissue concentrations of contaminants can be difficult to
314 establish and verify in free-living animals. Sessile animals (e.g. mussels), therefore offer important
315 advantages (National Research Council, 1991). Thus, they have been used to monitor and study toxicity
316 response to a range of radionuclides, including tritium (Dallas et al., 2016a; Jha et al., 2006, 2005; Pearson
317 et al., 2018). As summarised in Table 5 and Figure 4, molluscs are the main aquatic invertebrates used to
318 study bioaccumulation and biological effects of tritium, as HTO or OBT (T-Gly) either individually or in
319 combination with other stressors or contaminants like metals. Jaeschke and Bradshaw (2013) provided
320 evidence for the accumulation of organic tritium into the mussel tissues via tritiated-phytoplankton,
321 suggesting a transfer pathway of tritium and its potential biomagnification. The tritium activity in foot,
322 gills, digestive gland and mantle tissues showed a linear increase with the number of feedings, and the
323 digestive gland had the highest incorporation of tritium compared to the other tissues. The induction of
324 micronuclei in haemocytes was also observed after exposure to HTO and T-Gly (Jaeschke et al., 2011).
325 The activity from HTO was depurated within one day, whereas T-Gly depurated relatively slowly.
326 Genotoxicity in mussels also appears to be temperature- and time-dependent, with induction of DNA
327 strand breaks after exposure to HTO observed after three days when exposed at 25°C but after seven
328 days at 15°C (Dallas et al., 2016b). In a co-exposure studies with zinc and HTO, Pearson et al.(2018)

329 observed a clear antagonistic effect of Zn on the genotoxicity (DNA strand break) of HTO at all Zn
330 concentrations used, possibly due to the importance of Zn in DNA repair enzymes. These observations
331 highlight the importance of assessing potential interactions of physical and chemical factors with tritium
332 in order to improve current and future risk assessment strategies for organism exposure in the
333 environment.

334 Variations in sensitivity to contaminants between life stages have been reported for fish and aquatic
335 invertebrates, and early life stages are considered more susceptible to toxic substances (Mohammed,
336 2013; Santos et al., 2018). Nevertheless, most of the available data on tritium impacts on invertebrates
337 refer to adults (Dallas et al., 2016a; Devos et al., 2015; Jaeschke et al., 2011; Jaeschke and Bradshaw, 2013;
338 Pearson et al., 2018). Potential effects in embryo-larvae of the marine bivalve molluscs, *Mytilus edulis* and
339 in goosse barnacle, *Pollicipes polymerus* are the only early life-stages of invertebrates studied (Hagger et
340 al., 2005; Abbott and Mix, 1979). In bivalve, cytogenetic damage, cytotoxicity, developmental
341 abnormalities and mortality were observed after acute exposure, which increased as a function of
342 radiation dose (Hagger et al., 2005). In barnacles, an exponential decrease in moulting index related to
343 HTO concentration was observed. The effects were evident at a concentration as low as 259 BqL⁻¹ (Abbott
344 and Mix, 1979).

345 **5. Biological effects**

346 Different biological tests and assays have been used according to the nature of the stressors involved in
347 various organisms or model species (Aliko et al., 2018). Potentially, alterations in any process may be
348 used as biomarkers and may be measured in tissues or body fluid samples, or at the level of whole
349 organisms, to provide evidence of exposure effects from one or more contaminant (Hagger et al., 2006).
350 Responses at each level of biological organisation provide information that helps to understand and
351 interpret the relationship between exposure and adverse effects (Hagger et al., 2006; Van der Oost et al.,
352 2003a). In particular, radiobiological effects have been commonly assessed through the “umbrella end-
353 points” which includes morbidity, mortality, reproductive and mutational effects on the organisms
354 (Garnier-Laplace et al., 2006; Real et al., 2004; Sazykina and Kryshev, 2003) as suggested in the FASSET
355 project framework (Coppelstone et al., 2008). Survival and reproduction represent both individual and
356 population-relevant end-points since variations in these parameters can affect the fitness of the
357 population and genetic diversity (King-Heiden et al., 2012; Marty et al., 2011). More often, however,
358 effects are more subtle, ultimately modulating organism fitness, because contaminants can act via

359 numerous mechanisms (Connon et al. 2009). Moreover, once an effect is manifested at an organism level,
360 remedial measures are often too late.

361 Over the past few years, the use of molecular, biochemical and physiological biomarkers have increased
362 while determining the potential impact of in tritium on the NHB (Figure 5). Considering that there is no
363 single biomarker that can unequivocally measure detrimental impact, many studies have implemented or
364 recommended application of an integrated multi-biomarker approach (Adams, 2005; Brenner et al., 2014;
365 Galloway et al., 2004a; Jha, 2008; Larsson et al., 2018; Linde-Arias et al., 2008; Turja et al., 2014). In the
366 framework of ERA, both short-term responses and long-term ecologically relevant end-points provide a
367 weight-of-evidence approach for establishing relationships between environmental stressors and
368 ecological effects (Galloway et al., 2004b; Hagger et al., 2006).

369 **5.1 Behavioural responses**

370 Behaviour analysis is considered to be a sensitive indicator that can reflect biochemical and/or
371 physiological disturbances. Behaviour can have a direct or indirect effect on population growth rate,
372 thereby representing an ecologically relevant response at organismal and supra-organism levels (Bertram
373 et al., 2015; Candolin and Wong, 2019; Scott and Sloman, 2004). For example, (Wang and Zhou, 1995)
374 found that mice offspring receiving 48.18 and 144.54 kBqg⁻¹ *in utero* (after pregnant adults received a
375 single intraperitoneal injection of HTO) had difficulties in both learning and memory retention for skill
376 performance. Similar results were found in rat offspring that were related to the induced degeneration
377 and malformation of hippocampal neurons observed in treated groups (Gao et al., 1999). More recent
378 studies have reported that HTO exposure can affect fish behaviour (Festarini et al., 2016; Li et al., 2021).
379 Li et al. (2021) observed that 120 hours post fertilization (hpf) zebrafish larvae exposed to HTO (3.7×10^6
380 BqL⁻¹, 3.7×10^7 BqL⁻¹, 3.7×10^8 BqL⁻¹) presented decreases in activity, swimming speed and total swimming
381 distance when compared to a control group. Altered swimming behaviour has also been reported in 96
382 hpf zebrafish larvae exposed to 1.10×10^8 and 1.35×10^9 BqL⁻¹ HTO and attributed to developmental
383 abnormalities (Arcanjo et al., 2020).

384 **5.2 Reproduction and development**

385 Reproduction is known to be one of the most radiosensitive biological functions and might be impaired at
386 doses corresponding to less than 10% of the dose causing mortality (Adam-Guillermin et al., 2018). It is
387 suggested that actively dividing cells are highly sensitive, highest radiosensitivity is therefore likely to be
388 found in cell systems undergoing rapid cell division for either reproduction (e.g. spermatogonia) or growth
389 (e.g. the developing embryo) (UNSCEAR, 1996).

390 In rodents, tritium reproductive and developmental effects have been assessed experimentally after
391 different exposure scenarios that include acute, chronic, *in utero* and transgenerational (see Table 4 for
392 an overview). Reproductive impairment, dose-dependent decreases in oocyte numbers and various
393 neuronal effects associated with an abnormal development are the main effects observed in
394 transgenerational studies (Table 4). In rats, sterile offspring have been reported after *in utero* continuous
395 exposure to HTO (1850 and 3700 x 10⁶ BqL⁻¹ body water in pregnant adults) (Cahill and Yuile, 1971),
396 whereas reductions in testes and ovary weights were observed in F1 after being exposed to a lower dose
397 of HTO (370 x 10⁶ BqL⁻¹ body water) (Cahill and Yuile, 1971; Laskey et al., 1973; Laskey and Bursian, 1976).
398 In a multigenerational study, constant prenatal exposure to HTO showed significant effects on F2 rat
399 neonates, including reductions in relative brain weight after HTO *in utero* exposure (3.7, 37 and 370 x
400 10⁶ BqL⁻¹ body water in pregnant adults) and decreased body weight in F2 from females exposed to HTO
401 concentrations of 37 and 370 x 10⁶ BqL⁻¹ body weight (Laskey et al., 1973). Brain abnormalities and
402 alterations in the establishment of conditional reflexes were also reported in rats that received different
403 doses of tritium (92 x 10³ µGy and 273 x 10³ µGy) during gestation (Gao et al., 1999). In mice, males
404 injected with tritiated thymidine (0.185 x 10⁶ Bq mg⁻¹ body weight) presented a decrease of spermatocytes
405 after four days (Johnson and Cronkite, 1959). Decrease in viability was observed in embryos from mice
406 maintained on drinking water containing 111 x 10⁶ BqL⁻¹ of tritiated water (Carsten and Commerford,
407 1976), and F1 exposed *in utero* to HTO (3.1 x 10⁶ BqL⁻¹ body water in pregnant adult) presented a
408 significantly decreased in oocytes (Lowry Dobson and Cooper, 1974).

409 Most of the above studies have focused on reproduction and potential neurohistopathologies. Even
410 though these represent sub-lethal effects, they clearly have an important effect at organism and
411 population levels, especially considering that some developing neurons and female sex cells are both
412 irreplaceable in adult mammals (Bharti et al., 2021; Stifani, 2014). It is also important to appreciate that
413 doses applied are above proposed screening values for vertebrates, and data are only available for mice
414 and rats. It would be useful to have additional information on the effects induced by tritium exposure in
415 other mammalian species and across a range of concentrations in order to improve ecological risk
416 assessment.

