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Integrated Research and Experimental Plan under STAR-WP4

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Table of Contents

1 Introduction

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 are 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 radiological assessments.
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.
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.
4. Identify appropriate tools for Ecological Risk Assessments (ERA) of mixtures containing radionuclides, assess the degree of conservatisms and apply selected ERA methods to a limited number of case studies.

Deliverable 4.1 (Critical review of existing approaches, methods and tools for mixed contaminant exposure, effect and risk assessment in ecotoxicology and evaluation of their usefulness for radioecology, Vandenhove et al., 2012) built on the outcomes of programmes and reviews dealing with multiple stressor issues in ecotoxicology. The aim of Deliverable 4.1. was to give an overview of mixed contaminant approaches and to critically evaluate the usefulness and applicability of these approaches for mixed contaminant conditions that include ionising radiation or radionuclides as one of the contaminants. Approaches and tools were discussed focussing on (i) the impact of co-contaminants on the environmental availability and uptake, (ii) the assessment of effects and possible interactions between contaminants (iii) the risk assessment framework and methods.

We intend to establish an experimental plan, using (adapted) approaches applied in ecotoxicology, to evaluate whether radiation protection criteria for wildlife need to consider contaminant mixtures by first determining if interactions exist among several plausible binary mixtures including radionuclides or radiation. If no interaction occur then consideration of mixtures in an additive way may be considered acceptable (though it should be recognized that it may be difficult to make firm conclusions based on a low number of experiments). If, however, significant interactions do exist, then additional consideration may be required when evaluating protection criteria for radiation in the presence of other contamination.

Interactions between pollutants in a mixture may occur in four ways: (1) influencing each other's speciation and mobility in the environmental media and hence, each other's availability to organisms; (2) blocking or enhancing each other's uptake into the organism (toxicokinetic-adsorption interactions); (3) once inside the organism, blocking or enhancing each other's detoxification (toxicokinetic-metabolism interactions); (4) altering the nature of their toxic actions, and/or impacting on repair capacities (toxicodynamic interactions). Understanding the exposure and effects of chemical mixtures in real ecosystems requires knowledge of how biological and non-biological parameters may affect interactions for all of the four interaction types listed above.

Within the initial proposal under WP4, experiments were suggested in two domains:

1. 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.
2. 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.

For each of these topics, it was proposed to conduct preliminary tool testing or experiments, but it was decided that the final decision on the experimental set-up would depend on the outcome of the review conducted under Deliverable 4.1. (Vandenhove et al., 2012) and consultation workshops with experts.

Some of the conclusions of the WP4 May 2011 workshop were that (i) contrary to what was initially proposed, we should consider binary mixtures instead of ternary mixtures, (ii) mixtures should be representative of existing or planned situations and (iii) the selected co-contaminants should include those with similar and dissimilar modes of action compared to radiation. We then also decided that some effort should be dedicated to ecological risk assessment in a multiple contaminant context which included radionuclides or radiation.

In their recommendations the External Advisory Board (EAB) in turn suggested that the focus should be on a few exemplary binary mixtures, chosen on the basis of modes of action (for the choice of binary mixtures we refer to section 3.3). The decision was based on the observation that ternary interaction effects are seldom larger than those of binary mixtures (Svendson and Cedergreen, submitted). Using information on mechanisms/mode of action, the EAB considered it possible to create mixtures that are most likely to result in synergistic, antagonistic, or additive effects. The EAB recommended approaches to understand the mechanisms underlying the interactive effects. They also felt that STAR should contact experts within mechanistic toxicology to determine the potential range of functional tools that can be used to address the hypotheses proposed and whether such an approach will adequately address the questions proposed and contribute added value (see 3.4.4). They also strongly favoured hypothesis driven research.

Based on an additional STAR workshop (November 2011) and a STAR-expert consultation workshop (January 2012), a final research and experimental plan was established with the aim to evaluate if and how co-contaminants may affect environmental availability, bioavailability and effects induced by radionuclides and if existing risk assessment approaches for mixtures are adaptable to situations where radionuclides are among the mix to give an answer to the objectives 2 to 4 above.

Through the proposed work plan we also endeavoured to favour integration between the STAR partners involved by selecting similar experimental approaches and conditions, applying uniform data treatment, by choosing for knowledge and expertise exchange and exchange of scientists.

The proposed approach for hypothesis driven experiments and risk assessment under WP4 is presented in the following sections.

2. Testing the feasibility and applying existing approaches and tools for radionuclide (bio)availability assessment under mixed contaminant conditions (Task leaders: NERC – UMB)

2.1 Environmental availability under mixed contaminant conditions

We wanted to evaluate whether co-contaminants would impact the speciation and hence environmental availability of the radionuclides of interest using the selected geochemical speciation models. Levels of co-contaminants present in the environment are high for the uranium mining and milling industry and we selected two case studies for which we had relevant and adequate information on environmental characteristics to perform geochemical speciation calculations. The two case studies were also rather different in chemical composition: the French Ritord scenario and the Beaverlodge Lake in Canada. The Ritord scenario has high levels of Mn and Ba, and levels of Fe and Al in monitored surface water values were above French guidelines values. Therefore, Fe and Al could also be considered as contaminants. The Ritord ecosystem is slightly acidic. For the Beaverlodge lake case there are high heavy metal concentrations (As, Ba, Cu, Ni, Pb, Se, Zn) in an ecosystem which is alkaline and has a high carbonate content (providing a contrast to Ritord).

Preliminary assessments using geochemical speciation models presented in Deliverable 4.1 (Vandenhove et al., 2012) did not show a significant effect of these co-contaminants on the speciation of selected radionuclides (U, Th, Po, Ra, Pb). These tests were extensive, involving four different geochemical speciation tools and, in total, six geochemical databases. We therefore concluded that there was comparatively little additional benefit in continuing to extend this element of the programme. Leo Posthuma (personal communication) also found for metal mixtures in soils that extractable amounts of compound A were hardly affected by the presence of compound B, i.e. hardly any evidence for environmental-chemical interactions upon equilibrium concentrations in various fractions (like $CaCl_2$-extractable fractions). We therefore decided to collate data for two additional example cases for U-mining sites and report the outcomes of the entire study in a peer reviewed journal article.

2.1.1 Availability 1. Paper project – Influence of co-contaminants on the speciation of natural radionuclides at uranium mining sites

We concluded that the finding that co-contaminants do not have a significant effect on the speciation of radionuclides (RNs) in the example studied, is scientifically interesting and relevant to communicate to the scientific community. For elaborating and populating this paper, information will be collated from 2 (3) additional U mining sites (Australia and Germany; Central Asia: a JER Special issue devoted to Central Asian U mining legacy sites will be published in 2012, and speciation data related to RN and metals can be provided by UMB).These data will be compared with the speciation modeling data and enhance the evidence by additional analysis and interpretation.

Partners involved

NERC (lead), IRSN, SCK•CEN, UMB

Timing

Paper submitted: December 2012

Availability 2. Preliminary characterisation and environmental availability assessment at Observatory Site(s)

At the Observatory Site(s) (still to be identified and selected) we intend to carry out a detailed site characterisation at one well-defined location. This characterisation would include analysis of (i) radionuclides and co-contaminants (heavy metals), (ii) solid (soil and/or sediment) characteristics including texture, mineralogy, organic matter content, oxides/hydroxides of Al, Fe, Mn, exchangeable cations, CEC, Eh, pH, (iii) liquid compartment characteristics including geochemically active pool (i.e. pore water composition), surface water composition, *in situ* fractionation of water and water quality. Where relevant, the analyses will be supplemented with solid phases sequential extractions for evaluating potential availability. Environmental availability of the radionuclides will be assessed by both experimental measurements and geochemical speciation simulations.

