EUROPEAN COMMISSION

Community research

STAR

(Contract Number: Fission-2010-3.5.1-269672)

DELIVERABLE (D-N°4.4)

Critical evaluation of Robustness of Protection Levels in a Multiple Contaminant Context

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Editor: **Laureline Février**

Reporting period: 01/02/14 – 31/07/15

Date of issue of this report: 28/07/2015

Start date of project: 01/02/2011 Duration: 54 Months

STRATEGY FOR ALLIED RADIOECOLOGY

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Project co-funded by the European Commission under the Seventh Euratom Framework Programme for Nuclear Research &Training Activities (2007-2011)		
Dissemination Level		
PU	Public	PU (after 01/07/2017)
RE	Restricted to a group specified by the partners of the [STAR]	RE (before 01/07/2017)
CO	Confidential, only for partners of the [STAR] project	

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Foreword

The overarching goal of the STAR Work Package 4 "Radiation Protection in a Mixed Contaminant Context" is to determine if radiation protection criteria for wildlife are robust, even within a mixed contaminant context.

To achieve this goal, four specific objectives were pursued:

1. Critically review existing approaches, methods and tools developed in ecotoxicology for assessing exposures, effects and risks in a mixed contaminant context and evaluate their applicability for radioecological research and radioecological risk assessments (task 1, D-N°4.1, Vandenhove et al., 2012).
2. Test and improve selected ecotoxicological approaches and tools for reliable radionuclide (bio)availability and exposure assessment under mixed contaminant conditions, and improve the understanding of underlying mechanisms and processes (task 2, D-N°4.2).
3. Apply selected approaches developed in ecotoxicology to assess the impact of mixed contaminant conditions on radiation induced effects, and improve the understanding of underlying mechanisms and processes (task 3, D-N°4.3).
4. Integration of all research and technology development results for a critical evaluation on how mixed contaminant conditions may affect radiation protection standards (task 4).

This document is the final deliverable for this work package studying mixed exposure situations in which radiation or radionuclides are one of the contaminants in the mixture. It deals mainly with task 4. Hence, it aims at providing a synthesis of the experimental results obtained during this project (mainly in task 3). Additionally, three questions are addressed:

- What are the implications for the future of multiple stressor research in a radioecological context?
- What can we say about the robustness of radiation/environmental protection benchmarks in a multiple stressor context?
- Is further research needed and if yes what should the focus of future multiple stressor research be?

It is written as a paper and will be submitted in the course of 2016 after the data papers that form the basis of this deliverable are accepted for publication.

1 Introduction

Increased industrialization and population densities have led to humans and the environment being exposed to a multitude of contaminants, for which little is known about their combined health and ecological consequences. The issue of multiple contaminants has been addressed in a number of international projects (e.g. NoMiracle (Lokke, 2010), BEAM (Backhaus et al., 2010), PHIME (2011), SOLUTIONS (Brack et al., 2015)) and reviews (Kortenkamp et al., 2009; Van Gestel et al., 2011). However, those approaches still do not consider radioactive contaminants, nor integrate the recent derivation of environmental radiation protection criteria by international organizations (e.g. IAEA, 1992; ICRP, 2008; UNSCEAR, 2008; EC, 2014) and EURATOM projects (ERICA, Larsson, 2008; PROTECT, Howard et al., 2010).

The issue of multiple contaminants is also of concern for radionuclides. Their occurrence in the environment is, in many situations, concomitant with other contaminants such as in routine liquid releases from nuclear power plants (Garnier-Laplace et al., 2008), high-level radioactive waste disposal (Harju-Autti and Volckaert, 1995), uranium mining and milling (Geletneky et al., 2002; Salbu et al., 2011) and the NORM industry (Tayibi et al., 2009; Müller et al., 2000). In addition to the above controlled and planned releases of radionuclides by industries, radionuclides have been released to the global environment following a series of historic events (nuclear weapon tests, use of depleted uranium ammunition, nuclear weapons accidents, nuclear reactor accidents, dumping of nuclear waste at sea). Adding to the list is the use of radionuclides for medical purposes, research, or specific uses in industry. This shows that radionuclide releases in the environment are expected to occur in a range of widely varying situations where also other non-radioactive contaminants are present.

In the framework of the radiological protection of the environment, recent consideration has been dedicated to the mixture issue under the umbrella of the IUR (2011) and IAEA (IAEA, 2011) working groups on Multiple Stressors. Although about three-quarters of the papers reviewed suggested some form of interaction of effects existed among the stressors, a review paper (Vanhoudt et al., 2012) highlighted that conclusions were mostly based on the incorrect principle of effect summation or on own judgment of the authors. In many cases this stems from the fact that the studies were not specifically designed to investigate mixture or interacting effects (dose-response curves not fully covered, confounding environmental factors, lack of systematic quantitative assessment of exposure concentrations/doses, lack of mechanistic understanding…) and from misunderstandings (or misuses) of concepts for the description of combined effects.