417 In aquatic biota, end-points measured at the organismal level have included mortality, hatching success
418 of embryos and developmental parameters, and the focus has mainly been on fish (Table 5). Suyama and
419 Ichikawa (1974) studied the effect of tritium exposure in the development of two marine fishes: flounder
420 (*Paralichthys olivaceus*) and puffer (*Fugu niphobles*). Neither showed any significant decrease in

421 hatchability after exposure to tritiated water (up to $370 \times 10^6 \text{ BqL}^{-1}$), and effects of HTO on the hatchability
422 and growth of puffer embryo, including smaller eye size and swelled abdomen on hatching, were only
423 evident at a very high concentration (370×10^8 to $370 \times 10^9 \text{ BqL}^{-1}$). Similar effects have been observed in
424 zebrafish larvae after exposure to a lower concentration of HTO ($100 \times 10^6 \text{ BqL}^{-1}$) (Gagnaire et al., 2020).
425 Here, eggs were exposed after three hours of being fertilised, whereas puffer eggs had been exposed 19
426 hours post fertilisation. Bondareva (Bondareva, 2017) observed an increase in incidence of abnormalities
427 after larvae of *Carassius gibelio* (6 hpf) were chronically exposed to HTO (50, 500, 5000 and 50000 BqL^{-1}),
428 although this was not correlated with increasing exposure level. These studies suggest a differential
429 sensitivity between developmental stages, as previously reported in zebrafish (Arcanjo et al., 2018).
430 However, differences in species sensitivity or tritium behaviour (e.g. chemical activity) in freshwater and
431 seawater cannot be ruled out.

432 Few studies have assessed the impacts of tritium in the reproduction of aquatic biota, and the most
433 commonly studied reproductive end-point is fecundity (i.e., the ability of an organism to produce viable
434 gametes) (Table 5). In fry of medaka, it was observed that germ cell survival diminished exponentially
435 with tritium dose and female germ cells were more radiosensitive than male germ cells. These results
436 were obtained on eggs (2 hours post-fertilisation) that were kept in HTO ($1850\text{-}37000 \times 10^6 \text{ BqL}^{-1}$) for ten
437 days (Hyodo-Taguchi and Etoh, 1986). Similarly, in adult males of medaka, a decrease in the number of
438 germ cells was reported upon the addition of tritiated water at a concentration of $370 \times 10^6 \text{ BqL}^{-1}$ after
439 ten days exposure (Hyodo Taguchi and Egami, 1977), apparently indicating a higher sensitivity of germ
440 cells in adult fish. Because long-term survival of a species depends on its reproductive success, alterations
441 to this process are among the most significant sublethal effects. However, it is also known that the normal
442 reproductive pattern of natural species can be highly influenced by different factors, such as temperature,
443 age, food availability, and seasonality (Jha, 2008; Rizzo and Bazzoli, 2019). This makes laboratory
444 measurements of reproduction logistically challenging (Jha, 2008), and more difficult to extrapolate
445 between laboratory results and ecosystem effects (Hook et al., 2014).

446 Although data on reproductive effects arising from tritium exposure are scarce, early life-stage studies
447 assessing developmental parameters provide valuable information that is just as ecologically relevant as
448 a decrease in fecundity (Connon et al., 2012; Dallas et al., 2012; McArdle et al., 2020). Sublethal
449 developmental abnormalities can compromise the ecological fitness of individual organisms or a
450 population since the individual must be able to avoid predation, reproduce, compete with other
451 organisms for food and cope with other environmental stressors. Moreover, if development is significantly
452 delayed by tritium exposure, as reported in barnacles (Abbott and Mix, 1979) and different fish species

453 (Arcanjo et al., 2018; Gagnaire et al., 2020; Suyama and Ichikawa, 1974), organisms may spend a greater
454 time at more vulnerable stages where they are more likely to be predated (Paradis et al., 1999) or infected
455 with pathogens (Sweet and Bateman, 2015).

456 **5.3 Cytotoxicity**

457 The energy from internal radioactive decay generates reactive oxygen species (ROS). Since these species
458 are powerful oxidants with short half-lives, oxidative stress responses have been used as end-points to
459 assess DNA damage and cellular death (Isaksson, 2010; Lionetto et al., 2019; Turja et al., 2014).

460 Oxidative stress has been frequently evaluated in studies using bioindicator species in both the field and
461 laboratory based studies (Amachree, 2014; Lourenço et al., 2016; Van der Oost et al., 2003b), and with
462 respect to tritium, in zebrafish (Arcanjo et al., 2018; Gagnaire et al., 2020), fathead minnow (Beaton et
463 al., 2019; Gagnaire et al., 2018, 2017) and mice (Kelsey-Wall et al., 2006). The study in mice reported no
464 induction of activities of antioxidant enzymes (catalase [CAT], glutathione peroxidase [GPx], and
465 superoxide dismutase [SOD]) after mice had been exposed to tritiated drinking water (activity about
466 300,000 BqL⁻¹) for a period of two weeks. In a field study, Beaton et al. (Beaton et al., 2019) observed that
467 GPx activity in fathead minnows was negatively correlated with tritium dose in the liver and positively
468 correlated with dose in the brain, but these trends were not evident in the laboratory. The authors
469 therefore suggested that GPx activity might respond to the environment and not to tritium concentration.
470 In an independent study, enzymatic activities of SOD, CAT and GPx showed no changes after fathead
471 minnows had been exposed to HTO but ROS index was negatively correlated with tritium dose rate in
472 tissues (Gagnaire et al., 2018).

473 Another common biomarker to assess the health status of cells is the measurement of lysosomal
474 membrane stability (LMS). In zebrafish, Gagnaire et al. (Gagnaire et al., 2020) observed that LMS was
475 positively correlated with the tritium dose in tissues, while a multi-stressor exposure with tritium and
476 metals showed an increase of LMS in the spleen of fathead minnows that was attributed to a protective
477 effect of tritium towards harmful metals (Gagnaire et al., 2017).

478 **5.4 Genotoxicity**

479 Since genetic damage could be induced at a much lower dose rate and be determined using a range of
480 techniques immediately after exposures to ionising radiation, relevant end-points have been employed in
481 exposures to tritium. Specifically, genotoxic effects of tritium exposure have been reported in molluscs
482 (Dallas et al., 2016a; Devos et al., 2015; Hagger et al., 2005; Jha et al., 2005; H. Pearson et al., 2018), insects

483 (Blaylock, 1971), mammals (Ichimasa et al., 2003; Lee et al., 2019) and fish (e.g., Arcanjo et al., 2019;
484 Festarini et al., 2016; S. Li et al., 2021) (Tables 4 and 5).

485 One of the most common assays to assess genotoxicity is comet or single cell gel electrophoresis assay
486 [SGCE] which is used to detect DNA single/double strand breaks (SSB/DSB) (Jha, 2008; Lee and Steinert,
487 2003). The micronucleus (MN) test has also been used to detect genotoxic effects of environmental
488 radionuclides (Beaton et al., 2019; Gagnaire et al., 2018; Jha et al., 2005; Poblete-Naredo and Albores,
489 2016; Samanta et al., 2018; Vernon et al., 2020). The MN test detects non-repairable damage while SCGE
490 detects recent lesions that can be repaired, such as breaks and alkali labile sites (Frenzilli et al., 2009).
491 Concordance between the genotoxic effects assessed through both SCGE and MN have been reported for
492 HTO (Jha et al., 2005), although it is suggested that the comet assay presents higher sensitivity (Frenzilli
493 et al., 2009). DNA damage in haemocytes have been assessed and detected in mussels exposed to dose
494 rates as low as $20 \mu\text{Gyh}^{-1}$ ($15 \times 10^6 \text{BqL}^{-1}$ HTO) (Dallas et al., 2016a) and $4.9 \mu\text{Gyh}^{-1}$ ($1.48 \times 10^6 \text{BqL}^{-1}$ T-Gly)
495 (Jaeschke et al., 2011) following seven day exposures. In mussel larvae, Hagger et al. (2005) reported
496 chromosomal aberrations, induction of sister chromatids exchanges (SCEs) and changes in the random
497 amplified polymorphic DNA (RAPD) profile after being exposed to HTO (3.70×10^3 - $370 \times 10^3 \text{BqL}^{-1}$; 30 –
498 150 μGy ; 18.6 h). DNA damage using comet assay was also detected in oysters exposed to a dose rate of
499 7.5 and $113.9 \mu\text{Gyh}^{-1}$ ($1 \times 10^6 \text{BqL}^{-1}$ and $15 \times 10^6 \text{BqL}^{-1}$ HTO) following a 14-day exposure (Devos et al.,
500 2015). In the fathead minnow, a dose rate of $0.65 \mu\text{Gyh}^{-1}$ ($180 \times 10^3 \text{BqL}^{-1}$ HTO) for 60 days increased DNA
501 damage in gonads and MN frequency in blood (Gagnaire et al., 2018). In the same species, γH2AX foci
502 detection (reflecting DNA double strand break) was positively correlated with the internal dose rate after
503 exposure to tritium in two forms: HTO and OBT-spiked feed (Gagnaire et al., 2017). Conversely, Festarini
504 et al. (2019) reported no differences in cell viability, DNA breaks and DNA repair activity in adult trout
505 chronically exposed to HTO activity concentrations that were close to the current Canadian Drinking
506 Water Guideline of 7000BqL^{-1} . However, changes in response to a subsequent acute high dose of gamma
507 radiation delivered *in vitro* were noted when evaluating DNA repair activity. In comparison to non-tritiated
508 fish tissues, after exposure to a 4 Gy challenge dose, the response of liver and heart was enhanced
509 compared to the other tissues tested. This highlights the presence of differential DNA repair activities and
510 sensitivities of tissue to tritium radiation. Similarly, in zebrafish embryo-larvae, γH2AX detection revealed
511 no difference between controls and groups exposed to 1.22×10^2 and $1.22 \times 10^3 \text{BqL}^{-1}$ of HTO (0.40 and 4
512 mGyh^{-1}) (Arcanjo et al., 2020). However, under the same exposure scenario, the authors observed an up-
513 regulation of *h2afx* gene (coding for histone H2A) which is involved in the response to DNA damage. It

514 was hypothesised that these results may reflect an enhancement of DNA repair pathways to balance the
515 effects of ionising radiation at a higher level of biological organisation.

