



Review

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

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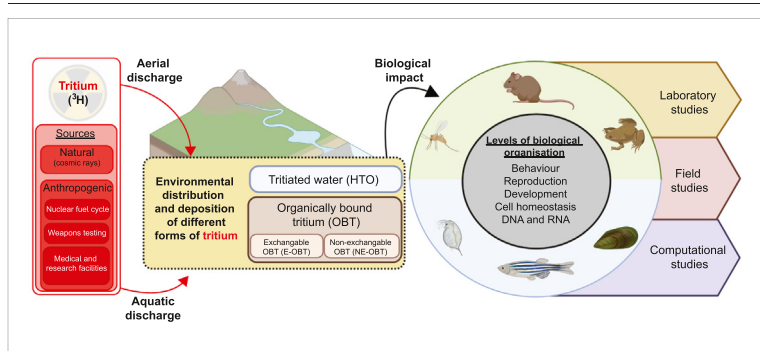
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HIGHLIGHTS

- Tritium (³H) emissions likely to further increase due to expansion of nuclear processes.
- ³H is quickly integrated into the environment and biological systems.
- Sources, properties and effects of ³H in non-human biota (NHB) critically examined.
- Studies in NHB are inclined towards bivalves, fish and rodents.
- Integrated approaches required to more comprehensively assess the impact of ³H.

GRAPHICAL ABSTRACT



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ABSTRACT

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

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1. Introduction

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

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

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

Once in the environment, tritium reacts with oxygen and is quickly integrated into numerous cycles of the biosphere as tritiated water (HTO) (Fig. 1). Due to its chemical properties, it is extremely mobile in biological systems (Bay et al., 2020; Hanslík et al., 2017; Larsen and Babineau, 2020) and may be found in all hydrogenated molecules and associated water in the biosphere (Ducros et al., 2018; Eyrolle et al., 2018). Tritium exists in tissues in three forms: (i) tissue free water tritium (TFWT), and associated with organic matter (OBT) when (ii) bound to oxygen and nitrogen atoms in tissue as exchangeable organically bound tritium (E-OBT) and (iii) bound to carbon atoms in tissue as non-exchangeable organically bound

tritium (NE-OBT) (Baumgaertner et al., 2009; Jaeschke et al., 2011; Nie et al., 2021).

It is generally accepted that E-OBT equilibrates very quickly with TFWT, which is itself at equilibrium with the water molecules in the surrounding environment. The metabolism of the NE-OBT mostly leads to the production of tritiated water at rates dependent on the nature of the tritiated organic molecules and their function in the body. Then, it is expected that tritium as NE-OBT presents an extended biological half-life since it remains bound until the catabolism of the tritiated molecule. The NE-OBT is, therefore, more stable than E-OBT in tissues and it can provide useful information regarding longer term exposures to tritium (Antonova et al., 2022; Baumgaertner et al., 2009; Eyrolle et al., 2018; Jaeschke et al., 2011; Le Goff et al., 2014). Given sustained and future increased discharges of tritium and its unique behaviour in different compartments of the environment, tritium represents an increasing concern for the health of both humans and non-human biota (NHB). However, compared to human studies, little is known about tritium characteristics in NHB (Beresford et al., 2008; Tornero and Hanke, 2016). In this context, the present review aims to critically analyse the available literature regarding the sources, environmental relevance and biological effects of tritium in NHB. We also aim to highlight knowledge gaps for future tritium research.

Literature surveys were carried out through keyword and key-phrase searches in Google scholar, ScienceDirect and PubMed, including “tritium radiation”, “tritium sources”, “fission energy”, “tritium effects”, “tritium dosimetry”, “tritium in the environment”, “tritium in biota”, “tritiated water”, “organically bound tritium” and any combinations thereof. Studies on NHB were grouped according to computational, field or laboratory-based studies. Reported effects were grouped according to exposure (acute versus chronic), taxa and biological level of organisation.

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

2. Tritium in the environment

Considering that about 99 % of the tritium present in the atmosphere is in the form of tritiated water, it is expected that it enters the ocean rapidly through vapour exchange, precipitation and river runoff (Liger et al., 2018; Oms et al., 2019). Levels of tritium in the aquatic environment vary according to latitude, season and proximity to urbanisation and nuclear facilities (Ansari et al., 2018; Chae and Kim, 2018; Harms et al., 2016). Fig. 2 shows the average global concentration of tritium and its distribution

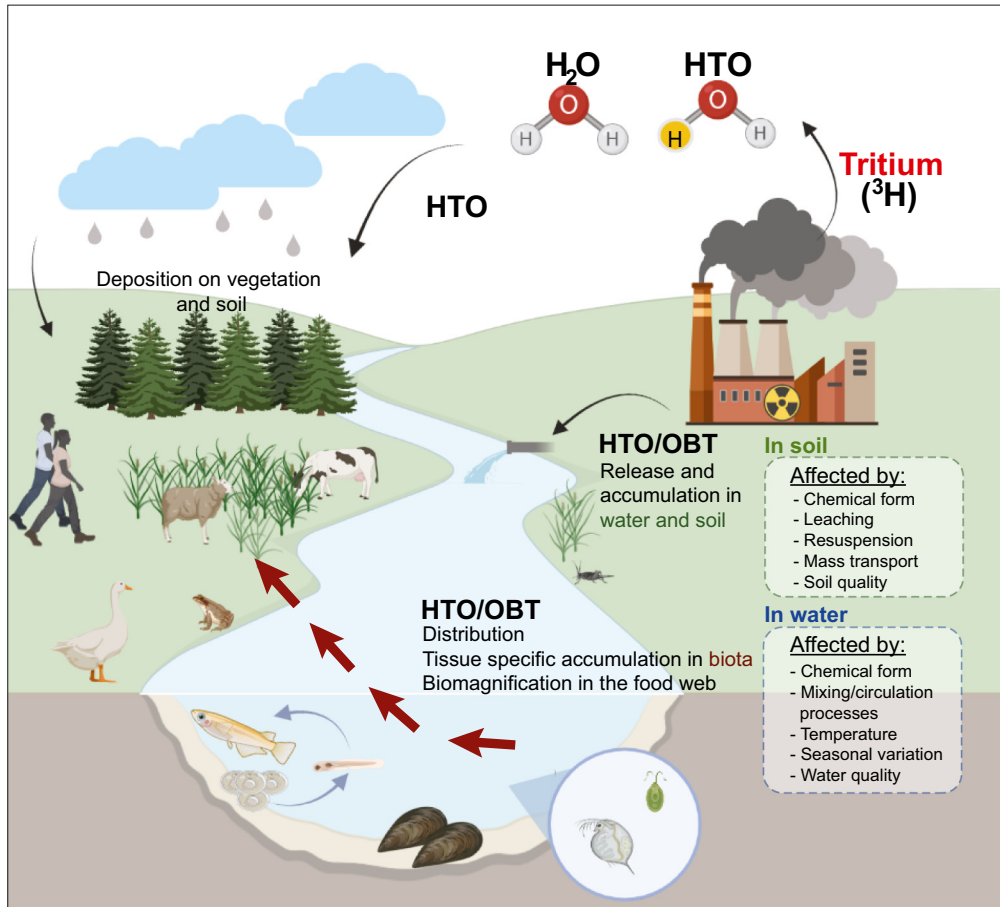


Fig. 1. Tritium sources, transport and dispersal in the environment. Created with BioRender.com

between the northern and southern hemispheres between 1996 and 2016 (Oms et al., 2019; UNSCEAR, 2016). More than half of the world's reactors are located in North America and Western Europe, with less than 10 % in developing countries (Adamantiades and Kessides, 2009; Oms et al., 2019), and this is reflected in the higher concentration present in the northern hemisphere.

In Canada, three of the largest reactors in the country are located on the shores of the Great Lakes. The maximum level of tritium was reported along the northern shores of Lake Ontario with a concentration (8.4 Bq L^{-1}) more than twice as high as offshore waters (3.5 Bq L^{-1}) (Dove et al., 2021). In

France, tritium levels ranging from about 3 to 4 Bq L^{-1} were reported in non-nuclearised coastal rivers but in the River Rhône, characterised by a high density of nuclear facilities, concentrations fluctuated between 2.50 and 12.85 Bq L^{-1} , with a mean of 6.31 Bq L^{-1} (Jean-Baptiste et al., 2018). At Fourmile Branch, US, an area that received contaminated effluent from nuclear weapons material production facilities, tritium concentrations in water from ponds adjacent to the contaminated stream ranged from 1570 to 1920 Bq L^{-1} , with an average concentration (1790 Bq L^{-1}), or approximately twenty times higher than the average (70 Bq L^{-1}) measured above the stream (Yu et al., 2020).

