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ORIGINAL ARTICLE

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Adverse outcome pathways (AOPs) for radiation-induced reproductive effects in environmental species: state of science and identification of a consensus AOP network

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ABSTRACT

Background: Reproductive effects of ionizing radiation in organisms have been observed under laboratory and field conditions. Such assessments often rely on associations between exposure and effects, and thus lacking a detailed mechanistic understanding of causality between effects occurring at different levels of biological organization. The Adverse Outcome Pathway (AOP), a conceptual knowledge framework to capture, organize, evaluate and visualize the scientific knowledge of relevant toxicological effects, has the potential to evaluate the causal relationships between molecular, cellular, individual, and population effects. This paper presents the first development of a set of consensus AOPs for reproductive effects of ionizing radiation in wildlife. This work was performed by a group of experts formed during a workshop organized jointly by the Multidisciplinary European Low Dose Initiative (MELODI) and the European Radioecology Alliance (ALLIANCE) associations to present the AOP approach and tools. The work presents a series of taxon-specific case studies that were used to identify relevant empirical evidence, identify common AOP components and propose a set of consensus AOPs that could be organized into an AOP network with broader taxonomic applicability.

Conclusion: Expert consultation led to the identification of key biological events and description of causal linkages between ionizing radiation, reproductive impairment and reduction in population fitness. The study characterized the knowledge domain of taxon-specific AOPs, identified knowledge gaps pertinent to reproductive-relevant AOP development and reflected on how AOPs could assist applications in radiation (radioecological) research, environmental health assessment, and radiological protection. Future advancement and consolidation of the AOPs is planned to include structured weight of evidence considerations, formalized review and critical assessment of the empirical evidence prior to formal submission and review by the OECD sponsored AOP development program.

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1. Introduction

The deposition of energy and subsequent ionization of biological macromolecules and intracellular water are typical initial effects of ionizing radiation (Rossi 1960). This deposition of energy and reactive oxygen species (ROS) production represent the first interactions with key biological molecules that cause a subsequent cascade of molecular and cellular events leading to an adverse outcome of human or environmental concern (Helm and Rudel 2020; Song, Xie, Lee, Brede et al. 2020; Tanabe, O'Brien et al. 2022b). Adverse effects of ionizing radiation display large diversity and span from acute (e.g. mortality) to chronic (e.g. growth, development and reproduction) effects.

Reproduction is one of three biological processes (with survival and growth) that are critical for population dynamics. Reproductive disturbances are often the focus of ecological risk assessment as perturbations may impact wildlife populations in the long-term, and consequently affect

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ecosystem functioning (Anderson and Wild 1994; Dallas et al. 2012). Studies addressing biological effects of chronic radiation in a wide range of non-human species have also shown that reproduction is the most radiosensitive phenotypic endpoint, compared to endpoints such as morbidity and mortality (Garnier-Laplace et al. 2010; Adam-Guillermin et al. 2018). In addition, species sensitivity and resilience to chronic radiation might differ, depending on species physiology and reproduction strategies (e.g. sexual, asexual, parthenogenetic, hermaphroditic, etc.) (Shuryak 2020). To date, developmental disorders and altered reproductive endpoints have been observed following radiation exposure. Although some effects on individual endpoints may appear of minor importance (Kryvokhyzha et al. 2019; Beresford et al. 2020), their combination may lead to ecologically significant consequences such as decrease in population abundance and/or extinction. Moreover, studies to address such reproductive perturbations are particularly relevant in multi-generational exposure scenarios since they may alter the ability of offspring to adapt to environmental stressors and to survive (Lynch 1992; Alonzo et al. 2008; Dutilleul et al. 2015).

Not surprisingly, literature shows that a substantial variability in sensitivity exists among species (Garnier-Laplace et al. 2013), with more than five orders of magnitude difference reported in the 10% effective dose rate (EDR10) for reproduction, mortality and growth. The rationale for these differences are not fully understood (Shuryak 2020), but factors related to taxa, life stages, size, age, environmental adaption and nutritional status have been proposed as explanations for the substantial interspecies differences in radiosensitivity (Sazykina 2018; Real and Garnier-Laplace 2020). It is envisioned that better qualitative and quantitative mechanistic understanding of causality between radiation exposure and effects at different levels of biological organization will enhance our understanding of radiosensitivity.

This type of scientific knowledge is also required by stakeholders in order to perform proportionate environmental impact assessment and regulation. A number of European Commission (EC), United Nations (UN) and International Commission on Radiological Protection (ICRP) projects have contributed significantly to such efforts (see section "Applications of AOPs"). However, the data on causal dose–effect relationships remain limited and findings generated from field studies are scarce. In this context, Adverse Outcome Pathways (AOPs), and especially AOPs focused on reproductive endpoints have the potential to: (i) deliver a mechanistic understanding to enable establishment of causal links (not simply associations) between radiation dose (or dose rate) and effect on specific reproductive endpoints; and (ii) organize and evaluate research findings that demonstrate lines of evidence underpinning regulatory benchmarks.

1.1. Adverse outcome pathway (AOP)

The AOP concept was originally proposed by US EPA (Ankley et al. 2010) as a conceptual knowledge framework to capture, organize, evaluate, and report causal linkages between the initial interaction of a stressor with its biological target (i.e. the molecular initiating event, MIE) and the onset of an adverse effect (adverse outcome, AO) of relevance for environmental or human health. Each AOP (Figure 1) represents a unit of development and evaluation that characterizes a single, linear sequence of events proceeding from the MIE to the AO through a series of intermediate key events (KEs) at different levels of biological organization (Villeneuve et al. 2014). The KEs are required to be essential and measurable, and the connection between the KEs is characterized by specific key event relationships (KERs) where biological plausibility, concordance of empirical evidence, essentiality of the KEs and the quantitative relationship between two adjacent KEs are evaluated by modified Bradford–Hill (B–H) criteria (Becker et al. 2015). These assessments provide an opportunity to evaluate and assess the weight of evidence (WOE) for both the KEs and KERs, and define the stressor, taxonomic, life stage and sex (gender) applicability domains. The WOE is a key requisite for ensuring that qualitative and quantitative evidence are included in the AOP descriptions, and where uncertainty of causality or lack of coverage are reflected and acknowledged. The AOPs were originally developed on the basis of effect information obtained from studies with prototypical chemicals, and the AOPs themselves considered to be representative for all chemicals and non-chemical stressors acting in a similar way (i.e. being stressor agnostic). Although originally developed for chemical interactions, there have been efforts over the last few years to expand AOPs to radiation (Chauhan et al. 2019; Chauhan, Wilkins et al. 2021; Chauhan et al. 2022a; Tanabe, O'Brien et al. 2022b). A key feature of AOPs is the reuse of AOP events into new AOPs that share one or more KEs and KERs to generate AOP networks (Chauhan, Hamada, Wilkins et al. 2022c; Knapen et al. 2015). These AOPs and AOP networks are in principle living documents that undergo scientific evolution through collaborative efforts to consolidate available toxicological consensus information and reflect common understanding of toxicity pertinent to a given regulatory challenge (Villeneuve et al. 2014). The development of AOPs follows structured data organization and evaluation guidelines (OECD 2018), submission to the AOP repository "AOP-wiki" (https://aopwiki.org) and internal and external review processes toward potential endorsement by the Organization for Economic Cooperation and Development (OECD) AOP developmental programme (OECD 2021). It is envisioned that AOPs could facilitate research developments through defining the AOP knowledge domain, identifying knowledge gaps and prioritizing research efforts, while providing flexible and mechanistically-informed assessment and evaluation of events spanning multiple levels of biological organization (Ankley et al. 2010; Vinken et al. 2017; Chauhan, Wilkins et al. 2021). There are currently 14 (out of 459) AOPs and 16 (out of 2021) KEs representing ionizing radiation in the AOP-Wiki, although none of these have reached the level of OECD endorsement

