Current evidence for a role of epigenetic mechanisms in response to ionizing radiation in an ecotoxicological context

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Manuscript Details

Manuscript number	ENVPOL_2019_714_R1
Title	Current evidence for a role of epigenetic mechanisms in response to ionizing radiation in an ecotoxicological context
Article type	Review Article

Abstract

The issue of potential long-term or hereditary effects for both humans and wildlife exposed to low doses (or dose rates) of ionising radiation is a major concern. Chronic exposure to ionising radiation, defined as an exposure over a large fraction of the organism's lifespan or even over several generations, can possibly have consequences in the progeny. Recent work has begun to show that epigenetics plays an important role in adaptation of organisms challenged to environmental stimulae. Changes to so-called epigenetic marks such as histone modifications, DNA methylation and non-coding RNAs result in altered transcriptomes and proteomes, without directly changing the DNA sequence. Moreover, some of these environmentally-induced epigenetic changes tend to persist over generations, and thus, epigenetic modifications are regarded as the conduits for environmental influence on the genome. Here, we review the current knowledge of possible involvement of epigenetics in the cascade of responses resulting from environmental exposure to ionising radiation. In addition, from a comparison of lab and field obtained data, we investigate evidence on radiation-induced changes in the epigenome and in particular the total or locus specific levels of DNA methylation. The challenges for future research and possible use of changes as an early warning (biomarker) of radiosensitivity and individual exposure is discussed. Such a biomarker could be used to detect and better understand the mechanisms of toxic action and inter/intra-species susceptibility to radiation within an environmental risk assessment and management context.

Keywords	Epigenetic marks; Radioecology; gamma radiation; Chronic exposure; multi-transgenerational; Nuclear accidents

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Suggested reviewers	Etiene Bucher, David Copplestone, Almudena Real, Carmel Mothersill, Peter Kille

Submission Files Included in this PDF

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Ref: ENVPOL_2019_714
Title: Current evidence for a role of epigenetic mechanisms in response to ionizing radiation in an ecotoxicological context
Journal: Environmental Pollution

Dear editor,

Thank you and your reviewers for giving us the opportunity to revise our manuscript entitled *Current evidence for a role of epigenetic mechanisms in response to ionizing radiation in an ecotoxicological context* (ENVPOL_2019_714). We have tried to address all remarks made by the reviewers as well as journal recommendations. Below you can find our rebuttal specifying the changes made to the text and tables. We hope that our manuscript will now meet the standards of Environmental Pollution and gets approval of the reviewers.

Looking forward to hearing from you,

Kind regards

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Rebutal (ENVPOL_2019_714)

Comments from the editors and reviewers:
-Reviewer 1

The authors reviewed the current knowledge of possible involvement of epigenetics in the cascade of responses resulting from environmental exposure to ionizing radiation. They also summarized the evidence on whether changes in the epigenome and in particular the total or locus specific levels of DNA methylation may serve as an early warning (biomarker) of radio-sensitivity and individual exposure, from a comparison of lab and field obtained data. This review paper could help understand the mechanisms of toxic action and inter/intra-species susceptibility to radiation within an environmental risk assessment and management context.
This review paper is certainly interesting and presents valuable information. However it may be suitable for publication in a more subject focused journal, such as Radiation Physics and Chemistry, Radiation Research, Radiation Protection Dosimetry, and the like.

We would like to thank the reviewer for his comments and suggestion to submit our current paper to another journal which is more focused on radiation protection. However, we deliberately chose for Environmental Pollution as this journal, in contrast to the once suggested by the reviewer, is focused on the consequences of pollutants in our natural environment. This review paper specifically deals with radiation as an environmental polluter and the possible long-term consequences to the environment of enhanced exposures. We therefore truly believe it falls within the scope of the journal.

-Reviewer 2
The authors in this review, they bring together and present in a relatively nice way the current knowledge of possible involvement of epigenetics in the responses resulting from environmental exposure to ionising radiation (IR). The need for reliable radiation exposure biomarkers in order to evaluate or even predict the stochastic effects of IR in this case for example mutations, genomic instability 'transmitted' to next generations is certianly important. Now if the epigenetic markers for wildlife or even humans are the best that remains to be seen. The authors need to revise and correct several issues as described below.

The major issues are that they need to embrace the knoweldge of radiation bystander effects, the role of IR-stress manifested to the complexity of DNA damage (and probably lipds, proteins etc.). These issues starting from the early work by Munira Kadhim (2004: Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response. Mutation research, 568, 21-32) to more recent analytical reviews explaining all these (Pouget, J.P., et al. 2018) Targeted and Off-Target (Bystander and Abscopal) Effects of Radiation Therapy: Redox Mechanisms and Risk-Benefit Analysis. Antioxid Redox Signal). Because the DNA damage response is the main regulator of all further effects so the complexity of damage is probably the most prominent parameter for this reviewer. Of course

cases where direct damage to methylating protein genes naybe involved but how possible is this directly at least from radiation only to these genes?

We have added some lines on the possible links between non-targeted effects and epigenetics. We share the view of the recent review by Schofield, that the understanding of the mechanisms linking these two processes is still poorly understood.

"A number of papers have proposed a link between epigenetic effects and non-targeted effects (NTE) such genomic instability and bystander effects (Schofield and Kondratowicz, 2018). However, while the existence of non-targeted effects is well established (Morgan, 2002, Kadhim et al 2004, Pouget et al 2018), and studies have shown an association between the two effects (e.g., Kaup et al., 2006; Wang et al 2018), evidence of a causal relationship is more elusive, since NTE could be either a mechanism or a consequence of epigenetic changes (Schofield and Kondratowicz, 2018)"

Another point and based on several discussion on the radiation ecology communities etc. is for example how one can differentiate between IR-effects and for example airborne particles from wild fires or chemical pollutants? How sure are the authors that their markers are exclusive to IR-exposure? This the reason that probably they need to invest in the unique feature of IR as discussed above and below as a type of environmental stress.

We agree with the author that it in natural environments it will remain questionable whether a specific marker for IR-exposure or any other stressor can be found. In this review we look at evidence for the appearances of changes in methylation or other epigenetic marks after exposure to radiation in either field or lab conditions. It is the first step in the quest whether or not these can serve as biomarkers. Hence we only shortly touch upon the possibility of biomarkers in this review and this mainly in paragraph 9. Within this section it was already indicated at two places that it will be challenging if not impossible to exclude the influence of confounding factors:
- line 671 to 673: However, it is also recognised that significant challenges related to the effects of genetic background and the influence of confounding factors also exist.
- line 680 to 683: Specific challenges relate to working with some autochtonous species for which genome resources may be lacking and, the influence of confounding factors which may mask the causal response between ionising radiation exposure and epigenetic changes.
We have added the review of Pernot E, et al. (Mutation Research-Reviews in Mutation Research. 2012;751(2):258-286) as the authors describe the challenges on the specificity of biomarkers for IR.
We do believe that it will be necessary to combine different observations that lead to an adverse outcome in order to identify a set of changes that will be specific for IR as is indicated in the text: epigenetic approaches may be more powerful indicators of effects when linked to known biomarkers using, for example, transcriptional analysis. When used in conjunction with other mechanistic measurements, epigenetic analysis

has the potential to enhance the ecological relevance of molecular biomarkers, as described in the Adverse Outcome Pathway concept (Groh et al. 2015) Line 683-687. We also suggest a thorough comparison between field and lab studies line 696-697. We also added in paragraph 9 the paper of Lourenco J, et al. (J Hazard Mater. 2016;317:503-542) as this is a comprehensive review on the use of biomarkers or bioindicator species for humans and non-human species for IR.

Other specific (not minor) comments:
1. In the abstract, bullets, whole manuscript , the authors need to be very specific. For example when they say radiation they must at least explain initially , what type of ionizing radiation they refer to, what type of exposure acute or chronic as assumed.

To address this remark we have added some more information in table 2 and 3 we have included in the text that exact amounts can be found in the tables (line 333). Throughout the text we also tried to be more precise on this matter.

2. Abstract and elsewhere: "... have consequences in the progeny, which may not be attributable to increased mutation rates alone". The question here is really crucial and based on the long discussion of the systemic effects of ionizing radiation. When they refer to progeny, is the progeny exposed to radiation, or for example the parents have left the irradiation area and the offsprings have been born to a background radiation level area?

We agree with the reviewer that it is important to indicate whether or not the offspring itself is exposed. We have defined this (line 697 to 701) as mutligenerational exposure (both parents and offspring are exposed, transgenerational: only parental line is exposed. It is also true that care must be taken that sometimes for example in pregnant female organisms it is the F3 generation that is the first true transgenerational exposed generation, this concern is also included in the text (Line 313-317)

3. The Figure is way too large.

We agree with the reviewer that the figure is too large, but just provided this figure in the intention to rescale it the size desired for the graphical abstract.

4. Low doses and chronic exposures must be defined.Lines 59-61: One question that arises, is how it is possible radiation accidents like Chernobyl and Fukushima to be considered as low doses exposure examples? For time periods and areas around the accidents, doses where high to very high. Examples and clarifications must be given.

We agree that for Chernobyl and Fukushima the term low dose is not correct for the first and second period after the accident. However dose rates of the last 30 years are estimated to be maximally 0.5 mGy/h. Examples of the dose rates are now included in the text. For the current situation the highest contaminated areas like the Red Forest for CEZ has about 0.5 mGy/h (Beresford personal communication) to 0.1 mGy/h ambient dose rates (Horemans N, et al. Journal of Environmental

Radioactivity. 2018;192:405-416). As it is impossible for these field conditions to exclude the memory effect of previous higher exposures even if it is more than 30 years ago and to avoid confusion we have omitted the use of the term low dose in the text. This review deals with long-term consequences of chronic exposures: we have as suggested included a definition for chronic exposure conditions both in the abstract as in the first paragraph of the introduction.