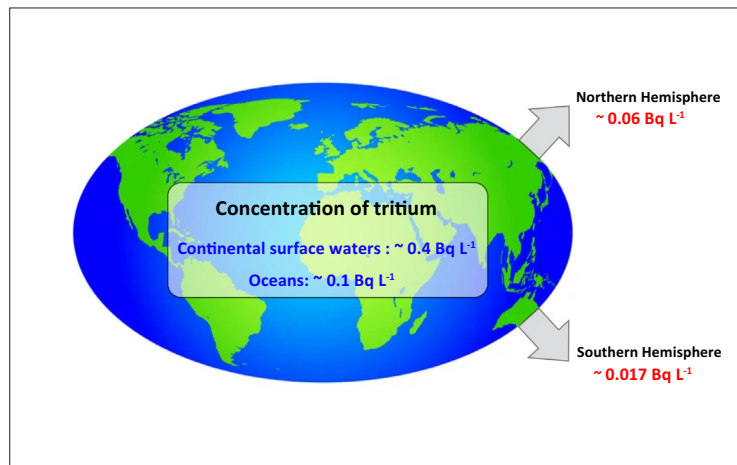


Fig. 2. Global distribution of tritium in water (average values from UNSCEAR, 2016; Oms et al., 2019).

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

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

In terrestrial animals, HTO is up taken from contaminated water in drink and food, and can be produced by the catabolism of organic molecules. Animals can also incorporate OBT into their tissues and fluids by consuming tritiated food. The retention time and incorporation into the dry matter of tissues/organs are generally higher for constitutive and storage molecules than for water (Le Goff et al., 2014; Takeda, 1991). Previous studies have also reported OBT in some reservoirs where organic matter is preserved, such as in soils (Thompson et al., 2015) and aquatic surface sediments (Eyrolle-Boyer et al., 2014; Eyrolle et al., 2019), raising concerns about the transfer of OBT stocks to the water cycle and living organisms (Galeriu et al., 2008; Ota et al., 2017).

Evaluation of terrestrial wildlife inhabiting areas with a history of radiation contamination is important to provide information on the bio-availability and dynamics of radionuclides in the environment (Cleary et al., 2021; Kelsey-Wall et al., 2005). However, few data are available in this regard (Beresford et al., 2016; Kelsey-Wall et al., 2005), and laboratory based studies are often employed to fill the gaps (Galeriu et al., 2005a,b; Melintescu and Galeriu, 2011). In the past few decades, computational assessments and transfer models have been developed that aim to predict and calculate the movement of tritium through the environment and

Table 1

Tritium limits in drinking water (Bq L^{-1}) proposed by different organisations and countries. Adapted from (Canadian Nuclear Safety Commission, 2008).

| Organisation/Country | Tritium concentration limit (Bq L^{-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 ^a |
| Finland | 30,000 |
| Russia | 7700 |
| Switzerland | 10,000 |
| United States | 740 |

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

Table 2

Average concentrations of tritium (Bq L^{-1}) in areas with a high density of nuclear facilities, and site-specific limits established for tritium waterborne discharges (Bq year^{-1}). Darlington: Heavy-Water moderated and cooled reactor; Hartlepool: nuclear power station; La Hague: fuel reprocessing plant; Olkiluoto and Loviisa: nuclear power plant reactors; Sellafield: fuel reprocessing plant; Wolsong: pressurised heavy water reactor.

| Location | Tritium in areas with high density of nuclear facilities (Bq L^{-1}) | References |
|-----------------------|---|---|
| Fourmile Branch (USA) | 1790 | (Yu et al., 2020) |
| Lake Ontario (Canada) | 4.76 | (Dove et al., 2021) |
| Rhône river (France) | 6.31 | (Jean-Baptiste et al., 2018) |
| Location | Site-specific limits for nuclear facilities (Bq year^{-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) (Schneider and Marignac, 2008) |
| La Hague (France) | 1.85×10^{16} | (Masionis et al., 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) |

plant and animal tissues (Dallas et al., 2016b; Galeriu et al., 2005a,b; Keum et al., 2006; Nie et al., 2021; Vives et al., 2022) (Fig. 3). Models assessing the exposure of wildlife to radiation would benefit from OBT-HTO data for biota to improve accuracy when studying phylogenetically different organisms (Kim et al., 2019; Vives et al., 2022). This would also allow comparisons to be made of spatial and temporal effects on bioaccumulation or uptake as well as potential species-specific impacts (Beresford et al., 2016; Kim et al., 2013a).

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

In common with other radionuclides, biota can be exposed externally and/or internally to tritium (Goodhead et al., 2004; Melintescu and Galeriu, 2011). It is, therefore, critical to accurately determine the absorbed radiation dose that could be linked to observed effects in order to improve environmental risk assessment (ERA) and protection (Adam-Guillermin et al., 2012; Nushtaeva et al., 2020). In contrast to human health, where physical, biological and clinical dosimetry have been well established, there has been very limited progress in estimating radiation doses in

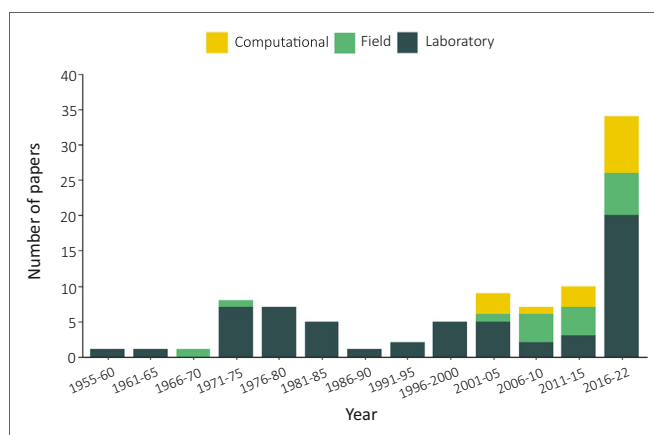


Fig. 3. Papers published evaluating the effects of tritium on biota from 1955 to 2022. Publications were excluded if no English translation was available.

NHB, with efforts mainly aiming to filter out situations of minimal risk to the individual or population (Andersson et al., 2009; Beaugelin-Seiller et al., 2020; Beresford et al., 2020; Garnier-Laplace et al., 2010; Mothersill et al., 2020; Real and Garnier-Laplace, 2020).

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

In common with other types of contaminant, an important concern is tissue-specific accumulation, which has implications for radiation dose and hence risk assessments. In rats, for example, a single oral administration of HTO showed that tritium was rapidly and uniformly distributed among tissues (Lee et al., 2019). In this study, OBt and tissue-free HTO concentrations (Bq g^{-1} and both wet and dry samples) showed no significant differences among different tissues (e.g., heart, lung, liver, gonads) after 7 and 13 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). Similar results in tissue specific analyses were obtained for OBt (Bq g^{-1} for both wet and dry samples) on

days 1, 3 and 5 after rats had received a single oral administration of HTO ($3.7 \times 10^3 \text{ Bq per gram of body weight}$) (Takeda et al., 1985). However, the distribution of OBt (Bq g^{-1} both wet and dry samples) among tissues was not uniform in rats exposed to tritiated wheat ($0.074 \times 10^3 \text{ Bq per gram of body weight}$) and tritium excreted as urine and faeces was less after the ingestion of tritiated wheat than HTO (Takeda et al., 1985). These observations, along with results of a comparative biokinetic study in rats chronically exposed to tritiated water and tritium-labelled food (Takeda et al., 2001), support the assertion that tritiated organic compounds remain for longer periods of time in the body than tritiated water. Moreover, the distribution pattern of OBt among tissues depends not only on the chemical or biochemical character of each tritiated compound but also on the metabolic activity of each tissue (Kim et al., 2013a; Le Goff et al., 2014; Takeda, 1991; Takeda et al., 1985).