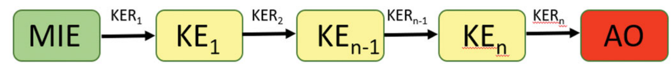

Figure 1. Example of a generic linear Adverse Outcome Pathway (AOP) that is the basis for developing sets of interlinked AOPs into AOP networks (AOPNs), schematics adapted from the user's handbook for AOP development (OECD 2018).

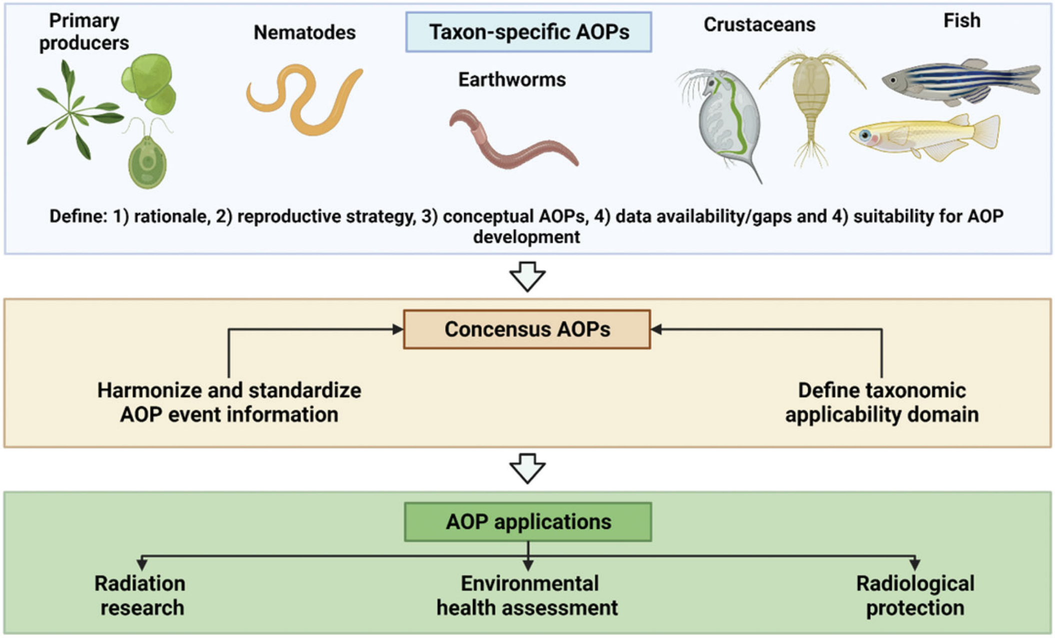

Figure 2. Workflow for integrating individual taxon- or species group-specific adverse outcome pathways (AOPs) into a consensus AOP (cAOP) and potential applications. Created with BioRender.com.

(accessed 6 July 2022). Out of these AOPs, seven are focused on reproduction and population relevant toxicity pathways.

1.2. MELODI–ALLIANCE workshop on radiation AOPs

Adverse outcome pathways for non-chemical stressors, as compared to chemical stressors, have had a slow development, but have in recent years been picking up a substantial momentum in the field of low dose radiation research (Chauhan et al. 2019). A dedicated workshop was co-organized by the Multidisciplinary European Low Dose Initiative (MELODI; https://melodi-online.eu/) and the European Alliance in Radioecology (ALLIANCE; https://radioecology-exchange.org/) 12–15 April (2021) to facilitate interactions between different research communities, to stimulate AOP discussions and support development of AOP relevant for health and environmental effects within the field of radiation (Chauhan, Hamada et al. 2021; Chauhan, Wilkins et al. 2021; Chauhan et al. 2022a). The present paper reports the developments of one of total four working groups (WGs) that focused on developing AOPs with human and/or environmental relevance. This specific WG, focusing on the development of AOPs for reproductive effects of radiation in non-human model species, was composed of experts in the field of low dose radiation, chemical research, reproductive biology, computational science, environmental assessment and AOP development. The diversity of composition, with experts working with different taxa (i.e., primary producers, nematodes, annelids, arthropods and fish) and methods spanning different levels of biological organization, were considered well suited to cover the area of relevance in a broad and complementary manner.

1.3. Objectives

The aim of the present work was to review the available data for radiation effects in a range of organisms to assess the potential for developing AOPs for radiation-mediated effects on reproduction and subsequent population changes. The work focused on a few model organisms that represent different reproductive strategies and sensitivities to radiation, and for which data from laboratory studies on radiation-mediated effects were well developed. The work (Figure 2) consisted of: (1) developing a set of taxon-specific AOPs; (2) identifying common AOP components (events) to develop consensus AOPs (cAOPs) with a broad taxonomic applicability; and (3) discussing how reproductive-relevant AOPs could be applied in radiation (radioecological) research, environmental health assessment and radiological protection.

2. Case Studies

A set of case studies (Figure 2) were developed to illustrate the diversity and commonalities in AOP components across laboratory model species representing different taxa (primary producers, nematodes, earthworms, crustaceans and fish). Each case study (see Supplementary Table S1 for details) represented a different taxonomic group and was developed using available data collected through narrative reviews. The species groups covered were chosen on basis of available knowledge from literature and data generated by the WG participants, rather than defining a broad as possible taxonomic applicability domain *a priori*. Available data were organized within an AOP framework and assessed for conformity to the B–H criteria at the taxon-specific level using the Guidance Document for developing and evaluating AOPs (OECD 2018) and the resultant AOPs were submitted to the AOP-wiki, when considered sufficiently mature (see Supplementary Table S1).