5. Lines 86-87: "...Long-term exposures to low levels of environmental stressors have been linked to lasting responses in organisms within, but also over multiple exposed generations". Again here wild uncertainties, long term, low levels, stressors of what type? Type of stressors for DNA have been discussed analytically and differentiated from endogenous and a discussion must be done since the main difference between environmental IR stress and endogenous oxidative stress in all living organisms is the complexity of damage (DNA mainly: Nikitaki, Z. et al. 2015. Stress-induced DNA damage biomarkers: applications and limitations. Frontiers in chemistry, 3, 35.).

For this part of the manuscript we have not followed the suggestion by the reviewer to include a discussion on the type of stressor as this sentence in the introduction refers to different stressors (not including IR) as evidenced by the references: (Mirbahai and Chipman 2014:review on different chemical stressors; Schultz et al. 2016 on Ag ions, nanopaticles and sulphadised nanoparticles; Jimenez-Chillaron et al. 2015 (different xenobiotics); Marczylo et al. 2016 and Hanson and Skinner 2016 ((again reveiws on different contaminants but not IR). We included this sentence to show that the fact that long-term exposure to an environmental stressor can have consequences potentially over generations and that this has been noted before for different pollutants. We do have removed low dose and added sub-lethal concentrations in order to avoid confusion with IR that is expressed in dose.

6. The Tables are nice but the type of assumed or measured or estimated radiation type and levels must be incorporated.
We have tried to follow this request and added were appropriate radiation dose rate (with indication whether it is ambient or total dose rate including internal exposure) and metal concentrations in the tables 2 and 3

7. How one can be sure that animals and living species have not migrated from irraiated areas and through sometype of systemic effects alter the epigenome to animals in background levels areas?

We agree with the reviewer that for field studies it is impossible to exactly estimate the exposed dose and one can never be certain whether an organism has not migrated. For the field studies in which the co-authors were involved care was taken to include control sites that are further away from the contaminated sites than the normal action radius of the organism itself. Dosimetry was carefully performed taken into account as much as possible the habitat and frequency of an organism to reside in that habitat. This is described in the references cited in this review. As however you can never exclude changes in exposure we recommend to compare lab and field conditions as much as possible.

8. Earlier studies suggest in many cases no real diferences between high- and low-radation areas (see for example : https://inis.iaea.org/collection/NCLCollectionStore/_Public/23/039/23039160.pdf)

We have tried when describing the field studies to include whether or not the response followed the radiation gradient, for some cases a correlation between the measured endpoint and the gradient is found indicating differential responses to be radiation level dependent (see e.g. for plants Kovalchuk et al. 2003,2004) other plant studies did not find a correlation (Horemans et al 2018) or showed a transient respons (Volkova et al 2018). Hence, it seemed study-dependent and the underlying mechanisms have not been identified yet. Hence, in the manuscript we did not make conclusions on radiation gradient dependencies but included it as a challenge for further research to find the underlying mechanism explaining this if you want to use epigenetic marks as a biomarker.

From the suggested IAEA report we picked out the following definition and included it in the manuscript (line first paragraph of the introduction):
An acute exposure is one which is delivered in a time period which is short compared with the time over which any obvious biological response develops. A chronic exposure is one which could continue over a large fraction of the natural life of the organism. These descriptors as a function of time lead naturally to qualifiers of total dose: A high exposure is one which leads to an acute response, usually a severe (and obvious) pathological reaction, with the primary one being mortality. A low exposure would have only marginal and late effects on the normal mortality/time relationship of the organism, but it may produce detectable effects in the normal biological processes of the organism without necessarily producing any obvious harm to the individual.

-Reviewer 3
There are few minor suggestions i have:

1. "Chernobyl" is generally used spelling given that this nuclear catastrophe had happen in Soviet Union, where official language was Russian. It is, however, a Ukrainian city and proper spelling for it is "Chornobyl". This is, however, my recomendation and i would leave it up to the authors to take a decision on it.

We agree with the author that Chornobyl is the correct current name for this city. We are willing to change the names accordingly. We are, however, a bit reluctant as we are afraid Chornobyl is not widespread as a search term such reducing the visibility of our manuscript once accepted and therefore left it as Chernobyl for the time being.

2. Page 6, line 106: should be "as" instead of "a" in front of "cladocerans".

Changed in the text

3. Page 7, lines 135-136: nucleosome positioning is currently considered as one of the major and driving epigenetic mechanisms, along with the other three - DNA methylation, histone modifications and non-coding RNAs.

We have modified the sentence on this in the text as follows:
The epigenetic landscape is shapen by three epigenetic marks; DNA methylation, histones and it's post translation modifications and small RNA interactions. Together they shape the structure of the DNA called chromatin (C. David Allis and Thomas Jenuwein, Nature, 2016, The molecular hallmarks of epigenetic control).

4. Page 9, lines 182 - 185. It is now generally accepted that DNMT1, besides it role in maintenance of DNA methylation patterns, can be also involved in de novo methylation; similarly, DNMT3A and 3B have been shown to partake in maintenance as well.

We agree with the reviewer that this might be added to the text and therefore altered the section as follows:

"In vertebrates, maintenance methylation by DNMT1 occurs during the S-phase of mitosis, where the newly synthesized DNA strand is methylated using the original strand as template. De novo DNA methylation is undertaken DNMT3 family members, although recent insights have shown redundancy between to two DNMT family members Lyko F. Nature Reviews Genetics. 2018;19(2):81-92.

- Review on long term effects of exposure of wildlife to chronic low dose radiation
- Inter-, multi- and transgenerational studies for both lab and field exposures
- Changes found in epigenetic marks induced by chronic exposure to ionising radiation
- DNA methylation possibly transfers the response from one generation to the next

Arabidopsis
Daphnia
Earthworms
Pine tree
Salmon
Shephered's purse
Treefrog
Zebrafish…

F_0 F_1 F_2 …F_x

intergenerational multigenerational transgenerational

? ? ?

Δ Epigenetic marks:

DNA methylation, histone modifications, miRNA expression

Current evidence for a role of epigenetic mechanisms in response to ionizing radiation in an ecotoxicological context

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27 **Declaration of interest:** none

28 **Keywords:** Epigenetic marks; Radioecology; DNA methylation; gamma radiation; Chronic

29 exposure; multi-transgenerational; Wildlife; Chernobyl; Chernobyl; Fukushima; Nuclear

30 accidents

Abstract

The issue of potential long-term or hereditary effects for both humans and wildlife exposed to low doses (or dose rates) of ionising radiation is a major concern. Chronic exposure to ionising radiation, defined as an exposure over a large fraction of the organism's lifespan or even over several generations, can possibly have consequences in the progeny. Recent work has begun to show that epigenetics plays an important role in adaptation of organisms challenged to environmental stimulae. Changes to so-called epigenetic marks such as histone modifications, DNA methylation and non-coding RNAs result in altered transcriptomes and proteomes, without directly changing the DNA sequence. Moreover, some of these environmentally-induced epigenetic changes tend to persist over generations, and thus, epigenetic modifications are regarded as the conduits for environmental influence on the genome.

Here, we review the current knowledge of possible involvement of epigenetics in the cascade of responses resulting from environmental exposure to ionising radiation. In addition, from a comparison of lab and field obtained data, we investigate evidence on radiation-induced changes in the epigenome and in particular the total or locus specific levels of DNA methylation. The challenges for future research and possible use of changes as an early warning (biomarker) of radiosensitivity and individual exposure is discussed. Such a biomarker could be used to detect and better understand the mechanisms of toxic action and inter/intra-species susceptibility to radiation within an environmental risk assessment and management context.

Capsule:

Review: possible changes in epigenetic marks in wildlife exposed to ionising radiation suggests DNA methylation changes as a key to transfer the response from one generation to the next.

1 Introduction

Activities like ore mining and milling, nuclear accidents and production and testing of nuclear weapons have resulted in enhanced concentrations of radionuclide pollutants in the environment. This can lead to long-term or chronic exposures of organisms defined as an exposure over a considerable fraction of the lifespan of the organism (IAEA 1992). The issue of biological effects induced by chronic sub-lethal doses of ionising radiation along with the question on the potential hereditary effects for both humans and wildlife is a topic of considerable debate and concern. This has been reinforced after the Chernobyl and Fukushima accidents, especially with respect to the quantification (and reduction if possible) of the magnitude of risk to ecosystems when exposed chronically for multiple generations. This concerns both short-term and chronic exposure over several generations and heritable effects on unexposed progeny. To improve the scientific basis for risk assessment for both human and environment in chronic exposure scenarios as observed e.g. in Chernobyl and Fukushima exclusion zones (CEZ and FEZ), an enhanced understanding of the mechanisms that underpin these responses is needed. This will lead to a better understanding of the complex interplay between exposure, organism physiology and phenotypic response over extended timescales (e.g., Marczylo et al. 2016). Comprehensive reviews of the observed phenotypic effects observed in wildlife in CEZ and FEZ have been published e.g. by Hinton et al. (2007), Geras'kin et al. (2008), Lourenco et al. (2016) Steinhauser et al. (2014), Strand et al. (2014), Batlle (2016) and Beresford et al. (2016). The amounts of radionuclides released into the environment after the Chernobyl accident (5300 PBq, excluding noble gases) were about tenfold of those of the accident in Japan (520 PBq) (Steinhauser et al. 2014). Despite this difference both exclusion zones have common features such as (i) for both areas the exposure can be divided in 3 time-periods depending on the exposure rates as described in paragraph

6, (ii) the degree to which spatial and temporal heterogeneity is present in the distribution of the radionuclides (including the presence of hot particles); (iii) the presence of other additional pollutants (e.g. from historical land use); (iv) the challenge of finding comparable control conditions and (v) the difficulty to estimate the exact exposure dose rates. Additionally and of importance for interpreting observations made in these contaminated regions, both exclusion zones have undergone changes induced by the removal of human presence and occupancy leading to specific ecological changes that are hard to distinguish from the possible radiological impact (Beresford and Copplestone 2011). The unique nature of these study areas means that the interpretation of field data from these sites needs careful contextual consideration and have led to contrasting and sometimes conflicting reports on effects observed in the CEZ and FEZ (Beresford and Copplestone 2011; Garnier-Laplace et al. 2013).

