The ALLIANCE Workshop on epigenetic factors and long-term effects of ionising radiation on organisms (Paris 4th-6th April 2018)

In recent years, a large amount of work has been undertaken to assess primarily the role of epigenetic mechanisms in the field of human disease. However, epigenetics have also been considered for ecologically relevant issues. The question of the role of epigenetic factors in trans-generational effects of ionising radiation exposure of wildlife was a core component of the European project COMET (http://www.radioecology-exchange.org/content/comet). Epigenetic modifications were studied in various organisms (plants, earthworms, frogs, fish) exposed to ionising radiation in the laboratory and/or in the field.

An international workshop on Transgenerational and Epigenetic Mechanisms of Radiation Toxicity at Chronic Doses was previously held on the 10-12 December 2014 at St Catherine's College (Oxford, UK) (https://www.radioecology-exchange.org/sites/default/files/Deliverable%205-3%20COMET%20Oxford%20meeting_Final.pdf). It was intended as a forum to discuss the results arising from EURATOM funded work and also to allow ecotoxicologists and radiobiologists to meet in an open forum to discuss current developments in epigenetics fields This meeting built on the output from the MELODI meeting 7-9 October 2014 (www.melodi-online.eu/ws6.html) in Barcelona on epigenetics and included full feedback of the outcomes from that meeting.

An ALLIANCE (http://www.er-alliance.org/) sponsored workshop on 'Epigenetic factors and long-term effects of ionising radiation on organisms' (agenda, Appendix 1) was held from the 4th to the 6th of April in Paris, gathering together more than 30 researchers. The presentations from this workshop can be accessed from the Radioecology Exchange website (https://radioecology-exchange.org/content/workshops-0) and are linked to the appropriate speakers in Appendix 1. The overall aim of the meeting was to bring together scientists involved in epigenetic studies, in the field of ionising radiation, environmental risk assessment, ecology and ecotoxicology, and to also have representation from regulators.

The work programme comprised four main topics (see full program in Appendix 1):

- Risk assessment and epigenetics

- Observations of epigenetic modifications in exposed organisms

- Epigenetic and genetic mechanisms

- Epigenetics and transgenerational effects

Two break-out sessions were included in the programme and notes from these are summarised.

Break-out session 1

Question 1: should epigenetic changes be targeted? Consensus answer - **YES**

- Ionising radiation (IR) → DNA damage → repair mechanisms will not reconstruct methylome → direct link between IR and methylation
- IR → free radicals → they will use the pool of methyl groups → both histone modification and DNA methylation are affected.
- However, no receptor for IR (like there is for UV)
- Non-targeted effects?
- Important to find the targets so you know where to look at

- Effect on gene expression will probably come from effect on epigenetic level
- Necessary to look at epigenetic changes for regulatory reasons?
- Risk Assessment: standardised test method necessary that everyone does the test at the same way. Or adapt existing test for IR
- Can epigenetic changes be used as biomarkers for radiation?
- Will digging into the mechanisms help us change the environmental protection criteria?
- Epigenetic changes have to be addressed with other biological responses (need to obtain multi-level data). Do we need to look at other phenotypic marks (e.g. behavior)?
- Need to establish cause and effects, over long time series

Questions 2 and 3 are considered together as they are linked. Question 2. Which epigenetic markers should be targeted? Question 3. Transfer of findings to non-model species?

- All epigenetic marks can be important
- Global methylation is probably not the way to go
- Look at chromatin state/structure. Can be done for all sequenced species.
    - Important since open chromatin can be more easily targeted by radiation. And eg. open chromatin in irradiated ovaries can lead to effects in offspring
- Histone modifications are more evolutionary conserved than DNA methylation (eg. overlap in flies, C. elegans, etc) and can be more easily transferred to non-model species. DNA methylation is very different between different species
- We need models, but, we have to keep in mind that every model has its issues
- Non-coded RNAs are important since they can transfer information between generations.
    - They all have different names in different species (even if the sequence is more or less the same) but they are epigenetic marks that we can use across species→ important to look at the targets of the microRNAs → interesting to look at if similar pathways are affected in different species
- What about species sensitivity? Why are some species more sensitive than others? Is it, genetics, epigenetic, genome size ..?
- Epigenetic changes are not necessarily a clear physiological endpoint
- A biomarker is not necessarily a biological response bringing any molecular understanding (exposure vs effect biomarker)
- Model species are not always a good starting point for extrapolation to non-model species.
- Need more basic knowledge in non-model species
- It is not necessary to have the same epigenetic mark for all organisms

Question 4. Can we include epigenetics in Environmental Risk Assessment?

- Important to link what is happening at the phenotype with lower levels of biological complexity
- Make guidelines/standardized protocols. However, this is difficult since epigenetics is a relatively new field and there are new techniques continuously being developed
- Quality assurance like we have e.g. for qPCR data?
  Use existing guidelines and adapt those for ionising radiation → radiation is just another stressor so we just have to look at what already has been done for chemicals
- Important to look at mixtures → adverse outcome pathway (AOP) networks

- We need adverse effects before regulators will do something.
- But what is a change/adverse effect? What is relevant? → sublethal effects that affect later generations are important! → transgenerational effects?
- Need more fundamental work
- Epigenetics can be useful to give more weight to decisions for the regulators
- Translation is needed between science and regulators

Break-out session 2

What are our priority research questions for the effects of radiation on the environment/wildlife?