Discussions to formulate common MIEs, KEs and AOs for the different taxon-specific AOPs were initially undertaken to harmonize and standardize AOP components to those found in the AOP-wiki as well as to identify events that may be applicable to larger groups of organisms. The MIE, the first interaction between stressor and the biological targets (Allen et al. 2014), was defined as the physical step of "Deposition of energy" and expressed in gray, Gy (J kg^{-1}). This step is widely accepted by both physicists and radiobiologists/radioecologists as it precedes ionization of cell components such as water, biomolecules (DNA, protein, lipids, etc.) and appears to be credibly linked to reported biological effects (Nikjoo et al. 1998). The initiation of biological effects depends on both ionization and non-ionization events (such as excitation), giving rise to both direct (e.g. double or single strand DNA breaks, production of ROS and reactive nitrogen species (RNS)) and indirect (e.g. ROS/RNS-mediated oxidative DNA damage, lipid peroxidation, protein deactivation, etc.) effects. Although direct damage is often studied as the earliest biological events of radiation, it is increasingly acknowledged that indirect damage caused by ROS (and potentially RNS) from radiolysis of water is also contributing to radiation-induced pathologies (Tanabe, O'Brien et al. 2022b). The role and magnitude of the impact of direct and indirect effects is dependent on the radiation type, e.g. alpha, beta, gamma or X-rays (Nikjoo et al. 2016). This transition from earlier efforts to define the MIE as increase in ROS formation and increase in DNA damage (Song, Xie, Lee, Brede et al. 2020) thus seems reasonable, and complies with harmonization efforts for other radiation-relevant AOPs (Tanabe, Beaton et al. 2022a; Tanabe, O'Brien et al. 2022b). Data used to develop the individual taxon-specific AOPs originated from acute and chronic studies under controlled laboratory conditions predominantly with external gamma radiation (low LET – linear energy transfer) that spanned fairly large dose rate and dose ranges. The studies used to populate the individual taxon-specific AOPs in this study were typically from a limited set of laboratory model species that due to ease of handling, standardization, short to moderate life cycles, well-documented biology and well-characterized toxic responses to radiation, provided data of suitable quality and coverage of relevant effects spanning different levels of biological organization. These organisms also displayed substantial diversity in terms of evolutionary classification (plants, invertebrates, vertebrates), habitat, position in the food chain, life cycle length, time to sexual maturity and reproductive strategies. Many of the taxon- or species group-specific AOPs (e.g. for primary producers, androdioecious nematodes, parthenogenesis crustaceans and earthworms) were used to revise existing or submit new entries to the AOPwiki as part of this work (see Supplementary Table S1 for details).

2.1. Ionizing radiation effects on primary producer reproduction

2.1.1. Rationale

As organisms that can derive energy from sunlight and abiotic sources, primary producers, such as plants, algae, moss, lichens and specific bacteria, play a key role in ecosystems. The reproductive state within the life cycle of a plant is known to be sensitive to different stress factors and is influenced by various environmental cues (Blümel et al. 2015). While the main interest on studying the effects of ionizing radiation in plants originally came from agriculture research on improving plant yield (Posner and Hillman 1960; Bowen et al. 1962; Sax 1963), nuclear weapons testing in the 1950–1970s as well as the nuclear accidents of Kyshtym, Chernobyl and Fukushima motivated environmental studies on the effects on different primary producers for hazard and risk assessment (Copplestone et al. 2003; Real et al. 2004; Brechignac et al. 2016; Beresford et al. 2020; Shuryak 2020). Among the biological effects observed, failure of reproduction was one of the most severe effects reported, and to date 30% of the publications addressing ionizing radiation in primary producers implicate reproduction as a major contributor to the effects observed (Real et al. 2004). Studies of model primary producers, such as flowering plants, aquatic macrophytes and algae, have demonstrated dose- and dose rate-dependent inhibition of reproduction after chronic exposures to gamma radiation in plants displaying different reproductive strategies.

2.1.2. Reproductive strategy

Generally, there are two types of reproduction strategies in plants, sexual and asexual production. Sexual reproduction produces offspring by fusion of gametes involving meiosis and fertilization in their reproductive organs (Hamilton et al. 1990). In contrast, plant asexual reproduction uses plant roots, stems, leaves, and other nutritional organs to reproduce genetically identical offspring plants by cell division or fragmentation (Barrat-Segretain 1996).

2.1.3. Conceptual AOPs

An AOP network consisting of four interconnected AOPs has been proposed and submitted to the AOPwiki (AOP #386, #387, #388 and #435) for radiation-induced reproductive effects in primary producers. This AOP network (see Supplementary Table S1 for details) was developed using data from studies of a few model species, and can be potentially applied to other types of ionizing radiation (e.g. X-rays) or non-ionizing radiation (e.g. UV radiation), that display similar toxicity mechanisms involving oxidative stress and DNA damage. As the KEs were highly recapitulative and relevant to common biological processes, the taxonomic applicability domain can potentially cover most terrestrial and aquatic primary producers that contains chloroplasts and mitochondria at any life stage. The AOP network was in general considered gender (sex) unspecific because most plants are hermaphroditic (Christopher et al. 2019).

2.1.4. Brief evaluation of data availability/gaps

Supporting evidence for this AOP network were captured from various studies with external beta (0.08–97 mGy h^{-1}) and gamma (^{137}Cs and ^{60}Co, 0.08–1500 mGy h^{-1}) radiation on the microalgae *Chlamydomonas reinhardtii*, the macrophyte *Lemna minor*, and the higher plants *Pinus sylvestris*, *Arabidopsis thaliana* and *Oryza sativa*. However, different primary producers may display species/taxon-specific reproduction strategies that give rise to larger diversity in AOP events than that proposed herein. The current AOPs were predominantly concerned with energy supply and genotoxicity induced by radiation. Other sexual reproduction strategies of primary producers to radiation, including oogenesis, flowering, and seed germination, were not included in the proposed AOPs and require additional investigation.

2.1.5. Assessment of suitability for AOP development

The overall evidence support of the current AOP network was considered "moderate" due to the incomplete literature review and predominance of in-house studies. Although individual AOPs have been proposed, they are still in the early stages of development and WOE assessment of a larger domain of literature is currently being undertaken.

2.2. Ionizing radiation effects on nematode reproduction

2.2.1. Rationale

Nematodes, or roundworms, are a diverse animal phylum inhabiting a broad range of environments. Of the many species of nematodes, *Caenorhabditis elegans* is an important ecotoxicological test organism, representative of soil ecosystem nematode species. *Caenorhabditis elegans* is a free living, non-parasitic, bactivorous species as well as an extensively studied model within life sciences including neurology, molecular biology, and genomics (Johnson and Hartman 1988; Daly 2009; Corsi et al. 2015). Reprotoxic effects in nematodes have been observed as decreased number of progeny, following both chronic and multigenerational exposure of wild type (N2) *C. elegans* to gamma radiation (Daly 2009; Buisset-Goussen et al. 2014; Dubois et al. 2018; Maremonti et al. 2019; Dufourcq-sekatcheff et al. 2021; Guédon et al. 2021). Reproduction is a radiosensitive process in *C. elegans*, relevant for population fitness, and thus represents a suitable adverse effect in hazard and risk assessment.

2.2.2. Reproductive strategy

Caenorhabditis elegans is androdioecious and consists of hermaphrodites, producing both oocytes and spermatocytes, and rare males (under optimal conditions, a population of wild-type N2 consists of mostly self-fertilizing hermaphrodites (99%) and a few males). Its main reproductive strategy is therefore self-fertilization but hermaphrodites can also mate with males. In addition, *C. elegans* hermaphrodites are protandrous, first producing a defined number of sperm (300), before switching to oogenesis, thus gonadal development and gamete production proceed via a defined developmental program. Therefore, while oocytes are constantly produced and experience physiological programmed cell death (~50%), which is increased by gamma radiation (Maremonti et al. 2019), sperm is a limiting factor in hermaphrodites. The developmental cycle from embryo fertilization to sexually mature L4-stage adult takes 3–4 days.