516 The genotoxic potential of chemicals depends on the properties of the cell or tissue and the location of
517 contaminant accumulation (Jha, 2008). Thus, information on tritium distribution and bioaccumulation in
518 different tissues would be useful to improve our understanding of tissue sensitivity and DNA repair
519 pathways.

520 **5.5 “Omics” studies**

521 To the best of our knowledge, neither proteomics nor metabolomics have been applied to explore tritium
522 effects in NHB, and only a few studies have assessed the effects of tritium exposure in biota transcriptome.
523 These have all been performed in fish models (Table 5). Zebrafish larvae (24 and 96 hpf) exposed to HTO
524 (1.22×10^8 and 1.22×10^9 BqL⁻¹) presented changes in expression of genes involved in muscle contraction,
525 eye opacity, circadian and oxidative stress responses, and in pathways involved in muscle development,
526 and skeletal and cardiac muscle contraction (Arcanjo et al., 2018). These effects were confirmed at a
527 higher biological scale after electron microscopic observations of 96 hpf larvae, indicating that muscle
528 integrity could be considered as a good biomarker of HTO exposure in early developmental stages of
529 zebrafish (Arcanjo et al., 2018). Changes in gene expression involved in eye opacity were not confirmed
530 at a higher biological scale using stages from 24 hpf to 96 hpf (Gagnaire et al., 2020), but mis-regulation
531 of genes involved in muscle contraction were linked to effects in locomotion (Arcanjo et al., 2020). Li et
532 al. (2021) observed changes in the expression of genes involved in cardiac, cardiovascular and nervous
533 system development and the metabolism of xenobiotics in zebrafish embryo (120 hdp) after HTO
534 exposure to a lower dose (3.7×10^7 BqL⁻¹).

535 Even if molecular changes are not always translated into effects at a higher level, transcriptomics
536 represent a “discovery” tool to characterise responses or to further explore biological process (Hook et
537 al. 2014). Moreover, these types of information could be taken together to predict the susceptibility of
538 other species of interest that share similar adverse outcome pathways (AOPs) (McArdle et al., 2020).

539 It is realised that the application and integration of omics approaches for ERA poses significant challenges
540 in the real world. This is in contrast to human health arena where omics approaches are widely adopted
541 to assess the biological responses following exposures to ionising radiations (Brackmann et al., 2020; Fang
542 et al., 2022). In the natural environment, factors such as spatial and temporal variability in the physico-
543 chemical, hydro morphological and seasonal characteristics in addition to species specific factors (e.g. sex,

544 age, reproductive and life stages) are known to influence molecular responses to environmental stressors
545 (Garcia-Reyero and Murphy, 2018; Martyniuk, 2018). Nevertheless, it has been recognised that use of the
546 AOPs as a conduit between omics and ecological responses could facilitate the transition toward a more
547 mechanistically informed hazard and risk assessment (Martyniuk, 2018; Tollefsen et al., 2022; Yahya et
548 al., 2021) . A recent study developed AOP networks for radiation effects on reproduction using data from
549 studies of different model species of primary producers, roundworms, earthworms, crustacean, and fish
550 (Tollefsen et al., 2022). These taxon-focused AOPs were then used to define a common set of events into
551 a consensus AOPs as an effort to gather information spanning different levels of biological organisation
552 which included a high number of species into a common knowledge framework (Tollefsen et al., 2022). In
553 this context, it is suggested that the formulation of a radiation specific AOP will form a framework within
554 which data and knowledge from different organisms are synthesised to obtain useful information for ERA
555 under different environmental conditions (Beresford et al. 2020). Further studies combining the existing
556 information are however required to populate these AOPs with a more diverse set of data. This effort
557 should also aim to reduce the bias towards a few model species. This will provide weight of evidence
558 based assessments in a complex environment (Halappanavar et al., 2020; Portugal et al., 2022; Tollefsen
559 et al., 2022).

560 Other studies have assessed the impact of tritium exposure in the expression of target genes. Devos et
561 al. (2015) studied the impact of chronic exposure of HTO in oysters, and analysed nine genes for heat
562 shock chaperone proteins and members of the detoxification, oxidative stress and cell cycle regulation
563 machineries. However, after 14 days no statistical changes for the genes considered were detected.
564 Clearance rate remained unchanged after tritium exposure and although DNA damage increased, this was
565 likely in the range of sustainable DNA repairing capacity. In mussels, Dallas et al. (2016a) analysed
566 expression of six genes responsible for detoxification, oxidative stress, protein folding, DNA double strand
567 break repair and cell cycle checkpoint. They found that *hsp70*, *hsp90* and *mt20* were upregulated in gills
568 after the mussels were exposed for one hour to HTO (15,000 BqL⁻¹) at 15°C; after 72 hours, *rad51* and *p53*
569 also increased. Interestingly, *hsp70*, *hsp90*, *mt20*, *rad51* and *p53* were downregulated when mussels were
570 exposed to HTO at 25°C. Gene expression results were also correlated with DNA damage, and correlations
571 varied with time but not with temperature. For example, *p53* (gene associated to DNA repair) showed a
572 negative and a positive correlation with DNA damage after 72 h and 168 h exposure, respectively. Further
573 research is required to determine whether these effects are translated to responses at a higher biological
574 level.

575 **6. Knowledge-gaps and future directions**

576 The use of nuclear power is expected to increase in the future, as it is considered essential in the transition
577 to low-carbon economies (Adam-guillermin et al., 2012; Kadiyala et al., 2016; Nie et al., 2019). In
578 particular, tritium (^3H) demand and production is expected to increase due to its potential role in nuclear
579 fusion technology (Jean-Baptiste and Fourné, 2013; Singh et al., 2012). In this context, many nations face
580 having to develop long-term strategies to manage tritiated radioactive waste and develop tools to assess
581 its environmental impact (Bay et al., 2020; Di et al., 2012; Hanslík et al., 2017; Lainetti, 2016; Lamego
582 Simões Filho et al., 2013; Stamper et al., 2014) .

583 Radiological protection for NHB have received increasing attention in the last twenty years, with different
584 international guidelines being developed (Andersson et al., 2009, 2008; IAEA, 2005; ICRP, 2017; UNSCEAR,
585 2016). However, the proposed no-effect dose rate limit (0.24 mGy d^{-1} , $10 \mu\text{Gyh}^{-1}$, Table 3) appears to be
586 inappropriate for some species (Dallas et al., 2016a; Hagger et al., 2005; Jha et al., 2005). This review has
587 shown that available data are heavily biased towards marine bivalves, fish and mammals (i.e. rodents),
588 and mainly cover laboratory rather than field studies and with a focus on tritium exposure as HTO. Lacking
589 are investigations exploring the uptake pathways and consequences of other forms of tritium like OBT
590 (Kim et al., 2013a; Roch-Lefèvre et al., 2018) or tritiated particles (Grisolia et al., 2019; Liger et al., 2018;
591 Smith et al., 2022). The latter include tritium associated with steel and cement particles arising from
592 decommissioning or dismantling of nuclear reactors.

593 For assessing ecotoxicological impact, future research should consider more diverse keystone species
594 from different ecological niches and perform comparative studies, since it has been noted that data
595 available exist for only a few taxonomic groups (Adam-Guillermin et al., 2012). Results from diverse and
596 ecologically relevant species will improve not only the datasets to establish appropriate benchmarks for
597 relative sensitivities of the different species but will also facilitate the development of models used for
598 more general environmental assessments and to study future exposure scenarios (Galeriu and
599 Melintescu, 2011; Melintescu et al., 2011). Research should also investigate aspects of combined
600 anthropogenic and natural stressors, including chemicals (e.g. metals, organics) and physical (e.g.
601 temperature, oxygen and dissolved organic carbon), in risk assessment of radionuclides in the
602 environment (Dallas et al., 2012; Pearson et al., 2018; Vanhoudt et al., 2012).