Effect Characterization in support of Ecological Risk Assessment (ERA) under mixed contaminant exposure conditions is a major challenge (Eggen et al., 2004). One of the ways to consider mixtures, e.g. for predictive and first tier ERA framework, is to use mathematical models for the prediction of combined effects, based on the known individual effects of contaminants (Groten et al., 2001). This approach has the advantage of allowing the use of knowledge on single contaminants ecotoxicology, as well as being compatible with most of the ERA frameworks (EC, 2011). Two mathematical reference models, "concentration addition" (CA) and "independent action" (IA), are generally accepted for the prediction of the combined effects of contaminants (Jonker et al., 2004; Kortenkamp et al., 2009; D N°4.1 Vandenhove et al., 2012).

The review performed by the STAR project (Vandenhove et al., 2012) concluded that there is no theoretical or conceptual limit that would prevent the application of the general concepts as proposed in ecotoxicology to mixtures where radiation or radionuclides are one or more of the contaminants. Particularly, CA and IA models are potentially scientifically valid approaches that can support component-based Cumulative Risk Assessments (CRA) for mixture including radionuclides under the assumption of no interactions between the stressors, and provide a basis for the consideration of mixtures with radioactive substances. However, from an experimental point of view, the exploration of mixtures including radioactive substances may be challenging. The existence of non-monotonous effect patterns in the data of gamma irradiation at low doses (such as an hormesis-like growth response), the combination of chemical and radiological modes of action for some radionuclides and the scarcity of irradiation facilities are among the factors hampering assessment of mixed exposure situation. Additionally, incorporating external radiation doses into the existing conceptual framework that is constructed on the basis of contaminants having an effect once taken up into the organism is not evident.

The underlying assumption of CA and IA models is the additivity of the individual stressor effects, *i.e.* no interaction between the contaminants. There is considerable evidence from research on non-radioactive contaminants that the effects of multiple contaminants are frequently additive, although there are some exceptions where mixture effects are less or more than those predicted by the models, thus jeopardising the robustness of mixtures ERA methodologies (Kortenkamp et al., 2009; Baas et al., 2010a,b). The challenge remains to identify the exceptions from additivity, *i.e.* cases where interactions (especially synergistic interactions) occur in mixtures including radioactive substances. Further when such case are identified, there is a need to establish the mechanistic bases for these interactive effects in order to understand if these interactions can be generalised for untested chemical and radiological combinations.

In this context, the overarching goal of the STAR work package on “Radiation Protection in a Mixed Contaminant Context” was to provide new and robust experimental data for the assessment of the combined exposure and toxic effects of radioactive and stable substances to the ecosystems. Our aim was to evaluate if the joint toxicity of such mixtures is predictable from single substance toxicity data, according to the additivity concepts now established in the literature for stable contaminants. When interaction was identified, further analysis considering different aspect of exposure, (extractable, modelling exposure at uptake sites and internal concentrations) was used to identify the potential cause of this interaction. A limited set of exemplary binary mixtures were considered ionizing gamma irradiation (γ) or uranium (U), both in mixture with stable cadmium (Cd) or the organic compound fluoranthene (FL). The joint effects of UxCd and γxCd were being studied for five different species groups: the nematode worm *Caenorhabditis elegans*; the aquatic plant *Lemna minor*; the fish *Salmo salar*; the crustacean *Daphnia magna*; the unicellular algae *Pseudokirchneriella subcapitata*.

Due to practical and time restraints the combinations with FL were not that extensively studied. As such, UxFL was only studied in *C.elegans* whereas work on γxFL was limited to *D. magna* and *P. subcapitata*. As the work on FL was not performed on all species this is not considered further in this review.