The observatory sites should be identified by November 2012 (after the Berlin meeting of June 2012), there are no candidates yet, but multiple sites can be included. It was agreed that at least one of the sites should be a site where uranium is present (due to e.g. U-mining or NORM-industry) so it fits within WP4. At least one observatory site needs to have measurable uranium and heavy metal concentrations in order to fulfill the requirements for *Availability 2.*

Sampling strategy (how and how many samples at the observatory sites are best taken) and associated analyses will depend on the site selected. It was agreed that care would be taken so that sampling and associated analysis results would feed many goals.

Which variables to be monitored is to be carefully discussed. NERC will e.g. give recommendations for which parameters to measure to enable the geochemical speciation modeling for the observatory site. By carefully selecting the sites and afterwards the sampling/testing design, the data can also be used for the validation of the Biotic Ligand Model (BLM) (*Availability 3*) and to apply the tiered approach we will develop in *Risk 1* on real data (*Risk 2).*

Partners involved

STUK, BfS and UMB for site characterisation.

NERC, IRSN, SCK•CEN for application of the geochemical speciation models

Timing

When the sites are selected, a sampling plan will be established (with input from NERC) and the sampling/analyses will start (presumably July 2013). The study is expected to last for 1 year, till July 2014.

2.2 Bio-availability (uptake) under mixed contaminant conditions

In ecotoxicology, models have been developed for assessing metal bioavailability, some of which have been tested for mixtures of metals. For example, the BLM (Biotic Ligand Model) has been proposed as a tool to quantitatively predict the manner in which water chemistry affects the speciation and the biological availability of cationic metals in aquatic systems. Chen et al. (2010) evaluated the validity of the BLM for bioavailability assessment of Pb and Cu present as a metal mixture. Biotic Ligand Models for mixtures are in general rare and do not include radionuclides.

The review (D4.1 Vandenhove et al., 2012) revealed that currently there are no well-developed models for assessment of the impact of co-contaminants on the toxicity of radionuclides. Therefore we decided to conduct experiments to assess the effect of a contaminant on the bioavailability and toxicity of a cationic

uranic radionuclide (U(VI), the dominant oxidation state of U in oxic media) under different physical-chemical conditions.

Within this task, we hope to show that the combination of geochemical speciation models with BLMs is a valuable tool for assessing the influence of varying environmental conditions and mixed contaminant conditions on U speciation, bioavailability and toxicity.

Elaboration of a Biotic Ligand Model is a complex task and therefore we decided to limit our effort to one radionuclide, uranium.

2.2.1 Hypotheses

The hypotheses formulated were:

H1: The bioavailability of U(VI) on organisms will exhibit statistically significant variations in response to the exposure medium used.

H2: The variation in U(VI) bioavailability can be described by organism-specific Biotic Ligand Models (BLMs) that take into account the chemical speciation of U(VI) and competition of UO_2^{2+} with major cations.

H3: Cationic trace metal co-contaminants will influence the bioavailability of U. The magnitude of this influence will be consistent with a BLM-based description of uptake competition.

2.2.2 Experiments proposed: Availability 3: Development of a Uranium Biotic Ligand Model for aquatic organisms under mixed contaminant conditions

We decided to develop a BLM model for U and study the influence of a trace metal (Cd) on the model parameters. The choice of U and Cd is partially driven by the presence of high concentrations of these elements at a number of sites (so it is realistic), partially by the available body of data and expertise on U uptake, including on how its uptake is influenced by environmental features. Moreover, there is adequate information in geochemical databases on the behaviour of a number of different species of U, although information on colloidal species is scarce. BLM for Cd are existing (e.g. Fatheard minnow, Rainbow trout and *Ceriodaphnia dubia* are already implemented within the BLM v2.2.3 tool proposed by http://www.hydroqual.com/). Section 3.3 presents additional justification for the selection of U and Cd.

The BLM-experiments will be conducted with three organisms: a plant, an invertebrate and a vertebrate – *Lemna minor, Daphnia magna* and Atlantic salmon (*Salmo salar)* [For Atlantic Salmon, the juvenile stage (parr) will be used to obtain U-BLM information, as the experiments can then be based on standardised acute exposure protocols (48-96 hrs. of exposure), and accumulation in different organs (i.e., gills, liver and kidney) can be followed.]

For each organism selected adequate concentration response relationship for U will be derived in the relevant reference test media. Once the U-dose response curve is known for control conditions and considering the growth requirements of the organism of interest, an experimental plan will be proposed and discussed together with NERC. A bioavailability model will be developed (if possible similar to a biotic ligand model) and the effect of Cd on U-speciation, bioavailability and toxicity will be assessed. Geochemical speciation modelling will be used to predict speciation under all experimental conditions, and compared with experimental data.

U toxicity test will be repeated in the presence of potential competing ions (including pH (H^+), Ca^{2+}, Mg^{2+}, Na^+, K^+) (addressing H1). Phosphate species ($H_x(PO_4)_y^{-z}$) are known to affect environmental availability of U, at least for plants, and effect of P on U uptake will be tested for plants. It was decided to select 5 pHs, and

mes for Na, Mg, Ca and K (and for plants also P). We will first evaluate effects of extremes (e.g. [illegible] and low Mg) and then look in more detail if the ion has an important influence. If so, more experiments will be set up to evaluate the influence on U uptake and toxicity induced by that respective ion.

The U uptake and toxicity tests in the presence of these ions, will make it possible to derive parameter values that account for the effects of each competing ion on U uptake and toxicity in a BLM and this for the 3 test organisms (addressing H2). The complexity of U may complicate BLM development: BLM is generally developed for a specific elemental species (e.g. the free ion form). However, for U many physico-chemical forms exist depending on the source and environmental conditions and uptake by organisms is not solely of a single U species. Furthermore, different organisms may have a different affinity for uptake of different U-species.

In the next phase, we will evaluate how Cd affects the parameters of the U BLM and hence affect uptake and toxicity (linked with H3). First a literature search to find more data on the existing BLMs for the selected species and for the co-contaminant of interest (Cd) will be performed. A likely experimental set-up to test the effects of Cd, the U concentration and medium conditions will be kept constant while the concentration of Cd is varied

In order to validate our experimental findings, it was agreed to use the surface water from the observatory site and evaluate the effects observed on test organisms and see if the BLM can predict the observed accumulation and toxicity.

Partners involved

Experiments: UMB/NRPA (*S. salar*), BfS and STUK (*L. minor*) and IRSN (*D. magna*); NERC assistance within experimental design; SCK•CEN knowledge transfer *Lemna* and guest lab. (STUK and BfS will carry out experiments at SCK•CEN). STUK/BfS will take care of all U-analysis of the *Lemna* BLM experiment for the parts of the experiments they are involved in. Possibly STUK will carry out experiment at STUK facilities after training at and knowledge transfer by SCK•CEN.

Development of BLM for U (in presence of co-contaminants): NERC + UMB/NRPA (BLM on *S. salar*) + BfS, STUK and SCK•CEN (BLM on *L. minor*) + IRSN (BLM on *D. magna*)).

U-speciation calculations: NERC, SCK•CEN, IRSN (a common model used by all partners still needs to be selected)

Tests at Observatory sites: same task distribution as for the tests with artificial medium

Dosimetry/dose distribution, U analysis: NRPA

Timing

Perform literature review on BLM for Cd for selected test organisms and growth condition range for the test organisms – September 2012.

U-dose-response curve for organisms of interest in selected reference medium – September 2012

Course on BLM-experimental set-up and modelling: October 2012 (organised by NERC)

Establishment of experimental plan for first series of experiments for each test organism – December 2012

Start of BLM experiments: Jan 2013

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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 leaders: IRSN – SCK•CEN)

3.1 General comments

Set ups to enable the application of CA and IA models to the effects data under mixture conditions were preferred for the majority of the experiments. Since the DEBtox model is experimentally highly demanding, it was suggested to use a limited experimental set up.

In the initial proposal we provisionally planned to perform 'Something from Nothing' experiments (better formulated as 'Something from something very small'). Though perhaps very relevant from a regulatory or risk assessor perspective, given the workload and resources within this WP4, and following the suggestion of the EAB to limit the scope of our research, we decided not to carry out these experiments.