Long-term exposures to environmental stressors have been linked to lasting responses in organisms within, but also over multiple exposed generations (Mirbahai and Chipman 2014; Schultz et al. 2016; Jimenez-Chillaron et al. 2015; Marczylo et al. 2016; Hanson and Skinner 2016). Yet, the outcome of a long term-exposure to pollutants is not always predictable. For example, chronic exposure to pollutants or adverse conditions has been shown to lead to changed phenotypes (Singer et al. 2016; Gonzalez et al. 2016; Potters et al. 2007) resulting in adaptation within a population (Costa et al. 2012; Coors et al. 2009; Bible and Sanford 2016). In contrast, there is also evidence suggesting that long term exposures to environmental stressors can lead to an increased population sensitivity (Parisot et al. 2015) that may result in population declines (Vasseur and Cossu-Leguille 2006). This makes predicting the long-term and/or transgenerational consequences of exposure to a stressor a particular challenge for estimating risks to populations (Groh et al. 2015).

Selection has been recognised as a major mechanism through which adverse environmental conditions can impact the phenotypes of successive generations. Selection of alleles associated with tolerance can lead to changes in the phenotypic characteristics within a population and, hence, is known to be a key driver of changes in population level sensitivity to pollutant effects (Van Straalen and Roelofs 2007). Detailed studies of populations inhabiting polluted sites have identified numerous cases of modified phenotypes and also of specific genetic selection at loci that lead to biochemical changes that underpin adaptation. Examples cover exposure to radionuclides, trace metals and persistent organic pollutants and taxa such as cladocerans (Hochmuth et al. 2015; Jansen et al. 2015), collembola (Costa et al. 2012; Nota et al. 2013), chironomids (Groenendijk et al. 1999; Loayza-Muro et al. 2014), terrestrial and freshwater annelids (Kille et al. 2013; Langdon et al. 2003; Levinton et al. 2003), fish (Wirgin et al. 2011; Shaw et al. 2014; Reid et al. 2016; Theodorakis and Shugart 1997), plants, birds (Ellegren et al. 1997) and small mammals (Theodorakis et al. 2001). Although selection for enhanced tolerance is a commonly observed phenomenon, some data have shown that rapid adaptation towards heavy-metals or radionuclides in organisms cannot be explained only by increased mutation rates, but could also be due to non-genetic changes in the activity of functional genes and these might be heritable over generations (Geras'kin et al. 2013; Kovalchuk et al. 2003; Mirbahai and Chipman 2014; Kille et al. 2013; Wang et al. 2017). This has revealed further levels of complexity probably provided by relevant epigenetic mechanisms relating to structure and regulation of gene expression and splicing that have the potential to transfer information over generations.

In this paper an overview is given of epigenetic changes induced after long-term (within and over generations) exposure to ionising radiation. Although different epigenetic mechanisms

125 will be discussed the main focus of the current review will be on comparing the evidence from
126 both lab and field studies on changes in DNA methylation.
127
128 **2. *Overview of epigenetic mechanisms***
129 The first definition of epigenetics, as 'the causal interactions between genes and their
130 products, which brings the phenotype into being', was provided by Waddington (1939) long
131 before any mechanistic understanding of the relevant processes had developed. This
132 definition has since been refined. For example, Wu and Morris (2001) defined epigenetics as
133 'Nuclear inheritance which is not based on changes in DNA sequence' or Bird (2007) as 'the
134 structural adaptation of chromosomal regions so as to register, signal or perpetuate altered
135 activity states'. This reflects that epigenetics is now widely seen as 'the study of the landscape
136 of mitotically and/or meiotically heritable changes in gene activity and transcript architecture,
137 including splicing variation, that cannot be explained solely by changes in DNA sequence
138 (Vandegehuchte and Janssen 2011; Allis et al. 2007; Berger et al. 2009).
139 The epigenetic landscape is shapen by three epigenetic marks; DNA methylation, histones and
140 it's post translation modifications and small RNA interactions. Together they shape the
141 structure of the DNA called chromatin (Allis and Jenuwein 2016). These major epigenetic
142 players are engaged in a network of interconnected 'cross-talk' (Irato et al. 2003; Iorio et al.
143 2010) and orchestrate gene expression that "...underpins the differences between species,
144 ecotypes and individuals" (Mattick et al. 2009; Brautigam et al. 2013). Well established as a
145 key mechanism involved in the aetiology of human disease (Huang et al. 2003), it is only
146 relatively recently that the significance of epigenetic mechanisms in toxicology (Szyf 2007),
147 ecology (Bossdorf et al. 2008) and evolutionary biology (Rapp and Wendel 2005), has begun
148 to emerge. Within ecology, it has been suggested that epigenetics could define "... where the

149 environment interfaces with genomics ... (and could provide a) rapid mechanism by which an
150 organism can respond to its environment without having to change its hardware" (Pray 2004).
151 Studies on plants have indicated that epigenetic systems provide functional links between the
152 detection of environmental change and regulation of gene expression (Bossdorf et al. 2008;
153 Grativol et al. 2012; Whittle et al. 2009; Rasmann et al. 2012; Verhoeven et al. 2016; Sahu et
154 al. 2013; He and Li 2018). Similarly in animals, the role of specific components or changes of
155 the epigenome in species responses to environmental stress has been demonstrated
156 (Vandegehuchte and Janssen 2014; Schott et al. 2014; Marsh and Pasqualone 2014; Mirbahai
157 and Chipman 2014; Wang et al. 2017; Marczylo et al. 2016). Thus epigenetic mechanisms
158 appear to play an important role in determining the physiological responses of species to long-
159 term multigenerational exposure, including to persistent stressors such as radionuclides.
160 To integrate emerging understanding of epigenetic mechanisms with existing mechanistic
161 knowledge in radioecology, a clear understanding of long-term effects induced by ionizing
162 radiation exposure of non-human species and their potential (epigenetic) mechanistic basis is
163 needed. To provide this, we here give a brief overview of the evidence of trans- and
164 multigenerational effects in organisms exposed to ionising radiation. The potential role and
165 value of epigenetic analyses in site-specific studies in radioecology will be discussed, including
166 their relevance for future radiological risk assessment. As the most widely studied mechanism
167 and its potential to be transferred to the next generation, special attention will be given to
168 changes in DNA methylation (locus-specific or total) as a possible marker for exposure to
169 ionising radiation, including under field conditions.
170
171 ***3. The biology of epigenetic mechanisms***

DNA methylation, histone modifications, and small non-protein coding RNA molecules are the major known epigenetic mechanisms. DNA methylation is the addition of a methyl group to the one of the DNA bases (cytosine or adenine). Most prevalent DNA methylation is on the fifth position of the cytosine ring (5-methyldeoxycytidine, mC). In vertebrates this usually but not exclusively located at in CpG sites. For example, in *Drosophila* methylation is mostly found in the context of CpT dinucleotides (Feil and Fraga 2012), in honey bees there appears to be a clear distinction of CpG sites in exons and non-CpG sites in introns (Cingolani et al. 2013) and in plants and embryonic stem cells also at CHG and CHH sites (H=A,T or C) in addition to CpG (Feil and Fraga 2012; Cingolani et al. 2013).

In vertebrates, around 60% of genes are associated with CpG islands that occur at or near the transcription start site of, particularly, housekeeping genes (Gardiner-Garden and Frommer 1987). The hypermethylation in CpG rich promoters can be associated with the repression of gene expression (Bock 2012). In invertebrates, methylation is targeted more towards gene body, potentially playing a role in alternative splicing and gene function diversification (Flores et al. 2012; Asselman et al. 2016). Cytosines can be methylated via maintenance and *de novo* methyltransferase enzymes (Law and Jacobsen 2010). In vertebrates, maintenance methylation by DNMT1 occurs during the S-phase of mitosis, where the newly synthesized DNA strand is methylated using the original strand as template. De novo DNA methylation is undertaken DNMT3 family members, although recent insights have shown redundancy between to two DNMT family members (Lyko 2018). *De novo* DNA methylation is undertaken DNMT3 family members. In plants the homologues of DNMT3, DOMAINS REARRANGED METHYLTRANSFERASE 1/2 (DRM1/DRM2) are responsible for the de novo methylation whereas maintenance of CG methylation is conducted by DNA METHYLTRANSFERASE 1 (MET1) which is a homolog for DNMT1 (Law and Jacobsen 2010; Chan et al. 2005). In addition