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

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

Table 3

Numerical screening values ($\mu\text{Gy h}^{-1}$) proposed by different organisations and directives for the protection of diverse organism groups.

| | Aquatic organisms | | Terrestrial organisms | | References |
|-------------------|---|---|-----------------------|-------------------|--------------------------|
| | Freshwater | Marine | Invertebrates | Vertebrates | |
| ERICA | 10 | 10 | 10 | 10 | (Beresford et al., 2004) |
| FASSET | 100 | 100 | 100 | 100 | (Larsson, 2004) |
| IAEA | 400 | | 40 | 40 | (IAEA, 1992) |
| ICRP ^a | 4–40 ^b , 40–400 ^c | 400–4000 ^d , 40–400 ^e | 400–4000 ^g | 4–40 ^h | (ICRP, 2008) |
| NCRP | 400 | 400 | | | (Templeton et al., 1991) |
| PROTECT | 2; 200 ^f | 2; 200 ^f | 200 | 2 | (Andersson et al., 2009) |
| UNSCEAR | | | 400 | 100 | (UNSCEAR, 2008) |

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

^a Range values are not intended to be regarded as dose limits, but as dose rates at which evaluation of the situation would be warranted.

^b Dose level for reference frog.

^c Dose level for reference freshwater fish (trout).

^d Dose level for reference crab.

^e Dose level for reference marine fish (flatfish).

^f Dose levels for vertebrates ($2 \mu\text{Gy h}^{-1}$) and invertebrates ($200 \mu\text{Gy h}^{-1}$).

^g Dose levels for reference bee and earthworm.

^h Dose levels for reference deer, rat and duck.

4. Laboratory and field exposure studies

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

Although Fig. 3 indicates that data are available for different organisms from field studies, most of these have investigated tritium accumulation and very few have been published on the effects of tritium on NHB (Audette-Stuart et al., 2011; Beaton et al., 2019; Gagnaire et al., 2017). Near an operating nuclear site in Canada, Audette-Stuart et al. (2011) reported that frogs collected in areas with above-background levels of tritium (Duke Swamp, 2800 Bq L^{-1}) presented lower levels of DNA damage in liver cells compared to frogs inhabiting areas with background levels (214 Bq L^{-1}) after an in vivo exposure to a challenging dose of 4 Gy ionising radiation. Decreased sensitivity to radiation damage was also observed in cultured liver cells from frogs collected in Duke Swamp when the radiation dose was delivered in vitro. The authors suggested that the stress present in the area with a higher concentration of tritium could induce a protection of DNA or a cellular defence mechanism as an adaptive response to radiation. However, further studies are required as these results were based on a small number of samples. In the same region, another study revealed that tritium exposure induced genotoxicity, DNA repair activity, changes in fatty acid composition, and immune, neural and antioxidant responses in fathead minnows fish (Gagnaire et al., 2017). In a subsequent controlled laboratory study in which fish were chronically exposed to a gradient of HTO (12×10^3 , 25×10^3 , and $180 \times 10^3 \text{ Bq L}^{-1}$) and OBT (tritiated amino acids; $27 \times 10^3 \text{ Bq L}^{-1}$), similar observations were reported to those found at the field sites (Gagnaire et al., 2018). Beaton et al. (2019) found that DNA damage in fish exposed in the laboratory was higher than fish exposed in field, whereas enzymatic activities (e.g., SOD and catalase) in the liver were lower in fish exposed under laboratory conditions. A positive monotonic relationship between DNA damage and internalised tritium was observed in both experiments, but no correlation was found between tritium internal concentration and enzymatic activities. This is not surprising considering that organisms in the environment are exposed to a mixture

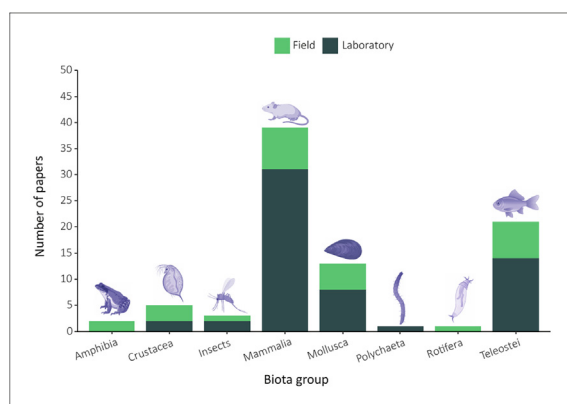


Fig. 4. Taxa used in field and laboratory studies from papers published between 1955 and 2022. Articles examining two or more species were counted and/or categorised multiple times.

of contaminants and a range of variables (e.g., temperature, pH, salinity), and oxidative stress biomarkers are known to be non-specific (Lourenço et al., 2016; Van der Oost et al., 2003). An integrative approach, where investigations in the field are followed by controlled laboratory experiments, provides a better understanding of tritium behaviour and is able to identify suitable biomarkers for tritium exposure in order to assess and predict the impact of current and future radiation exposure (Dallas et al., 2016b; Parisot et al., 2015).

4.1. Chronic and acute exposures

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

In mice, both HTO and OBT were found to induce increased levels of chromosomal aberrations in peripheral blood lymphocytes at concentrations of 1 and $20 \times 10^6 \text{ Bq L}^{-1}$ following a one-month chronic exposure. However, excess damage was not observed for HTO when the exposure was protracted to eight months, suggesting that a longer exposure could trigger some compensatory repair mechanism (Roch-Lefèvre et al., 2018). By contrast, Bannister et al. (2016) found no evidence for cytotoxicity or genotoxicity in mouse spleen following chronic exposures (one and eight months) to HTO up to $20 \times 10^6 \text{ Bq L}^{-1}$, while Saito (2002) observed that DNA-bound tritium in cells from mouse spleen was lower than in nucleus from liver and brain cells. This highlights the importance of tritium accumulation being tissue- and time-specific (Dallas et al., 2016a; Jha et al., 2005; Pearson et al., 2018a).

In studies of aquatic biota, effects reported after acute tritium exposure include alteration in gene expression levels, and development, genotoxic and cytotoxic effects. Conversely, sub-chronic and chronic exposures have been performed to assess the impact of tritium in physiological, behavioural responses and genotoxic effects (Table 5). Responses at different level of organisation may be better understood over different timescales of exposure, as acute and chronic exposures may target different metabolic routes, physiological processes or different life stages.

4.2. Studies on different animal groups

An overview of studies aiming to assess potential impact of tritium on different animal groups has been summarised in Table 4. Rodents, such as mice and rats, have long served as laboratory research models due to the anatomical, physiological and genetic similarity to humans. They have been considered preferred models also because of their short gestation period, short life cycle, small size and ease of maintenance. Accordingly, Fig. 4 and Table 4 show that mammals, and mainly mice and rats, are predominant in laboratory studies involving tritium. Although these studies have been performed using high concentrations and with the aim to address potential toxicity to human health, they provide valuable data and generate mechanistic information that could help understanding tritium effects in mammals and other biota. Molecular to behavioural effects have been reported under laboratory settings after acute (Li et al., 2021b) and chronic (including transgenerational) exposure scenarios (Bannister et al., 2016; Gao et al., 1999; Roch-Lefèvre et al., 2018) (Table 4). Observed effects include a decrease in offspring survival (Cahill et al., 1975; Clerici et al., 1984), a reduction in weight of reproductive organs (Cahill and Yuile, 1971; Laskey et al., 1973) and sterile F1 (Cahill et al., 1975). Alterations in the nervous system at molecular (Laskey and Bursian, 1976) and