2.2.3. Conceptual AOPs

Based on data obtained from lab studies, a network of AOPs for reproductive effects of chronic exposure to radiation leading to reduced reproduction in *C. elegans* has previously been proposed and submitted to the AOPwiki (AOP #396). This AOP network (see Supplementary Table S1 for details) was developed for chronic external gamma radiation (low LET, ^{60}Co, ^{137}Cs) at a total dose >2.8 Gy (dose rates mostly ranging from 40 to 100 mGy h^{-1}, with 16–65 h exposure). It applies to androdioecious species of nematodes consisting of hermaphrodites and rare males, that display similar reproductive strategy as *C. elegans.* No data were available for dioecious nematode species, with separate male and female individuals, thus limiting the ability to extend the applicability of the AOP network to the whole nematode taxon at the time of development. This AOP focused on hermaphrodites as very few studies exist on males only and have a life stage applicability to larval development (from egg to young adult stage).

2.2.4. Brief evaluation of data availability/gaps

In this AOP network, most KEs were directly measurable, thus making it possible to find direct evidence (e.g. sperm number, apoptosis). However, some data gaps remained regarding effects on meiosis for which there is transcriptomic evidence only, and on KER (e.g. link between DNA damage and meiosis impairment). Moreover, data gaps remain for the extrapolation of laboratory studies to outcomes at the population level and inclusion of other nematode species than *C. elegans*.

2.2.5. Assessment of suitability for AOP development

The overall evidence support of the current AOP network was considered moderate/high, as the supportive data were obtained from multiple experiments and a variety of methodologies performed by different teams. Although this conceptual AOPs has been submitted to the AOPWiki, the WOE of all KE/KER remains to be completed.

2.3. Ionizing radiation effects on earthworm reproduction

2.3.1. Rationale

Earthworms are predominantly test organisms in ecotoxicology, as well as a key species in terrestrial ecology. Earthworms are also one of the organisms receiving high doses in contaminated environments, due to their residence in soils, and population level effects have been documented after both the Mayak and the Chernobyl Accidents (Krivolutzkii et al. 1992; IAEA 2006; Hinton et al. 2007; Allen et al. 2014; Bonzom et al. 2016). Earthworms are on the list of International Commission on Radiological Protection (ICRP) Reference Animal and Plants (RAP), making them an appropriate test organism for assessing effects of radiation both in the laboratory and in field assessments (ICRP 2008). Among these, the earthworm *Eisenia fetida* represents a model species used for acute and chronic (reproductive) toxicity tests for various pollutants in terrestrial systems and is the recommended species in standardized test guidelines for toxicity to soil organisms (OECD 1984; Reinecke et al. 2001).

2.3.2. Reproductive strategy

Eisenia fetida is a hermaphrodite earthworm species, with cross-fertilization presenting separate testes and ovaries that function simultaneously and during copulation they can both donate and receive spermatozoa. Mature ova and spermatozoa are discharged into the cocoon where fertilization takes place. Cocoons continue to be formed until the stored seminal fluid has been used (Edwards and Bohlen 1996; Jamieson 1992).

2.3.3. Conceptual AOP

Based on data obtained from laboratory studies, a conceptual AOP was proposed and submitted to the AOPwiki (AOP #444) for reproductive effects of radiation on earthworms. This AOP (see Supplementary Table S1 for details) primarily focused on effects ranging from embryos to that of sexually mature adults of *E. fetida*, as early spermatogenesis and developmental stages are more sensitive to radiation. The AOP can be extended to other hermaphrodite earthworm species however, other species of earthworms have different reproductive strategies (e.g. parthenogenesis), and different levels and rates of reproduction, and may thus show different responses to radiation. The supporting evidence came mainly from chronic studies (8–13 weeks exposure of consecutive life stages) with external gamma radiation (^{60}Co) exposure in the dose rate range 4–10 $mGy\,h^{-1}$, although acute studies were undertaken for some toxicity endpoints such as apoptosis.

2.3.4. Brief evaluation of data availability/gaps

In this AOP, most KEs were directly measurable and providing potential evidence for connections between different events. However, as the data have been generated in a limited number of studies and with a limited number of species (*E. fetida*), additional studies to strengthen the empirical evidence for KEs, KERs for *E. fetida* and other earthworm species as well as extrapolation of laboratory studies to outcomes at the population level are required.

2.3.5. Assessment of suitability for AOP development

The overall evidence support for this AOPs was considered moderate and additional work on expanding the AOP for other species of earthworms with similar reproductive strategy and performing WOE consideration remains to be performed.

2.4. Ionizing radiation effects on crustacean reproduction

2.4.1. Rationale

Many crustacean species are key primary consumers (herbivores) connecting primary producers and higher consumers (carnivores) in aquatic food webs (Burns and Schallenberg 1996; Covich et al. 1999). Crustaceans such as the water flea *Daphnia magna* have been widely used as indicators for ecosystem health and as standard species in regulatory toxicity tests for ecological hazard and risk assessment (OECD 2012). Freshwater crustaceans, including *Asellus aquaticus* and *Daphnia pulex*, occur in contaminated lakes and ponds in Chernobyl, although reported variations in effects observed in the field do not seem to correlate to estimated dose rates (Fuller et al. 2017; Goodman et al. 2019). In both laboratory and field, the number of studies reporting on adverse effects of radiation has increased over the past decades, revealing declines in reproduction, offspring fitness and body size and mass, and alterations in molting patterns, reviewed in Dallas et al. (2012) and Fuller et al. (2015).

2.4.2. Reproductive strategy

Crustaceans form a large taxon of arthropods, including a diversity of marine, freshwater and terrestrial animals such as copepods, decapods (crabs, shrimps, prawns, lobsters, crayfish), euphausiids (krill), isopods, amphipods, woodlice, barnacles, etc. and showing an extremely wide range of reproductive strategies. Although the vast majority of crustaceans reproduce sexually, planktonic species of the genus *Daphnia*, from which a large amount of radiation effect data originate, are known to alternate between sexual and asexual reproduction across the seasonal cycle (Ebert 2005). After fertilization, a pair of eggs is deposited in a protective shell known as the ephippium, which undergo a diapause in the sediment over the winter. Hatching occurs under favorable environmental conditions. During spring and summer, parthenogenetic females can produce diploid eggs that develop directly into genetically identical daughters, without meiosis and fertilization. Under autumnal conditions, some diploid asexual eggs develop into males and females by producing haploid oocytes which require fertilization. Regulatory toxicity tests, focusing on reproductive output, are achieved using parthenogenetic cultures of daphnids (OECD 2012).