2 Materials and methods

The STAR experimental strategy was to derive dose-response curves for each of the single substances in the mixture, and then to apply the general concepts of CA/IA to mixtures including ionising radiation or radionuclides, both to make predictions on mixture effects addition as well as to assess deviations from addition. Chemical analysis and exposure modelling was also used to understand the mechanisms responsible for interactions in relation to environmental exposure, toxicokinetics and toxicodynamics. The choice was made to experimentally test a limited number of binary mixtures. The toxicity of binary mixtures of UxCd and γxCd was systematically tested on five representative species: the nematode worm *Caenorhabditis elegans* (growth and reproduction after 11-days exposure; Margerit et al., 2015), the aquatic plant *Lemna minor* (growth inhibition after 7-days exposure; Horemans et al., 2015), the fish *Salmo salar* (Parr survival after 3-days, or egg survival and development after 92-days exposure; Teien et al., 2015), the crustacean *Daphnia magna* (immobility, carbon incorporation and growth after 3-days; Nascimento et al., 2015) and the unicellular algae *Pseudokirchneriella subcapitata* (a range of subcellular, cellular and population level endpoints after 3-days exposure; Bradshaw et al., 2014). The ranges of tested exposure levels for each species are given in Table 1. In order to be able to test the validity of the mixture toxicity models CA and IA, it is needed to have a dose response curve ideally with a number of points going up to levels above 50% effect for at least one of the components. This indicates the need to test within a concentration range that elicits effects in the tested endpoints. That explains why some high concentrations or dose rates were tested here.

Table 1: Ranges of exposure level tested on the different species (nominal tested concentration) and type of mixture design tested for the study of binary mixtures

Radioactive substance	***Uranium***	***Gamma irradiation***
Stable substance	***Cadmium***	***Cadmium***
C. elegans (Margerit et al., 2015)	*Partial factorial* [U]=0.95-1.3 mM [Cd]=6-40 µM	*Full factorial* γ=1-1500 mGy/h [Cd]=0.1-100 µM
L. minor (Horemans et al., 2015)	*Ray design* [U]=3-75 µM [Cd]=3-67 µM	*Full factorial* γ=26 -1500 mGy/h [Cd]= 4-32 µM
S. salar (Teien et al., 2015)	*Partial factorial* [U]=4.2-14.7µM [Cd]=0.9-35 nM	*Partial factorial* γ=0.4-422 mGy/h [Cd]= 2.7-267 nM
D. magna (Nascimento et al., 2015) + *P. subcapitata* (Bradshaw et al., 2014)	*Not tested*	*Full factorial* γ=2.5-100 Gy [Cd]=0.1-8.9 µM *Full factorial* γ=5-100 Gy [Cd]=0.09-8.9 µM

The obtained joint toxicity results were synthesized in regard to the typical levels of exposure in unaffected and contaminated ecosystems, as well as screening values for the protection of ecosystems. Those values are shortly summarized in **Erreur ! Source du renvoi introuvable.**2 and below:

- Natural background levels of gamma irradiation in the environment originate from primordial (U and Th-series, K-40) and cosmogenic (C-14, H-3, Be-7) radioisotopes and cosmic radiation. Typically, the estimated total weighted whole-body absorbed dose rates levels in natural environments ranges from 0.07 µGy/h for pine trees (Beresford et al., 2008) up to 60 µGy/h for small mammals lungs in radon-rich soils (Macdonald and Laverock, 1998). The level of ecosystems exposure is increased by artifically produced radionuclides (routine releases from nuclear and other industries. For example, calculated total weighted whole-body absorbed dose rates to soil invertebrates (nematodes) were estimated at 200 to 400 µGy/h in the Chernobyl Exclusion Zone 25-year after the accident (Lecomte-Pradines et al., 2014), i.e. two orders of magnitude lower than the estimated dose rates one year after the accident (up to ca. 50 mGy/h; Geras'kin et al., 2008). Similar exposure dose rates (around 100 µGy/h) were estimated for some marine fish species 5 months after the Fukushima accident in 2011 (Vives i Batlle et al., 2014), as well as for aquatic organisms in lakes of the southern Urals (up to 400 µGy/h) after the Mayak accident (Kryshev et al., 1996). In comparison, generic (and organism group-specific) predicted no-effect dose rate (Andersson et al. 2009) ranges from 10 to 200 µGy/h.
- Uranium is a naturally occurring, long half-life radioelement. Its levels are increased by nuclear industries (e.g. mining and milling). For uranium, background concentrations in soils are from 0.5 to 5 mg/kg in soils, 20 mg/kg in freshwater sediments and 0.1 to 20 µg/L in freshwaters (Ribera et al., 1996; Ragnarsdottir and

Charlet, 2000; Uralbekov et al., 2011). It may reach 10-1000 mg/kg soil and 20-500 µg/L in freshwaters (Carvalho et al. 2007; Gongalsky, 2006; Lottermoser et al., 2005; Ragnarsdottir and Charlet, 2000; Uralbekov et al., 2011). Uranium toxicity is linked to complex uptake, toxicokinetic process, and interaction with other cations and chemical complexation in the exposure media that influences its bioavailability (Markich, 2002, 2013). As a function of its bioavailability, uranium screening values ranges from 0.03 to 30 µg/L (Sheppard et al., 2005; MEDE, 2007; RIVM, 2014).