Table 4

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

| Concentration/dose | Duration/time point | Observed effects | Reference |
|---|---|--|-----------------------------------|
| Insects- <i>Chironomus riparius</i> (transgenerational) | | | |
| $9250 \times 10^6 \text{ Bq L}^{-1}$ | 20 days | Chromosomes aberrations in larvae from adults developed in HTO. | (Blaylock, 1971) |
| $18,500 \times 10^6 \text{ Bq L}^{-1}$ | 20 days | Chromosomes aberrations in larvae from adults developed in HTO. | (Blaylock, 1971) |
| Mammalia- <i>Mus musculus</i> | | | |
| $18.5 \times 10^3 \text{ Bq g}^{-1}$ (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. | (Li et al., 2021a) |
| $10 \times 10^3 \text{ Bq L}^{-1}$ (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) |
| $1 \times 10^6 \text{ Bq L}^{-1}$ (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) |
| $20 \times 10^6 \text{ Bq L}^{-1}$ (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) |
| $0.01 \times 10^6 \text{ Bq L}^{-1}$ (HTO or OBT) | 1 and 8 months | No chromosome aberrations. | (Roch-Lefèvre et al., 2018) |
| $1 \times 10^6 \text{ Bq L}^{-1}$ (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) |
| $20 \times 10^6 \text{ Bq L}^{-1}$ | 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) |
| $0.3 \times 10^6 \text{ Bq L}^{-1}$ | 15 days | No induction of oxidative stress. | (Kelsey-Wall et al., 2006) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 90, 330, 500, 560, 700 days | Increased in chromosome aberration frequency in all groups except 500, 560 days exposure. | (Brooks et al., 1976) |
| $0.01 \times 10^6 \text{ Bq L}^{-1}$ | 1 and 8 months | No effect in spleen weight or DNA inversions in spleen. | (Bannister et al., 2016) |
| $1 \times 10^6 \text{ Bq L}^{-1}$ | 1 and 8 months | No effect in spleen weight or DNA inversions in spleen. | (Bannister et al., 2016) |
| $20 \times 10^6 \text{ Bq L}^{-1}$ | 1 and 8 months | No effect in spleen weight or DNA inversions in spleen. | (Bannister et al., 2016) |
| Mammalia- <i>Mus musculus</i> (embryo) | | | |
| $0.37 \times 10^6 \text{ Bq L}^{-1}$ (OBT) | 96 h | Decrease in survival | (Clerici et al., 1984) |
| $3.7 \times 10^6 \text{ Bq L}^{-1}$ (OBT) | 96 h | Decrease in survival | (Clerici et al., 1984) |
| $37 \times 10^6 \text{ Bq L}^{-1}$ (OBT) | 96 h | Decrease in survival | (Clerici et al., 1984) |
| Mammalia- <i>Mus musculus</i> (transgenerational) | | | |
| $100 \times 10^3 \mu\text{Gy}$ | 56 days | Reduction in the pyramidal cell densities. | (Sun et al., 1997) |
| $200 \times 10^3 \mu\text{Gy}$ | 56 days | Reduction in the pyramidal cell densities. | (Sun et al., 1997) |
| $400 \times 10^3 \mu\text{Gy}$ | 56 days | Reduction in the pyramidal cell densities. Decrease in brain weight and reduction of thickness of cerebral cortex. | (Sun et al., 1997) |
| $800 \times 10^3 \mu\text{Gy}$ | 56 days | Reduction in the pyramidal cell densities. Decrease in brain weight and reduction of thickness of cerebral cortex. | (Sun et al., 1997) |
| $0.111 \times 10^9 \text{ Bq L}^{-1}$ bw IP injection + $0.074 \times 10^9 \text{ Bq L}^{-1}$ TDW | 17th 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) |
| $0.011 \times 10^9 \text{ Bq L}^{-1}$ bw IP injection + $0.074 \times 10^9 \text{ Bq L}^{-1}$ TDW | 17th day of gestation until parturition | Intensified damage in cerebellum in terms of degeneration and loss of Purkinje cells. | (Jain and Bhatia, 1996) |
| $50 \times 10^3 \mu\text{Gy}$ (IP injection) | 21 days | No neurobehavioural effects. | (Wang and Zhou, 1995) |
| $100 \times 10^3 \mu\text{Gy}$ (IP injection) | 21 days | Difficulties in learning and memory retention for skill performance. | (Wang and Zhou, 1995) |
| $300 \times 10^3 \mu\text{Gy}$ (IP injection) | 21 days | Difficulties in learning and memory retention for skill performance. | (Wang and Zhou, 1995) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 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) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 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) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 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) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | F0 treated before pregnancy until bith of F1. | 60 % of newborns (F1) with hematomas and edemas. | (Zamenhof and Van Marthens, 1979) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | F0 treated before pregnancy and after birth (F1). | Increase in alkaline phosphatase in blood (F1). | (Zamenhof and Van Marthens, 1979) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 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) |
| $111 \times 10^6 \text{ Bq L}^{-1}$ | 98 days | Reduction in viable embryo | (Carsten and Commerford, 1976) |
| $3.145 \times 10^6 \text{ Bq L}^{-1}$ | From conception until 14 days of age | Decrease in oocytes in all treated groups | (Lowry Dobson and Cooper, 1974) |
| $31.45 \times 10^6 \text{ Bq L}^{-1}$ | From conception until 14 days of age | Decrease in oocytes in all treated groups | (Lowry Dobson and Cooper, 1974) |
| $314.5 \times 10^6 \text{ Bq L}^{-1}$ | From conception until 14 days of age | Decrease in oocytes in all treated groups | (Lowry Dobson and Cooper, 1974) |
| $0.0185 \times 10^6 \text{ Bq L}^{-1}$ bw | 4 days | No observed effect. | (Johnson and Cronkite, 1959) |
| $0.037 \times 10^6 \text{ Bq L}^{-1}$ bw | 4 days | No observed effect. | (Johnson and Cronkite, 1959) |
| $0.185 \times 10^6 \text{ Bq L}^{-1}$ bw | 4 days | Decrease of spermatocytes. | (Johnson and Cronkite, 1959) |
| $0.37 \times 10^6 \text{ Bq L}^{-1}$ bw | 4 days | Decrease of spermatocytes. | (Johnson and Cronkite, 1959) |
| $0.74 \times 10^6 \text{ Bq L}^{-1}$ bw | 4 days | Decrease of spermatocytes. | (Johnson and Cronkite, 1959) |
| Mammalia- <i>Rattus norvegicus</i> (transgenerational) | | | |
| $74 \times 10^6 \text{ Bq L}^{-1}$ | 14 and 21 days. | No differences in MN frequency in blood samples. | (Lee et al., 2019) |
| $740 \times 10^6 \text{ Bq L}^{-1}$ | 14 and 21 days. | No differences in MN frequency in blood samples. | (Lee et al., 2019) |
| $0.24 \times 10^6 \text{ Bq g}^{-1}$ bw | One IP injection on day 13th of gestation | Delay in learning ability and decrease in memory. Lower number of hippocampal pyramidal cells. | (Gao et al., 1999) |

(continued on next page)

Table 4 (continued)

| Concentration/dose | Duration/time point | Observed effects | Reference |
|---|---|--|----------------------------|
| $0.48 \times 10^6 \text{ Bq g}^{-1} \text{ bw}$ | One IP injection on day 13th of gestation | Delay in learning ability and decrease in memory. Lower number of hippocampal pyramidal cells. | (Gao et al., 1999) |
| $1.44 \times 10^6 \text{ Bq g}^{-1} \text{ bw}$ | One IP injection on day 13th 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) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 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) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 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) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 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) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 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) |
| $37 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 22 days (gestation period) | No effect observed in lifespan, overall neoplasia incidence, incidence rate or onset of mammary fibroadenomas. | (Cahill et al., 1975) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 22 days (gestation period) | No effect observed in lifespan, overall neoplasia incidence, incidence rate or onset of mammary fibroadenomas. | (Cahill et al., 1975) |
| $1850 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 22 days (gestation period) | Sterile F1. Low incidence of mammary adenomas. | (Cahill et al., 1975) |
| $3700 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 22 days (gestation period) | Sterile F1. Lower incidence of mammary adenomas and overall neoplasia. Reduced mean lifespan. | (Cahill et al., 1975) |
| $0.37 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | From conception until 125 days of age | Reduction in liver and kidney weight (F2). | (Laskey et al., 1973) |
| $3.7 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | From conception until 125 days of age | Reduction in brain weight (F2). | (Laskey et al., 1973) |
| $37 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | From conception until 125 days of age | Reduction in brain weight and body weight (F2). | (Laskey et al., 1973) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 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) |
| $37 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Increased length in both sexes, liver and heart weight. | (Cahill and Yuile, 1971) |
| $370 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Reduction of testes and brain. | (Cahill and Yuile, 1971) |
| $740 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Reduction of testes, brain, spleen, thymus, kidney and heart. | (Cahill and Yuile, 1971) |
| $1850 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart. | (Cahill and Yuile, 1971) |
| $2775 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart. | (Cahill and Yuile, 1971) |
| $3700 \times 10^6 \text{ Bq L}^{-1} \text{ bw}$ | 21 days | Sterility. Reduction of gonads size, brain, spleen, thymus, kidney and heart. Reduction in litter size. | (Cahill and Yuile, 1971) |

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

behavioural levels (Gao et al., 1999; Wang and Zhou, 1995) have also been reported. The availability of the complete genome sequence for rats and mice also opens up possibilities to implement genetic techniques and develop transgenic mice and use them in toxicology studies (Ankley et al., 2010; Kratchman et al., 2018) and, regarding tritium research, monitor DNA inversion frequency and assess genotoxicity (Bannister et al., 2016).