603 Different responses to tritium exposure, from molecular to behavioural, have been reported in various
604 biota during early life stages, with the potential transmission of effects across generations proposed
605 through epigenetic mechanisms. Although little is known about these mechanisms (Merrifield and

606 Kovalchuk, 2013; Thaulow et al., 2020), as mentioned earlier “omics” techniques could help to fill
607 knowledge gaps and elucidate the relation between molecular and organismal level responses (Dallas et
608 al., 2012; Hurem et al., 2017; Parisot et al., 2015). These tools are utilised to study modes of action and
609 intracellular signalling pathways and do not require a priori molecular targets to specific stressors, making
610 them suitable in exploratory studies (Cambiaghi et al., 2017; del Mar Amador et al., 2018). Despite the
611 “omics” field rapidly evolving, its application to tritium radiobiology is still at a relatively early stage, and
612 only a limited number of studies have implemented them (Arcanjo et al., 2020, 2018; Gagnaire et al.,
613 2017; Li et al., 2021). In addition, post-1990 ICRP Recommendations have identified (i) induced genomic
614 instability (ii) bystander effects and (iii) minisatellite mutation induction in germ line as novel, real
615 radiobiological phenomenon (Goodhead et al., 2004). These biological phenomenon need to be
616 elaborated while assessing impact of tritium on NHB. Furthermore, in common with other radionuclides,
617 potential epigenetic effects also need to be explored (Horemans et al., 2018, 2019).

618 There is also an increasing interest in developing ecosystem-based approaches to environmental risk
619 management (Dallas et al., 2012; Bréchnac, 2017; Mothersill et al., 2020; Rhodes et al., 2020). To the
620 best of our knowledge, there is no study assessing the impact of tritium at the ecosystem level.
621 Furthermore, most studies reviewed have been carried out using very high concentrations of tritium,
622 which makes it possible to discover mechanisms of action and detect potential effects at higher levels of
623 biological organisation. These studies however not always reflect the damage observed in a realistic
624 exposure scenario (Brechignac et al., 2004; De Smet et al., 2017). Scientists from the ecological and
625 radiological fields have been deliberating the feasibility and challenges of reintegrating ecosystem science
626 into radioecology (Beresford et al., 2020; Mothersill et al., 2020; Rhodes et al., 2020). Ecological studies
627 in areas with above-background levels of radiation are limited, atypical and often inconclusive or
628 controversial (Dallas et al., 2012; Fuller et al., 2015; Beresford et al., 2020; Rhodes et al., 2020). The
629 challenges in the field of environmental radioactivity require to be addressed through adoption of robust
630 scientific approaches, unambiguous reporting and sharing of expertise (Beresford et al., 2020). Studies
631 carried out in microcosms, mesocosms and natural field conditions can produce appropriate data for the
632 construction of mathematical and computational models when planned in a coordinated way. Finally,
633 beyond these modelling approaches, current developments in artificial intelligence including machine
634 learning approaches offer novel opportunities to gain insights from large data sets in complex
635 environments contaminated with radionuclides (Shuryak, 2017, 2022). These will represent potentially
636 useful tools to assess biological impact of tritium and other environmentally relevant radionuclides
637 (Mothersill et al., 2020; Rhodes et al., 2020).

638 **Declaration of competing interest:** The authors declare that they have no known competing financial
639 interests or personal relationships that could have appeared to influence the work reported in this paper.

640 **Acknowledgements:** The work has been carried out within the TRANSAT project (<https://transat->
641 [h2020.eu/](https://transat-h2020.eu/)) which received funding from the Euratom research and innovation programme 2014–2018
642 (grant agreement No. 754586). The opinions expressed herein reflect only the authors' views and do not
643 necessarily reflect those of the European Commission.

644 Author Contribution:

645 **MFF:** data curation, methodology, writing-original draft, validation, reviewing and editing

646 **AT:** visualization, formal analysis, reviewing and editing

647 **ELV:** data curation, writing-original draft

648 **CG:** reviewing and editing; funding acquisition, project administration

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650 **VM:** reviewing and editing; funding acquisition, validation, project administration

651 **ANJ:** conceptualisation, visualization, formal analysis, validation, reviewing and editing, supervision,
652 funding acquisition, project administration

653

654

655 **Figure Legends**

656 **Figure 1.** Tritium sources, transport and dispersal in the environment.

657 **Figure 2.** Global distribution of tritium in water (average values from UNSCEAR 2016; Oms et al.
658 2019).

659 **Figure 3.** Papers published evaluating the effects of tritium on biota from 1955 to 2022.
660 Publications were excluded if no English translation was available.

661 **Figure 4.** Taxa used in field and laboratory studies from papers published between 1955–2022.
662 Articles examining two or more species were counted and/or categorised multiple times.

663 **Figure 5.** End-points used at different levels of biological organisation to study effects of tritium in
664 papers published between 1955-2022. Articles examining end-points at two or more
665 levels of organisation were counted and/or categorised multiple times.

666

667 **Table Captions:**

668

669 **Table 1.** Tritium limits in drinking water (BqL^{-1}) proposed by different organisations and countries.
670 Adapted from (Canadian Nuclear Safety Commission, 2008).

671 **Table 2.** Average concentrations of tritium (BqL^{-1}) in areas with a high density of nuclear facilities, and
672 site-specific limits established for tritium waterborne discharges (Bqyear^{-1}). Darlington :
673 Heavy-Water moderated and cooled reactor ; Hartlepool: nuclear power station; La
674 Hague: fuel reprocessing plant; Olkiluoto and Loviisa: nuclear power plant reactors;
675 Sellafield: fuel reprocessing plant.

676 **Table 3.** Numerical screening values (μGyh^{-1}) proposed by different organisations and directives for the
677 protection of diverse organism groups.

678 **Table 4.** Overview of the effects of tritium observed in laboratory studies involving different terrestrial species.
679 Exposure levels represent nominal concentrations of HTO unless otherwise stated (OBT was
680 contained in amino acids).

681 **Table 5.** Overview of the effects of tritium observed in laboratory studies involving different aquatic species.
682 Exposure levels represent nominal concentrations of HTO unless otherwise stated (OBT was
683 contained in amino acids).

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- 1353

1354 **Tables: Ferreira et al. (Tritium review)**

1355 **Table 1.** Tritium limits in drinking water (BqL⁻¹) proposed by different organisations and countries.
 1356 Adapted from (Canadian Nuclear Safety Commission, 2008).

1357

Organisation/ Country	Tritium concentration limit (BqL ⁻¹)
Canada Nuclear Safety Commission	7000
United States Environmental Protection Agency	740
World Health Organization	10,000
Australia	76,100
Canada	7000
EU	10,000 ¹
Finland	30,000
Russia	7700
Switzerland	10,000
United States	740

1358

1359 ¹ The EU Commission did not make the requirements for radioactivity mandatory, but only indicative.
 1360 Tritium was cited as an indicator parametric value at 100 BqL⁻¹. The 100 BqL⁻¹ parameter is effectively a
 1361 screening value, providing an indication of the possible presence of other, potentially more harmful,
 1362 artificial radionuclides discharged into the environment. Both the tritium concentration and the total
 1363 indicative dose have a similar status, indicating a potential radiological problem when exceeded, and
 1364 should not be regarded as limit values.

1365

1366 **Table 2.** Average concentrations of tritium (BqL⁻¹) in areas with a high density of nuclear facilities, and
 1367 site-specific limits established for tritium waterborne discharges (Bqyear⁻¹). Darlington : Heavy-Water
 1368 moderated and cooled reactor ; Hartlepool: nuclear power station; La Hague: fuel reprocessing plant;
 1369 Olkiluoto and Loviisa: nuclear power plant reactors; Sellafield: fuel reprocessing plant.

1370

Location	Tritium in areas with high density of nuclear facilities (BqL ⁻¹)	References
Fourmile Branch (USA)	1.79	(Yu et al., 2020)
Lake Ontario (Canada)	4.76	(Dove et al., 2021)
Rhône river (France)	6.31	(Jean-Baptiste et al., 2018)
	Site-specific limits for nuclear facilities (Bqyear ⁻¹)	References
Darlington (Canada)	4.3×10^{18}	(Canadian Nuclear Safety Commission, 2012)
Hartlepool (UK)	1×10^{13}	(RIFE, 2019)
Loviisa (Lithuania)	1.50×10^{14}	(Masionis et al., 2008)
La Hague (France)	1.85×10^{16}	(Schneider and Marignac, 2008)
Olkiluoto (Lithuania)	1.83×10^{13}	(Masionis et al., 2008)
Sellafield (UK)	1.8×10^{16}	(RIFE, 2019)
Wolsong (South Korea)	3.48×10^{14}	(Feng and Zhuo, 2022)

1371

1372 **Table 3.** Numerical screening values (μGyh^{-1}) proposed by different organisations and directives for the
 1373 protection of diverse organism groups.