- Cadmium is a stable trace element, ubiquitous in all NORM contaminated sites. The European background level of Cd is 0.3 mg/kg in soils, and 0.1 µg/L in freshwaters (EC, 2007). Its levels are increased by mining, smelting, refining, fuel combustion, etc. and Cd is a priority substance under the Water Framework Directive. In contaminated areas, e.g. at the vicinity of former lead smelters (Bernard et al., 2010), soil concentration can be as much as 7 mg/kg. Concentrations encountered in freshwaters reaches 20 µg/L downstream Cd producing/processing sites or Ni/Cd battry recyclers (EC, 2007). At polluted sites such as smelters and mines however examples for Cd concentrations as high as 500 mg/kg have been documented (Bundy et al., 2007; Spurgeon and Hopkin, 1996, 1999; Spurgeon et al., 2005). The Predicted No Effect Concentrations of Cd depends on water hardness and pH and ranges from 0.08 to 0.3 µg/L in freshwater (SCA, 2011).

Table 2: Typical levels of exposure and screening values of the selected substances for binary mixtures experiments within the STAR project (bold values used in Figures 1 and 2)

		Ionizing radiation		Uranium			Cadmium		
Typical level in unaffected ecosystems		*µGy/h (internal+external)*	*ref*	*µg/L(µg/kg)*	*µmol/L*	*ref*	*µg/L(µg/kg)*	*µmol/L*	*ref*
	Soil	$\mathbf{7.10^{-2}}$ - 6.10^{-1} (up to 6.10^{1})	a b	5.10^{2} - 5.10^{4}	$\mathbf{2.10^{0}}$-2.10^{1}	d	3.10^{2}	$\mathbf{3.10^{0}}$	g
	Seawater	1.10^{-1} - 6.10^{0} (up to 3.10^{1})	c	2.10^{1}	1.10^{-2}	e	1.10^{-3} - 4.10^{-2}	$\mathbf{1.10^{-5}}$ - 4.10^{-4}	h
	Freshwater	4.10^{-1} - 4.10^{0} (up to $\mathbf{6.10^{1}}$)	b	1.10^{-1} - 2.10^{1}	$\mathbf{4.10^{-4}}$- 10^{-1}	e f	1.10^{-1}	1.10^{-3}	g
contaminated ecosystems									
	Soil	2.10^{2} - 4.10^{2} (up to >$\mathbf{5.10^{4}}$)	i j	1.10^{4} - 1.10^{6}	4.10^{1} -$\mathbf{4.10^{4}}$	m	7.10^{3}	$\mathbf{7.10^{1}}$	o
	Seawater	1.10^{2}	k						
	Freshwater	$\mathbf{3.10^{1}}$ – 4.10^{2}	l	2.10^{1} - 5.10^{2}	$\mathbf{10^{-1}}$-8.10^{1}	n	2.10^{1}	$\mathbf{2.10^{-1}}$	p
Screening values	Soil	1.10^{1} (2.10^{0} to 2.10^{2})	q	1.10^{5}	4.10^{2}	r	1.10^{3} – 2.10^{3}	1.10^{1} – 2.10^{13}	t
	(fresh)water	$\mathbf{1.10^{1}}$ (2.10^{0} to $\mathbf{2.10^{2}}$)	q	3.10^{-1} – 5.10^{-1}	$\mathbf{10^{-3}}$ – $\mathbf{10^{-1}}$	s	8.10^{-2} – 3.10^{-1}	$\mathbf{7.10^{-4}}$ – $\mathbf{2.10^{-3}}$	t