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

Tritium studies in aquatic mammals are scarce, and fish represents the main source of data available for vertebrates (Adam-Guillermin et al., 2012; Beresford et al., 2016) (Fig. 2, Table 5). Due to the well-known physiology and life cycle of certain fish species, different approaches, from molecular (e.g., Arcanjo et al., 2018; Festarini et al., 2019) to behavioural

studies (e.g., Arcanjo et al., 2020; Festarini et al., 2016), have been conducted in laboratory (e.g., Arcanjo et al., 2018; Festarini et al., 2016, 2019; Gagnaire et al., 2020) or field settings (Gagnaire et al., 2017). These models share some advantages with rodents, such as known and short life cycles, but, to our knowledge, no study has assessed transgenerational effects of tritium exposure in fish.

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

Relationships between ambient (water) and tissue concentrations of contaminants can be difficult to establish and verify in free-living animals. Sessile animals (e.g. mussels), therefore offer important advantages (National Research Council, 1991). Thus, they have been used to monitor and study toxicity response to a range of radionuclides, including tritium (Dallas et al., 2016a; Jha et al., 2005, 2006; Pearson et al., 2018a). As summarised in Table 5 and Fig. 4, molluscs are the main aquatic invertebrates used to study bioaccumulation and biological

Table 5

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

| Concentrations/Dose | Duration/time point | Observed effects | Reference |
|---|---------------------|---|-------------------------------|
| Polychaeta- <i>Ophryotrocha diadema</i> (transgenerational) | | | |
| 2240 × 10 ⁶ Bq L ⁻¹ | 22 days | Reduction in survival of eggs. | (Knowles and Greenwood, 1997) |
| Crustacea- <i>Crassostrea gigas</i> | | | |
| 1 × 10 ⁶ Bq L ⁻¹ | 14 days | DNA damage in haemocytes. | (Devos et al., 2015) |
| 15 × 10 ⁶ Bq L ⁻¹ | 14 days | DNA damage in haemocytes. | (Devos et al., 2015) |
| Crustacea- <i>Daphnia magna</i> (transgenerational) | | | |
| 0.5 × 10 ² Bq L ⁻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 × 10 ⁶ Bq L ⁻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 × 10 ⁶ Bq L ⁻¹ | 17 days (F1) | Reduction in growth rate. 100 % of offspring mortality. | (Gudkov and Kipnis, 1996) |
| Crustacea- <i>Pollicipes polymerus</i> (larvae) | | | |
| 0.00037 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| 0.0037 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| 0.037 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| 0.37 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| 3.7 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| 37 × 10 ⁶ Bq L ⁻¹ | 32 days | Alteration in molting index. | (Abbott and Mix, 1979) |
| Mollusca- <i>Mytilus galloprovincialis</i> | | | |
| 5 × 10 ⁶ Bq L ⁻¹ | 14 days | DNA damage in haemocytes. | (Pearson et al., 2018a) |
| 5 × 10 ⁶ Bq L ⁻¹ (HTO) + 383 nM (Zn) | 14 days | No genotoxic effect. | (Pearson et al., 2018a) |
| 5 × 10 ⁶ Bq L ⁻¹ (HTO) + 1913 nM (Zn) | 14 days | No genotoxic effect. | (Pearson et al., 2018a) |
| 5 × 10 ⁶ Bq L ⁻¹ (HTO) + 3825 nM (Zn) | 14 days | No genotoxic effect. | (Pearson et al., 2018a) |
| 15 × 10 ⁶ Bq L ⁻¹ at 15 °C | 12, 72, 168 h | DNA damage in haemocytes only at 168 h. Gene expression of hsp-70, hsp-90, mt20 and p53 was upregulated at 72 h. | (Dallas et al., 2016a) |
| 15 × 10 ⁶ Bq L ⁻¹ at 25 °C | 12, 72, 168 h | DNA damage in haemocytes. Gene expression of hsp-70, hsp-90, mt20 and p53 was down-regulated at 72 h. | (Dallas et al., 2016a) |
| Mollusca- <i>Mytilus edulis</i> | | | |
| 0.122 × 10 ³ μGy h ⁻¹ | 7 days | Induction of micronuclei in haemocytes. | (Jaeschke et al., 2011) |
| 0.079 × 10 ³ μGy h ⁻¹ | 14 days | Induction of micronuclei in haemocytes. | (Jaeschke et al., 2011) |
| 0.0049 × 10 ³ μGy h ⁻¹ (OBT) | 7 days | Induction of micronuclei in haemocytes. | (Jaeschke et al., 2011) |
| 0.012 × 10 ³ μGy h ⁻¹ | 96 h | DNA damage in haemocytes. | (Jha et al., 2006) |
| 0.121 × 10 ³ μGy h ⁻¹ | 96 h | DNA damage in haemocytes. | (Jha et al., 2006) |
| 0.485 × 10 ³ μGy h ⁻¹ | 96 h | DNA damage in haemocytes. | (Jha et al., 2006) |
| Mollusca- <i>Mytilus edulis</i> (embryo-larvae) | | | |
| 0.37 × 10 ⁶ Bq L ⁻¹ | 24 h | Differential pattern of random amplified polymorphic DNA. Decrease of proliferative rate index. Decrease of normal larvae and survival. | (Hagger et al., 2005) |
| 3.7 × 10 ⁶ Bq L ⁻¹ | 24 h | 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 × 10 ⁶ Bq L ⁻¹ | 24 h | 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 × 10 ⁶ Bq L ⁻¹ | 24 h | 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 × 10 ³ Bq L ⁻¹ | 672 h | Abnormal larvae. Greater length than control. | (Bondareva, 2017) |
| 0.5 × 10 ³ Bq L ⁻¹ | 672 h | Abnormal larvae. Greater length than control. | (Bondareva, 2017) |
| 5 × 10 ³ Bq L ⁻¹ | 672 h | Abnormal larvae. Greater length than control. | (Bondareva, 2017) |
| 50 × 10 ³ Bq L ⁻¹ | 672 h | Abnormal larvae. Greater length than control. | (Bondareva, 2017) |
| Teleostei- <i>Danio rerio</i> (embryo-larvae) | | | |
| 3.7 × 10 ⁶ Bq L ⁻¹ | 60 and 120 hpf | Decrease in hatching rate. Decrease in swimming activity. | (Li et al., 2021b) |
| 37 × 10 ⁶ Bq L ⁻¹ | 60 and 120 hpf | Decrease in hatching rate, swimming activity and hormone (T3, T4) concentration. Alteration in gene expression. | (Li et al., 2021b) |
| 370 × 10 ⁶ Bq L ⁻¹ | 61 and 120 hpf | Increase in heartbeat. Decrease in swimming activity. | (Li et al., 2021b) |
| 0.4 × 10 ³ μGy h ⁻¹ | 24, 72, 96 h | Lower swimming velocity in 96 hpf larvae. | (Arcanjo et al., 2020) |
| 4 × 10 ³ μGy h ⁻¹ | 24, 72, 96 h | No effect in swimming behaviour. | (Arcanjo et al., 2020) |
| 10 × 10 ⁶ Bq L ⁻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) |

(continued on next page)

Table 5 (continued)