2.4.3. Conceptual AOPs

Based on data obtained from laboratory studies, an AOP network consisting of four conceptual AOPs has been proposed and submitted to the AOPWiki (AOP #216, #238, #299 and #311) for radiation-induced reproductive effects in crustaceans. The AOP network (see Supplementary Table S1 for details) was in general female specific because supporting evidence covers radiation effects on oocytes, ovaries and egg production only. The majority of studies were conducted on clonal females which reproduce via asexual parthenogenesis (without meiosis, without fertilization), including *D. magna* and other species of the daphnid family (Gilbin et al. 2008;

Marshall 1962, 1966; Parisot et al. 2015; Trijau et al. 2018; Song, Xie, Lee, Brede et al. 2020). The proposed AOPs were considered applicable to female crustaceans that reproduce sexually, as similar decline in egg production was reported in the Kuruma prawn *Marsupenaeus japonicus*, the giant freshwater prawn *Macrobrachium rosenbergii* and the marine copepod *Paracyclopina nana* (Sellars et al. 2005; Won and Lee 2014; Stalin et al. 2019). The supporting evidence came from studies with external gamma radiation (^{137}Cs and ^{60}Co, 6.5 μGy h^{-1} to 43.1 mGy h^{-1}). The proposed AOPs might be applicable to other types of radiation, including ionizing (e.g. X-ray) and some non-ionizing radiation (e.g. ultraviolet B radiation) (Song, Xie, Lee, Tollefsen et al. 2020).

2.4.4. Brief evaluation of data availability/gaps

Key events and key event relationships at the molecular and cellular levels (e.g. links between energy deposition, DNA strand breaks, ROS production, and oxidative DNA damage) are considered canonical. At higher levels of biological organization, the weight of evidence was particularly strong for parthenogenetic *D. magna*, with many studies addressing gamma radiation effects at multiple levels of biological organization, over the whole asexual cycle and across multiple generations (Gilbin et al. 2008; Parisot et al. 2015; Trijau et al. 2018; Song, Xie, Lee, Brede et al. 2020). In other species of the crustacean subphylum, however, studies on gamma radiation-induced effects on reproductive success were comparatively well documented in females and extremely scarce in males, with only one example reported for Kuruma prawn *M. japonicus* (Sellars et al. 2005; Fuller et al. 2015). In this context, additional studies are required to strengthen the plausibility of radiation effects on the gonad, gametes and fertility in crustacean males and inclusion of other species than daphnids to expand the taxonomic applicability of the AOP network.

2.4.5. Assessment of suitability for AOP development

The overall evidence support for this AOP network was considered moderate, although additional WOE considerations are considered instrumental for consolidating the AOPs submitted to the AOPwiki.

2.5. Ionizing radiation effects on fish reproduction

2.5.1. Rationale

Fish occupy fresh and marine waters and may potentially be exposed to sufficiently high levels of ionizing radiation to cause reproductive effects. Significant effects in fish gonads from chronic radiation exposure would be unlikely at dose rates less than 1 mGy h^{-1} (UNSCEAR 1996), and sufficiently mechanistically informative reproductive studies are limited to a few model species such as Zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*). The primordial gonads in developing fish embryo and the newly hatched fry have been found to be somewhat more sensitive to acute radiation exposure than adult fish. However, reduced reproductive success was observed in the range between 0.04 and 0.4 mGy h^{-1} and indicates that reproductive processes may be particularly susceptible to ionizing radiation (ICRP 2008; Hurem, Gomes et al. 2017; Guirandy et al. 2022). Although radiosensitivity differs between fish species, zebrafish has displayed high sensitivity to ionizing radiation (Hurem, Gomes et al. 2017; Guirandy et al. 2022) during gametogenesis (development of the germ cells) and embryogenesis (including gonad development). Irradiation of reproductively active male and female medaka, which have a comparable generation interval, conditioning and reproductive strategy (asynchronous spawning) as zebrafish, also increased the number of deformed and dead embryos resulting from subsequent matings at a dose of 5Gy (ICRP 2008). Exposure of juvenile and adult *D. rerio* has caused negative effects on both male and female reproductive capacity (Hurem, Gomes et al. 2018; Hurem, Martín et al. 2018; Guirandy et al. 2019). These exposures did not cause any acute effects to the adults, which were able to maintain reproductive behavior and generate gametes. However, exposure to 53 mGy h^{-1} (28 days) and 50 mGy h^{-1} for 10 days caused 100% mortality to resulting embryos occurring at the gastrulation stage. Exposure of parental generation during gametogenesis 8.7–50 mGy h^{-1} also induced reprotoxic effects measured as reduced number of offspring. The offspring also showed elevated frequency of developmental defects accompanied by persistently elevated ROS, lipid peroxidation, DNA damage, genomic instability, and bystander effects. Furthermore, the exposed parents developed complete senescence of reproductive organs within 1.5 years post irradiation.

2.5.2. Reproductive strategy

As most teleost fish, zebrafish and medaka are oviparous, meaning that the females lay unfertilized oocytes, which are externally fertilized by the males. Furthermore, they are asynchronous spawners and ovulate regularly over prolonged period of time, in contrast to many other fish species that are synchronous spawners and reproduce once per year (Jalabert 2005). Gametogenesis cycles (oogenesis and spermatogenesis) consist of mitotic, meiotic and post-meiotic cells that ultimately lead to sperm and oocyte formation. Up to the end of a period of 25–35 days post-fertilization (dpf), zebrafish gonads are not gender-differentiated and exist as a juvenile ovary structure (Maack and Segner 2004), which is known as juvenile hermaphroditism (Uchida et al. 2002; Slanchev et al. 2005). Oogenesis begins in all individuals, regardless their future sex, while actual sex differentiation begins later in gonadal development. During sex differentiation, immature oocytes undergo degeneration through apoptosis in presumptive males, while oogenesis proceeds to completion in presumptive females at 50 dpf (Uchida et al. 2002; Koç et al. 2008). In medaka, male and female gonads are determined by XY–XX sex chromosomes and the presumptive male and female germ cells develop after hatching. Oogonia continue to proliferate actively, while the proliferation of the male germ cells is arrested for 2 weeks, until they resume mitotic activity and proliferate. A part of the female germ cells enters meiosis just after hatching, while this is delayed in the developing testes for 40–50 dpf (Schartl 2004).

2.5.3. Conceptual AOPs

Based on data obtained from lab studies, an AOP network was proposed for reproductive effects of radiation on *D. rerio* and *O. latipes* and submitted to the AOPWiki (AOP #461). This AOP network (see Supplementary Table S1 for details) had taxonomic applicability to asynchronous fish species and potentially also to other oviparous fish exposed to ionizing radiation during sensitive life stages. The AOP network displayed male and female applicability (Hurem, Gomes et al. 2018), as radiation affects both oogenesis and spermatogenesis. The supporting evidence came predominantly from controlled chronic exposure studies (10–30 days) with external gamma radiation using ^{60}Co (5–53 mGy h^{-1}) and ^{137}Cs (0.05–50 mGy h^{-1}).

2.5.4. Brief evaluation of data availability/gaps

Key event and key event relationships associated with molecular and cellular effects (i.e. deposition of energy, ROS production, oxidative stress, DNA damage, etc.) were considered generally applicable to most fish species as being conserved across taxa. However, supporting evidence for the tissue and organ effects captured in this AOP network was developed based on the data from a limited number of studies with the laboratory zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). Fish species display a considerable diversity in reproductive strategies, sexual development programs and time to reach sexual maturity, that would necessitate a more thorough assessment of the AOP applicability beyond the studies assessed herein. Although disruption of oogenesis is well supported by evidence, knowledge of radiation-mediated effects on the endocrine system and spermatogenesis may require additional evaluation.