3.2 Hypotheses

For the effects assessment, the following hypotheses were that:

H1: The effects of chemical contaminants and ionising radiation are additive.

H2: If there are interactions between the effects induced by ionising radiation and chemical contaminants, these interactions will be independent of test organism.

We are going to test our hypothesis with 2 different methods: classical ecotox testing using CA/IA on fixed endpoint dose-response curves (CA/IA) (see Section 3.4) and Dynamic Energy Budget modelling for Toxicity testing (see Section 3.5).

3.3 Choice of binary mixture

The following binary mixtures were selected for effects directed research:

- External gamma irradiation + Cd
- External gamma irradiation + fluoranthene
- U(VI) + Cd
- U(VI) + fluoranthene.

Mixtures were selected based on the likelihood of occurrence and/or expected mode of action of the different mixture components. Below we describe in more detail the reasons for the selection.

After agreement obtained at the January 2012 meeting where the global experimental set-up was discussed, some organisations insisted to perform also U+gamma and Cd+fluoranthene experiments. At the moment of writing, this is not taken up as obligatory to all groups participating to the effects experiments.

3.3.1 Gamma

General occurrence: All organisms are continuously exposed to gamma radiation, ranging from background levels of fractions of µGy/h to ten thousands µGy/h in accidental scenarios.

Mode of action: Gamma radiation has a short wavelength and consequently a high energy giving it a high penetration capacity in different tissues as well as the ability to cause ionizations and excitations. Most radiobiological studies use ecologically irrelevant high doses of gamma radiation, but the effect of low or chronic dose rates is of interest here. An overview of the effects induced by low-level chronic gamma radiation in plants and animals has been compiled by Real et al. (2004). More recent studies not included in the review are also available: Gilbin et al. (2008); Vandenhove et al. (2009); Pereira et al. (2011); Simon et al. (2011). Biological effects induced by radiation in organism originate from the deposition of energy from the radioactive material to biomolecules (DNA, proteins, etc.). Ionising radiation can be genotoxic as it interacts with DNA either directly, by deposition of energy in the DNA molecule, or indirectly by formation of free radicals that via recombination produce reactive oxygen species (ROS) leading to excitations and ionisations. More details are available in section 4.1.2. of Deliverable 4.1 (Vandenhove et al., 2012).

Basis for the selection of gamma radiation for mixture effects experiments: Since we are dealing with the effect of co-contaminants in a radiation context, the selection of a gamma emitter is logical. External gamma is the most controllable radiation type, it can be applied continuously and homogenously for the duration of the experiment and the exposure does not include confounding factors associated with uptake and inhomogeneous dose distribution. Moreover it is easy to quantify. Within STAR, gamma exposure platforms are available at UMB (^{60}Co), IRSN (^{137}Cs) and at SCK•CEN (^{137}Cs and ^{60}Co).

3.3.2 Uranium

General occurrence: Uranium is a radioactive metal that is generally present in low amounts in rocks, soil, water, plants, and animals, except in geologically anomalous areas (U mining areas). Uranium and its decay products contribute to the natural background radiation levels in the environment. It occurs in numerous minerals such as pitchblende, uraninite, carnotite and autunite, and phosphate rock, lignite and monazite sands from which it can be mined commercially. Natural uranium occurs as three radioactive isotopes, ^{238}U, ^{235}U and ^{234}U, with respective abundance of 99.27%, 0.72% and 0.0055% and half-lives of 4.47 x 10^9, 7.04 x 10^8 and 2.46 x 10^5 years respectively. Given its long half-life, uranium is more chemotoxic than radiotoxic. Exploitation of uranium bearing materials has resulted in highly contaminated waste streams which need to be adequately managed and controlled. These waste streams usually contain both U-decay products, ^{232}Th decay series radionuclides, arsenic and a range of heavy metals.

Mode of Action: There is a greater risk of chemical toxicity than of radiological toxicity when the physical decay half-life of an isotope is very long as occurs for ^{238}U with a half-life of 4.47 10^6 years. Indeed, the relative importance of these two toxicities was shown to differ among the various U isotopic compositions (i.e. natural, enriched, depleted – Matthews et al., 2009).Therefore natural U needs to be enriched with ^{235}U to present a radiotoxicological problem (Ribera et al., 1996). Chemical toxicity is particularly significant in compounds containing natural U. Mechanisms of U toxicity have been predominantly studied on man and some animal species (Ribera et al., 1996). Chemical toxicity of U is predominantly caused by the aqueous hexavalent uranyl ion, UO_2^{2+}. The biochemical reactivity of UO_2^{2+} is linked with it seeking oxygen binding centres. Hence, it resembles Ca^{2+} and Mg^{2+}, but complexes of higher stability are formed with U. Ca^{2+} stabilizes cell membranes (Nieboer et al., 1979) and since UO_2^{2+} binds more strongly than Ca^{2+} to Ca-binding sites, it has the potential to increase the K^+ permeability by inflicting structural changes in cell membranes (Boileau et al., 1985). Replacement of Ca from critical enzymes or Mg from phosphorylated compounds appears to explain why U can cause enzyme inactivation (Nieboer et al., 1980). Mg^{2+} plays a major role in living organisms because it stabilizes the macromolecules RNA and DNA including those involved in ATP and chlorophyll synthesis. Exchange by the uranyl cation may affect these molecules. The uranyl ion may induce cell surface inhibition by interaction with membrane proteins. These postulates are reasonable since

UO_2^{2+} is known to form strong complexes with carboxylic groups of glutamate and aspartate residues in proteins and occasionally with the hydroxide side chains of threonine and serine (Blundell and Jenkins, 1977). U toxicity can also be caused by changes in membrane permeability due to binding of uranyl ions to phosphate ligands (see Riethmuller et al., 2000 and references therein). UO_2^{2+} has a strong affinity for phosphate moieties and sugar alcohol groups of nucleotides and polynucleotides and this may explain DNA damage observed after U exposure. Reviews of ecotoxicology thresholds for uranium have shown that for aquatic vertebrates, Ca^{2+}, Mg^{2+} (through competition) and carbonate content (through complexation) all have a significant impact on U toxicity, with water hardness (as $CaCO_3$) being a dominant factor (Environment Canada, 2003).

In plants, U has been shown to induce lipid peroxidation and oxidative stress responses (Vanhoudt et al., 2008; Vanhoudt et al., 2011a, 2011b). Uranium was also shown to be genotoxic on fish embryonic cells (Pereira et al., 2012), at least partly through disturbing the repair rate of Double Strand Breaks (DSB). In adult zebrafish, hepatic antioxidant defences, red blood cells, DNA integrity and brain acetylcholinesterase activity were found to be significantly altered (Barillet et al., 2011), as well as the mitochondrial function in brain and skeletal muscles (Lerebours et al., 2010). Experiments also showed that uranium exposure induced a variety of histological impairments in fish (e.g. gill disruption; muscle and gonadal tissue alterations) (Barillet et al., 2010).

Basis for the selection of U for mixture effects experiments: U(VI) is predominantly an alpha-emitter, but is more chemotoxic than radiotoxic. Its daughters need to be considered for the dose calculations so it has a complex dosimetry. Selection of U will be unlikely to enable testing of the robustness for radiation protection criteria. It is also a complex element with many species of which a number form precipitates (e.g. U-P). However, it is a very ecologically relevant radionuclide. It is present in the environment in high concentrations together with a number of co-contaminants. In addition, U is easy to handle so there are limited radiation protection issues and will deliver a full-dose response in realistic concentrations (which is needed for CA/IA analysis, see 3.4.3). Finally, a number of STAR partners already have large experience with U.

3.3.3 Cadmium

Miscellaneous: Cd and Cu were initially shortlisted as the potential heavy metal co-contaminant, because of their occurrence at NORM and U mining and milling sites and their presence in NPP aquatic releases. This was discussed at the January 2012 meeting and there was, a slight preference for Cd over Cu as Cu is also a micronutrient.