| Concentrations/Dose | Duration/time point | Observed effects | Reference |
|---|---------------------|---|---------------------------------|
| 10^8 Bq L ⁻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 Bq L ⁻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) |
| 0.4×10^3 μ 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) |
| 4×10^3 μ Gy h ⁻¹ | 24, 96 h | 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) | | | |
| 37×10^5 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^7 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^9 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^{10} Bq L ⁻¹ | 130 h | Inactive larvae. Smaller bodies with swollen abdomens. Smaller eye diameter. | (Suyama and Ichikawa, 1974) |
| Teleostei- <i>Oncorhynchus mykiss</i> | | | |
| 0.030×10^6 Bq L ⁻¹ (OBT) | 126, 146 days | No effect observed. | (Festarini et al., 2019) |
| 0.007×10^6 Bq L ⁻¹ | 126, 146 days | No effect observed. | (Festarini et al., 2019) |
| 0.03 (OBT) + 0.007 (HTO) $\times 10^6$ Bq L ⁻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) | | | |
| 3700×10^6 Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| 7400×10^6 Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| $14,800 \times 10^6$ Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| $18,500 \times 10^6$ Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| $27,750 \times 10^6$ Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| $37,000 \times 10^6$ Bq L ⁻¹ | 10 days | Decrease in the number of germ cells. | (Hyodo-Taguchi and Etoh, 1986) |
| 370×10^6 Bq L ⁻¹ | 240 days | Decrease in the number of germ cells. | (Hyodo Taguchi and Egami, 1977) |
| Teleostei- <i>Paralichthys olivaceus</i> (embryo) | | | |
| 370 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^3 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^5 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| 37×10^7 Bq L ⁻¹ | 180 h | No effect in hatchability. | (Suyama and Ichikawa, 1974) |
| Teleostei- <i>Pimephales promelas</i> (embryo) | | | |
| 0.012×10^6 Bq L ⁻¹ | 60 and 120 days | Increased DNA damage in gonad at 120 days. | (Gagnaire et al., 2018) |
| 0.025×10^6 Bq L ⁻¹ | 60 and 120 days | Increased DNA damage in gonad at 120 days. | (Gagnaire et al., 2018) |
| 0.18×10^6 Bq L ⁻¹ | 60 and 120 days | Increased DNA damage in gonad at 60 and 120 days. Increased micronuclei frequency in blood. Increased phagocytosis activity at 120 days. | (Gagnaire et al., 2018) |
| 27×10^3 Bq L ⁻¹ (OBT) | 60 and 120 days | Increased DNA damage in gonad at 120 days. | (Gagnaire et al., 2018) |
| 25×10^3 Bq L ⁻¹ (OBT, HTO) | 60 and 120 days | Increased DNA damage in gonad and micronuclei frequency in blood. | (Gagnaire et al., 2018) |
| Teleostei- <i>Salmo gairdneri</i> | | | |
| 0.4×10^{-3} μ Gy | 20 days | Irreversible suppression of immune capacity. | (Strand et al., 1977) |
| 4×10^{-3} μ Gy | 20 days | Irreversible suppression of immune capacity. | (Strand et al., 1977) |
| 40×10^{-3} μ Gy | 20 days | Irreversible suppression of immune capacity. | (Strand et al., 1977) |
| 400×10^{-3} μ Gy | 20 days | Irreversible suppression of immune capacity. | (Strand et al., 1977) |
| Teleostei- <i>Salmo gairdneri</i> (juvenile) | | | |
| 0.4×10^{-3} μ Gy | 20 days | No effect in immune response. | (Strand et al., 1977) |
| 4×10^{-3} μ Gy | 20 days | No effect in immune response. | (Strand et al., 1977) |
| 40×10^{-3} μ Gy | 20 days | Reduction of immune response. | (Strand et al., 1977) |
| 400×10^{-3} μ Gy | 20 days | Reduction of immune response. | (Strand et al., 1977) |

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

effects of tritium, as HTO or OBT (T-Gly) either individually or in combination with other stressors or contaminants like metals. Jaeschke and Bradshaw (2013) provided evidence for the accumulation of organic tritium into the mussel tissues via tritiated-phytoplankton, suggesting a transfer pathway of tritium and its potential biomagnification. The tritium activity in foot, gills, digestive gland and mantle tissues showed a linear increase with the number of feedings, and the digestive gland had the highest incorporation of tritium compared to the other tissues. The induction of micronuclei in haemocytes was also observed after exposure to HTO and T-Gly (Jaeschke et al., 2011). The activity from HTO was depurated within one day, whereas T-Gly depurated relatively slowly. Genotoxicity in mussels also appears to be temperature- and time-dependent, as induction of DNA strand breaks was observed after

three days when exposed to HTO at 25 °C but after seven days at 15 °C (Dallas et al., 2016b). In co-exposure studies with zinc and HTO, Pearson et al. (2018a) observed a clear antagonistic effect of Zn on the genotoxicity (DNA strand break) of HTO at all Zn concentrations used, possibly due to the importance of Zn in DNA repair enzymes. These observations highlight the importance of assessing potential interactions of physical and chemical factors with tritium in order to improve current and future risk assessment strategies for organism exposure in the environment.

Variations in sensitivity to contaminants between life stages have been reported for fish and aquatic invertebrates, and early life stages are considered more susceptible to toxic substances (Mohammed, 2013; Santos et al., 2018). Nevertheless, most of the available data on tritium impacts on

invertebrates refer to adults (Dallas et al., 2016a; Devos et al., 2015; Jaeschke et al., 2011; Jaeschke and Bradshaw, 2013; Pearson et al., 2018a). Potential effects in embryo-larvae of the marine bivalve molluscs, *Mytilus edulis* and in goosse barnacle, *Pollicipes polymerus* are the only early life-stages of invertebrates studied (Hagger et al., 2005; Abbott and Mix, 1979). In bivalves, cytogenetic damage, cytotoxicity, developmental abnormalities and mortality were observed after acute exposure, which increased as a function of radiation dose (Hagger et al., 2005). In barnacles, an exponential decrease in moulting index related to HTO concentration was observed. The effects were evident at a concentration as low as 259 Bq L^{-1} (Abbott and Mix, 1979).

5. Biological effects

Different biological tests and assays have been used according to the nature of the stressors involved in various organisms or model species (Aliko et al., 2018). Potentially, alterations in any process may be used as biomarkers and may be measured in tissues or body fluid samples, or at the level of whole organisms, to provide evidence of exposure effects from one or more contaminant (Hagger et al., 2006). Responses at each level of biological organisation provide information that helps to understand and interpret the relationship between exposure and adverse effects (Hagger et al., 2006; Van der Oost et al., 2003). In particular, radiobiological effects have been commonly assessed through the “umbrella end-points” which includes morbidity, mortality, reproductive and mutational effects on the organisms (Garnier-Laplace et al., 2006; Real et al., 2004; Sazykina and Kryshev, 2003) as suggested in the FASSET project framework (Coplestone et al., 2008). Survival and reproduction represent both individual and population-relevant end-points since variations in these parameters can affect the fitness of the population and genetic diversity (King-Heiden et al., 2012; Marty et al., 2011). More often, however, effects are more subtle, ultimately modulating organism fitness, because contaminants can act via numerous mechanisms (Connon et al. 2009). Moreover, once an effect is manifested at an organism level, remedial measures are often too late.

Over the past few years, the use of molecular, biochemical and physiological biomarkers have increased while determining the potential impact of in tritium on the NHB (Fig. 5). Considering that there is no single biomarker that can unequivocally measure detrimental impact, many studies have implemented or recommended application of an integrated multi-biomarker approach (Adams, 2005; Brenner et al., 2014; Galloway et al., 2004a; Jha, 2008; Larsson et al., 2018; Linde-Arias et al., 2008; Turja et al., 2014). In the framework of ERA, both short-term responses and long-term ecologically relevant end-points provide a weight-of-evidence

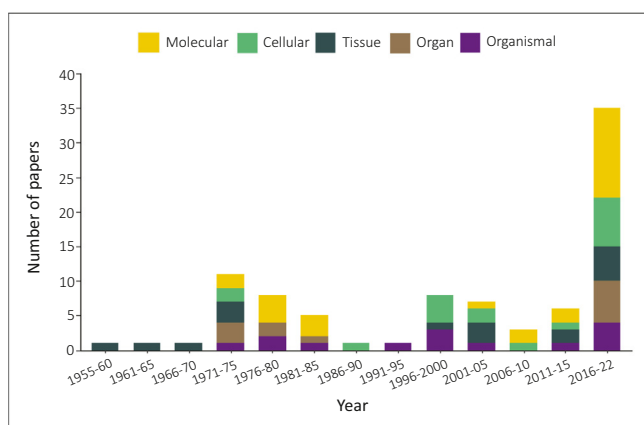


Fig. 5. End-points used at different levels of biological organisation to study effects of tritium in papers published between 1955 and 2022. Articles examining end-points at two or more levels of organisation were counted and/or categorised multiple times.

approach for establishing relationships between environmental stressors and ecological effects (Galloway et al., 2004b; Hagger et al., 2006).

5.1. Behavioural responses

Behaviour analysis is considered to be a sensitive indicator that can reflect biochemical and/or physiological disturbances. Behaviour can have a direct or indirect effect on population growth rate, thereby representing an ecologically relevant response at organismal and supra-organism levels (Bertram et al., 2015; Candolin and Wong, 2019; Scott and Sloman, 2004). For example, (Wang and Zhou (1995)) found that mice offspring receiving 48.18 and $144.54 \times 10^3 \text{ Bq g}^{-1}$ in utero (after pregnant adults received a single intraperitoneal injection of HTO) had difficulties in both learning and memory retention for skill performance. Similar results were found in rat offspring that were related to the induced degeneration and malformation of hippocampal neurons observed in treated groups (Gao et al., 1999). More recent studies have reported that HTO exposure can affect fish behaviour (Festarini et al., 2016; Li et al., 2021b). Li et al. (2021b) observed that 120 h post fertilisation (hpf) zebrafish larvae exposed to HTO ($3.7 \times 10^6 \text{ Bq L}^{-1}$, $3.7 \times 10^7 \text{ Bq L}^{-1}$, $3.7 \times 10^8 \text{ Bq L}^{-1}$) presented decreases in activity, swimming speed and total swimming distance when compared to a control group. Altered swimming behaviour has also been reported in 96 hpf zebrafish larvae exposed to 1.10×10^8 and $1.35 \times 10^9 \text{ Bq L}^{-1}$ HTO and attributed to developmental abnormalities (Arcanjo et al., 2020).