2.5.5. Assessment of suitability for AOP development

The supporting evidence of these AOP network was considered moderate, supported by current data obtained from independent laboratory exposures. However, this AOP network is in the early stages of development and WOE assessment is required to expand on and consolidate the AOP submitted to the AOPwiki.

3. Consensus AOPs

AOP organizes a chain of events leading from a MIE via measurable KEs on different levels of biological organization to an adverse outcome at the organism or population level. In the case of radiation-induced reproductive effects, the AOPs should reflect a causal progression from an initial deposition of energy in a cell to a series of KE at higher levels of biological organization. It was necessary to reduce a complex biology spanning multiple species and taxon, such as species or taxa-specific differences in biology and sexual reproductive strategies to reach this objective. In this respect, the taxon-specific AOPs were used to propose a set of consensus AOPs that represent causal events in radiation-induced reproductive disturbances associated with reduction of population growth rate across a broad taxonomic applicability domain (Figure 3). Supporting evidence spanning multiple levels of organization and taxa were summarized for each AOP event (Table 1).

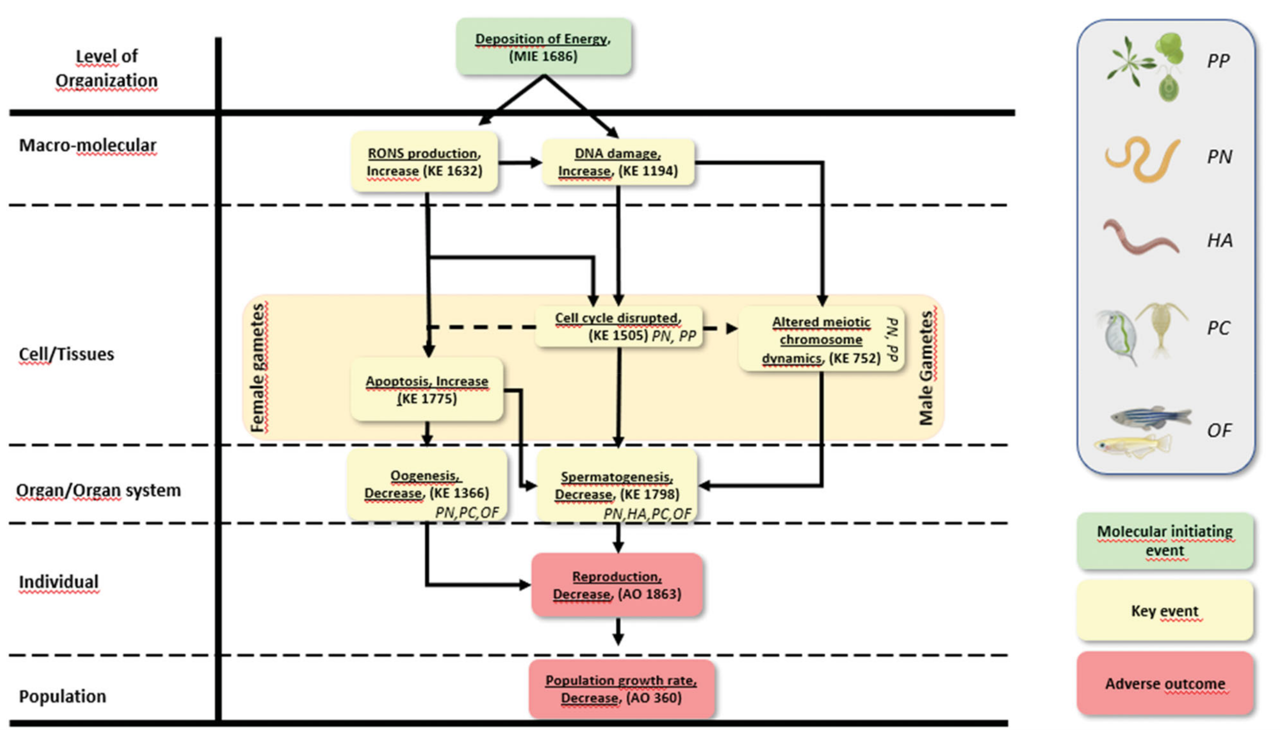

Figure 3. Integration of data from individual taxa into a network of consensus Adverse Outcome Pathways (cAOPs) spanning primary producers (PP), androdioecious nematodes (PN), hermaphroditic annelids (HA), parthenogenic crustaceans (PC) and oviparous fish (OF) on basis of existing model species data. The solid lines indicate key event relationships (KERs) with broad taxonomic applicability, whereas the broken arrows represent KERs with limited empirical evidence from different species. Created with BioRender.com.

Table 1. Definition of events, detections methods and studies used to populate a consensus Adverse Outcome Pathway Network (AOPN) for reproductive effects in environmental species.