General occurrence: Cadmium is a ubiquitous co-contaminant in all NORM contaminated sites (see also Annex 1). Co-occurrence of radiation, U and cadmium is therefore common.

Cadmium is a metal predominantly present as Cd^{2+}. It is ubiquitously present but its natural concentrations are relatively low (Nriagu et al., 1988). However, due to industrial activities such as smelting and refining of non-ferrous metals, fossil fuel combustion and municipal waste incineration its concentration has considerably increased in many locations (Valko et al., 2005).

Mode of Action: In both plants and animals cadmium induces DNA damage and oxidative stress. This leads to various DNA base modifications, enhanced lipid peroxidation and altered calcium and sulfhydryl homeostasis. It is not a redox active compound and is therefore unable to directly induce the formation of free radicals. However, it induces reactive oxygen species in many tissues (Cuypers et al., 2010 and references therein), probably by inhibition of complexes in the mitochondrial electron transport chain (i.e. complex II and III) (Wang et al., 2004). Cadmium toxicity is further explained by its high affinity for

ydryl groups and its ability to replace the essential ions Ca^{2+} and Mg^{2+}(Verbruggen et al., 2009), thereby inactivates many important proteins such as DNA repair enzymes (Hartwig and Schwerdtle, 2002).

Basis for the selection of Cd for mixture effects experiments: As well as being a ubiquitous metal in the environment, Cd is an important toxicant and its toxicity has been intensively studied (for reviews, see Sharma et al., 2005; Valko et al., 2005; Strydom et al., 2006; Hasan et al., 2009; Verbruggen et al., 2009; Cuypers et al., 2010). Cd has no known biological role in organisms. This is in contrast to many other metals (e.g. Cu) which are biologically essential in trace amounts, thus complicating toxicity studies.

3.3.4 Organic contaminant - fluoranthene

Fluoranthene is a three-ringed Polycyclic Aromatic Hydrocarbon (PAH) with the chemical formula $C_{16}H_{10}$. The molar mass is 202.26 g mol^{-1} and log K_{ow} of 5.33 (i.e. it is lipophilic). The solubility in water at 25 °C is 0.26 mg l^{-1}. Fluoranthene is on the priority list of EPA as one of the 16 EPA PAHs (EPA, 1979).

General occurrence: Natural sources of PAHs and hence of fluoranthene include forest fires, volcanic eruptions, diagenesis and biosynthesis (EC, 1994). PAHs also occur naturally in coal derivatives and petroleum products and are released upon incomplete burning of these fossil fuels (NRCC, 1983 as cited in EC, 1994). Anthropogenic activities including the manufacturing of coal tar, asphalt, tyres and other oil-containing products from petroleum as well as accidental spills of petroleum products, natural seeps and run off from mining sites may have led to persistent higher local concentrations of PAHs.

Mode of action: Polycyclic aromatic hydrocarbons are in general classified as type I narcotic chemicals (Veith and Broderius, 1990, Di Toro and McGrath, 2000). For such compounds the mode of toxic action is non-polar narcosis, which disturbs normal cell functioning through non-specific binding to cell membranes. The binding of the chemicals are reversible, but under continuous exposure the effect on biota is observed as decreased activity, limited reaction to stimuli and eventually death. Non-polar narcosis is also termed “baseline toxicity” as it represents the minimum toxicity that may be caused by a range of chemicals (Van Wezel and Opperhuizen, 1995). Whether fluoranthene may act as a carcinogenic or mutagenic compound is unclear (EPA, Integrated Risk Information System (IRIS) search on fluoranthene CAS 206-44-0), but it does induce oxidative stress (Pathiratne and Hemachandra, 2010)

Not many papers on the toxicity in plants of fluoranthene in plants are available yet. However, it is shown that it clearly interferes with plant hormone metabolism. As such exposure to 5 mg/l of fluoranthene in combination with the plant hormones either indole-3-acetic acid (IAA, 0.1 mg l^{-1}) or a combination of IAA and N6-benzyladenine (BA, both 0.1 mg l^{-1}) caused reduced growth, increased production of ethylene and ethane as well as decreased production of CO_2 in *Pisum sativum* L. cultivated for 21 days (Kummerova et al., 2010). In addition, the level of the endogenous fytohormone abscisic acid (ABA) significantly increased with increasing fluoranthene concentrations in the presence of both IAA and IAA plus BA (Vanova et al., 2009). In addition to interaction with plant hormone metabolism photosynthesis processes of both *P. sativum* and the symbiotic algae in the lichens species *Lasallia pustulata* and *Umbilicaria hirsute* are affected by exposure to fluoranthene (Kummerova et al., 2007; Vanova et al., 2009)

Model predictions (DEBtox) show that exposure to fluoranthene negatively affects the production of somatic and reproductive tissue in *C. elegans*, but ultimately, the final body size of adult specimens were reached (Swain et al., 2010). In another study, treatment with 5 mg/ml fluoranthene was shown to shorten the life spans of ad libitum fed nematodes, and if put under dietary restrictions (DR) the sensitivity to fluoranthene increased (Schleit et al., 2011).

Fluoranthene is, like other PAHs, readily metabolized by fish (Schuler et al., 2007; Hillenweck et al., 2008). In the fathead Minnow (*Pimephales promelas*), the least sensitive stage is the eggs, while the juvenile growth was most severely affected by exposure to fluoranthene (Schuler et al., 2007).

Basis for selecting fluoranthene for the mixture effect experiments: Fluoranthene was one of the [illegible] organic compounds that came out of a survey among the STAR partners. All compounds were compared based on their polarity, solubility, the ease of measuring, their toxicity on a realistic deterministic endpoint, the ease of handling in lab-based experiments and their persistence in the environment. Further the mode of action and the realism of presence together with radiation or radionuclides in the environment were used for discrimination between possible candidates. We refer to Annex 1 for the details of the evaluation. Fluoranthene came out of this physicochemical and toxicity comparison together with another PAH (phentanthrene) as the two possible candidates. As described above the toxicity and mode of action of both PAH compounds and their presence as pollutants in the environment are in general very comparable. The presence of hands-on experience in toxicity studies with fluoranthene within the STAR network (Swain et al., 2010) gave a final decisive argument to choose fluoranthene as the organic compound of choice for the mixture toxicity studies performed in WP4.

3.3.5 Testing for alpha/gamma mixture

The advantage of testing an alpha/gamma mixture would be that this would provide a direct link with STAR's WP5 where single dose response curves for external gamma and ^{241}Am will be established for a number of test organisms defined under WP4. It is generally assumed that effects of the doses from different types of radiation are additive and that there is no interaction, though this has never been proven (or disproven). There are clear differences in exposure between gamma and alpha radiation. Gamma is continuous and monotonous from the start till the end of the experiment, largely homogeneous over the whole organisms, and easy to quantify; alpha's have first to be taken up before they exert their effect (except for the outer cell layer in direct contact with the source), their distribution is not homogenous in time and internally of the organisms and hence produces an inhomogeneous dose.

We decided to await the outcome of the single dose-response curves being performed in WP5 before reconsidering whether to conduct an alpha/gamma mixture experiment.

3.4 Effect 1: Binary mixture exposure experiments applying classical ecotoxicological settings and CA/IA as reference models

3.4.1 Exposure conditions

We decided to test binary combinations using classical ecotoxicological test experiments and CA/IA as reference models to assess possible interactions of the contaminants.

As mentioned, the following binary mixtures were selected for effects directed research:

- External gamma irradiation + Cd
- External gamma irradiation + fluoranthene
- U(VI) + Cd
- U(VI) + fluoranthene.

3.4.2 Test organisms

Selecting different species groups and levels of biological organisation is expected to aid in the extrapolation process. Thus, the following were chosen to assess the impact of co-contaminants:

Invertebrate: *C. elegans*

Plant: *L. minor*

Vertebrate: Fish -*S. salar*

All test organisms selected (except the community test organisms) are used in standard ecotoxicological tests. Having standard test protocols will simplify experimental design and allow for easier comparison with other studies.