5.2. Reproduction and development

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

In rodents, tritium reproductive and developmental effects have been assessed experimentally after different exposure scenarios that include acute, chronic, in utero and transgenerational (see Table 4 for an overview). Reproductive impairment, dose-dependent decreases in oocyte numbers and various neuronal effects associated with an abnormal development are the main effects observed in transgenerational studies (Table 4). In rats, sterile offspring have been reported after in utero continuous exposure to HTO (1850 and $3700 \times 10^6 \text{ Bq L}^{-1}$ body water in pregnant adults) (Cahill and Yuile, 1971), whereas reductions in testes and ovary weights were observed in F1 after being exposed to a lower dose of HTO ($370 \times 10^6 \text{ Bq L}^{-1}$ body water) (Cahill and Yuile, 1971; Laskey et al., 1973; Laskey and Bursian, 1976). In a multigenerational study, constant prenatal exposure to HTO showed significant effects on F2 rat neonates, including reductions in relative brain weight after HTO in utero exposure (3.7 , 37 and $370 \times 10^6 \text{ Bq L}^{-1}$ body water in pregnant adults) and decreased body weight in F2 from females exposed to HTO concentrations of 37 and $370 \times 10^6 \text{ Bq L}^{-1}$ body weight (Laskey et al., 1973). Brain abnormalities and alterations in the establishment of conditional reflexes were also reported in rats that received different doses of tritium ($92 \times 10^3 \mu\text{Gy}$ and $273 \times 10^3 \mu\text{Gy}$) during gestation (Gao et al., 1999). In mice, males injected with tritiated thymidine ($0.185 \times 10^9 \text{ Bq g}^{-1}$ body weight) presented a decrease of spermatocytes after four days (Johnson and Cronkite, 1959). Decrease in viability was observed in embryos from mice maintained on drinking water containing $111 \times 10^6 \text{ Bq L}^{-1}$ of tritiated water (Carsten and Commerford, 1976), and F1 exposed in utero to HTO ($3.1 \times 10^6 \text{ Bq L}^{-1}$ body water in pregnant adult) presented a significantly decreased in oocytes (Lowry Dobson and Cooper, 1974).

Most of the above studies have focused on reproduction and potential neurohistopathologies. Even though these represent sub-lethal effects, they clearly have an important effect at organism and population levels, especially considering that some developing neurons and female sex cells are

both irreplaceable in adult mammals (Bharti et al., 2021; Stifani, 2014). It is also important to appreciate that doses applied are above proposed screening values for vertebrates, and data are only available for mice and rats. It would be useful to have additional information on the effects induced by tritium exposure in other mammalian species and across a range of concentrations in order to improve ecological risk assessment.

In aquatic biota, end-points measured at the organismal level have included mortality, hatching success of embryos and developmental parameters, and the focus has mainly been on fish (Table 5). Suyama and Ichikawa (1974) studied the effect of tritium exposure in the development of two marine fishes: flounder (*Paralichthys olivaceus*) and puffer (*Fugu niphobles*). Neither showed any significant decrease in hatchability after exposure to tritiated water (up to 370×10^6 Bq L⁻¹), and effects of HTO on the hatchability and growth of puffer embryo, including smaller eye size and swelled abdomen on hatching, were only evident at a very high concentration (370×10^8 to 370×10^9 Bq L⁻¹). Similar effects have been observed in zebrafish larvae after exposure to a lower concentration of HTO (100×10^6 Bq L⁻¹) (Gagnaire et al., 2020). Here, eggs were exposed after three hours of being fertilised, whereas puffer eggs had been exposed 19 h post fertilisation. Bondareva (2017) observed an increase in incidence of abnormalities after larvae of *Carassius gibelio* (6 hpf) were chronically exposed to HTO (50, 500, 5000 and 50,000 Bq L⁻¹), although this was not correlated with increasing exposure level. These studies suggest differential sensitivity between developmental stages, as previously reported in zebrafish (Arcanjo et al., 2018). However, differences in species sensitivity or tritium behaviour (e.g., chemical activity) in freshwater and seawater cannot be ruled out.

Few studies have assessed the impacts of tritium in the reproduction of aquatic biota, and the most commonly studied reproductive end-point is fecundity (i.e., the ability of an organism to produce viable gametes) (Table 5). In fry of medaka, it was observed that germ cell survival diminished exponentially with tritium dose and female germ cells were more radiosensitive than male germ cells. These results were obtained on eggs (2 h post-fertilisation) that were kept in HTO ($1850\text{--}37,000 \times 10^6$ Bq L⁻¹) for ten days (Hyodo-Taguchi and Etoh, 1986). Similarly, in adult males of medaka, a decrease in the number of germ cells was reported upon the addition of tritiated water at a concentration of 370×10^6 Bq L⁻¹ after ten days exposure (Hyodo Taguchi and Egami, 1977), apparently indicating a higher sensitivity of germ cells in adult fish. Because long-term survival of a species depends on its reproductive success, alterations to this process are among the most significant sublethal effects. However, it is also known that the normal reproductive pattern of natural species can be highly influenced by different factors, such as temperature, age, food availability and seasonality (Jha, 2008; Rizzo and Bazzoli, 2019). This makes laboratory measurements of reproduction logistically challenging (Jha, 2008) and more difficult to extrapolate between laboratory results and ecosystem effects (Hook et al., 2014).

Although data on reproductive effects arising from tritium exposure are scarce, early life-stage studies assessing developmental parameters provide valuable information that is just as ecologically relevant as a decrease in fecundity (Connon et al., 2012; Dallas et al., 2012; McArdle et al., 2020). Sublethal developmental abnormalities can compromise the ecological fitness of individual organisms or a population since the individual must be able to avoid predation, reproduce, compete with other organisms for food and cope with other environmental stressors. Moreover, if development is significantly delayed by tritium exposure, as reported in barnacles (Abbott and Mix, 1979) and different fish species (Arcanjo et al., 2018; Gagnaire et al., 2020; Suyama and Ichikawa, 1974), organisms may spend a greater time at more vulnerable stages where they are more likely to be predated (Paradis et al., 1999) or infected with pathogens (Sweet and Bateman, 2015).

5.3. Cytotoxicity

The energy from internal radioactive decay generates reactive oxygen species (ROS). Since these species are powerful oxidants with short half-

lives, oxidative stress responses have been used as end-points to assess DNA damage and cellular death (Isaksson, 2010; Lionetto et al., 2019; Turja et al., 2014).

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

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

5.4. Genotoxicity

Since genetic damage could be induced at a much lower dose rate and be determined using a range of techniques immediately after exposures to ionising radiation, relevant end-points have been employed in exposures to tritium. Specifically, genotoxic effects of tritium exposure have been reported in molluscs (Dallas et al., 2016a; Devos et al., 2015; Hagger et al., 2005; Jha et al., 2005; Pearson et al., 2018a), insects (Blaylock, 1971), mammals (Ichimasa et al., 2003; Lee et al., 2019) and fish (e.g., Arcanjo et al., 2019; Festarini et al., 2016; Li et al., 2021b) (Tables 4 and 5).