[a] calculated total weighted absorbed dose rates to small mammals lungs in radon-rich soils (Macdonald and Laverock, 1998)
[b] calculated total weighted whole-body absorbed dose rates (Beresford et al., 2008)
[c] calculated total weighted whole-body absorbed dose rates (Brown et al., 2004)
[d] Ribera et al. (1996)
[e] Ragnarsdottir and Charlet (2000)
[f] concentrations measured in the vicinity of uranium mines in Kazakhstan (Uralbekov et al., 2011)
[g] regional (European) background (EC, 2007)
[h] baseline concentrations in European coastal waters (Santos-Echeandía et al., 2012)
[i] calculated total weighted whole-body absorbed dose rates to soil invertebrates (nematodes)) in some areas of the Chernobyl Exclusion Zone 25-year after the accident (Lecomte-Pradines et al., 2014)
[j] estimated dose rates during the early phase (1986) and long term (2008) in some areas of the Chernobyl Exclusion Zone (Geras'kin et al., 2008)
[k] early phase maximum total whole-body dose rate calculated for marine fish 5 months after the Fukushima accident (2011) (Vives i Batlle et al., 2014)
[l] early phase (1957) and long term (1992) maximum total whole-body dose rate calculated for aquatic organisms in lakes of the southern Uralsafter the Mayak accident (Kryshev et al., 1996)
[m] Carvalho et al. (2007), Gongalsky (2006), Lottermoser et al. (2005)
[n] concentrations measured in the vicinity of uranium mines (Ragnarsdottir et Charlet, 2000 ; Uralbekov et al., 2011)
[o] soil concentration in the vicinity of the former lead Metaleurop Nord smelter (Bernard et al., 2010)
[p] predicted environmental concentrations downstream NiCd battry recycler or Cd producing/processing sites (EC, 2007)
[q] generic (and organism group-specific) predicted no-effect dose rate (Andersson et al.; 2009)
[r] ecotoxicity thresholds for uranium (Sheppard et al., 2005)
[s] interim EQS in France (MEDE, 2007) and EQS in The Netherlands (RIVM, 2014)
[t] Predicted No Effect Concentrations depending on water hardness and pH (SCA, 2011)

3 Results and discussion

3.1 *Synthesis of the binary mixture toxicity data obtained within the STAR project*

This work showed that the joint effects of radioactive and stable substances could be predicted in a robust way from single substance toxicity data, according to CA or IA concepts. For all cases tested, considering only the effect of one of the toxicants was not sufficient to explain the observed effects and led to an underestimation of the effects compared to CA/IA predictions. It is therefore concluded that a joint mixture effect was present in all tested cases. As such, for all species and tested conditions CA and/or IA gave a significant better fit of the data often explaining 68 up to 94% of the variation in the data. This indicates that the conceptual models CA/IA worked well for the data sets. However, several deviations (synergistic or antagonistic) were highlighted in in regard to the addition assumption. Some of the data showed interactions at different levels that may result in deviation of mixture effects from the reference model predictions. Those interactions were further assessed for the two tested binary mixtures.

The results are represented below in Figure 1 (UxCd mixture) and Figure 2 (γxCd mixture). These figures show the typical levels of exposure and screening values (bold numbers in Table 2) and the observed interactions for each tested species (from antagonism in the green zones, to synergism in the red zones), in comparison to the IA predictions.

- **Uranium x Cadmium joint effects**

Despite the different toxic potency of both U and Cd between species (U/Cd EC_{50} ratios were 1, 83 and 623, respectively for *L. minor* 7-days growth inhibition, *C. elegans* 11-days reproduction and *S. salar* 3-days Parr survival), an overall antagonism between U and Cd was identified for all tested organism and almost all toxicity endpoints (Figure 1). The explored exposure ranges were driven by the sensitivity of each species and experimental conditions. For example, fish species are known to be very sensitive to Cd, and on the other hand *C. elegans* is a more robust organism and was exposed in solid media (thus, less bioavailability of the metals could be expected). Selection of concentration ranges included concentration at the higher exposure levels that were sufficient to elicit a response in measure endpoints sufficient to support concentration response modelling.

The overall antagonism could be attributed to a protective U effect, especially for species where U/Cd ratios are in favour of competitive effects for *e.g.* binding to a biotic ligand. Hence, this antagonism was further explained by interactions in the exposure media, and/or interactions for bioavailability and bioaccumulation of the metals. To approach possible competition between U and Cd considerable effort was made to develop a biotic ligand model (BLM) for uranyl species for *S. salar, D. magna* and *L. minor* including modelling the effect of Cd along on U chemistry. Generally it was shown that U toxicity and its relationship to U chemistry are more complex than are typically seen for non-radionuclide metals such as copper or cadmium. Despite this complexity the developed BLM models generally described the UxCd mixture effects fairly well, suggesting that accounting for competition between uranium and metallic co-contaminants using a BLM-type approach has considerable promise. For both *S. salar* and *L. minor* mixture effects were also evaluated on internal concentrations of the metals. For both species a clear toxicokinetic interaction of U on Cd uptake was demonstrated in this way. However, toxicodynamic processes are also important for both species exposed to UxCd as antagonistic interactions compared to the reference models are still present when data are expressed on internal concentrations (for details see D N° 4.3, Gilbin et al. 2015).