	Aquatic organisms		Terrestrial organisms		References
	<i>Freshwater</i>	<i>Marine</i>	<i>Invertebrates</i>	<i>Vertebrates</i>	
ERICA	10	10	10	10	(Beresford et al., 2004)
FASSET	100	100	100	100	(Larsson, 2004)
IAEA	400		40	40	(IAEA, 1992)
ICRP¹	4-40 ² , 40-400 ³	400-4000 ⁴ , 40-400 ⁵	400-4000 ⁷	4-40 ⁸	(ICRP, 2008)
NCRP	400	400			(Templeton et al., 1991)
PROTECT	2; 200 ⁶	2; 200 ⁶	200	2	(Andersson et al., 2009)
UNSCEAR			400	100	(UNSCEAR, 2008)

1374

1375 ¹Range values are not intended to be regarded as dose limits, but as dose rates at which evaluation of the
 1376 situation would be warranted. ²Dose level for reference frog. ³Dose level for reference freshwater fish
 1377 (trout). ⁴Dose level for reference crab. ⁵Dose level for reference marine fish (flatfish). ⁶Dose levels for
 1378 vertebrates ($2 \mu\text{Gyh}^{-1}$) and invertebrates ($200 \mu\text{Gyh}^{-1}$). ⁷Dose levels for reference bee and earthworm.

1379 ⁸Dose levels for reference deer, rat and duck.

1380

1381 NCRP=National Council on Radiation Protection; IAEA= International Atomic Energy Agency; FASSET=
 1382 Framework for ASSESSment of Environmental impact; ICRP= International Commission on Radiological
 1383 Protection; UNSCEAR= United Nations Scientific Committee on the Effects of Atomic Radiation. ERICA
 1384 project= Environmental Risk from Ionising Contaminants: Assessment and Management; PROTECT project
 1385 = Protection of the Environment from Ionising Radiation in a Regulatory Context.

1386

1387 **Table 4.** Overview of the effects of tritium observed in laboratory studies involving different terrestrial species.
 1388 Exposure levels represent nominal concentrations of HTO unless otherwise stated (OBT was contained in amino
 1389 acids).

Concentration/ dose	Duration/ time point	Observed effects	Reference
Insects- <i>Chironomus riparius</i> (transgenerational)			
9250 x 10 ⁶ Bq ⁻¹	20 days	Chromosomes aberrations in larvae from adults developed in HTO.	(Blaylock and Griffith, 1971)
18500 x 10 ⁶ Bq ⁻¹	20 days	Chromosomes aberrations in larvae from adults developed in HTO.	(Blaylock and Griffith, 1971)
Mammalia- <i>Mus musculus</i>			
18.5 x 10 ³ Bqg ⁻¹ (IP injection)	2, 4, 7 ,14 days	Increased MN (except at 14 days) and % tail DNA. Decrease of white blood cells and platelet count on day 2.	(H. Li et al., 2021)
10 x 10 ³ BqL ⁻¹ (HTO or OBT)	1 and 8 months	Decrease in red blood cells (Rbc) and iron deprivation was seen in all OBT exposed groups after 1 month. Rbc decrease and increase in mean globular volume after 8 months.	(Bertho et al., 2019)
1 x 10 ⁶ BqL ⁻¹ (HTO or OBT)	1 and 8 months	Decrease in red blood cells (Rbc) and iron deprivation was seen in all OBT exposed groups after 1 month. Rbc decrease and increase in mean globular volume after 8 months.	(Bertho et al., 2019)
20 x 10 ⁶ BqL ⁻¹ (HTO or OBT)	1 and 8 months	Decrease in red blood cells (Rbc) and iron deprivation was seen in all OBT exposed groups after 1 month. Rbc decrease and increase in mean globular volume after 8 months.	(Bertho et al., 2019)
0.01 x 10 ⁶ BqL ⁻¹ (HTO or OBT)	1 and 8 months	No chromosome aberrations.	(Roch-Lefèvre et al., 2018)
1 x 10 ⁶ BqL ⁻¹ (HTO or OBT)	1 and 8 months	Increased levels of chromosome aberrations after 1 month (exposed to HTO and OBT) and 8 months (exposed to OBT).	(Roch-Lefèvre et al., 2018)
20 x 10 ⁶ BqL ⁻¹	1 and 8 months	Increased levels of chromosome aberrations after 1 month (exposed to HTO and OBT) and 8 months (exposed to OBT).	(Roch-Lefèvre et al., 2018)
0.3 x 10 ⁶ BqL ⁻¹	15 days	No induction of oxidative stress.	(Kelsey-Wall et al., 2006)
111 x 10 ⁶ BqL ⁻¹	90, 330, 500, 560, 700 days	Increased in chromosome aberration frequency in all groups except 500, 560 days exposure.	(Brooks et al., 1976)
0.01 x 10 ⁶ BqL ⁻¹	1 and 8 months	No effect in spleen weight or DNA inversions in spleen.	(Bannister et al., 2016)
1 x 10 ⁶ BqL ⁻¹	1 and 8 months	No effect in spleen weight or DNA inversions in spleen.	(Bannister et al., 2016)
20 x 10 ⁶ BqL ⁻¹	1 and 8 months	No effect in spleen weight or DNA inversions in spleen.	(Bannister et al., 2016)
Mammalia- <i>Mus musculus</i> (embryo)			

0.37 x 10 ⁶ BqL ⁻¹ (OBT)	96 hours	Decrease in survival	(Clerici et al., 1984)
3.7 x 10 ⁶ BqL ⁻¹ (OBT)	96 hours	Decrease in survival	(Clerici et al., 1984)
37 x 10 ⁶ BqL ⁻¹ (OBT)	96 hours	Decrease in survival	(Clerici et al., 1984)
Mammalia- <i>Mus musculus</i> (transgenerational)			
100 x 10 ³ μGy	56 days	Reduction in the pyramidal cell densities.	(Sun et al., 1997)
200 x 10 ³ μGy	56 days	Reduction in the pyramidal cell densities.	(Sun et al., 1997)
400 x 10 ³ μGy	56 days	Reduction in the pyramidal cell densities. Decrease in brain weight and reduction of thickness of cerebral cortex.	(Sun et al., 1997)
800 x 10 ³ μGy	56 days	Reduction in the pyramidal cell densities. Decrease in brain weight and reduction of thickness of cerebral cortex.	(Sun et al., 1997)
0.111 x 10 ⁹ BqL ⁻¹ bw IP injection + 0.074 x 10 ⁹ BqL ⁻¹ TDW	17 th day of gestation until parturition	Cerebellum alterations in 1, 2 and 3 week age groups of mice in terms of degeneration and loss of Purkinje cells.	(Jain and Bhatia, 1996)
0.011 x 10 ⁹ BqL ⁻¹ bw IP injection + 0.074 x 10 ⁹ BqL ⁻¹ TDW	17 th day of gestation until parturition	Intensified damage in cerebellum in terms of degeneration and loss of Purkinje cells.	(Jain and Bhatia, 1996)
50 x 10 ³ μGy (IP injection)	21 days	No neurobehavioural effects.	(Wang and Zhou, 1995)
100 x 10 ³ μGy (IP injection)	21 days	Difficulties in learning and memory retention for skill performance.	(Wang and Zhou, 1995)
300 x 10 ³ μGy (IP injection)	21 days	Difficulties in learning and memory retention for skill performance.	(Wang and Zhou, 1995)
111 x 10 ⁶ BqL ⁻¹	F0 treated before pregnancy until birth of F1.	Decrease in DNA and protein content in specific parts of the brain in newborns (F1).	(Zamenhof and Van Marthens, 1981)
111 x 10 ⁶ BqL ⁻¹	F0 treated before pregnancy and after birth (F1).	Decrease in DNA and protein content in specific parts of the brain adolescence (F1).	(Zamenhof and Van Marthens, 1981)
111 x 10 ⁶ BqL ⁻¹	30 days before pregnancy through 5 generations.	Decrease in DNA and protein content in specific parts of the brain in adults.	(Zamenhof and Van Marthens, 1981)
111 x 10 ⁶ BqL ⁻¹	F0 treated before pregnancy until birth of F1.	60% of newborns (F1) with hematomas and edemas.	(Zamenhof and Van Marthens, 1979)
111 x 10 ⁶ BqL ⁻¹	F0 treated before pregnancy and after birth (F1).	Increase in alkaline phosphatase in blood (F1).	(Zamenhof and Van Marthens, 1979)