C. elegans was selected for its short life cycle (shorter than that of daphnids). A possible factor in favour of daphnids could be that more toxicity literature is readily available on daphnids than for *C. elegans*. Number of effect studies on *C. elegans* are readily increasing in number and as said, the short life cycle of this species, made us select for *C. elegans*.

Lemna minor is a well-established aquatic plant ecotox test with standard endpoint parameters related to growth and photosyntehsis e.g. chlorophyll a and b levels.

Atlantic salmon (*S. salar)* is a salmonid fish species which have been demonstrated to be one of the most sensitive fish species and is very important environmentally as well as economically. Complete life-cycle studies with salmonid fish are impractical since it takes two to five years for these fish to reach maturity (Environment Canada, 1998). The early developmental stages (i.e. embryo, larvae and early juveniles) are generally considered to be equally or more sensitive to contaminant exposure than adults (EC, 1998). The toxicity experiments with *S. salar* will study the effects on development, growth and mortality of early fish life stages, from fertilization of the egg to hatching. The exposure protocol will be based on a standardized protocol developed by Environment Canada (1998) (for studying effects on early life stages of a closely related salmonid fish (rainbow trout).

The community to be used is under discussion. A simple (e.g. 3 species) mix of phytoplankton and zooplankton species is one possibility. Experiments with external gamma + Cd and gamma + fluoranthene will be done first before deciding whether to proceed with U experiments. Working with such a community increases the complexity of the experiment and we may need to simplify the set up. However, these tests will add a level of environmental realism and complexity to the other single species tests.

3.4.3 Endpoints

For *C. elegans*, *L. minor* and *S. salar:* classical ecotox test endpoints will be considered (ie. survival, growth and reproduction parameters). For *Lemna* the classical endpoints can be extended to respiration, photosynthesis.

For the community, the endpoint will focus on community function. Individual species sensitivities, using single species exposure experiments, will not be determined, for practical reasons. However, responses of individual species in the community may be analyzed (e.g. counts of individuals within a species).

3.4.4 Approach

For each of the mixture components a dose-response curve will be set up for the selected test organisms in an ecotox set-up. The establishment of the dose-response curves and the derivation of the curve using statistics will be carried out as described in D5.1. (Garnier-Laplace et al., 2011). Mixture experiments will be set up based on the shape of the dose-response curve (under guidance from NERC and constrained by the number of experimental “units” that is possible to be run for any experiment). When few units are possible, then designs such as a simple replicated Control, 1 TU Comp 1, I TU Comp 2 and ½ TU comp 1 + ½ TU comp 2 type experiment can be chosen. The ability to cope with more “units” allows more complex isobole and single ray designs to be used. When many units can be used simultaneously (10s of experimental units) factorial designs are possible. A fixed-ratio design will probably be used as it is less time and resource-demanding and still provides valuable data (Jonker et al., 2011). The following ratios of toxic unit could be

for the experiments: 1:0; 3:1; 1:1; 1:3; 0:1 as reported in Svendsen et al. (2010) and Jonker et al. (2011). For the same reasons, the assay will probably be reduced to partial lifespan (eg. until spawning of nematodes stops). TU generally used are 0, 0.25, 0.5, 1, 2 and 4 (Jonker et al., 2011) but this choice "requires careful consideration" as stated by Jager et al. (2010).

The detailed experimental set-up will be described once the final experimental plan is agreed upon (March-May 2012).

Effects data will be analysed according to the CA or IA concept. Based on the single dose-response curves, the expected mixture toxicity according to CA and IA will be calculated using the formulae described in Chapter 4 of Deliverable 4.1 (Vandenhove et al., 2012). Possible synergistic or antagonistic effects will be subsequently investigated by statistical tests comparing measured mixture toxicities with the predicted values.

The approach for the calculations, the mathematical methodology, should be the same for all partners. It was agreed to organize a training session within WP6 (Education and training) to teach the mathematical methodology to assess mixture effects so the involved partners use the same approach. It was preferred to do this via a web presentation and on line problem solving. NERC (Claus Svendsen) and IRSN (Claire Della-Vedova) will together prepare the web-training.

If interacting effects are observed in the binary mixtures, experimental testing for the underlying mechanisms will be set up (*Effects 1-supplement)*. The EAB advices to contact experts in mechanistic toxicology to determine the potential range of functional tools that can be used to best address the underlying mechanisms of effects observed and to best address the hypotheses proposed. Our preliminary opinion is to rather evaluate e.g. specific biomarkers for DNA damage and oxidative stress than to engage in holistic full 'omics' assessments.

3.4.5 Partners involved

IRSN will be involved in CA/IA experiments for *C. elegans* (in interaction with UMB) and NERC will help establish the D-R curves for Cd and fluoranthene; SCK•CEN will perform the experiments with *L. minor;* UMB (with NRPA) will work with *S. salar* and SU will work with communities. NERC will assist with the experimental design and data treatment for all partners. They will share their expertise on effects studies with organics.

SU does not have the facilities to perform effects experiments with external low-dose chronic radiation and they do not have permission to work with uranium. SU will therefore perform its experiments at the facilities of one of the other partners (which has the added benefit of promoting integration and sharing of facilities).

3.4.6 Timing

Effect 1: Start March 2012. First step is discussion on detailed experimental design. As the experimental setup will be different for the different partners it is agreed to first set up a protocol for the individual D-R curves (cfr D5.1., Garnier-Laplace et al., 2011) and circulate it between the partners in order to have the protocol as similar as possible [e.g. w.r.t. form of Cd or U-salt used, U-composition (depleted, natural) etc.].

First, individual D-R curves will be established (starting June 2012) and results communicated to the different partners. Based on the form of the D-R curves and a realistic number of experimental units, the experimental design will be established in consultation with NERC.

NERC and IRSN will prepare a web-based course to teach the mathematical methodology to assess mixture effects so the involved partners use the same approach (June 2012).

mechanistic studies of observed combination effects will be established only if clear interaction effects observed.

3.5 Effects 2: Binary mixture exposure experiments applying the DEBtox approach

Although CA/IA approaches on single endpoint dose response curves are generally accepted and used, there are associated limitations. The CA and IA models provide a prediction of the mixture based on single chemical dose-response curve relationships assuming no interaction. They do not take into account interactions between co-contaminants that may affect the mobility, absorption, distribution, storage, biotransformation and elimination of other contaminants (although these factors can be tested at specific time steps). They cannot explain observed interactions and they do not allow for mixture effects differing with time (Baas et al., 2007), with endpoint considered (Cedergreen and Streibig, 2005) or that there may be dose-dependent variation in interactions. A biology-based approach can be used to estimate the toxic effects of mixtures on growth, reproduction and survival over the life cycle of exposed organisms (Baas et al., 2009a, b).

To understand organisms' integrated responses to contaminant mixtures, methods such as the Dynamic Energy Budget (DEB) theory can be used to link feeding, growth, development and reproduction over the organism's life cycle (Jager et al., 2010). The DEB theory explains how organisms acquire and use resources over their life cycle, based on a set of simple rules for metabolic organization. DEBtox could give additional evidence for non-interaction. It would also give supra-individual information and allows for determining No Effect Concentrations. Chapter 4 of Deliverable 4.1 evaluates these aspects in more detail.

3.5.1 Approach

DEBtox experiments are labour-intensive as the DEBtox parameters still need to be derived for many toxicants and organisms. Therefore, only a limited number of organisms and exposure conditions (*C. elegans*, *L. minor*; gamma+Cd; if time allows also U+Cd) will be used to evaluate the impact of co-contaminants in a DEBtox approach (to evaluate among others how and if the co-contaminants affect the DEBtox derived No Effect Dose Rate). As the DEB model is not yet described for *L. minor*, the potential for applying this approach will depend on a successful parameterisation of a DEB and DEBtox model for this organism under WP5.