Type	Event ID	Title	Parameter measured	Taxon	Reference
MIE	1686	Deposition of Energy	Fluorescent Nuclear Track Detector (FNTD); Nanodots dosimetry; Liquid scintillation counting; RPL dosimetry; TLDs dosimetry	PP, PN, HA, PC, OF	Dubois et al. 2019; Dufourcq-sekatcheff et al. 2021; Gomes et al. 2017; Guédon et al. 2021; Hurem, Gomes et al. 2018; Maremonti, Brede et al. 2020; Maremonti et al. 2019; Maremonti, Eide et al. 2020; Nilsson and Brahme 1983; Rahmanian et al. 2017; Smith et al. 2012; Song, Xie, Lee, Brede et al. 2020; Van Hoeck et al. 2015a, b; Xie et al. 2019
KE	1632	Increase in RONS	Fluorescent probe assays (H2DCFDA, DHR123); Antioxidant gene expression; Antioxidant enzyme activities; GSSG/GSH imbalance, In vivo measurements of H2O2; Indirect measurement as oxidative DNA damage	PP, PN, HA, PC, OF	Gagnaire et al. 2021; Gagnaire et al. 2015; Gomes et al. 2017; Guédon et al. 2021; Guirandy et al. 2019; Hertel-Aas, Oughton et al. 2011; Hurem, Gomes et al. 2017; Hurem, Gomes et al. 2018; Hurem, Martín et al. 2018; Kariuki et al. 2019; Maremonti, Brede et al. 2020; Maremonti, Eide et al. 2020; Olsvik et al. 2010; Reisz et al. 2014; Shin et al. 2011; Song et al. 2014; Song, Xie, Lee, Brede et al. 2020; Song, Xie, Lee, Tollefsen et al. 2020; Van Hoeck et al. 2015a, b; Xie et al. 2019
KE	1194	Increase, DNA damage	Comet assay; Expression of DNA damage related genes and proteins; Increase, Oxidative damage to DNA; 8-oxo-G ELISA assay	PP, PN, HA, PC, OF	Adam-Guillermin et al. 2012; Azzam et al. 2012; Blagojevic et al. 2019; Dubois et al. 2019; Gagnaire et al. 2015; Gomes et al. 2017; Guédon et al. 2021; Hertel-Aas, Oughton et al. 2011; Hurem, , Martín et al. 2017; Kariuki et al. 2019; Maremonti et al. 2019; Oladosu et al. 2016; Pereira et al. 2011; Song et al. 2014; Van Hoeck et al. 2015a, b; Vanhoudt et al. 2014
KE	1505	Cell cycle, disrupted	Cell cycle arrest; Expression of relevant genes and proteins	PN, OF	Dubois et al. 2019; Guédon et al. 2021; Hurem, Martín et al. 2017; Hurem, Martín et al. 2018; Maremonti, Brede et al. 2020; Maremonti et al. 2019; Maremonti, Eide et al. 2020
KE	1864	Increase, Programed cell death	TUNEL assay; Cell division and cell cycle arrest relevant genes; Flow cytometry of endoploidy levels; Atrophy of seminal vesicles and testes; CED-1 fluorescent reporter strain for DNA damage-induced germ cell apoptosis	PP, PN, HA, PC, OF	Biermans et al. 2015; Guédon et al. 2021; Hertel-Aas, Oughton et al. 2011; Hurem, Martín et al. 2017; Hurem, Martín et al. 2018; Kariuki et al. 2019; Maremonti, Brede et al. 2020; Maremonti, Eide et al. 2020; Rusin et al. 2019; Scaldaferro et al. 2013; Song, Xie, Lee, Brede et al. 2020; Song, Xie, Lee, Tollefsen et al. 2020; Sowmithra et al. 2015; Van Hoeck et al. 2015a, b; Xie et al. 2020
KE	1366	Decrease, Oogenesis	Egg-laying rate; Increase in pre-vitellogenic follicles; Regression of gonads; Reduction in visual eggs	PN, PC, OF	Dufourcq-sekatcheff et al. 2021 ; Hurem, Gomes et al. 2018; Hurem, Martín et al. 2018
KE	752	Altered, Meiotic chromosome dynamics	Cytological analysis; Expression of relevant genes and proteins	PP, PN	Chu and Shakes 2013; Maremonti et al. 2019; Rao and Rao 1977
KE	1798	Decrease, spermatogenesis	Sperm count; atrophy of seminal vesicles and testes; Regression of testis; Reduction in spermatogonia; Increase in pollen abnormalities; Decrease pollen viability; Pollen shape analysis	PP, PN, HA, OF	Dufourcq-sekatcheff et al. 2021; Guédon et al. 2021; Hertel-Aas et al. 2007; Hurem, Martín et al. 2018; Hyodo Taguchi and Egami 1976; Maremonti et al. 2019; Møller et al. 2016; Nurmansyah et al. 2018
AO	1863	Decrease, Reproduction	Reduced fertility, Reduced egg count, Reduced fertility, Reduced fecundity, changes to germination characteristics	PP, PN, HA, PC, OF	Amirikhah et al. 2021; Cheng et al. 2018; Dubois et al. 2018; Dufourcq-sekatcheff et al. 2021; Guédon et al. 2021; Guirandy et al. 2022; Guirandy et al. 2019; Hertel-Aas, Brunborg et al. 2011; Hinton et al. 2004; Hurem, Martín et al. 2018; Maremonti et al. 2019; Song, Xie, Lee, Brede et al. 2020; Sowmithra et al. 2015; Xie et al. 2019
AO	360	Decrease, Population growth rate	Reduction in plant area and numbers; Reduction in offspring over several generations; Dynamic energy-based predictive modeling; population modeling	PP, PN, HA, PC, OF	Alonzo et al. 2016; Cheng et al. 2018; Guédon et al. 2021; Guirandy et al. 2022; Guirandy et al. 2019; Hertel-Aas et al. 2007; Hurem, Gomes et al. 2018; Hurem, Martín et al. 2018; Lecomte-Pradines et al. 2017; Xie et al. 2019

MIE: molecular initiating event; KE: key event; AO: adverse outcome.

At the molecular scale, energy deposition (MIE 1686) in all taxa reviewed led to direct ionization of biomolecules including DNA (KE 1194) and free radical (ROS) generation (KE 1632) presumably through water radiolysis. The observed RONS production, predominantly detected as an increase in intracellular ROS, induced indirect DNA damage notably through oxidation of DNA (KE 1194), increase in programmed cell death or apoptosis in sufficiently damaged reproductive cells (KE 1775) in all taxa assessed. Evidence from studies with flowering plants and androdioecious nematodes suggested that disruption of reproductive cell cycle processes (KE 1505) and alteration in meiotic chromosome dynamics (KE 752) were also relevant events in the progression from DNA damage to disruption of gamete development. Although substantial commonality was observed across different taxa in earlier events of the AOP network, considerable differences were observed in the intermediate events associated with reproductive cell development. This diversity was large due to different reproductive strategies, where both sexual (e.g. fish, some primary producers, crustaceans and hermaphrodism in earthworms and androdioecious nematodes) and asexual (e.g. many primary producers, some parthenogenic crustaceans such as *D. magna*) reproduction was relevant. Despite this disparity in reproductive strategies, subsequent events were associated with specific germ or reproductive cell development, broadly defined as female gametes (oocytes) and male gametes (spermatocytes or pollen). Although terminology of cell types differed, disturbances of the reproductive cells development and differentiation were closely associated with the disruption of oogenesis (KE 1366) and spermatogenesis (KE 1798) in the investigated taxa. The radiation-mediated disruption of oogenesis was predominantly relevant for parthenogenetic crustaceans (*D. magna)*, oviparous *fish* (*D. rerio* and *O. latipes*), androdioecious nematodes (*C. elegans*), whereas disturbance of spermatogenesis was found relevant for *C. elegans, E. fetida*, *D. rerio*, and primary producers undergoing cell division or plant fragmentation. Reduction or interference in either oogenesis or spermatogenesis were both expected to affect the overall reproduction (AO 1863) that was found to be applicable to all taxa at different dose rates and doses.

Data generated from controlled laboratory studies with ionizing radiation and reproductive endpoints have additionally been used to parametrize population models to characterize potential population impacts of reduced reproduction in *C. elegans*, *D. magna*, and *E. fetida* (Alonzo et al. 2016; Lecomte-Pradines et al. 2017). These studies represented a limited albeit good set of examples of how laboratory-based studies can be used to predict population-relevant effects and support individual to population extrapolations. Such modeling efforts could also contribute to predicting population dynamics under ecologically relevant situations, although this would require that field conditions, including high level of biological variation, complex population and community interactions, changing environmental conditions and co-exposure to multiple stressors, are considered. Reproduction is together with growth and survival the most relevant apical endpoints critical for population dynamics and evolutionary fitness (Stearns 1992). Toxicity due to stressor exposure can cause changes through a diversity of mechanisms, leading to decline in population growth rate and changes to population demographics (Kooijman 2010). The AOP does despite its shortcomings to address complex ecological interactions, represent a flexible knowledge aggregation framework that is designed to be "fit for purpose" and evolve through critical evaluation and inclusion of new information from international collaborative efforts as those described herein.