111 x 10 ⁶ BqL ⁻¹	30 days before pregnancy through 5 generations.	Decrease of DNA content (F1,F3,F4) and protein content (F1,F2,F4, F5) in brain.	(Zamenhof and Van Marthens, 1979)
111 x 10 ⁶ BqL ⁻¹	98 days	Reduction in viable embryo	(Carsten and Commerford, 1976)
3.145 x 10 ⁶ BqL ⁻¹	From conception until 14 days of age	Decrease in oocytes in all treated groups	(Lowry Dobson and Cooper, 1974)
31.45 x 10 ⁶ BqL ⁻¹	From conception until 14 days of age	Decrease in oocytes in all treated groups	(Lowry Dobson and Cooper, 1974)
314.5 x 10 ⁶ BqL ⁻¹	From conception until 14 days of age	Decrease in oocytes in all treated groups	(Lowry Dobson and Cooper, 1974)
0.0185 x 10 ⁶ BqL ⁻¹ bw	4 days	No observed effect.	(Johnson and Cronkite, 1959)
0.037 x 10 ⁶ BqL ⁻¹ bw	4 days	No observed effect.	(Johnson and Cronkite, 1959)
0.185 x 10 ⁶ BqL ⁻¹ bw	4 days	Decrease of spermatocytes.	(Johnson and Cronkite, 1959)
0.37 x 10 ⁶ BqL ⁻¹ bw	4 days	Decrease of spermatocytes.	(Johnson and Cronkite, 1959)
0.74 x 10 ⁶ BqL ⁻¹ bw	4 days	Decrease of spermatocytes.	(Johnson and Cronkite, 1959)
Mammalia- <i>Rattus norvegicus</i> (transgenerational)			
74 x 10 ⁶ BqL ⁻¹	14 and 21 days.	No differences in MN frequency in blood samples.	(Lee et al., 2019)
740 x 10 ⁶ BqL ⁻¹	14 and 21 days.	No differences in MN frequency in blood samples.	(Lee et al., 2019)
0.24 x 10 ⁶ Bqg ⁻¹ bw	One IP injection on day 13 th of gestation	Delay in learning ability and decrease in memory. Lower number of hippocampal pyramidal cells.	(Gao et al., 1999)
0.48 x 10 ⁶ Bqg ⁻¹ bw	One IP injection on day 13 th of gestation	Delay in learning ability and decrease in memory. Lower number of hippocampal pyramidal cells.	(Gao et al., 1999)
1.44 x 10 ⁶ Bqg ⁻¹ bw	One IP injection on day 13 th of gestation	Delay in learning ability and decrease in memory. Lower body and brain weight. Degeneration and malformation of neurons cultured. Decrease of Ca ²⁺ conductance in hippocampal pyramidal cells.	(Gao et al., 1999)
370 x 10 ⁶ BqL ⁻¹ bw	42 days starting from conception	At 300 days of age, increased concentration of FSH and decreased concentrations of both NE and DA. Reduction in weight of the testes and sperm count. Fewer offspring (F2) but with increased body weight.	(Laskey and Bursian, 1976)

370 x 10 ⁶ BqL ⁻¹ bw	42 days starting the day of birth	At 300 days of age, increased concentration of FSH, and decreased concentrations of both NE and DA. Reduction in weight of the testes and sperm count. Reduction in weight of the ovaries. Increased body weight and decreased of brain weight of offspring (F2)	(Laskey and Bursian, 1976)
370 x 10 ⁶ BqL ⁻¹ bw	42 days starting at 42 days of age	At 300 days of age, increased concentration of FSH, and decreased concentrations of both NE and DA. Alteration in pituitary weight	(Laskey and Bursian, 1976)
370 x 10 ⁶ BqL ⁻¹ bw	42 days starting at 74 days of age	Decreased concentrations of both NE and DA at 300 days. Increased concentration of FSH. Increased body weight of offspring (F2). Alteration in body weight and relative brain weigh.	(Laskey and Bursian, 1976)
37 x 10 ⁶ BqL ⁻¹ bw	22 days (gestation period)	No effect observed in lifespan, overall neoplasia incidence, incidence rate or onset of mammary fibroadenomas.	(Cahill et al., 1975)
370 x 10 ⁶ BqL ⁻¹ bw	22 days (gestation period)	No effect observed in lifespan, overall neoplasia incidence, incidence rate or onset of mammary fibroadenomas.	(Cahill et al., 1975)
1850 x 10 ⁶ BqL ⁻¹ bw	22 days (gestation period)	Sterile F1. Low incidence of mammary adenomas.	(Cahill et al., 1975)
3700 x 10 ⁶ BqL ⁻¹ bw	22 days (gestation period)	Sterile F1. Lower incidence of mammary adenomas and overall neoplasia. Reduced mean lifespan.	(Cahill et al., 1975)
0.37 x 10 ⁶ BqL ⁻¹ bw	From conception until 125 days of age	Reduction in liver and kidney weight (F2).	(Laskey et al., 1973)
3.7 x 10 ⁶ BqL ⁻¹ bw	From conception until 125 days of age	Reduction in brain weight (F2).	(Laskey et al., 1973)
37 x 10 ⁶ BqL ⁻¹ bw	From conception until 125 days of age	Reduction in brain weight and body weight (F2).	(Laskey et al., 1973)
370 x 10 ⁶ BqL ⁻¹ bw	From conception until 125 days of age	Reduction weight of testicles but no impairment of growth or reproductive ability (F1). Reduction in brain and body weights, reduction of litter size (F2).	(Laskey et al., 1973)
37 x 10 ⁶ BqL ⁻¹ bw	21 days	Increased length in both sexes, liver and heart weight.	(Cahill and Yuile, 1971)
370 x 10 ⁶ BqL ⁻¹ bw	21 days	Reduction of testes and brain.	(Cahill and Yuile, 1971)
740 x 10 ⁶ BqL ⁻¹ bw	21 days	Reduction of testes, brain, spleen, thymus, kidney and heart.	(Cahill and Yuile, 1971)
1850 x 10 ⁶ BqL ⁻¹ bw	21 days	Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart.	(Cahill and Yuile, 1971)
2775 x 10 ⁶ BqL ⁻¹ bw	21 days	Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart.	(Cahill and Yuile, 1971)
3700 x 10 ⁶ BqL ⁻¹ bw	21 days	Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart. Reduction in litter size.	(Cahill and Yuile, 1971)

1390 Bw = body weight, TDW = tritiated drinking water, NE = norepinephrine, DA = dopamine, IP = intraperitoneal,
 1391 MN = micronucleus, FSH = follicular stimulating hormone.

1392
 1393 **Table 5.** Overview of the effects of tritium observed in laboratory studies involving different aquatic species.
 1394 Exposure levels represent nominal concentrations of HTO unless otherwise stated (OBT was contained in amino
 1395 acids).
 1396

Concentrations/ Dose	Duration/ time point	Observed effects	Reference
Polychaeta- <i>Ophryotrocha diadema</i> (transgenerational)			
2240 x 10 ⁶ BqL ⁻¹	22 days	Reduction in survival of eggs.	(Knowles and Greenwood, 1997)
Crustacea- <i>Crassostrea gigas</i>			
1 x 10 ⁶ BqL ⁻¹	14 days	DNA damage in haemocytes.	(Devos et al., 2015)
15 x 10 ⁶ BqL ⁻¹	14 days	DNA damage in haemocytes.	(Devos et al., 2015)
Crustacea- <i>Daphnia magna</i> (transgenerational)			
0.5 x 10 ² BqL ⁻¹	5 generations	Reduction in growth rate. No alteration in the onset of sexual maturation. Decrease in fecundity and offspring survival. Increase in abnormalities during embryogenesis. Increase in number and diameter of nucleoli.	(Gudkov and Kipnis, 1996)
0.5 x 10 ⁶ BqL ⁻¹	5 generations	Reduction in growth rate. No alteration in the onset of sexual maturation. Decrease in fecundity and offspring survival. Increase in abnormalities during embryogenesis. Increase in number and diameter of nucleoli.	(Gudkov and Kipnis, 1996)
500 x 10 ⁶ BqL ⁻¹	17 days (F1)	Reduction in growth rate. 100% of offspring mortality.	(Gudkov and Kipnis, 1996)
Crustacea- <i>Pollicipes polymerus</i> (larvae)			
0.00037 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
0.0037 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
0.037 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
0.37 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
3.7 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
37 x 10 ⁶ BqL ⁻¹	32 days	Alteration in molting index.	(Abbott and Mix, 1979)
Mollusca- <i>Mytilus galloprovincialis</i>			
5 x 10 ⁶ BqL ⁻¹	14 days	DNA damage in haemocytes.	(Pearson et al., 2018)

5 x 10 ⁶ BqL ⁻¹ (HTO) + 383 nM (Zn)	14 days	No genotoxic effect.	(Pearson et al., 2018)
5 x 10 ⁶ BqL ⁻¹ (HTO) + 1913 nM (Zn)	14 days	No genotoxic effect.	(Pearson et al., 2018)
5 x 10 ⁶ BqL ⁻¹ (HTO) + 3825 nM (Zn)	14 days	No genotoxic effect.	(Pearson et al., 2018)
15 x 10 ⁶ BqL ⁻¹ at 15°C	12, 72, 168 hours	DNA damage in haemocytes only at 168h. Gene expression of hsp-70, hsp-90, mt20 and p53 was upregulated at 72 h.	(Dallas et al., 2016)
15 x 10 ⁶ BqL ⁻¹ at 25°C	12, 72, 168 hours	DNA damage in haemocytes. Gene expression of hsp-70, hsp-90, mt20 and p53 was down-regulated at 72 h.	(Dallas et al., 2016)
Mollusca- <i>Mytilus edulis</i>			
0.122 x 10 ³ µGy h ⁻¹	7 days	Induction of micronuclei in haemocytes.	(Jaeschke et al., 2011)
0.079 x 10 ³ µGy h ⁻¹	14 days	Induction of micronuclei in haemocytes.	(Jaeschke et al., 2011)
0.0049 x 10 ³ µGy h ⁻¹ (OBT)	7 days	Induction of micronuclei in haemocytes.	(Jaeschke et al., 2011)
0.012 x 10 ³ µGy h ⁻¹	96 hours	DNA damage in haemocytes.	(Jha et al., 2006)
0.121 x 10 ³ µGy h ⁻¹	96 hours	DNA damage in haemocytes.	(Jha et al., 2006)
0.485 x 10 ³ µGy h ⁻¹	96 hours	DNA damage in haemocytes.	(Jha et al., 2006)
Mollusca- <i>Mytilus edulis</i> (embryo-larvae)			
0.37 x 10 ⁶ BqL ⁻¹	24 hours	Differential pattern of random amplified polymorphic DNA. Decrease of proliferative rate index. Decrease of normal larvae and survival.	(Hagger et al., 2005)
3.7 x 10 ⁶ BqL ⁻¹	24 hours	Differential pattern of random amplified polymorphic DNA. Induction of Sister chromatid exchange and chromosomal aberration. Decrease of proliferative rate index. Decrease of normal larvae and survival.	(Hagger et al., 2005)
37 x 10 ⁶ BqL ⁻¹	24 hours	Differential pattern of random amplified polymorphic DNA. Induction of Sister chromatid exchange and chromosomal aberration. Decrease of proliferative rate index. Decrease of normal larvae and survival.	(Hagger et al., 2005)
370 x 10 ⁶ BqL ⁻¹	24 hours	Differential pattern of random amplified polymorphic DNA. Induction of Sister chromatid exchange and chromosomal aberration. Decrease of proliferative rate index. Decrease of normal larvae and survival.	(Hagger et al., 2005)
Teleostei- <i>Carassius gibelio</i> (larvae)			