One of the most common assays to assess genotoxicity is comet or single cell gel electrophoresis assay [SCGE] which is used to detect DNA single/double strand breaks (SSB/DSB) (Jha, 2008; Lee and Steinert, 2003). The micronucleus (MN) test has also been used to detect genotoxic effects of environmental radionuclides (Beaton et al., 2019; Gagnaire et al., 2018; Jha et al., 2005; Poblete-Naredo and Albores, 2016; Samanta et al., 2018; Vernon et al., 2020). The MN test detects non-repairable damage while SCGE detects recent lesions that can be repaired, such as breaks and alkali labile sites (Frenzilli et al., 2009). Concordance between the genotoxic effects assessed through both SCGE and MN have been reported for HTO (Jha et al., 2005), although it is suggested that the comet assay presents higher sensitivity (Frenzilli et al., 2009). DNA damage in haemocytes have been assessed and detected in mussels exposed to dose rates as low as $20 \mu\text{Gy h}^{-1}$ (15×10^6 Bq L⁻¹ HTO) (Dallas et al., 2016a) and $4.9 \mu\text{Gy h}^{-1}$ (1.48×10^6 Bq L⁻¹ T-Gly) (Jaeschke et al., 2011) following seven day exposures. In mussel larvae, Hagger et al. (2005) reported chromosomal aberrations, induction of sister chromatids exchanges (SCEs) and changes in the random amplified polymorphic DNA (RAPD) profile after being exposed to HTO ($3.70 \times 10^3\text{--}370 \times 10^3$ Bq L⁻¹; 30–150 μGy ; 18.6 h). DNA damage using comet assay was also detected in oysters exposed to a dose rate of 7.5 and 113.9 $\mu\text{Gy h}^{-1}$ (1×10^6 Bq L⁻¹ and 15×10^6 Bq L⁻¹ HTO) following a 14-day exposure (Devos et al., 2015). In the fathead minnow, a dose rate of 0.65 $\mu\text{Gy h}^{-1}$ (180×10^3 Bq L⁻¹ HTO) for 60 days increased DNA damage in gonads and MN frequency in blood (Gagnaire et al., 2018). In the same species, γH2AX foci detection (reflecting DNA double strand break) was positively correlated with the internal dose rate after exposure to tritium in two forms: HTO and OBT-spiked feed (Gagnaire

et al., 2017). Conversely, Festarini et al. (2019) reported no differences in cell viability, DNA breaks and DNA repair activity in adult trout chronically exposed to HTO activity concentrations that were close to the current Canadian Drinking Water Guideline of 7000 Bq L⁻¹. However, changes in response to a subsequent acute high dose of gamma radiation delivered in vitro were noted when evaluating DNA repair activity. In comparison to non-tritiated fish tissues, after exposure to a 4 Gy challenge dose, the response of liver and heart was enhanced compared to the other tissues tested. This highlights the presence of differential DNA repair activities and sensitivities of tissue to tritium radiation. Similarly, in zebrafish embryo-larvae, γ H2AX detection revealed no difference between controls and groups exposed to 1.22 × 10² and 1.22 × 10³ Bq L⁻¹ of HTO (0.40 and 4 mGy h⁻¹) (Arcanjo et al., 2020). However, under the same exposure scenario, the authors observed an up-regulation of *h2afx* gene (coding for histone H2A) which is involved in the response to DNA damage. It was hypothesised that these results may reflect an enhancement of DNA repair pathways to balance the effects of ionising radiation at a higher level of biological organisation.

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

5.5. “Omics” studies

To the best of our knowledge, neither proteomics nor metabolomics have been applied to explore tritium effects in NHB, and only a few studies have assessed the effects of tritium exposure in biota transcriptome. These have all been performed in fish models (Table 5). Zebrafish larvae (24 and 96 hpf) exposed to HTO (1.22 × 10⁸ and 1.22 × 10⁹ Bq L⁻¹) presented changes in expression of genes involved in muscle contraction, eye opacity, circadian and oxidative stress responses, and in pathways involved in muscle development and skeletal and cardiac muscle contraction (Arcanjo et al., 2018). These effects were confirmed at a higher biological scale after electron microscopic observations of 96 hpf larvae, indicating that muscle integrity could be considered as a good biomarker of HTO exposure in early developmental stages of zebrafish (Arcanjo et al., 2018). Changes in gene expression involved in eye opacity were not confirmed at a higher biological scale using stages from 24 hpf to 96 hpf (Gagnaire et al., 2020), but mis-regulation of genes involved in muscle contraction were linked to effects in locomotion (Arcanjo et al., 2020). Li et al. (2021b) observed changes in the expression of genes involved in cardiac, cardiovascular and nervous system development and the metabolism of xenobiotics in zebrafish embryo (120 hdp) after HTO exposure to a lower dose (3.7 × 10⁷ Bq L⁻¹).

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

It is realised that the application and integration of omics approaches for ERA poses significant challenges in the real world. This is in contrast to human health arena where omics approaches are widely adopted to assess the biological responses following exposures to ionising radiations (Brackmann et al., 2020; Fang et al., 2022). In the natural environment, factors such as spatial and temporal variability in the physico-chemical, hydro morphological and seasonal characteristics in addition to species specific factors (e.g., sex, age, reproductive and life stages) are known to influence molecular responses to environmental stressors (Garcia-Reyero and Murphy, 2018; Martyniuk, 2018). Nevertheless, it has been recognised that use of the AOPs as a conduit between omics and ecological responses could facilitate the transition towards a more mechanistically informed hazard and risk assessment (Martyniuk, 2018; Tollefsen et al., 2022; Yahya et al., 2021). A recent study developed AOP networks for radiation

effects on reproduction using data from studies of different model species of primary producers, roundworms, earthworms, crustacean, and fish (Tollefsen et al., 2022). These taxon-focused AOPs were then used to define a common set of events into a consensus AOPs as an effort to gather information spanning different levels of biological organisation which included a high number of species into a common knowledge framework (Tollefsen et al., 2022). In this context, it is suggested that the formulation of a radiation specific AOP will form a framework within which data and knowledge from different organisms are synthesised to obtain useful information for ERA under different environmental conditions (Beresford et al., 2020). Further studies are, however, required to populate these AOPs with more diverse sets of data. This effort should, therefore, aim to reduce the bias towards only a few model species. This will provide weight of evidence based assessments in a complex environment (Halappanavar et al., 2020; Portugal et al., 2022; Tollefsen et al., 2022).

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

6. Knowledge-gaps and future directions

The use of nuclear power is expected to increase in the future, as it is considered essential in the transition to low-carbon economies (Adam-Guillermin et al., 2012; Kadiyala et al., 2016; Nie et al., 2019). In particular, tritium (³H) demand and production is expected to increase due to its potential role in nuclear fusion technology (Jean-Baptiste and Fourré, 2013; Pearson et al., 2018b; Singh et al., 2012). In this context, many nations face having to develop long-term strategies to manage tritiated radioactive waste and develop tools to assess its environmental impact (Bay et al., 2020; Di et al., 2012; Hanslík et al., 2017; Lainetti, 2016; Filho et al., 2013; Stamper et al., 2014).

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

For assessing ecotoxicological impact, future research should consider more diverse keystone species from different ecological niches and perform comparative studies, since it has been noted that data available exist for only a few taxonomic groups (Adam-Guillermin et al., 2012). Results

from diverse and ecologically relevant species will improve not only the datasets to establish appropriate benchmarks for relative sensitivities of the different species but will also facilitate the development of models used for more general environmental assessments and to study future exposure scenarios (Galeriu and Melintescu, 2011; Melintescu et al., 2011). Research should also investigate aspects of combined anthropogenic and natural stressors, including chemicals (e.g., metals, organics) and physical parameters (e.g., temperature, oxygen and dissolved organic carbon), in risk assessment of radionuclides in the environment (Dallas et al., 2012; Pearson et al., 2018a; Vanhoudt et al., 2012).

Different responses to tritium exposure, from molecular to behavioural, have been reported in various taxonomic groups during early life stages, with the potential transmission of effects across generations proposed through epigenetic mechanisms. Although little is known about these mechanisms (Merrifield and Kovalchuk, 2013; Thaulow et al., 2020), “omics” techniques could help to fill knowledge gaps and elucidate the relationship between molecular and organismal level responses (Dallas et al., 2012; Hurem et al., 2017; Parisot et al., 2015). These tools are utilised to study modes of action and intracellular signalling pathways and do not require a priori molecular targets to specific stressors, making them suitable in exploratory studies (Cambiaghi et al., 2017; Del Mar Amador et al., 2018). Despite the “omics” field rapidly evolving, application to tritium radiobiology is still at a relatively early stage and only a limited number of studies have implemented them (Arcanjo et al., 2018, 2020; Gagnaire et al., 2017; Li et al., 2021b). In addition, post-1990 ICRP recommendations have identified (i) induced genomic instability (ii), bystander effects and (iii) minisatellite mutation induction in germ line as novel, real radiobiological phenomena (Goodhead et al., 2004). These need to be elaborated while assessing the impact of tritium on NHB, in addition to potential transgenerational and epigenetic effects (Beresford et al., 2020; Horemans et al., 2018, 2019).

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

CRediT authorship contribution statement

MFF: data curation, methodology, writing-original draft, validation, reviewing and editing. **AT:** visualization, formal analysis, reviewing and editing. **ELV:** data curation, writing-original draft. **CG:** reviewing and editing; funding acquisition, project administration. **LL-J:** reviewing and

editing. **VM:** reviewing and editing; funding acquisition, validation, project administration. **ANJ:** conceptualisation, visualization, formal analysis, validation, reviewing and editing, supervision, funding acquisition, project administration.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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