196 the plant specific CHROMOMETHYLASE 3 (CMT3) is responsible for maintaining methylation
197 in a context of CHG and together with DRM1/DRM2 for methylation in a CHH context (Chan
198 et al. 2005). Although the methyltransferase enzymes are the core proteins involved in
199 methylation, they are recruited and guided to their specific interaction targets by proteins,
200 such as UBIQUITIN-LIKE, CONTAINING PHD AND RING FINGER DOMAINS 1 (URHF1) and
201 PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) (Baubec et al. 2015). A further insight that
202 has recently emerged is that DNA methylation represents only one part of the DNA
203 methylation cycle. Recently, Tet methylcytosine dioxygenases (previously named ten-eleven
204 translocation (TET) proteins) have been identified as crucial proteins in putative
205 demethylation pathways (Coulter et al. 2013; Scourzic et al. 2015). Indeed, the dynamics
206 between methylation and hydroxymethylation exemplifies the balance of DNA methylation at
207 specific regions as well as globally during early developmental reprogramming (Wu and Zhang
208 2014).
209 Histone modifications occur as post-translational modifications predominantly to the N and C
210 terminal tails of histone proteins. Histone proteins are organised in octamer structures
211 forming nucleosomes as the fundamental units of chromatin (Berr et al. 2011). Initially
212 histones were thought of as primarily structural proteins. However, it is now recognised that
213 they play a pivotal role in regulating gene expression via structural changes of chromatin (Jung
214 and Kim 2012; Margueron et al. 2005). Major histone modifications include acetylation,
215 methylation, phosphorylation and ubiquitination (Bannister and Kouzarides 2011). A key role
216 played by histone isoforms and post-translational modifications that is highly relevant to
217 ionising radiation exposure, is their involvement in DNA damage repair (Hunt et al. 2013;
218 Mondal et al. 2016). DNA repair requires multiple steps, including the initial signalling of the
219 break, the opening of the compact chromatin to facilitate access for repair factors, and

afterwards the restoration of the chromatin state (Hunt et al. 2013; for details see Huertas et al. 2009). An authoritative overview of the post-translational modifications in histones triggered in response to DNA damage is given by Méndez-Acuña et al. (2010). Changes of histone modifications have also been linked to exposure to different pollutants in both mammalian and non-mammalian species (Kim et al. 2012b; Mendez-Acuna et al. 2010; Santos et al. 2011; Wang et al. 2017). Observations of heterochromatin state maintenance over multiple successive generations following exposure to heat or osmotic stress in *D. melanogaster* suggests a mechanism by which the effects of stress are inherited epigenetically via the regulation of chromatin structure (Seong et al. 2011).

Short interfering RNAs and microRNAs are functional non-coding RNA molecules. They are not translated into proteins and are involved in gene repression via RNA deactivation and degradation (Castel and Martienssen 2013). Single microRNAs may on average interact with ~400 different protein coding genes. Hence, changes in microRNA expression are proposed to be a key component of organism response to stressor exposure (see e.g. for plant responses Huang et al. 2016). Reduced expression of microRNA has been found in response to insecticide and fungicide exposure (Qi et al. 2014; An et al. 2013). MicroRNAs have been shown to be intimately involved in cellular response to metals such as cadmium and arsenic (Liu et al. 2016; Meng et al. 2011; Gielen et al. 2012). Important roles of non-coding RNAs in the epigenetic inheritance of DNA methylation through cell division and guiding de novo methylation after meiosis indicate key interactions between epigenetic pathways (Calarco et al. 2012; Larriba and del Mazo 2016). In plants e.g. DNA and histone methylation by DRM2 activity and subsequent gene silencing can also be mediated by siRNAs ARGONAUTE (AGO4) and polymerase V (POLV) (Holoch and Moazed 2015; Neeb and Nowacki 2018). Hence dynamic

243 interactions of different epigenetic mechanisms would be expected in response to
244 environmental challenge.
245 The relative role of the different epigenetic mechanisms can vary between species. The
246 majority of eukaryotic phyla possess cytosine methylation ranging from <<1% in some taxa
247 (e.g. many arthropods) to >10% for annelids, molluscs and vertebrates, with species such as
248 *C. elegans* even proposed to lack cytosine methylation completely (Regev et al. 1998) or to be
249 very low (~0.0033%) (Hu et al. 2015). Because of those variations in DNA methylation levels,
250 it was initially uncertain how important cytosine methylation may be among those phyla.
251 However, evidence of the importance of DNA methylation in heritable responses in
252 invertebrates following stressor exposure has begun to emerge, as well as for other epigenetic
253 mechanisms (Seong et al. 2011; Schultz et al. 2016; Stern et al. 2014; Klosin et al. 2017). For
254 some species, and particularly in *C. elegans*, a second DNA modification based on methylation
255 of the N-6 position on adenine may also act as an alternative form of DNA methylation (Greer
256 et al. 2015). In addition, the balance between DNA methylation, post-translational
257 modifications and types of microRNA molecules (both of which are species specific and highly
258 dynamic), presents a challenge to tease apart the roles that different epigenetic mechanism
259 play in gene expression dynamics and ultimately phenotypic responses to stress including
260 those in species exposed to radionuclides and other pollutants over extended timescales (Lim
261 and Brunet 2013).
262
263 ***4. Main methods used to detect DNA methylation changes***
264 This review will mainly focus on the evidence for DNA methylation changes induced by
265 radiation in different animals and plants and this in both lab and field conditions. The
266 measurement of total DNA methylation levels is now routine using molecular genetic and

biochemical protocols. These analyses provide a useful picture of overall methylation states. The methods have the advantages of reasonable cost per sample, established protocols, sensitivity to overall methylation pattern change and rapid sample processing (Table 1). Two global methylation methods that are commonly used are methylation sensitive amplified fragment length polymorphisms (meAFLP) and measuring the % of methylated cytosine by HPLC-MS/MS. The meAFLP technique is based on the use of two restriction enzymes, HpaII and MspI. Both HpaII and MspI recognize a CCGG sequence. MspI is able to cut both methylated recognition sites as well as unmethylated ones. In contrast, HapII is unable to cut at such locations when methylated (i.e. only unmethylated recognition sites are cut). Methylation of these restriction sites can be assessed by electrophoretic recording bands cut by MspI but not HapII on a fragment analyser (e.g. capillary sequencer). The method has been shown to demonstrate limited variability and has the benefit of an internal control (EcoRI) to account for variability in the amount of DNA input. The detection of methyl groups by HPLC-MS/MS allows highly sensitive quantification of methylated and hydroxymethyl cytosines (5 mC and 5-hmC) present in a hydrolysed DNA sample. The specific ability to detect and measure 5hmC is a specific advantage of this technique, given its recently demonstrated roles in development (Pastor et al. 2011; Song et al. 2011; Xu et al. 2011).

Although useful, application of global methylation analysis methods do not allow analysis of the specific methylation states needed to assess functional links between changes in site specific methylation, gene expression changes and phenotypic changes to be made. The use of methylation mapping techniques can provide improved resolution to identify and assess specific genes/regulatory regions of interest that are differentially methylated under specific treatment or exposure conditions. The number of options to study DNA methylation have become more diverse and methods such as reduced representation or whole genome bisulfite

sequencing, are now considered close to routine. The value of these genome wide methylation mapping techniques is that they go beyond the level of an overall change to identify the gene associated sites of differential methylation. These methods are of course limited when an organism reference genome is either not available or is poorly assembled or annotated. Hence, significant effort needs to be given to genome resource development before these methods can be used to study autochthonous species.

5. Laboratory evidence for multigenerational and transgenerational effects including those induced by ionising radiation

The interest in understanding the effects of persistent pollutants, including radionuclides, on population exposed for more than a single generation is ongoing. Therefore studies of multigenerational and transgenerational stressor effects on apical phenotypes have become more common. For multigenerational studies, exposure to the stressor in question is maintained in a continuously cultured and exposed population for successive generations (e.g., continuously exposed F0, F1, F2 etc.) to allow the consequences of multigenerational exposure to be assessed. Phenotypes are observed in those generations directly exposed. For these multigenerational cases, the simplest expectation is that the observed toxicity in the offspring is not greater than that in parents exposed over their full life-span (i.e. embryo until death), at least over initial generations, with possible development of tolerance over longer time-scales. Transgenerational experiments, on the other hand, consider not just effects on the exposed generation, but also effects on subsequent unexposed generation(s) reared after hatching in stressor free conditions (Skinner and Guerrero-Bosagna 2009; Skinner 2016; Groot et al. 2016). In such studies, stressor effects may be expected as a result of exposure of the F0 mothers in F1 embryo and F2 germline, but not in later offspring. The simplest expectation

315 from transgenerational experiments is thus of physiological effects no greater than those
316 observed in F0s, only in F1s (and possibly F2s), with no further such effects on the later (F3
317 etc.) generations.
318 There are cases where the simplest expectations of multigenerational and transgenerational
319 exposure are met, including examples for plants (Iglesias and Cerdan 2016; Groot et al. 2016;
320 Molinier et al. 2006), earthworms (Hertel-Aas et al. 2011), zebrafish (Baker et al. {2014)
321 (Schwindt et al. {2014) and mice (Ziv-Gal et al. {2015). However, critical analysis of reported
322 multigenerational exposures covering a range of stressor types including radionuclides,
323 metals, nanomaterials, organic chemical and antibiotics, suggests that, at least over the
324 durations used in the laboratory (usually < 10 generations) the simplest expectation of similar
325 sensitivity to F0 in later generations are not always be met. In a number of published cases,
326 an increasing sensitivity in later generations has been observed (see Table 2 and examples
327 below). While this prevalence may partly result from publication bias and from the clonal
328 organisms used, the high frequency of such responses does suggest that increased sensitivity,
329 at least over the initial generations of a multigenerational exposure, may be a common
330 phenomenon (see Table 2).
331 For exposure to radiation and radionuclides there are a number of multigenerational lab-
332 studies that have reported patterns of increased generational sensitivity for continuously
333 exposed populations (see Table 2 for exposure details). For daphnids it has been reported that
334 the progeny of organisms continuously exposed to gamma radiation, Am^{421} (and depleted
335 uranium) show higher sensitivity in the F1 and F2 generations than that for parents depending
336 on the endpoint measured (Pane et al. 2004; Biron et al. 2012; Alonzo et al. 2008b; Parisot et
337 al. 2015). Similarly, Zaka et al. (2004) exposed 5-day old *Pisum sativum* plants over three
338 generations to different acute doses of gamma radiation. Results indicated that doses

apparently harmless for the parental plants adversely affected the F2 generation. *Arabidopsis thaliana* plants exposed to different dose rates of gamma radiation during the vegetative growth stage for one or two generations also showed greater response in the later generation. In this case, increased responses of antioxidative enzyme activity were measured in multigenerationally exposed plants (van de Walle et al. 2016). This response was accompanied by phenotypic changes, such as accelerated flowering after multigenerational exposure (Horemans et al., pers. comm).