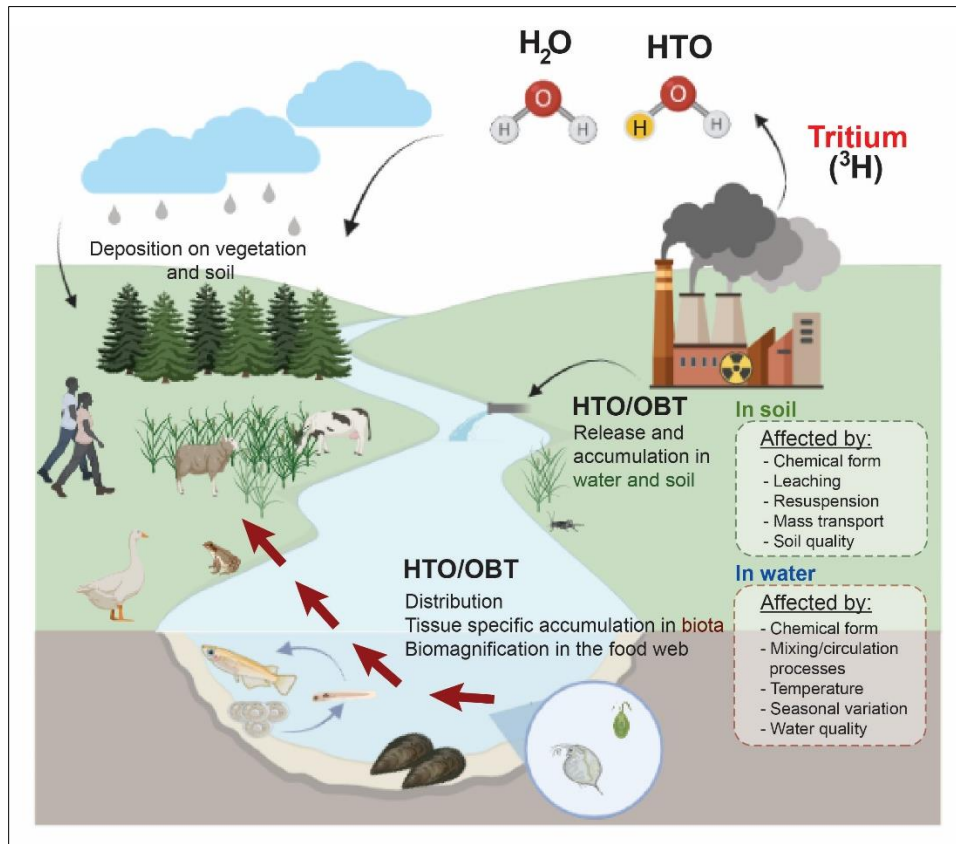
$0.05 \times 10^3 \text{ BqL}^{-1}$	672 hours	Abnormal larvae. Greater length than control.	(Bondareva, 2017)
$0.5 \times 10^3 \text{ BqL}^{-1}$	672 hours	Abnormal larvae. Greater length than control.	(Bondareva, 2017)
$5 \times 10^3 \text{ BqL}^{-1}$	672 hours	Abnormal larvae. Greater length than control.	(Bondareva, 2017)
$50 \times 10^3 \text{ BqL}^{-1}$	672 hours	Abnormal larvae. Greater length than control.	(Bondareva, 2017)
Teleostei- <i>Danio rerio</i> (embryo-larvae)			
$3.7 \times 10^6 \text{ BqL}^{-1}$	60 and 120 hpf	Decrease in hatching rate. Decrease in swimming activity.	(S. Li et al., 2021)
$37 \times 10^6 \text{ BqL}^{-1}$	60 and 120 hpf	Decrease in hatching rate, swimming activity and hormone (T3, T4) concentration. Alteration in gene expression.	(S. Li et al., 2021)
$370 \times 10^6 \text{ BqL}^{-1}$	61 and 120 hpf	Increase in heartbeat. Decrease in swimming activity.	(S. Li et al., 2021)
$0.4 \times 10^3 \mu\text{Gy h}^{-1}$	24, 72, 96 hours	Lower swimming velocity in 96 hpf larvae.	(Arcanjo et al., 2020)
$4 \times 10^3 \mu\text{Gy h}^{-1}$	24, 72, 96 hours	No effect in swimming behaviour.	(Arcanjo et al., 2020)
$10 \times 10^6 \text{ BqL}^{-1}$	24 hpf to 10 dpf	Higher egg diameter at 24 hpf, and higher yolk bag diameter at 3 and 7 dpf. Lower larvae length and less DNA damage at 4 dpf. Degradation of myofibrils with an alteration of actin and myosin filaments at 4 dpf.	(Gagnaire et al., 2020)
10^8 BqL^{-1}	24 hpf to 10 dpf	Higher DNA damage than controls at 4 dpf. ROS-stimulated levels were higher for 4-,7-, and 10-dpf larvae. Degradation of myofibrils with an alteration of actin and myosin filaments at 4dpf.	(Gagnaire et al., 2020)
10^9 BqL^{-1}	24 hpf to 10 dpf	Higher DNA damage and ROS stimulation index than controls at 4 dpf. Degradation of myofibrils in tail muscle at 4dpf.	(Gagnaire et al., 2020)
$0.4 \times 10^3 \mu\text{Gy h}^{-1}$	24, 96 h	Genes related to muscle contraction and eye opacity were upregulated after 24 h. Genes involved in ROS scavenging were differentially expressed after 24 h. Genes involved in DNA repair were enhanced at 96 h.	(Arcanjo et al., 2018)
$4 \times 10^3 \mu\text{Gy h}^{-1}$	24, 96 hours	Genes related to muscle contraction and eye opacity were down-regulated. Genes involved in DNA repair were enhanced at 24 and 96 h. Sarcomeres structure disruption after 96 h.	(Arcanjo et al., 2018)
Teleostei- <i>Fugu niphobles</i> (embryo-larvae)			

$37 \times 10^5 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^7 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^9 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^{10} \text{ BqL}^{-1}$	130 hours	Inactive larvae. Smaller bodies with swollen abdomens. Smaller eye diameter.	(Suyama and Ichikawa, 1974)
Teleostei- <i>Oncorhynchus mykiss</i>			
$0.030 \times 10^6 \text{ BqL}^{-1}$ (OBT)	126, 146 days	No effect observed.	(Festarini et al., 2019)
$0.007 \times 10^6 \text{ BqL}^{-1}$	126, 146 days	No effect observed.	(Festarini et al., 2019)
$0.03 \text{ (OBT) + } 0.007 \text{ (HTO)} \times 10^6 \text{ BqL}^{-1}$	126, 146 days	Different fatty acid composition when compared with the controls after 126 days.	(Festarini et al., 2019)
Teleostei- <i>Oryzias latipes</i> (embryo)			
$3700 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$7400 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$14800 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$18500 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$27750 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$37000 \times 10^6 \text{ BqL}^{-1}$	10 days	Decrease in the number of germ cells.	(Hyodo-Taguchi and Etoh, 1986)
$370 \times 10^6 \text{ BqL}^{-1}$	240 days	Decrease in the number of germ cells.	(Hyodo Taguchi and Egami, 1977)
Teleostei- <i>Paralichthys olivaceus</i> (embryo)			
370 BqL^{-1}	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^3 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^5 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
$37 \times 10^7 \text{ BqL}^{-1}$	180 hours	No effect in hatchability.	(Suyama and Ichikawa, 1974)
Teleostei- <i>Pimephales promelas</i> (embryo)			
$0.012 \times 10^6 \text{ BqL}^{-1}$	60 and 120 days	Increased DNA damage in gonad at 120 days.	(Gagnaire et al., 2018)
$0.025 \times 10^6 \text{ BqL}^{-1}$	60 and 120 days	Increased DNA damage in gonad at 120 days.	(Gagnaire et al., 2018)

1397 dpf = days post fertilisation; hpf = hours post fertilization.