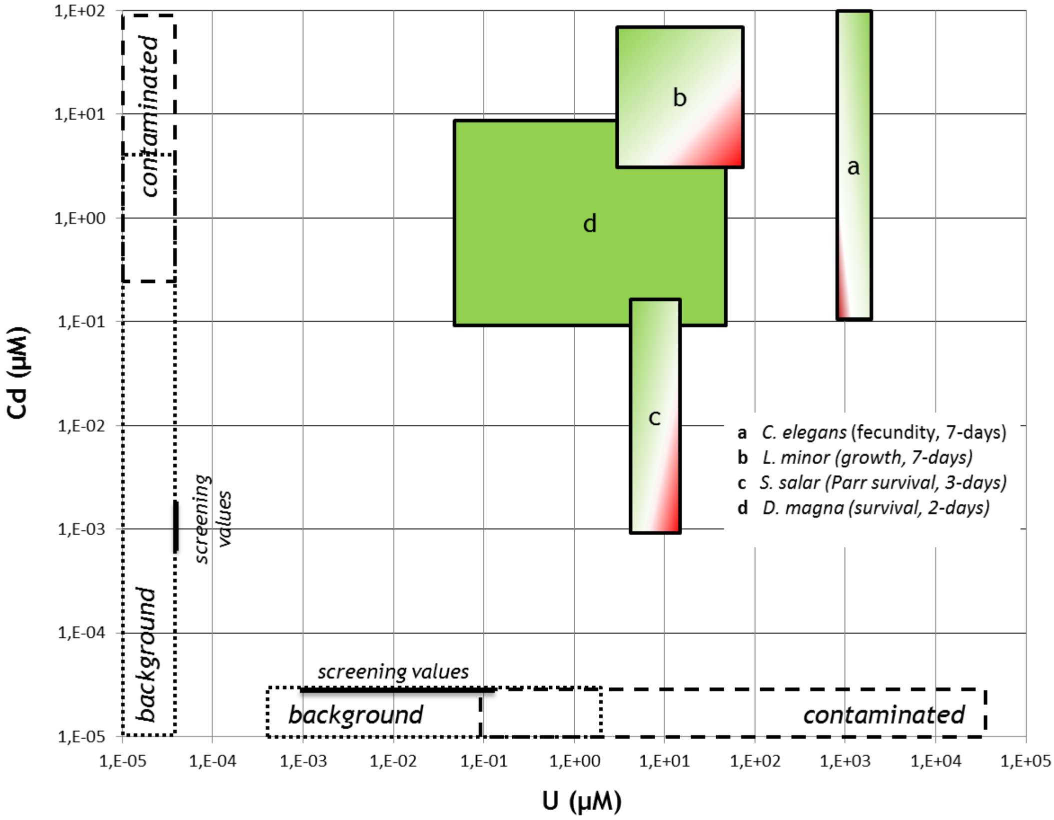

Figure 1: Synthesis of the observed joint effect of uranium and cadmium (expressed on nominal concentrations) and the identified interactions for the tested species (green=antagonism / red=synergism).

- **Ionizing gamma radiation x Cadmium joint effects**

The combined effects of gamma irradiation and cadmium showed mostly an additive or antagonistic interaction pattern among species, although less clear than the UxCd case (Figure 2). Interaction were further shown to depend on the endpoint tested for *D. magna.* For both acute immobilisation and growth antagonistic interactions compared to the reference model were present whereas for carbon incorporation antagonism was dominating at lower doses whereas synergism was present at higher doses. For *L. minor* growth inhibition the interaction switched to synergism at very high gamma dose rates but also, for the lower dose rates of gamma combined with the higher Cd concentrations. The high effect level synergistic interaction is unlikely to occur in the environment, while the synergy seen at combinations of lower Gamma (~30mGy/h) and middle range Cd (10-20 µM) may realistically be observed during accidental releases.

On the other hand, *S. salar* and *C. elegans* exposure to gamma irradiation and Cd mixture did not reveal any obvious synergistic or antagonistic effects. Those contrasted patterns may be the result of complex toxicodynamic interactions.

The synergistic areas observed at low irradiation effect dose rates like for *L. minor* (lower Gamma (~30mGy/h) and middle range Cd (10-20 µM)) may be the result of a potentiation of

Cd toxicity at non-toxic irradiation exposures. Inversely, at higher irradiation exposures, antagonisms may be due to oxidative stress compensations.