DEBtox experiments are scheduled to take place after the CA/IA experiments.

3.5.2 Partners involved

IRSN will be involved in mixDEBtox using *C. elegans* and SCK•CEN using *L. minor*.

3.5.3 Timing

DEBtox experiments will start after the classical ecotox CA/IA mixture experiments (Effects 1) around December 2013.

Evaluating selected risk assessment approaches for mixed exposure situations were radionuclides are involved (Task leaders: IRSN – SCK•CEN)

4.1 General

In the initial proposal, no further activity related to Ecological Risk Assessment (ERA) was foreseen. However, following the WP4-STAR meeting in May 2011, we decided to dedicate a limited effort to the application of risk assessment approaches for situations were radiation was present in a contaminant mix. Previous examples where the estimation of toxic pressure through a Cumulative Risk Assessment (CRA) approach (delta-PAF) has been applied to releases from NPP showed that this approach should be feasible in a broad application area (Garnier-Laplace et al., 2008).

4.2 Hypotheses

Though risk assessment in itself is not based on hypotheses, one working hypothesis was formulated:

H1: Cumulative Risk Assessment approaches for mixtures of radionuclides (or radiation) can be assessed according to CA and IA models in a consistent manner with other contaminants (for test species and communities and msPAF).

4.3 Proposed assessments

4.3.1 Risk 1: Application of a number of Cumulative Risk Assessment methodologies

We agreed to address Cumulative Risk Assessments for a limited number of case studies where radionuclides and other contaminants are of concern. Two broad sets of exposure scenarios will be considered: uranium mining and milling areas and releases from NPP
We agreed to implement a tiered approach for Cumulative Risk Assessment, the first approach being:

- Conservative red/green colour based on the most toxic compound, then
- Hazard Index approach based on the most toxic compound(s), then
- Sum of HI, then
- msPAF, then Refined msPAF, e.g. considering bioavailability (using e.g. geochemical speciation modelling, BLM for U)

The differences in CRA techniques will be highlighted for these situations (data requirements, data gaps, underlying hypotheses, assessment outcome, applicability to radioactively contaminated sites)

Partners involved

The different partners (IRSN, NERC, SCK•CEN) will contribute to the literature search to find data for the SSD's and defining the case studies for the run of the tiered approach, IRSN will make the calculations. Other partners (SCK•CEN, NERC) are interested to learn CRA methods and make calculations.

4.3.2 Risk 2: Application of CRA to Observatory sites

The tiered approach as in Risk 1 will be used for the observatory sites and same partners will be involved.

Partners involved:

For Observatory Sites, CRA methods will be applied by IRSN, (assisted by SCK•CEN, NERC) (cfr *Risk 1*).

5 Overview and Timing of Experimental Plan and Milestones

(Order of Tasks, Milestones and Deliverables, based on Delivery date)

Starting date	Delivery date	Task	Contributing groups Responsible for Milestones
Nov 2011	19/12/2012	Drafting experimental plan	SCK•CEN, IRSN, UMB, SU, NERC
Jan 19/20	Feb 7, 2012	Incorporating comments of EAB, experts, STAR partners and finalizing experimental plan	SCK•CEN, IRSN, UMB, SU, NERC
	Jan 31, 2012	MS43: Expert and stakeholder consultation and final integrated research program	SCK•CEN
Feb 2012	May 2012	Detailed experimental protocol and set up for multiple stressor effects experiments	NERC, IRSN, SCK•CEN, UMB/NRPA, SU, BfS, STUK
	Jul 31, 2012	MS44: Interim report on theoretical model runs to test effect of mixed contaminant conditions on exposure As annex in D4.1 *Model runs*	NERC/UMB
March 2012	Jun 2012	Web-based course on mathematical approach to assess mixture effects	IRSN and NERC
April 2012	Sept 2012	Perform literature review on BLM for Cd for selected test organisms and growth condition range for the test organisms; established U-dose-response curve for organisms of interest in selected reference medium.	UMB/NRPA, IRSN, SCK•CEN, BfS, STUK
Oct 2012	Oct 2012	Availability 3: Course on BLM-experimental set-up and modelling: (organised by NERC)	NERC
Oct 2012	Dec 2012	Availability 3: Establishment of experimental plan for first series of experiments for each test organism	NERC, UMB/NRPA, IRSN, SCK•CEN, BfS, STUK
Dec 2011	Dec 2012	Availability 1: Paper on comparison of speciation modeling on scenario's (Exposure)	NERC, IRSN, SCK•CEN, (UMB)
	Jan 2013	MS45: Interim report on availability/exposure related lab/field R&D and model runs and updated R&D plan *Midterm results from lab/field research and model run outcomes*	NERC/UMB
	Jan 2013 (was initially July	MS46: Parameterization of DEB model for all test organisms subjected to	SCK•CEN, IRSN

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	2012)	mixed exposure conditions *Interim note on DEB parameters, Model runs*	
	Jul 2013	MS47: Interim report on effects related lab R&D and model runs and updated R&D plan *Midterm results from lab research*	IRSN/SCK•CEN
Feb 2012	Feb 2014	Effects 1: CA/IA binary mixture experiments on ecotox tests	IRSN, SCK•CEN, UMB/NRPA, SU
	May 2014	MS48: Interim report on availability/exposure related lab/field R&D and model runs and updated R&D plan *Midterm results from lab/field research and model run outcomes*	NERC/UMB
Jul 2013	Jan 2015	Effects 1-supplement: Analyses and/or experiments for mechanistic understanding of interactions observed	IRSN, SCK•CEN, UMB/NRPA, SU
Jan 2013	Jun 2014	Availability 3: Experiments for building of U BLM model (+ under influence of Cd)	UMB/NERC, BfS/STUK, IRSN, NERC (help of SCK•CEN for *Lemna* tests)
	Jul 2014	MS49: Interim report on effects related lab R&D and model runs and updated R&D plan *Midterm results from lab research*	IRSN/SCK•CEN
Dec 2013	Dec 2014	Effects 2: Gamma/Cd DEBtox experiments for C elegans and if DEBtox has been derived also for L minor	IRSN, SCK•CEN
Jul 2013	Dec 2014	Risk 1: Modelling plus writing paper on msPAF/HI comparison for example cases (Risk Assessment)	IRSN, NERC, SCK•CEN, NRPA,
Jul 2013	Jul 2014	Availability 2: Characterisation of specific location at Observatory site	STUK, BfS, UMB
Jan 2014	Jan 2015	Risk 2: Modelling plus writing paper on msPAF/HI comparison (Risk Assessment) for Observatory sites	IRSN, NERC, SCK•CEN
Jan 2015	Mar 2015	Final reports on experimental parts	All

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Annex 1: Selection of organic compound for mixture experiments performed in WP4

In contrast to metal contaminants no obvious organic compound could be selected from the scenario's description performed in STAR D4.1. This difference in reporting between organic and metal components is probably due to fact that analysis of metallic compounds is generally easier than that of organics. The only organic routinely measured in nuclear power plant liquid releases is morpholine since it is a common additive for pH adjustments in nuclear power plant steam systems. However, morpholine is relatively intoxic to different organisms including humans (see for example the Pesticide Action Network (PAN) Pesticide Database (http://www.pesticideinfo.org/)) and was therefore not retained as a possible candidate for the multiple stressor studies.

In the January STAR WP4-WP5 workshop in Brussels criteria were listed that would be used to identify a possible organic toxicant as co-contaminant. These criteria included polarity, solubility of the compound, the ease to measure, the toxicity on a realistic deterministic endpoint, the ease of handling in a lab-based experiments and its persistence in the environment. It was further decided that if a decision could not be made based on these criteria we would further try to discriminate based on the possible mode of action of the compound and the realism of its presence together with radiation or radionuclides in the environment.