Transgenerational studies with radionuclides or after radiation-exposure have shown responses not just in continuously exposed generations, but also in later unexposed generations. A study of reproductive effects of gamma radiation in the nematode *C. elegans* exposed from F0 to F2, either continuously or only at F0 generation also found transgenerational effects in F2 organisms greater than in the initially exposed nematodes (Buisset-Goussen et al. 2014). Daughter cells of chronically gamma-radiation-exposed *Lemna minor* plants died off notwithstanding only a limited growth reduction in the exposed mother colonies (10-30%) indicating that the effects were, thus, greater in the recovering non-exposed plants than in the exposed F0s (Van Hoeck et al. 2017). These examples of transgenerational effects leading to increased sensitivity of progeny match similar results found for other stressors, suggesting a possible common mechanism (Schultz et al. 2016; Moon et al. 2017; Annacondia et al. 2018; Groot et al. 2016).

The current multigenerational and transgenerational toxicity literature is dominated by lab-studies with relatively high exposure dose rates (7-420 mGy/h, see table 2) and for ecotoxicological relevant species like *C. elegans*, *D. magna* and zebrafish (Table 2). For *C. elegans* and *D. magna*, the experimental populations that have been used in most laboratories, multigenerational and transgenerational exposure studies are clonal. Hence, the

potential for selection of alleles that may lead to evolution of tolerance in later generations in a multigenerational exposure experiment is limited. This is true especially because the majority of such experiments are conducted over only a relative limited number of generations (<10 and usually ≤ 3). Indeed, when nematodes were continuously exposed for 22 generations to U, adaptation was shown to occur (Dutilleul et al. 2014). Although many studies have shown generationally increased sensitivity and its transfer, the clonal nature of species may be accentuated, because the limited genetic variation of the inbred strains. In the study of Dutilleul et al. (2014) for nematodes discussed above, the population used that showed adaptation composed of wild isolates with increased genetic diversity above the clonal *C. elegans* strains used for previous multigenerational studies. Hurem et al (2018b) showed effects on the transcriptome in offspring from irradiated zebrafish that were even accentuated in offspring produced from the same parents does, however, indicated the potential to identify epigenetic responses in a genetically diverse population.

Multigenerational exposure experiments by their nature involve continuous incubation of populations with a toxicant or stressor, with generational phenotyping to allow detection of changes in sensitivity. In such studies, increased sensitivity in the progeny could theoretically arise if any toxicant induces "damage" that can be transferred to subsequent exposed generations. Indeed Parisot et al. (2015) highlighted a possible role of DNA damage in multigenerational effects by finding a correlation between increased sensitivity and the transmission of DNA damage in daphnids exposed to gamma radiation. This possible role of DNA damage and genome instability in multigenerational and transgenerational effects may lead to hypotheses about the type of stressors that may cause such phenomena.

The role of both paternal and maternal effects has received much research attention in ecology and toxicology (Frost et al. 2010; Wigle et al. 2007). Within these studies there is

strong evidence that indicate how the direct exposure of the developing embryo and germline can be adversely affected as a result of exposures to environmental pollutants. However, in addition to these more direct effects, there is evidence of a potential role of the epigenome in the transfer of aberrant phenotypes to F1 offspring and indeed to generations beyond (Bowman and Choudhury 2016; Chen and Baram 2016; Wang et al. 2017). For example, exposing *C. elegans* to nanoparticles resulted in aberrant phenotypes, that were persistent in future unexposed populations for 3 or more generations (Greer et al. 2011; Katz et al. 2009; Rechavi et al. 2014; Schultz et al. 2016). When transgenerational effects occur over these generation scales, germline exposures alone cannot be solely responsible, with the potential that epigenetic mechanisms may be intimately involved.

6. Evidence for long-term effects induced by radiation on the environment coming from field studies

The nuclear accidents of Chernobyl and Fukushima have made it possible to investigate possible effects of radiation on a whole range of organisms exposed to radionuclides under field conditions over extended timescales. The temporal changes that occurred in radiation exposure in the CEZ and the FEZ, have resulted in a specific time course of responses among non-human biota in the regions (IAEA 2006; Beresford et al. 2016; Beresford and Copplestone 2011; IAEA 2015). The most pronounced biological effects were seen in the first and second phases after the accident. In these early stages, the high doses experienced shortly after the accident by the forest located to the west of the Chernobyl reactor, later designated as the Red-forest. In this Red-forest massive death of pine trees was observed, while deciduous species survived despite an early loss of leaves and damage to woody tissues (Arkhipov et al. 1994; Kryshev et al. 2005). Similar morphological differences such as loss of apical dominance

411 were recently also reported in Japanese red pine in the FEZ (Yoschenko et al. 2016). In the first
412 phase after the nuclear accidents, direct effects such as a decrease in numbers of small
413 mammals as well as reduced development or survival of embryos was also seen (Geras'kin et
414 al. 2008) and the loss of specific groups of soil biota were also recorded in the most
415 contaminated areas (Krivolutsky 1996; IAEA 2006). These effect could also be linked to the
416 high levels of initial exposure that were experienced following both nuclear accidents. Initial
417 dose rates in the most contaminated areas of CEZ were as high as 5mGy/h (IAEA 2006)
418 The second phase characterised by a decrease in dose rates due to disapearence of short-lived
419 radioisotopes and wash-out and run-off (IAEA 2006). This phase started from two months
420 after the accidents, was associated with reductions (up to a factor of 30) in the density of
421 invertebrates living in the forest litter experiencing greatest contamination. These decreases
422 were linked to radionuclide exposure effects on reproduction and recruitment (Krivolutsky
423 and Pokarzhevskii, 1992; Krivolutsky et al., 1992).
424 In the third exposure phase resulting from the Chernobyl accident, most strongly affected
425 populations of species of pine trees and soil invertebrates were shown to slowly start to
426 recover (Arkhipov et al. 1994; Zelena et al. 2005). Recovery from the initial negative effects
427 was also found in birch pollen, embryonic cells of herbaceous plants like evening primrose
428 embryonic cells (Boubriak et al. 2008) and *Arabidopsis thaliana* (Kovalchuk et al. 2004) and in
429 exposed birds (Galvan et al. 2014). In this phase Cs-137 and Sr-90 are the main contributors
430 to the dose with some additional Am-241 and Pu-isotopes for CEZ and Cs-137/134 for FEZ
431 (Horemans et al. 2018; Saenen et al. 2017). Ambient dose rates now measured are maximally
432 0.5 mGy/h and these can be found in the forest western from the nuclear power plant
433 designated as the Red Forest (Beresford, personal communication).

In addition to changes observed at individual or population levels, the radiological impacts within both the CEZ and the FEZ, have also been reported at the sub-organismal level. Aberrant cell frequencies were found in the root meristem of plant seedlings (Geras'kin et al. 2011). Increased mutation rate (Kuchma et al. 2011) and gene deregulation (Zelena et al. 2005), have been seen in pine trees. Increased mitochondrial DNA haplotype and nucleotide diversity have been reported in bank voles (Matson et al. 2000; Baker et al. 2001), chromosomal aberrations in mice (Kubota et al. 2015) and in soil invertebrates, increased DNA damage in earthworms (Fujita et al. 2014). Most of these studies so far have, however, failed to find a link between these observed sub-organismal effects and impacts at higher level of biological complexity such as radiation-induced phenotypical changes and long-term effects on population dynamics (Meeks et al. 2009; Meeks et al. 2007).

The adaptive responses that have been indicated during the extended third phase of exposure following the two accidents at Chernobyl and Fukushima are at least in part due to the reduction over time in dose rates and, hence, exposure. Although a memory-effect of the early high exposures cannot be excluded, the decreased exposure in the third phase might allow both increased *in-situ* recruitment and survival leading to positive population growth, as well as the survival of inwardly migrating individuals (Jackson et al. 2004; Boubriak et al. 2008; Boubriak et al. 2016). Additionally it is also possible that increased tolerance, through selection and as a result of favourable mutations may make a contribution (Kovalchuk et al. 2003). However, in *Arabidopsis* no additional mutations compared to plants collected in control sites were found in the CEZ (Abramov et al. 1992). Ostensibly the probability of favourable mutations may be seen as unlikely. Assuming a germline mutation rate in plants of about 10^{-5} to 10^{-6} per gamete, one would expect only one mutation in 500,000 plants (Kovalchuk et al. 2003). Consequently it has been proposed that rapid adaptation may be more