1398 **Figures: Ferreira et al. (Tritium review)**

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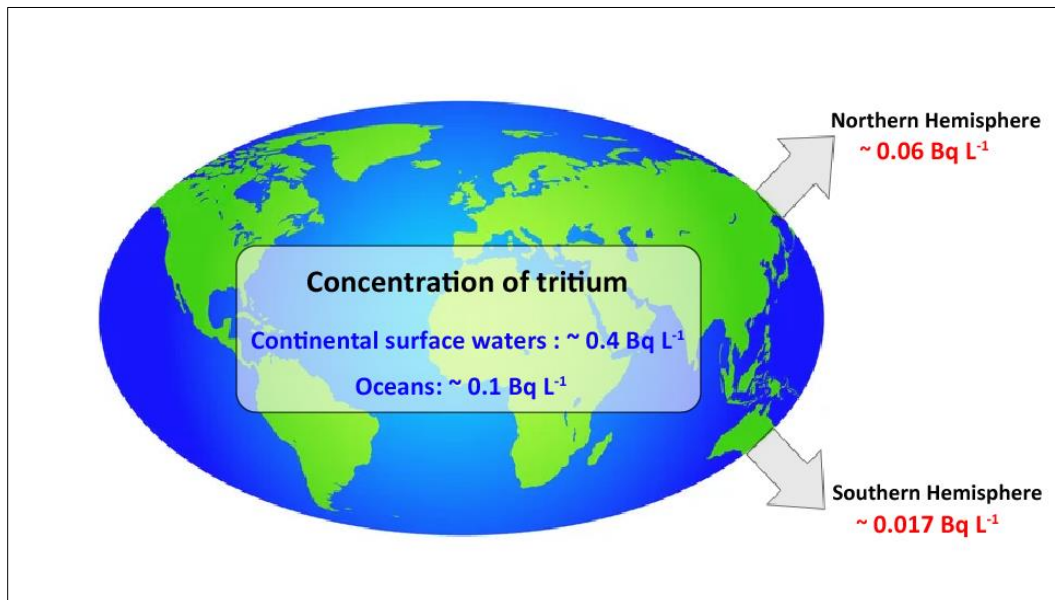


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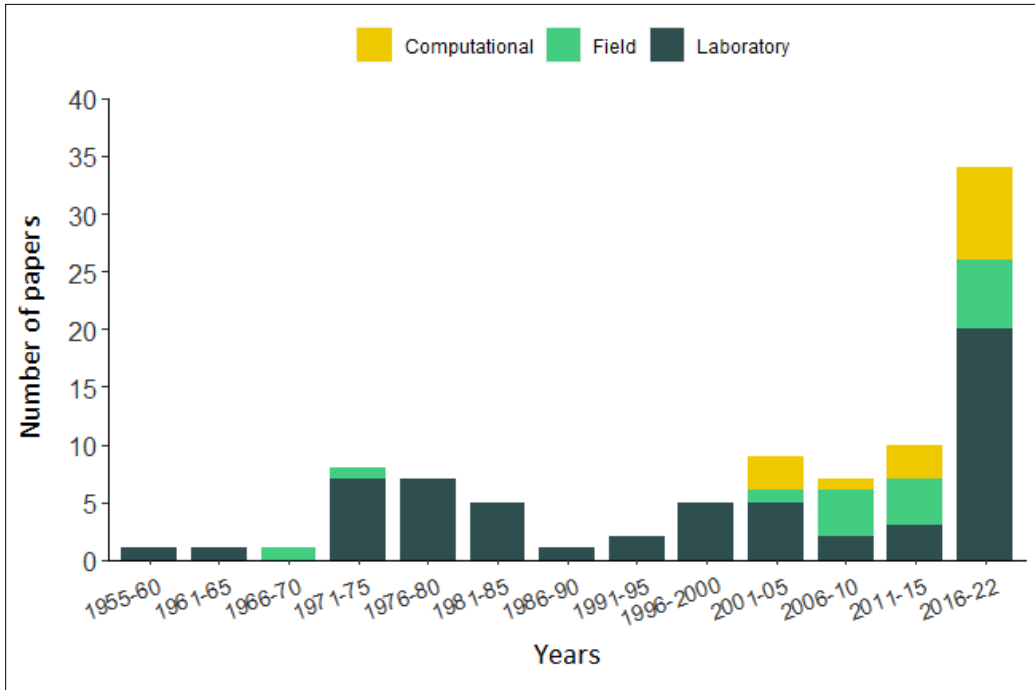
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Figure 1. Tritium sources, transport and dispersal in the environment.

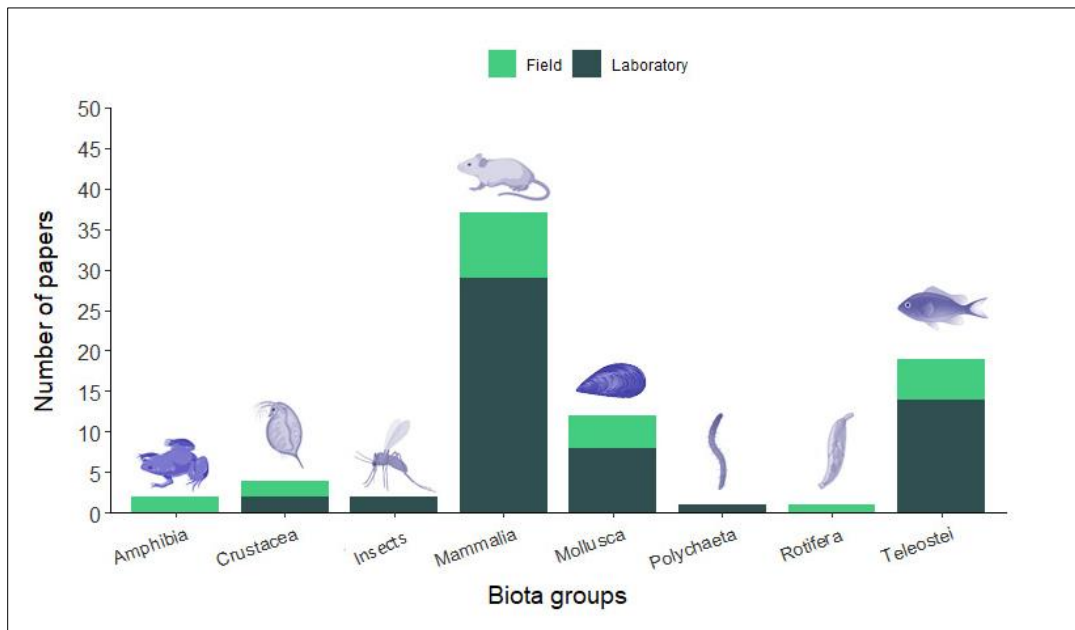


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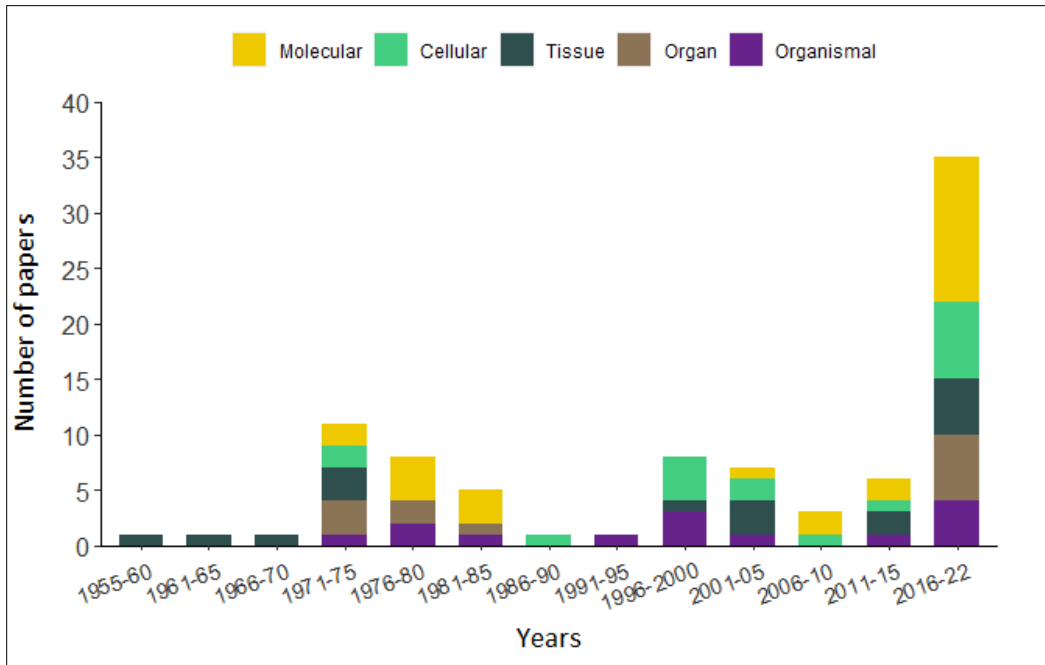
1404 **Figure 2.** Global distribution of tritium in water (average values from UNSCEAR 2016; Oms et al. 2019).
 1405



1406
 1407 **Figure 3.** Papers published evaluating the effects of tritium on biota from 1955 to 2022. Publications
 1408 were excluded if no English translation was available.



1409
 1410 **Figure 4.** Taxa used in field and laboratory studies from papers published between 1955–2022. Articles
 1411 examining two or more species were counted and/or categorised multiple times.



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Figure 5. End-points used at different levels of biological organisation to study effects of tritium in papers published between 1955-2022. Articles examining end-points at two or more levels of organisation were counted and/or categorised multiple times.

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