4. Applications of the AOPs

There has been increasing interest in the potential for the AOP framework to enhance radiation protection (Chauhan et al. 2022a). Such efforts would be expected to include advancing both radiation research as well as regulatory use of AOPs in context of hazard assessment and risk characterization (Chauhan et al. 2019, Chauhan, Stricklin et al. 2021; Chauhan, Wilkins et al. 2021; Chauhan, Hamada, Wilkins et al. 2022c). As environmental impact assessment and regulation should be proportionate and based on sound scientific evidence, they would need to be defensible to stakeholders (Schmidt et al. 2010; Environment Agency 2013). Achieving this requires the systematic acquisition and organization of scientific knowledge in a transparent manner that directly facilitates robust decision making (Carusi et al. 2022). Over the last two decades, there have been significant advances in the development of environmental radiation protection. Through projects such as the EC ERICA project (Larsson 2008; Brown et al. 2016), UN IAEA programmes (IAEA 2014) and the work of the ICRP (ICRP 2008, 2017), environmental radiation protection has already advanced to a stage where decision makers have software and tools, underpinned by robust datasets, to inform proportionate regulation. However, the data on dose–effect relationships remain limited for many organism groups and findings generated from field studies of radiation effects in the environment often deliver observations of associations with radiation exposure rather than demonstrating causality. Also, the focus of environmental protection is generally the protection of populations so evidence on the effects of radiation on reproductive endpoints is crucial.

In this context, AOPs, and especially AOPs focused on reproductive endpoints have the potential to meet two key requirements of a robust regulation: (i) delivering a mechanistic understanding to enable establishment of causal links (not simply associations) between radiation dose and effect on specific reproductive endpoints; and (ii) the organization and evaluation of research findings that enable clear demonstration of the WOE underpinning regulatory benchmarks. By organizing and evaluating the evidence in this way, regulators and industry will be able to respond more effectively to stakeholder challenges. More broadly, it may facilitate science and risk communication in relation to radiation in the environment, including comparison with other environmental stressors (Chauhan, Hamada, Garnier-Laplace et al. 2022b). This approach would also present other key opportunities to enhance regulation, including advancing understanding of radiosensitivity (of organism types and of life stages) to chronic radiation exposure and, by harmonization with the AOP approach for chemicals risk assessment, facilitate hazard and risk assessment of multi-stressors (Beyer et al. 2014; Salbu et al. 2019).

The benefits of AOPs to support and refine research has already been amply demonstrated in the chemical field and the present paper demonstrates how AOPs can also be used to mechanistically characterize the chain of events occurring for radiation-mediated toxicity of environmental relevance. The AOP networks developed herein describe a causal progression from the initial deposition of energy in a cell to a series of KE at higher levels of biological organization that ultimately cause reproductive disturbances and potential changes in population growth rate. It provides a demonstration of the reuse of several AOP events previously described in the literature for reproductive impairment of chemicals (Knapen et al. 2015) into radiation-relevant AOPs. This is a demonstration of the interest of capitalizing efforts in (eco)-toxicology of chemical and physical stressors. The AOP formalism displays potential usefulness in assisting researchers and regulators to share a common framework, to identify similarities and differences in the characterization of the hazard, and ultimately to progress toward an integrated approach for ecosystems health protection (Chauhan et al. 2019; Chauhan, Stricklin et al. 2021).

The work reduced a complex toxicology of radiation-mediated effects to a comprehensive, integrated, and biologically plausible synthesis of available knowledge, and identified knowledge gaps, with applicability to specific taxons as well as a broader taxonomic applicability domain. For the first time, consensus AOPs for radiation effects on reproduction was built through gathering knowledge from several taxon-specific AOPs. This effort could serve as a basis for evaluation of interspecies differences in radiosensitivity through development of quantitative AOPs (Perkins et al. 2019; Song, Xie, Lee, Tollefsen et al. 2020), and for identification of species-specific pathways that could explain differences in populations and individual radiosensitivity. Such development would be instrumental for thorough assessment of existing WOE considerations, consolidating the knowledge domain and fill data gaps in a transparent and reproducible way.

The information assembled into the AOP networks not only lends itself useful for enhancing the mechanistic understanding of effects underpinning adversity, but can be useful for identifying exposure or effect biomarkers that can specifically and quantitatively characterize stressor–response and response–response relationships for radiation-mediated effects on reproduction (Chauhan, Stricklin et al. 2021). The potential use of biomarkers has long been criticized if the linkage between the change in the biomarker and adversity is unclear or not direct (Hall et al. 2017), but it's anticipated that the AOPs would address this shortcoming by providing a scientifically credible source of information for causality between events of relevance for reproduction. The knowledge generated would also be supportive of identifying bioassays and methodological approaches that can used for non-model species and extend the applicability domain to species relevant for lab to field extrapolations. This would be an important development, as substantial controversy exists to the credibility of reported associations between effects at radiation dose rates within the range of natural background (Beresford et al. 2020). A key step in such development would be developing qAOPs by quantitating the stressor–response and response–response relationships for individual events identified in the present study. The AOPs and AOP networks discussed here would thus potentially be a source of information to enhance our understanding of interspecies differences in radiosensitivity and how individual AOPs and AOP networks are triggered in a dose rate- and dose-specific manner. Such efforts would ultimately facilitate the transition toward a more mechanistically informed hazard and risk assessment of reproductive impairment, including determination of Relative Biological Effectiveness (RBE) of the different radiation types (alpha, beta, gamma). However, it remains to be determined if our current mechanistic understanding, availability of data and linearization of complex biological mechanisms from controlled laboratory studies into AOPs provides a sufficiently robust model for predicting long-term population effects under dose rates and doses being ecologically relevant and conditions that reflect natural complexity. So does accounting for epigenetics, trans-generational, multi-generational and by-stander effects that all may modulate existing or trigger new toxicological pathways and/or AOPs.

5. Conclusions and future prospects

The current work has presented five individual reproductive- and taxon-focused cases studies of ecological relevance, and defined a common set of events into a set of cAOPs. The cAOPs represent the initial effort to capture and harmonize effect information spanning different levels of biological organization and a high number of species into a common knowledge framework that can be used to develop linearized AOPs with a broad taxonomic applicability. This effort, when undertaken, will take advantage of a well-developed scientific consortium, with experts representing diverse areas of expertise, to populate the individual AOPs with a more universal set of data to reduce the apparent bias toward a few well studied environmental model species. Future initiatives are expected to entail structured WOE assessments based on automated literature and data mining (Song, Xie, Lee, Tollefsen et al. 2020; Jornod et al. 2022), formalized review processes involving rapid, scoping or systematic reviews (Svingen et al. 2021) and critical assessment of the empirical evidence to evolve the AOPs toward formal submission, review and potential OECD endorsement in the AOP development program. Parallel efforts will be undertaken to develop qAOPs and support transition toward pragmatic applications in environmental health assessment and radiological protection for the different ionizing radiation types and multiple stressors.

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