458 strongly linked to epigenetic processes in the development of locally adapted phenotypes at
459 polluted sites (Kovalchuk et al. 2003).
460
461 ***7. Evidence for a role of epigenetics in long-term or transgenerational responses to***
462 ***radiation-induced stress***
463 Studies on the effects of stressors on the epigenome of organisms under environmentally
464 relevant exposure conditions have covered examples for ionising radiation exposure and for
465 a range of chemical and non-chemical stressors in different species. Within these studies, a
466 range of epigenetic mechanisms and endpoints have been considered (for review see e.g.
467 Aluru 2017; Bruce et al. 2007; Kim et al. 2012b; Mirbahai and Chipman 2014). Initial adaptive
468 changes resulting from exposure to these different stressors have been found for key
469 components of the epigenome, such as DNA methylation (Vandegehuchte and Janssen 2011;
470 Marczylo et al. 2016), non-coding RNAs (Kure et al. 2013; Wang et al. 2013; Song et al. 2012)
471 and histone modifications (Raut and Sainis 2012; Mondal et al. 2016). Changes in microRNA
472 expression have further been shown to be involved in metabolism following starvation and
473 the transfer of longevity (Greer et al. 2011; Katz et al. 2009; Rechavi et al. 2014). In plants,
474 small RNAs play an important role in chromatin remodelling and DNA methylation through
475 RNA-directed DNA methylation also in different abiotic stresses in plants (Hirayama and
476 Shinozaki 2010).
477 Although long a controversial issue and still not fully elucidated, recent evidence has
478 suggested that in plants, vertebrates and invertebrates, epigenetic marks induced by adverse
479 conditions encountered by the parents can be partly stable across generations (Uller et al.
480 2015; Klosin et al. 2017; Whittle et al. 2009; Saze 2012; Pecinka and Mittelsten Scheid 2012;
481 Sudan et al. 2018; Stassen et al. 2018; Norouzitallab et al. 2019). Such retention can potentially

lead to transgenerational heritable changes in offspring (Verhoeven et al. 2010; McCarrey 2012; Guerrero-Bosagna and Jensen 2015; Guerrero-Bosagna et al. 2012). Evidence has been accumulated for the transfer of DNA methylation patterns in the germline (Verhoeven et al. 2010; Verhoeven et al. 2016). As an example of the link between epigenetic mechanisms and transgenerationally altered phenotypes a study of transgenerational response to temperature in *C. elegans* has identified altered trimethylation of histone H3 lysine 9 as a mechanism for transgenerational inheritance (Klosin et al. 2017). On the other hand, in *Arabidopsis*, nickel chloride caused a change in DNA methylation patterns and some of this was inherited by the following generation (Li et al. 2015). In the offspring of mechanically wounded *Mimulus guttatus* plants changes in methylation could be associated with transgenerational plasticity (Colicchio et al. 2018). Depending on the methylation context, CG or non-CG methylation, these changes were found to be in gene coding regions or transposable elements, respectively (Colicchio et al. 2018). Dandelions (*Taraxacum officinale*) also showed altered DNA methylation that was largely inherited by the next generation of the asexually reproducing plants when exposed to a number of different stressors (Verhoeven and van Gurp 2012; Verhoeven et al. 2016).

A growing number of papers also indicate that exposure to ionising radiation will lead to changes in epigenetic markers (Table 3). For example, scots pine trees present in the most contaminated areas around the Chernobyl nuclear reactor have been found to have hypermethylated DNA, with this hypermethylation directly (Kovalchuk et al. 2003) or transiently associated with the radiation dose received (Volkova et al. 2018). Further work established that the genomes of young trees planted on contaminated soil showed higher levels of cytosine methylation than trees in uncontaminated soil. However, levels of cytosine methylations in plants grown in clean soil from seeds taken from previously exposed plants

were not found to differ significantly from controls Kovalchuk et al., (2003). Hence these results are suggestive of a within generation genome methylation effect, rather than of any multigenerational or transgenerational mechanism, as a result of exposure during the somatic development. However, since only overall levels of DNA methylation inheritance was addressed, the potential for loci specific cannot be discounted.

In a study of the progeny of *Arabidopsis sp.* sampled in three consecutive years from areas with different levels of contamination within the CEZ, higher resistance to mutagens in progeny of plants from the most contaminated sites compared to unexposed plants was identified (Kovalchuk et al. 2004). This difference in sensitivity could be attributed to higher expression of free radical scavenging enzymes and DNA-repair enzymes and was associated with global genome hypermethylation in the contaminated site plants. It was hypothesised from these data that epigenetic regulation of gene expression and genome stabilization may play a key role in the underlying processes that stabilise *Arabidopsis* genome architecture under exposure to ionizing radiation exposure (Kovalchuk et al. 2004). A number of papers have proposed a link between epigenetic effects and non-targeted effects (NTE) such genomic instability and bystander effects (Schofield and Kondratowicz 2018). However, while the existence of non-targeted effects is well established (Morgan 2002; Kadhim et al. 2004; Pouget et al. 2018; Burdak-Rothkamm and Rothkamm 2018), and studies have shown an association between the two effects (e.g., Kaup et al. 2006; Xu et al. 2015), evidence of a causal relationship is more elusive, since NTE could be either a mechanism or a consequence of epigenetic changes (Schofield and Kondratowicz 2018). Changes in the level of DNA methylation may be intimately linked with transcription remodelling in response to radiation exposures, including changes to the pathways involved in antioxidant defence and DNA repair. Confirmation of such effects would require the use of combined genome wide DNA

methylation mapping and transcriptomic approaches to allow loci specific methylation to be associated with gene expression phenotypes in exposed plants.

A study of the pale blue grass butterfly *Zizeeria maha* within the FEZ has provided a further indication of the potential for heritable epigenetic changes in a population exposed to ionising radiation (Hiyama et al. 2012; Hiyama et al. 2013). Mild morphological abnormalities were observed on some individuals of adult butterflies collected one month after the accident, but an increase of the severity of these abnormalities occurred in the F1 generation that were further inherited by F2 progeny. These abnormalities and their transgenerational transfer were proposed to be attributable either to random mutation on important genes or through epigenetic mechanisms. As the underlying mechanisms of these effects were not studied by the authors, leaving the mechanistic basis of the observed effects and their inheritance remain an open question.

Recently a number of European research groups have combined research efforts to study possible epigenetic changes in organisms exposed to ionizing radiation, in the laboratory or *in situ* (Chernobyl or Fukushima), in a range of species (plants, earthworms, fish, frogs) (Table 3). The focus of the combined efforts was to better understand the possible role of these mechanisms in the induction of long-term/transgenerational effects and their relevance as possible biomarkers of ionising radiation (Adam-Guillermin et al. 2013). The organisms chosen were all reproductive non-clonal organisms. Hence the work addresses multigenerational and transgenerational effects in genetically diverse populations. For example, in offspring of zebrafish that were exposed to ionising radiation during gametogenesis, a large number of differentially methylated regions were observed, with five specific loci showing a persistent effect up to the third generation (Kamstra et al. 2018). These methylation changes could be linked to changes in gene pathways and adverse effects found in progeny (Hurem et al. 2017;

554 Hurem et al. 2018b). In the same exposure study, miRNA expression was measured in first
555 filial offspring and histone marks H3K4me3, H3K9me4 and H3K27me3 at 3 specific
556 loci(Lindeman et al. 2019). There were 23 differentially expressed miRNAs indicating a
557 multifaceted response to ionising radiation exposure (Martin et al. 2019, in preparation).
558 Differentially enriched histone marks were observed as well at the three measures loci in F1
559 offspring, but interestingly these effects were diminished in F2 offspring (Lindeman et al. 2019,
560 submitted). Although only exposed embryo's were analysed similar changes in histone markes
561 were found for Atlantic salmon (*Salmo salar*) at higher dose rates (Lindeman et al. 2019).
562 A dose-rate dependent induction of total methylation levels was observed in *A. thaliana* plants
563 exposed in the lab to different levels of gamma radiation for up to three generations (Saenen
564 et al. 2017)). Moreover triple methyltransferase mutants (*drm1drm2cmt3*) of *A. thaliana*
565 showed increased sensitivity to irradiation including an increased induction of oxidative stress
566 (Saenen et al. 2017).
567 In the clonal cladoceran *Daphnia magna*, transgenerational inheritance of DNA methylation
568 changes were studied using bisulphite sequencing, after irradiation of generation F0 to 6.5
569 µGy/h or 41.3 mGy/h (Trijau et al. 2018). Significant methylation changes at specific CpG
570 positions in every generation were found, independent of dose rate and with a majority of
571 hypomethylation. The total number of common differentially methylated regions was greatest
572 between generations F2 and F3, with three specific persistent loci associated to genes known
573 to play a role during exposure to ionising radiation. The results above suggest a role of
574 enhanced methylation induced by chronic exposure to radiation in lab-conditions and indicate
575 the multi- and transgenerational natures of these responses.
576 For earthworms, studies of DNA methylation in the laboratory and CEZ have shown effects of
577 ionising radiation exposure on DNA methylation pattern as measured by methylated AFLP

analysis (Saenen et al. 2017). There are, however, specific challenges in the interpretation of the role of radionuclide exposure in these responses. Large differences in genetic diversity that may occur between morphological similar earthworm "species" may, for example, make it difficult to identify DNA methylation changes unless clades are assessed separately. Indeed clades of the earthworm *Lumbricus rubellus* were found to differ in the nature of their genetic and DNA methylation responses to soil contamination by copper and arsenic (Kille et al. 2013). A similar response was found within an analysed laboratory experiment, where both between and within species allelic differences precluded the identification of a clear DNA methylation profile response to exposure. In CEZ collected earthworm from two species *Aporrectodea caliginosa* and *Octolasion lacteum*, a clear site specific change in DNA methylation status was found (Saenen et al. 2017) in *Aporrectodea caliginosa*, while only limited separation was found for *Octolasion lacteum*. While these site specific changes in DNA methylation patterning may indicate a response to radionuclide exposure, a caveat is that the earthworms were collected from sites that differ in the prevailing ecosystem characteristics (wetland and garden sites).