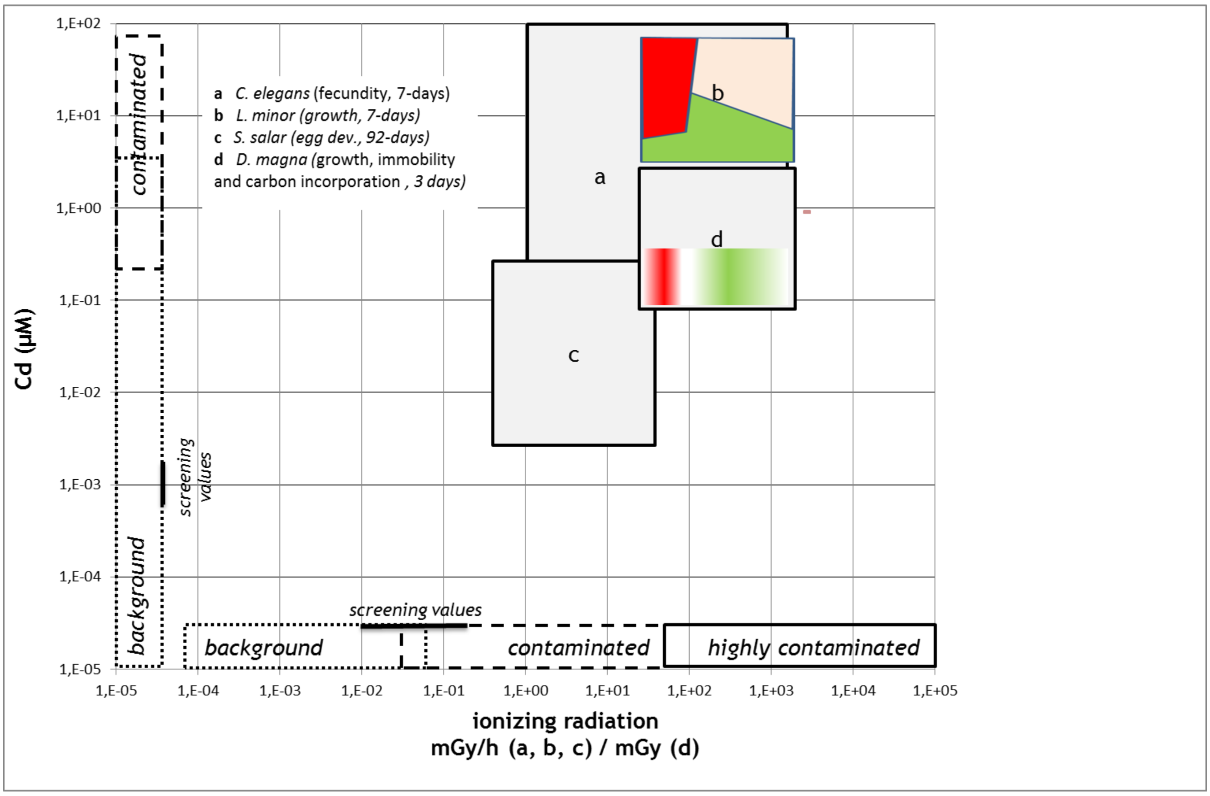

Figure 2: Synthesis of the observed joint effect of uranium and cadmium and the identified interactions for the tested species (grey follows IA/green=antagonistic compared to IA/ red=synergistic compared to IA)

3.2 *Implication for a cumulative risk assessment and future mixed exposure research*

Applying a common approach on five different organisms to study possible mixture effects between UxCd and γxCd resulted in the generation of an important dataset of new high-quality data which are available for others for additional analysis upon request. The successful integration among several laboratories was essential, since mixture effects studies are very demanding in time and require a multidisciplinary approach, the joint effort of experimentalists and modellers and shared infrastructure (chemistry, (molecular)biology, geochemical modelling, effects assessment models, irradiation facilities and facilities to work with radioactivity, …).

The results obtained form an interactive and integrative basis for future studies. They open the possibility to link and validate Cumulative Risk Assessment (CRA) predictions with in situ observed toxic effects under a multi-contamination context that includes radiation/radionuclides.

From a risk assessment perspective, our work confirms that for sites containing mixtures of pollutants including radionuclides, regulation on a single stressor basis (i.e. assuming only a single chemical alone present) may underestimate the ecosystem effects following multiple stressor exposures. Using those consensual concepts of CA and IA, the developments of an Ecological Risk Assessment framework for mixtures including radionuclides will remain consistent with the general ERA framework. However, integration in regulation is still needed.