Based on a small survey among the STAR partners a list was made of possible candidate organic compounds these included two alkylphenols (4-tert-octylphenol and nonylphenol), three polycyclic aromatic hydrocarbons (PAHs)namely benzo(a)pyrene, fluoranthene and phnenathren and finally 3 biocides: pentachlorophenol, trichlosan and glyphosate. Information on both chemical characteristics and ecotoxicological data of these substances was collected from different sources (www.ineris.fr/substances/ , http://circa.europa.eu/Public/irc/env/wfd/library, http://www.socopse.se/) and compiled in Tables A1 and A2.

Briefly, all the selected organics were environmentally relevant toxic compounds. Their mode of action is more or less generic except for the biocides. Although the low analytical limit for detection , special adapted techniques are necessary for detection of all compounds.

Based on the fact that glyphosate has a very low toxicity towards animals it was not retained as a candidate for the toxicity studies within WP4. The toxicity of all other compounds was generally high to all organisms of interest within STAR.

The solubility in aqueous solutions differs among the different compounds. For benzo(a)pyrene solubility is so low that for this reason benzo(apyrene was excluded as a candidate. Pentachlorophenol and trichlosan were not retained as candidates as both have a high photolysis (in the seven day Lemna test performed in light conditions it will degrade rapidly). Also octylphenol and nonylphenol show interaction with light with the production of highly toxic hydroxyl radicals during the breakdown process and some of the breakdown compounds being more toxic than the original compounds. In addition nonylphenol is quite volatile. For these reasons nor octylphenol nor nonylphenol were selected.

Hence, going through the list of considered organic compounds using the criteria listed in the January 2012 workshop, the two PAHs fluoranthene and phenanthrene seemed possible candidates for the mixture experiments. Fluoranthene is a little less volatile than phenanthrene but the volatility of phenanthrene was still low enough that it would not pose a problem in the toxicity experiments. The toxicity and presence of these two compounds at contaminated sites is generally the same and could therefore not be used as a criterium for selection. A literature search showed that more studies were available on the mode of action of phenanthrene in plants compared to fluoranthene. In contrast, in animal studies fluoranthene is the one most

studied to date. Among the STAR partners fluoranthene has already been used in a study on C. ele… that compared the dynamic energy budget (DEB) model with microarray data (Swain et al., 2010). Since, therefore, knowledge on the use of fluoranthene, its toxicity in one of the test organisms C. elegans and the parameters for the DEB model are already available, fluoranthene was selected as the organic compound for the mixture studies within WP4.

Reference

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Table A1: List of physicochemical properties of the different compounds selected as possible organic compounds for mixture studies in WP4. Depicted in red are values that differ strongly from the average of the considered characteristic. Information was compiled from www.ineris.fr/substances, http://circa.europa.eu/Public/irc/env/wfd/library, http://www.socopse.se/.

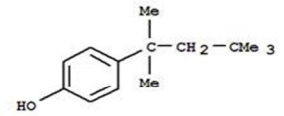
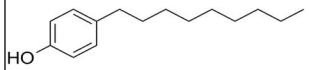
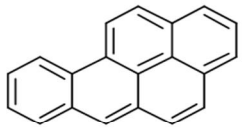
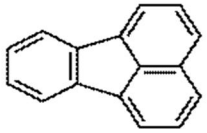
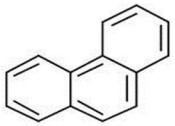
molecule description					physico-chemical properties					
CAS number	EU number	Name	Formula	Use	Solubility (mg/L at 25°C)	Hc (20°C)	Log Kow	Log Koc	persistance	Detection method, detection limit…
140-66-9		4-tert-Octylphenol (4-(1,1',3,3'-tetramethylbutyl)-phenol)		WFD priority list – production of resins, non-ionic surfactants and rubber additives, manufacturing of antioxidants, fuel oil stabilizers, adhesives, dyestuffs, fungicides, bactericides, and for vulcanizing synthetic rubber	5	0.699 (non volatile)	3,7	4,300008	Hydrolysis negligible in aquatic medium **Oxidation of HO radicals: T1/2 (d): 0.25 ; photodegradation (T1/2= 13.9h)**	GC-MS (LOD100 ng/L)
25154-52-3	246-672-0	Nonylphenol		WFD priority hazardous substance – manufacture of nonylphenol ethoxylates, nonionic surfactants that are used in a wide variety of industrial applications and consumer products	6	11.02 (moderately volatile)	4,48	4.35-5.69	SW persistance: T1/2 (d): 150 Photolysis & Hydrolysis negligible in aquatic medium **Oxidation of HO radicals: T1/2 (d): 0.3**	LC-MS, GC-MS (LOD 100 ng/L)
50-32-8	200-028-5	Benzo(a)pyrene		WFD priority hazardous substance (as PAH) – not manufactured, no industrial uses. Ubiquitously distributed. Primarily by-products of incomplete combustion (forest fires, volcanoes, combustion of fossil fuels, coke oven emissions and vehicle exhausts). Exfoliation from asphalt/bituminous liners may also contribute water contamination	0,0038	0(non volatile)	6,04	6,035	. PAH molecule stability and hydrophobicity are two primary factors which contribute to the persistence of HMW PAHs in the environment. Due to their lipophilic nature, PAHs have a high potential for biomagnification through trophic transfers	GC-FID, MS, PID HPLC -Fluo, UV (LOD ca 50 ng/L)
206-44-0	205-912-4	Fluoranthene		WFD priority list – used as an intermediate for dyes (fluorescent), pharmaceuticals and agrochemicals	0,26	0,8	5,1	5,160		
85-01-8	201-581-5	Phenanthrene		WFD priority hazardous substance (as PAH) – used in the synthesis of dyes, explosives and drugs. raw material of phenanthrenequinone (intermediate for pesticides) and diphenic acid (intermediate for resins)	1,2	2.9 (low volatile)	4,57	4,409		

Table A1 continued

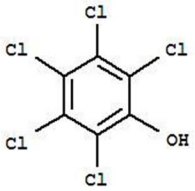
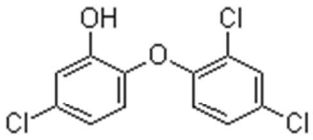
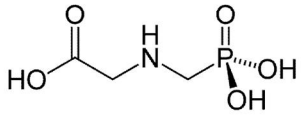
	201-778-6	Pentachlorophenol		WFD priority list – Fungicide. Banned use since 1994 but exemptions authorized for wood, fibres and historical buildings preservation. It is also used as intermediary of synthesis.	14	0.000001 (non volatile)	1.3(pH=10); 3.32(pH=7); 4.5(pH=4)	2.16-5.46	FW persistance: T1/2 (d): 4.9 Hydrolysis negligible in aquatic medium Photolysis T1/2 (d): 0.04 - 4.6	HPLC-UV (LOD 1µg/L), GC (LOD 0.1 µg/L)
3380-34-5		triclosan (2,4,4'- Trichloro-2'-hydroxyphenyl ether)		broad spectrum antibacterial agent used in personal care, veterinary, industrial and household products. infused in an increasing number of consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags	12	0.0152 (non volatile)	4,8	3,963788	no hydrolysys. Rapid photolysis (T1/2 <4h) with 2,4-dichlorophenol as main metabolite	HPLC, LC/MS, GC/MS, GC/EC (LOD 0.2 ng/L to 350 ng/L)
1071-83-6	213-997-4	Glyphosate (N-(phosphonomethyl)glycine)		Priority pesticide – broad-spectrum systemic herbicide (tradename Roundup). One of the most used herbicide both for agricultural market and home/garden market	10500	2,10E-07	-3,2	884-60000	slow hydrolysis and photolysis (T 1/2 > 30d). Not easily biodegradable	diffuse refractance spectro (LOD 7 µg/L) ; LC-MS/MS (LOD 0.5 ng/L)

Table A2: List of possible mode of action and toxicity concentrations of the different compounds selected as possible organic compounds for mixture studies in WP4. Depicted in red are values that differ strongly from the average for the considered characteristic. Information was compiled from www.ineris.fr/substances/ , http://circa.europa.eu/Public/irc/env/wfd/library, http://www.socopse.se/.