An *in situ* study of DNA methylation in frogs collected from a range of differently polluted sites within the Fukushima impacted area indicated that DNA methylation measured as methylated cytosines increased with total absorbed dose rate, up to 7 µGy/h. This increase was concomitant with increased levels of DNA damages (Saenen et al. 2017). As in the study for *A. thaliana* in the CEZ (Kovalchuk et al. 2004), this finding of higher DNA methylation associated with increased DNA damage and repair activity supports a functional role of the epigenome in maintaining DNA integrity. These results are in agreement with previous work done on zebrafish exposed to depleted uranium, where changes in DNA methylation patterns both at specific restriction sites and across the whole genome, were observed in F_0 adults and F_1 at the same time as DNA damages (Gombeau et al. 2016; Gombeau et al. 2017). A transient

602 increased methylation with the dose rate was also observed in needles of *Pinus sylvestris*
603 plants collected in radioactively contaminated areas of Belarus (Volkova et al. 2018). In
604 contrast no dose dependent changes in total methylation levels were observed for *C. bursa*
605 *pastoris* plants sampled in spring 2016 in contaminated areas of FEZ. For *A. thaliana* plants
606 collected in CEZ a decrease in global DNA methylation was found in the highest contaminated
607 fields (Horemans et al. 2018).
608 Overall the range of studies of the epigenetic response of species to radionuclide exposure in
609 the laboratory point to a role of the epigenome in adaptive responses. The field studies with
610 plants (pine trees and *Arabidopsis*) showed the potential for ionising radiation to induce
611 changes in DNA methylation levels under field conditions (Georgieva et al. 2017; Kovalchuk et
612 al. 2003; Kovalchuk et al. 2004). For invertebrates, the laboratory and studies in the CEZ and
613 FEZ have partially supported a role of increased methylation in response to radiation among
614 the majority of species studied to date. The challenge from these field studies remains to
615 unequivocally link the observed effects on the epigenome to radiation exposure, rather than
616 to other aspects of environmental variation across the CEZ and FEZ. Studies that specifically
617 investigate changes in mutant lines with reduced DNA methyltransferase activity, as outlined
618 above for *Arabidopsis*, provide initial causal evidence on the validity of such as link.
619
620 ***8. Knowledge gaps on epigenetic changes induced by ionising radiation***
621 Although all three different epigenetic layers have been implicated as key mechanisms
622 involved in determining the long-term and transgenerational responses of species to
623 pollutant, including ionising radiation exposure, a majority of studies have to date focussed
624 on the role of DNA methylation (Norouzitallab et al. 2019; Sun et al. 2018; Meehan et al. 2018;
625 Burgio et al. 2018). In cases where difference in DNA methylation response following exposure

626 to ionising radiation are observed, a number of aspects that need further consideration in
627 future work can be drawn.
628 (i) Global methylation alone may be too coarse a measure of epigenetic change to be able to
629 see all biologically relevant differences induced by exposure to low dose rates. As such,
630 differences in methylation might be located in specific sequences of the genome but cannot
631 be detected by global measurements. Therefore, it is important to also include other
632 techniques (e.g. whole genome or reduced representation sequencing) in order to identify
633 specific epigenetic changes and to link these observations to effects on gene expression and
634 physiological change (Paun et al. 2019).
635 (ii) Different DNA methylation response in function of cell type, tissues (as seen in the depleted
636 uranium exposure in zebrafish by Gombeau et al., 2015), or age (as seen in frogs exposed at
637 Fukushima (Saenen et al. 2017), could induce a mosaic of DNA methylation response at the
638 whole organism level, limiting the capability to identify a clear change in methylation pattern.
639 This argues for the analysis of more homogenous tissues or cell types.
640 (iii) Initial changes of DNA methylation resulting from an initial radiation exposure may be lost
641 in individuals exposed over generations of chronic exposure as found for pine trees by
642 Kovalchuk et al. (2003) and in the second generation of lab-exposed *A. thaliana* in a laboratory
643 exposure to gamma radiation. Such results suggest that DNA methylation may be a transient
644 acting potential as an intermediate state preceding later genetic selection and adaptation.
645 (iv) Genetic diversity of species between isolated local populations within the CEZ and FEZ may
646 mean that populations exposed to different levels of radiation may show markedly different
647 epigenetic responses, precluding the identification of a clear exposure response relationship.
648 The presence of natural and man-made barriers to dispersal, which may result in population
649 isolation, across these two zones, may accentuate such differences (Meeks et al. 2007).

650 (v) Although less commonly studied than DNA methylation, the work done to date on the
651 responses of other epigenetic mechanisms like microRNAs or histone modifications to ionising
652 radiation exposure, suggest that these complimentary epigenetic mechanisms may play roles
653 in the response to radiation that may even dominate over DNA methylation changes (Putiri
654 and Robertson 2011; Brautigam et al. 2013);
655 (vii) Long time exposure to radiation might result in selection of alleles linked to tolerance,
656 potentiated potentially by increased mutation (as is seen for frogs in FEZ) that may lead to
657 genetic adaptation that might negate differences in DNA methylation. An interplay between
658 epigenetic changes, notably DNA methylation, and the targeting of mutation has been
659 proposed mechanisms (Putiri and Robertson, 2011 ; Braütigam et al., 2013).
660 (vii) Confounding factors (habitat, soil type, water chemistry; climate etc.) may increase the
661 variability between the samples that may result in changes in DNA methylation that overlie
662 and obscure effects due to ionising radiation making it difficult to link epigenetic change to
663 exposure (see discussion, Garnier-Laplace et al. 2013).
664
665 ***9. Differential DNA methylated regions as possible biomarkers for exposure or effect of a***
666 ***pollutant and its use in risk assessment***
667 There is a strong interest in finding possible biomarkers for exposure and effects of radiation
668 and additionally those that can be markers for long-term effects. Loci specific changes of DNA
669 methylation have been proposed as possible biomarkers for different environmental cues
670 (Meehan et al. 2018) and could possibly be used as molecular fingerprints for e.g. genotoxicity
671 induced when exposed to ionising radiation. However, it is also recognised that significant
672 challenges related to the effects of genetic background and the influence of confounding
673 factors also exist (Pernot et al. 2012). Further studies at environmental realistic doses are

674 needed to assess the prevalence of such responses, including under field conditions. In
675 particular, the use of more targeted methods are needed that identify loci specific changes in
676 DNA methylation, histone modification and the expression of relevant miRNAs.
677 A clear conclusion that emerges from past and ongoing studies concerning the role of the
678 epigenome in response to chronic radiation exposure, lies in the interpretation of changes in
679 methylation patterns from field collected samples in respect to attribution of the principal
680 driver of effects. Specific challenges relate to working with some autochtonous species for
681 which genome resources may be lacking and, the influence of confounding factors which may
682 mask the causal response between ionising radiation exposure and epigenetic changes. In
683 efforts to attribute changes to specific stressor effects, epigenetic approaches may be more
684 powerful indicators of effects when linked to known biomarkers using, for example,
685 transcriptional analysis. When used in conjunction with other mechanistic measurements,
686 epigenetic analysis has the potential to enhance the ecological relevance of molecular
687 biomarkers, as described in the Adverse Outcome Pathway concept (Groh et al. 2015). Given
688 the critical need to establish the nature of effect of prolonged low level exposures, this
689 integrated approach seems a promising way forward, building as it does on existing
690 mechanistic knowledge.
691 The risk assessment process for radiation and radionuclides is largely based on using results
692 from short-term bioassays to predict the effects of exposures in the field. The validity of this
693 laboratory to field extrapolation is one of the key uncertainties in risk assessment (Lourenco
694 et al. 2016). A comparison of field vs laboratory studies has indeed shown that species
695 sampled in the field were 8 times more sensitive than those studied under laboratory
696 controlled conditions (Garnier-Laplace et al., 2013) indicating the need for further torough lab
697 to field studies. One of the largest differences between laboratory bioassays and field

698 exposures is exposure duration. This is true within a single generation (intergenerational
699 exposure), but even more so when subsequent generations are exposed to the same stressful
700 environment (multigenerational exposure) or when exposure of the parent generation has a
701 subsequent effect on the non-exposed offspring (transgenerational exposure). When
702 multigenerational exposures occur, these may result in effects in later generations that match,
703 and can even exceed those found in exposed F0s (see Table 2). The biological response of
704 species mediated through the genome and epigenome appear to play a role in the
705 development of such effects. Such findings may require a more refined understanding to
706 support and reduce the uncertainty in risk assessment for chronic low dose exposures. Hence,
707 the mechanisms that underlie differential responses within and over generations to previous
708 (sub-lethal) radiation-exposure require further studies to provide a baseline for the
709 development of new approaches such as Adverse Outcome Pathways on low dose radiation
710 exposure, to the risk assessment for both wildlife and human.
711
712 ***10. Conclusions and Recommendations for further development and application.***
713 Work reported to date in both lab and field have indicated changes in DNA methylation
714 resulting from chronic exposure to low dose of ionising radiation. A common conclusion from
715 this work is that both laboratory and field studies have demonstrated changes in overall
716 methylation in organisms exposed chronically to ionising radiation. Generally a chronic
717 enhanced ionising radiation level induced hypermethylation or methylation pattern change
718 which could be taken as a response to induce DNA stability. The main advantage of laboratory
719 studies is the ability to set up controlled multi/transgenerational studies, and avoid
720 confounding factors like local difference in soil characteristics, microclimate. Together with
721 the use of homogeneous populations, this allows for greater insight into the underling

722 mechanisms and processes. Field studies can provide the increased environmental realism of
723 the responses studied. Although data suggest that methylation changes can be observed in
724 different organisms a lower dose rates than those seen in laboratory experiments. The
725 challenge remains to unequivocally link such observations to a specific cause. Furthermore,
726 processes linked to the potential for population adaptation and interactions with other
727 environmental stressors can add a further level of complexity as compared to laboratory
728 studies. Improvements could be made by increasing site coverage and further targeted work
729 on molecular mechanisms, as well as data on the background levels and variations in
730 methylation changes.
731 From the studies presented here, it can be concluded that DNA methylation might be the key
732 to transferring the response to ionising radiation from one generation to the next. Whereas
733 measuring total DNA methylation can be performed without any prior information on genetic
734 background of the species, the rapid technical evolution and the decreasing cost of sequencing
735 analyses will offer a wider comparison of radiologically induced DNA methylation in different
736 biological models and provide greater insight into the underlying mechanisms. An important
737 step will be to compare the sensitivity, reliance and above all specificity of DNA methylation
738 as a possible biomarker of ionising radiation exposure at environmentally relevant levels, with
739 other epigenetic mechanisms such as histone modifications and microRNAs linked to
740 responses at higher level biological complexity e.g. changes in growth and reproduction.