Some remarks need to be taken into account. First, the most important thing to ascertain is whether there are potential interactive effects (especially synergistic), whereas CA and IA models are under the assumption of zero interactions. From the interactive effects observed most were antagonistic and not synergistic. For the UxCd tests a slight synergism around Cd screening value was observed for *S. salar*. On the other hand, a synergistic zone was identified at low Cd toxicity for nematode and plants. Despite its low amplitude, these synergistic areas can potentially question the robustness of a cumulative risk assessment. One should note however that these synergistic deviations were determined on the basis of IA additivity, which generally tends to predict less toxicity than CA. In a risk assessment perspective, it could be considered that the use of CA would be protective enough for a robust prediction of the joint effect of U and Cd. Secondly, for both UxCd and γxCd mixtures set ups the concentration/dose rate range tested was driven by the sensitivity of each species to the contaminant and the studied endpoints. Hence, these ranges are representative of highly contaminated areas (e.g. early post-accidental situations such as Chernobyl). The question remains about the extrapolation of the conclusion of those data at lower concentration levels or dose-rates. It has to be noted that if in a mixture levels of all contaminants are below levels inducing an effect the reference model IA will not work as it will predict no effect of the mixture as well, whereas for CA might be valid. Therefore the range of concentrations/doses chosen here was on the higher side to ensure some effects to be obtained on umbrella endpoints like growth. Further work on the same organisms could include more sensitive endpoints that will allow testing at lower, environmental relevant concentrations/doses. Finally interactions may remain at higher levels of organization (trophic/population) and long term exposures that were not address in the performed experiments.

4 Conclusions and way forward

Trends in UxCd and γxCd mixture effects were generally well described for very different organisms (plants, invertebrates, vertebrates) using general reference models of CA and IA. Hence it is shown that for the scenarios tested and based on the presently available data, it the observed effects could be predicted using CA/IA or deviations thereof. In all cases, taking account of the combined effects of the multiple radionuclide/radiation and other chemical stressor present provided a better prediction of observed hazard than considering one of the single stressor in isolation. Our data also demonstrated deviations from the CA/IA concepts possibly coming from interactions in the media for environmental availability; interactions at site of uptake and toxicokinetics or toxicodynamic interactions This indicates a requirement to view predictions from CA/IA models as central estimates of joint effects which have associated uncertainty for combination of radionuclides and chemicals for which specific relevant data is not available. In case where interactions are observed, then a need for mechanistic understanding of interactions at different process levels (interactions in the exposure media, interactions at uptake sites, toxicokinetic, or toxicodynamic interactions) is needed.

Using consensual concepts like CA and IA for the developments of an Ecological Risk Assessment framework for mixtures including radionuclides will remain consistent with the general ERA framework. The robustness of CRA (based on additivity) depends on the potential interactive effects between radionuclides and other stressors (especially if synergistic). In support to the refinement of Risk Assessment both quantifying the interaction amplitude and identifying their origins are required for the development of alternative mechanistic models (*e.g.* PBTK and dynamic models).

The number of scenarios, test organisms and mixture combinations, the end- and time points that could be tested in the frame of this project was limited and conclusions should be confirmed by additional experiments. In the future, the presence of synergisms would have to be investigated at lower realistic radiations levels and realistic concentrations of stable chemicals, for more sensible endpoints and time points and considering indirect effects (population, communities, etc.). For future mixture studies it would be useful to provide 'case studies' of mixture scenario's relevant to CRA, e.g., at concentrations measured at contaminated sites such as the STAR Observatory sites (NORM sites, Chernobyl, Fukushima...). Field validation of these approaches would help in the integration of such CRA approaches in future regulations.

Future mixture toxicity studies could also benefit from assessment of dynamic and biology based methods (eg. DEBtox, gene expression pathways) and, hence be more directed towards mechanistic understanding. For UxCd it was shown that BLM models have great promise in dealing with mixtures where radionuclides compete with other stressors for membrane binding sites. The developed model can now be used directly to predict the bioavailability of U by knowledge of level of influencing key water parameters and Cd. In order to expand the usefulness of this approach to CRA it still needs to be developed for chronical endpoints (salmon and daphnia BLM were fitted based on acute experiments), to be tested for case studies, and to be developed for other radionuclides with molar exposure ranges much lower than uranium. The validity of BLMs at very low concentration ranges would, however, be an additional challenge as BLMs have up to know been used mainly for toxic metals that are present at higher concentrations.

5 Acknowledgments

The questions raised by this work were debated during the final dissemination event, held in Aix-en-Provence (France), 9-11 June 2015. The authors would like to thank the panel of experts (Geert Biermans, Ronny Blust, Jan Baas Dave Spurgeon and Steve Lofts) as well as the whole audience who participated to this debate. The STAR project External Advisory Board members are also thanked for their contribution all along the project and the final

dissemination event (Rick Jones, Dick Roelofs, Nina Cedergreen, Mikhail Balonov, Satoshi Yoshida and Valery Forbes).

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