- Investigate effects of radiation at different levels
  - ➔ Extrapolation to humans
  - ➔ Extract new **therapies/treatments** because you know the affected pathways
- Important to look at **mixed pollutants**: this is the situation in nature! Looking at radiation alone is not the way forward. e.g. gamma radiation alone maybe 'OK' but gamma + Cd may be more sensitive
- Focus on **models and hypothesis** generation
  - ➔ Based on this, choose the correct species (e.g. small mammals because they are relatively sensitive), chemicals, etc.
  - ➔ Be aware of the fact that sometimes it is hard to see effects in the lab at low doses because you have limitations (e.g. capacity, not always possible to do very long exposures)
  - ➔ Important to take into account indirect effects: various observations of subtle reproduction endpoints which in themselves will not result in a significant effect – but if effect is combined for different organisms [e.g. plant and pollinators] then potentially may add-up to an effect that matters

- New tools/techniques
  - ➔ Look at chromatin structures. More conserved across species
    - Cross-species issues resolved
  - ➔ Dynamic processes (over time)
    e.g. mice with markers for DNA damage → real-time monitoring of what is happening to see dynamics of processes
    e.g. use transgenic lines of different species
    -> Also possible to do this for epigenetics (e.g. Akitake, Dev. Biol. 2011)
- Importance of **exposure route and internal exposure route**
- **Community structures** to look at impact on biodiversity → move away from individuals!

Summary conclusions and actions arising from the Workshop and Discussion groups

The conclusion of discussion are summarised below.

Epigenetic mechanisms are worthy of being investigated, even if they can be modulated by different factors (ionising radiation, UV, etc.); they are sensitive markers that can be measured in all organisms. Hence, as ionizing radiation effects are not specific (there is no receptor), the general approach developed for epigenetic studies in ecology or ecotoxicology can be used. All epigenetic marks should be investigated, as epigenetic mechanisms are not always the same in different

species. They can provide weight of evidence to assess the environmental risk associated with exposure. There is a need to link them to other physiological endpoints when using an approach such as the Adverse Outcome Pathway approach (Villeneuve et al., 2014).

We need to gain knowledge on more realistic situations (e.g. mixtures and long term exposure, non-model species) and to focus on hypothesis driven research to focus on the right species and exposure conditions. It will be important to study different epigenetic marks and to find to identify those that are general across species as well as transmittable to the next generation. In this respect it was suggested to put a future research focus on methylation induced transposable elements as these will be inherited, on chromatine remodelling or on processes that are important in the transmittance to the next generation.

The workshop gave the foundation for a project proposal in response to the EURATOM NFRP8 call (project ADAPT).

Appendix A

ALLIANCE Workshop on

Epigenetic factors and long-term effects of ionising radiation on organisms

(4th – 6th April 2018, Paris, Fontenay-aux-Roses, France)

AGENDA

Wednesday April 4th 2018

14:00-14:15	Christelle Adam, Nick Beresford and Nele Horemans	General introduction and objectives of the workshop
14:15-14:45	Nele Horemans	Position paper: Point of view from ALLIANCE
14:45-15:15	Sisko Salomaa	Epigenetic cross-cutting issues vs MELODI
Session 1	***Risk assessment and epigenetics***	
15:15-15:45	Carlos Barata	Overview of OECD multigenerational tests and potential value to detect epigenetic effects
15:45-16:00	*Coffee break*	
16:00:16:30	Jana Asselman	Epigenetic in risk assessment: lessons learned and future research directions
16:30-17:00	Ionna Katsiadaki	How oestrogenic is the contraceptive pill to fish?
Session 2	***Observations of epigenetic modifications in exposed organisms***	
17:00-17:30	Etienne Bucher	When the genome lowers its epigenetic shield: Transposable elements on the move

Thursday April 5th 2018

Session 2	***Observations of epigenetic modifications in exposed organisms***	
9:00-9:30	Peter Kille	Epigenetic changes induced by metals in invertebrates
9:30-10:00	Eline Saenen	Transgenerational and epigenetic effects in plants
10:00-10:30	Rémi Guedon	Multigenerational effects of chronic exposure to gamma radiation : From epigenome to phenotype
10:30-11:00	Christelle Adam	Genetic and epigenetic changes in the tree frog exposed to radionuclides in nuclear accident impacted area
11:00-11:15	*Coffee break*	
11:15-12:45	Breakout sessions	Two parallel sessions
12:45-14:15	*Lunch*	
Session 3	***Epigenetic and genetic mechanisms***	
14:15-14:45	Christophe Grunau	A systems biology view on genetic and epigenetic inheritance and its relationship to environmental cues and selection
14:45-15:15	Jean-Luc Ravanat	Recent advances in epigenetic DNA modifications
Session 4	***Epigenetics and transgenerational effects***	
15:15-15:45	Jorke Kamstra	Zebrafish as a model to assess transgenerational effects of ionising radiation via epigenetic mechanisms
15:45-16:00	*Coffee break*	
16:00-16:30	Frederic Alonzo	Transgenerational DNA methylation changes in daphnids exposed to gamma irradiation
16:30-16:45	Christine Fassert	HSS and Epigenetics : a few words of conclusion
	Dinner	

Friday April 6th 2018

Session 4	***Epigenetics and transgenerational effects***	
09:00-9:30	David Copplestone	Effects of radioactivity on plants and animals
09:30-10:00	Jess Goodman	Investigating the effects of chronic radiation on Daphnia pulex
10:00-10:15	*Coffee break*	
10:15-11:45	Break out sessions	Two parallel sessions
11:45-12:00	Jacqueline Garnier-Laplace and David Copplestone	View from the ICPR: View of epigenetic and long term effect studies and research requirements
12:00-12:15	Geert Biermans	Evaluating the impact of past and present NORM industry: a regulatory perspective
12:15-12:30	Nele Horemans, Nick Beresford, Christelle Adam	Wrap up and recommendation to the NFRP-8
12:30	*Lunch*	