Table 1. Pros and cons of DNA methylation methods. 5-mC (methylcytosine), 5-hmC (hydroxymethylcytosine), AFLP-MS (methylation specific amplification fragment length polymorphism), HPLC-MS/MS (high performance liquid chromatography coupled with tandem mass spectrometry), ELISA assay (enzyme-linked immunosorbent assay), MeDIP seq (methylated DNA immunoprecipitation coupled with next-generation sequencing), WGBS (whole genome bisulfite sequencing), RRBS (reduced representation bisulfite sequencing)

Method	**Principle**	**Methylated base detected**	**Pros**	**Cons**
AFLP-MS	Cut DNA with restriction enzymes and analyse on a fragment analyser	5-mC	Low cost per sample No need for sequenced genome Low DNA amount (250-500 ng) Low processing time	Detection of global methylation Specific equipment needed
HPLC-MS/MS	Detection of methyl groups on hydrolysed DNA sample	5-mC & 5-hmC	Medium cost per sample No need for sequenced genome Low processing time	Detection of global methylation High DNA amount (50-1000 ng) Specific equipment needed
5 mC ELISA assay	Use of monoclonal antibodies sensitive and specific for 5-mC	5-mC	Low cost per sample No need for sequenced genome No specific equipment needed Low processing time	Detection of global methylation High DNA amount (100-2000 ng)
MeDIP seq	Immunoprecipitation sequencing	5-mC	Detection of site specific methylation Low DNA amount (300 ng)	High cost per sample Need for sequenced genome Specific equipment needed High processing time
WGBS	Bisulfite conversion and DNA sequencing	5-mC & 5-hmC (oxBS-seq)	Detection of site specific methylation Low DNA amount (30 ng)	High cost per sample Need for sequenced genome Specific equipment needed High processing time
RRBS	Bisulfite conversrion and DNA sequencing	5-mC & 5-hmC (oxBS-seq)	Detection of site specific methylation	High cost per sample Need for sequenced genome High DNA amount (1000 ng) Specific equipment needed High processing time

Table 2: Overview of lab-based studies in which ecotoxicological relevant model organisms were exposed to radiation, radioisotopes or other toxins for multiple generations; F0=Parental organism, F...= offspring with the number indicating the generation

Species	**Chemical**	**Generations**	**Observed phenotype**	**Ref**
C. elegans	Gamma radiation 7-42 mGy/h	F0-F2	Greater reproduction effects in multigenerationally and transgenerationally exposed F2s than F0 generation	Buisset-Goussen et al. (2014)
D. magna	Gamma radiation 0.007-35 mGy/h	F0-F2	Toxicity on multiple traits increased from F0to F2	Parisot et al. (2015)
D. Rerio	Gamma radiation 9-53 mGy/h	F0-F1	Effect on DNA damage, transcription, lipid peroxidation and demographic endpoints in F1	Hurem et al. (2017), Hurem et al. (2018b), (2018a)
D. Rerio	Uranium 20-250 µg/L	F0-F1	Effect on DNA damage, transcription, DNA methylation and demographic endpoints in F1	Bourrachot et al. (2014), Gombeau et al. (2017)
D. magna	Americium 0.3-15 mGy/h	F0-F2	Threshold for effects on reproduction reduced from 1.5 $mGyh^{-1}$ in F0 generation to 0.3 $mGyh^{-1}$ in F2 and F3	Alonzo et al. (2008)
D. magna	Uranium 2-50 µg/L	F0-F1	Greater reduction in fecundity in F1 than F0at 50 □g/L	Plaire et al. (2013)
D. magna	Nickel 42-85 µg/L	F0-F1	Greater reduction of ATP levels in F1 compared to F0	Pane et al. (2004)
C. elegans	Ag nanoparticles EC30-value	F0-F10	Greater (10 fold) sensitivity in F2, F5, F8 and F10 generations compared to P generation	Schultz et al. (2016)
D. magna	Ag nanoparticles EC10-EC50	F0-F10	Population growth rate at 10 □g/L reduced by 80% in F2s compared to 21% in F0 generation	Volker et al. (2013)
D. magna	Penta-chlorophenol 0.0002-2 µmol/L	F0-F3	Population growth rate reduction increases from 28.2% to 34.9% to 46.3% in F0, F1, F2 generations	Chen et al. (2014)
D. magna	Tetracycline 0.1-5 mg/L	F0-F1	NOEC decreased from 5 mg/L to 0.1 mg/L from F0 to F3	Kim et al. (2012)
D. magna	Enrofloxacin 13 mg/L	F0-F1	Reproduction NOEC decreased from 30 mg/L to 3.1 mg/L from F0 to F1 generation	Bona et al. (2015)
C. elegans	Uranium 4-50 µg/L	F0-F16	Greater maximal length but increased sensitivity to uranium across the generations	Goussen et al. (2015)
C. elegans	Uranium 4.6 µg/L	F0-F22	Increase of sensitivity from F0 to F6 and subsequent adaptation until F22	Dutilleul et al. (2014)

Tabel 3: Overview of studies in which changes in epigenetic mechanisms (DNA methylation, histone modifications or miRNA's) are measured in organisms exposed to radiation in a long-term set-up (within or over generations) either in laboratory or field conditions. F0=Parental organism, F...= offspring with the number indicating the generation, CEZ: Chernobyl Exclusion Zone, FEZ: Fukushima Exclusion Zone

		Organism	Experimental conditions	Epigenetic changes	Additional endpoints	Reference
Laboratory exposed	Plants	*A. thaliana*	F1, F2, multigenerational (F0 from CEZ, 1.8-4.4µGy/h) methyl methane sulfonate (140 µM) or Rose Bengal (10 µM)	DNA methylation: hypermethylation in both F1 and F2	Higher resistance to mutagens, increased expression of ROS scavenging enzymes and DNA repair enzymes	Kovalchuk et al. (2004)
		P. sylvestris	F0, trans- and multigenerational set up, on contaminated soil both acute (~10Gy) and chronic (~80Gy) (F0 from CEZ, (absorbed dose 1986: >60Gy, 10-60Gy, 1-10, 0.1-10Gy),	DNA methylation: hypermethylation in exposed	-	Kovalchuk et al. (2003)
		A. thaliana	F1, F2 transgenerational, Progeny of plants collected at CEZ 1.8-4.4µGy/h	DNA methylation: hypermethylation	-	Kovalchuk et al. (2004)
		A. thaliana	F0, F1, F2, mutligenerational, 14 day exposure during vegetative state, 22, 38, 86, 457 mGy/h	DNA methylation: dose-dependent hypermethylation, strongest in F2	Changes in ROS-scavenging enzymes, DNA repair and developmental traits, mutants in methyltransferases showed increased sensitivity to radiation	van de Walle et al. (2016), Saenen et al. (2017)
	Invertebrates	*D. magna*	F0, F1, F2 and F3 transgenerational, F0 exposed for 25 days, 6,5 µGy/h or 41.3 mGy/h	DNA methylation: hypomethylation but dose-rate independent	Reduction in fecundity in F0, no adverse effects in F1, F2, F3	Trijau et al. (2018)
	Vertebrates	*D. rerio*	F0, F1, F2, F3, transgenerational, exposure during gametogenesis, 8.7 mGy/h, 28 days	DNA methylation: Genome-wide in F1,	Linked to gene pathways changes and adverse effects in progeny	Hurem et al. (2018b), Kamstra et al. (2018),

				locus-specific regions up to F3		Hurem et al. (2017)
		D. rerio	F0, F1, multigenerational, exposure during gametogenesis, 8.7 mGy/h, 28 days	miRNA expression in F1 embryos	-	Martin et al., in prep
		D. rerio	F0, F1, F2 transgenerational, gametogenesis, 8.7 mGy/h, 28 days	Histone modifications (hypermethylation) at specific loci in F0 and F1 but no longer in F2	-	Lindeman et al. (2019)
		S. salar	F0-embryo's, exposure from one-cell fertilized eggs till early gastrula stage, 1, 10, 20 or 30 mGy/h	Histone modification (hypermethylation) at specific loci at highest dose rate	-	Lindeman et al. (2019)
Field collected	Plants	*P. sylvestris*	F0, (Belarus, Chernobyl affected area), annual absorbed dose: 10-158 mGy or 1-14 µGy/h	DNA methylation: transient with dose, hypermethylation	-	Volkova et al. (2018)
		C. bursa pastoris	F0, FEZ : total dose rates: 0.13-38 µGy/h	DNA methylation : no change	-	Horemans et al. (2018)
		A. thaliana	F0, CEZ : total dose rates : 0.1-160 µGy/h	DNA methylation : Hypomethylation at highest dose rates	-	Horemans et al. (2018)
		G. max	F0, after 7 generations CEZ, total accumulated dose : 1-132 mGy	DNA methylation: slight increase (10%) in radio-	Increased levels of single and double DNA strand breaks	Georgieva et al. (2017)

				contaminated samples		
	Invertebrates	Earthworms (*A. calinginosa, O. lacteum*)	F0, CEZ, total dose rates 0.12-41 µGy/h	DNA methylation: site-specific differences *A. calinginosa.* for no or limited changes found for *O. lacteum*	-	Saenen et al. (2017)
	Verte brates	*H. arborea*	F0, FEZ, total dose rate 0.38-41,7 µGy/h	DNA-methylation: hypermethylation, dose- dependent	Concomitant with increased DNA damage	Saenen et al. (2017)

Acknowledgements

This work was supported by the EC's COMET (Coordination and implementation of a pan-European instrument for radioecology) program, Fission-2012-3.4.1-604794.

JK was supported by the Research Council of Norway through its Centers of Excellence funding scheme, project number 223268/F50.

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