molecule description			Excotoxicological data							
CAS number	**EU number**	**Name**	**Mode of action**	**accumiulation/depuration biokinetic**	**plants/algae**	**invertebrates**	**vertebrates**	**toxicity on C elegans**	**reference**	**PNEC or EQS**
140-66-9		**4-tert-Octylphenol (4-(1,1',3,3'-tetramethylbutyl)-phenol)**	Possible anti-androgen, by displacing androgen from the androgen receptor. Other possible non receptor-mediated modes of action for endocrine disruption (calcium-dependent apoptosis, inhibition of the testicular calcium ATPase enzyme …). One cellular mechanism reported involves the disruption of cytochrome P450 enzymes, which has consequential effects on steroidogenesis and potentially produce effects on the endocrine system.	BCF fish: 634	LC/EC50 algae (mg/L): 1.1 NOEC/EC10 algae (mg/L): 0.3	LC/EC50 (mg/L): 0.01 NOEC/EC10 (mg/L): 0.06	LC/EC50 fish (mg/L): 0.17 NOEC/EC10 fish (mg/L): 0.01	24h-LC100 (mg/L): 0.41	http://dx.doi.org/10.1248/jhs.48.555	PNEC FW (mg/L): 0.00012 PNEC SED (mg/kgww): / PNEC SOIL (mg/kgww): / EQS (mg/L) : 0.00012
25154-52-3	**246-672-0**	**Nonylphenol**	acute corrosivity, reproductive effects have been identified. mutagenicity and carcinogenicity are low. chronic effects: oestrogenic activity, perturbations in the reproductive system (oestrogenic activity of a potency that is between 3 to 6 orders of magnitude less than that of oestradiol). on plants: most likely interfered with photosynthesis and cell division.	BCF: 1280	LC/EC50 algae (mg/L): 0.06 NOEC/EC10 algae (mg/L): /	LC/EC50 (mg/L): 0.02 NOEC/EC10 (mg/L): 0.02	LC/EC50 fish (mg/L): 0.13 NOEC/EC10 fish (mg/L): 0.01	24h-LC50 (mg/L): 7.2	http://dx.doi.org/10.1248/jhs.48.555 http://dx.doi.org/10.1248/jhs.48.583	PNEC FW (mg/L): 0.00033 PNEC SED (mg/kgww): 0.039 PNEC SOIL (mg/kgww): 0.3 EQS (mg/L): 0.0003
50-32-8	**200-028-5**	**Benzo(a)pyrene**	forms DNA adduct, cancerogenic in animals, reported genotoxic in a many tests with procaryotes and eucaryotes	accumulates very slowly BCF algae: 3300 BCF invertebrates: 12800 (max) BCF fish: 2700 (max)	LC/EC50 algae (mg/L): 0.005 NOEC/EC10 algae (mg/L): 0.00078	LC/EC50 (mg/L): 0.0015 - 0.005 NOEC/EC10 (mg/L): 0.0005	LC/EC50 fish (mg/L): / NOEC/EC10 fish (mg/L): 0.0024 - 0.007	48h-LC50 (mg/L): 0.05 ; 0.174 72h-EC50repro (mg/L): 0.059	http://dx.doi.org/10.1248/jhs.48.583 http://dx.doi.org/10.1080/15287390903091814	PNEC FW (mg/L): 0.00005 PNEC SED (mg/kgww): 0.543 PNEC SOIL (mg/kgdw): 0.32 EQS (mg/L) : 0.00004
206-44-0	**205-912-4**	**Fluoranthene**	expected to show mainly narcotic toxicity. Mutagenicity results are ambiguous.	BCF fish : 1700 BCF mollusc: 10000	LC/EC50 algae (mg/L): 0.01 NOEC/EC10 algae (mg/L): /	LC/EC50 (mg/L): 0.01 NOEC/EC10 (mg/L): /	LC/EC50 fish (mg/L): 0.04 NOEC/EC10 fish (mg/L): 0.1	48h-LC50 (mg/L) : 2.7 72h-EC50repro (mg/L) : 0.352	http://dx.doi.org/10.1080/	PNEC FW (mg/L): 0.00012 PNEC SED (mg/kgww): 0.13-37 EQS (mg/L) : 0.0001
85-01-8	**201-581-5**	**Phenanthrene**	narcotic (act via a nonspecific baseline toxicity)	BCF aquatic: 5055	LC/EC50 lemna (mg/L): 0.94 NOEC/EC10 algae (mg/L): 0.03 - 0.13	LC/EC50 (mg/L): 0.15 - 0.7 NOEC/EC10 (mg/L): 0.01 - 0.11	LC/EC50 fish (mg/L): 0.15 - 0.25 NOEC/EC10 fish (mg/L): 0.028	48h-LC50 (mg/L) : 4.7 72h-EC50repro (mg/L) : 1.2	http://dx.doi.org/10.1080/15287390903091814	PNEC FW (mg/L): 0.00134 PNEC SED (mg/L): 0.00538 PNEC SOIL (mg/L): 0.00750

Table A2 continued

[illegible]	201-778-6	**Pentachlorophenol**	affects by uncoupling mitochondrial oxidative phosphorylation, thereby causing accelerated aerobic metabolism and increasing heat production. It causes loss of membrane electrical resistance. This mode of action explains why pronounced differences in the susceptibility of sensitive representatives of different taxonomic groups do apparently not occur.	BCF aquatic: 100 to 1000 pH dependent	LC/EC50 plant (mg/L): 0.08-7 NOEC/EC10 algae (mg/L): 0.005	LC/EC50 (mg/L): 0.24-2 NOEC/EC10 (mg/L): <0.015	LC/EC50 fish (mg/L): 0.02-0.6 NOEC/EC10 fish (mg/L):0.002	24h-LC50 (mg/L) : > 9.1 ?	http://dx.doi.org/10.1007/BF00203892	PNEC FW (mg/L): 0.00035 PNEC SED (mg/kgww): 0.0259 PNEC SOIL (mg/kgww): / EQS (mg/L): 0.0004
3380-34-5		**triclosan (2,4,4'- Trichloro-2'-hydroxyphenyl ether)**	in mammals, inhibitor of P450 and potent inhibitors of phase II enzymes. potentially weakly androgenic/estrogenic in fish and amphibians, effect on the immune function. Low genotoxicity. highly toxic to algae and reproductive and developmental effects in some fish	BCF 2.7-90	LC/EC50 algae (mg/L): 0.0012 NOEC/EC10 algae (mg/L): 0.0005	LC/EC50 (mg/L): 0.13 NOEC/EC10 (mg/L): 0.006	LC/EC50 fish (mg/L): 0.26 NOEC/EC10 fish (mg/L): 0.0341	/		PNEC FW (mg/L): / PNEC SED (mg/kgww): / PNEC SOIL (mg/kgww): / EQS (mg/L): 0.00005
1071-83-6	**213-997-4**	**Glyphosate (N-(phosphonomethyl)glycine)**	hibit an enzyme EPSPS involved in the synthesis of the aromatic amino acids (tyrosine, tryptophan and phenylalanine) in actively growing plants. highly toxic to plants, is **largely non-toxic to animals** (poorly absorbed, excreted essentially unmetabolized). No evidence was found to support glyphosate as a neurotoxicant, immunotoxicant or endocrine disruptor. The surfactants used in the formulation of glyphosate-based herbicides are far more acutely toxic than the active ingredient itself.	**BCF fish 0.52**	LC/EC50 algae (mg/L): 4.5 NOEC/EC10 algae (mg/L): 1.4	LC/EC50 (mg/L): 134 NOEC/EC10 (mg/L): 30	LC/EC50 fish (mg/L): >24 NOEC/EC10 fish (mg/L): 18.22	24h-LC50 = 5.7% glyphosate	http://dx.doi.org/10.1016/j.neuro.2011.02.002	PNEC FW (mg/L): 0.028 EQS (mg/L): 01