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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

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Executive Summary

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.

Within this framework, we have critically reviewed existing approaches, methods and tools developed in ecotoxicology for assessing exposures, effects and risks in a mixed contaminant context and evaluated their applicability for radioecological research and radiecological risk assessments.. The scope of the review is limited to multiple contaminant conditions (e.g. metals, organic contaminants as part of the mixture that includes radiation or radionuclides) and not to the wider context of multiple stressor conditions. A review of identified scenarios where such multiple contaminant conditions, may occur has been performed.

We have reviewed approaches and tools for assessing the impact of co-contaminants on environmental availability and uptake of contaminants of interest and theoretically evaluated their usefulness for conditions that include radionuclides. Speciation models are well established tools for predicting the chemical speciation of metals and radionuclides. The application of selected chemical speciation models to two test scenarios suggested that co-contaminants would not significantly affect the speciation (and hence environmental availability) of uranium or thorium. Models were also tested for radium and polonium, but the lack of available thermodynamic data excluded speciation prediction for these elements.

The modelling of contaminant bioavailability is most advanced for metals, where the Biotic Ligand Model (BLM) provides an established framework for understanding and predicting how the medium chemistry affects bioavailability. Examples of BLM models are presented. The structure of the BLM lends itself well to evaluate the influence of co-contaminants on the uptake and toxicity of the metal of interest, including radionuclides. Currently it has only been applied in a few cases as a tool to understand (non-radionuclide) metal mixture effects. Nevertheless, extension of the approach to understanding the effects of mixtures of radionuclides and metals is considered feasible.

Approaches and tools to assess or predict the effect of contaminant mixtures have also been reviewed. Their advantages and disadvantages and applicability in the context of assessing effects in relevant mixed contaminant scenarios that include radionuclides have been evaluated. In particular component-based approaches are described: principles of Concentration Addition (CA) and Independent Action (IA) are presented for their application to descriptive data (dose-response curves) and to dynamic and integrated DEBtox (Dynamic Energy Budget Model) approaches to assess effects of mixtures. Further, a number of whole mixture approaches is described. A comparative overview of the different methods, data requirements and applicability of these different approaches and their capacity to identify and predict mixture effects is provided. All the concepts considered have advantages and limitations for effects assessment of situations where radionuclides are present in the mixture.

Finally, an overview of the state of the art on Ecological Risk Assessment (ERA) of mixtures, including radionuclides, has been provided. ERA principles for chemicals and radionuclides are summarized. A general overview of different ERA approaches to deal with mixtures is

presented, with their application in ERA assessments illustrated by some examples of application to mixtures. No comprehensive guidelines for the ecotoxicological assessment of chemical mixtures have yet been developed. Currently, regulation for mixtures is poorly developed and mainly concerns humans. There seems to be consensus that ERA approaches based on component-based approaches (CA and IA) assuming no interactions between substances are valuable as a first tier approach to mixtures risk assessment, and CA appears a pragmatic and defendable default conservative approach. There remains, however, a need to consider uncertainty and variability within this modelling framework. For the generalization of this framework, there is still a clear need for a comprehensive classification scheme for contaminants (including exposure to ionising radiation or radionuclides) to support application of CA/IA models.

Both from the exposure/effect analysis and the risk assessment perspective, the challenge with mixtures remains to identify the cases where interactions, especially synergistic interactions, occur. To achieve this there is a need for a mechanistic framework that accounts for mixture interactions at different process levels, *e.g.* absorption, metabolism, target site, physiological process. For nuclides, mechanistic models are an option if some co-contaminants are proven to affect bioavailability, toxicokinetics or detoxification of radionuclides. As for other toxicants, toxicokinetic rather than toxicodynamic mechanisms would seem to be more likely sources of toxicologically significant interactions. This is also a domain where experimental research and modelling development would be needed to explore the possibilities for interactions with various relevant chemicals. As mixture science and risk assessment develops, the identification of assessment factors and uncertainty and validation exercises with real mixture scenarios are also major challenges.

List of Acronyms

AhR:	Hydrocarbon receptor
AR:	Androgen receptor
B[a]P:	Benzo[a]pyrene
BDF:	Bioassay-directed fractionation
BLM:	Biotic Ligand Model
BRN:	Biochemical Reaction Network
CA:	Concentration addition
CBR:	Critical body residue
CHESS:	Chemical Equilibrium with Species and Surfaces
CRA:	Cumulative Risk Assessment
CSA:	Chemical safety assessment
DBTK:	Data-based toxicokinetic
DEB:	Dynamic Energy Budget
DOM:	Dissolved organic matter
DOC:	Dissolved organic carbon
EAB:	External Advisory Board
EC:	European Commission
EDA:	Effect-Directed Analysis
EDF:	Electricité de France
EPIC:	Environmental Protection from Ionizing Contaminants in the Arctic
EQS:	Environmental Quality Standard
ER:	Estrogen receptor
ERA:	Ecological Risk Assessments
ERICA:	Environmental Risk from Ionising Contaminants: Assessment and Management
FASSET:	Framework for Assessment of Environmental Impact
FIAM:	Free Ion Activity Model
GSIM:	Gill Surface Interaction Model
GWB	Geochemist’s Workbench
HI:	Hazard index
IA:	Independent action
IAEA:	International Atomic Energy Agency
ILSI:	International Life Sciences Institute
IPPC:	Integrated Pollution Prevention and Control
IUPAC:	International Union of Pure and Applied Chemistry
LLM	Low Molecular Mass radionuclide species
MCS:	Multi-constituent substances
MOA:	Mechanism/mode of action
msPAF:	Multi-substance Probably Affected Fraction
NEC:	No Effect Concentration
NOECs:	No Observed Effect Concentrations
NORM:	Naturally Occurring Radioactive Materials

OM:	Organic matter
OSPAR:	European Oslo and Paris Commission
PAH:	Poly aromatic hydrocarbons
PBPK	Physiologically Based Pharmacokinetic
PBTK:	Physiologically Based Toxicokinetics
PCB:	Polychlorinated biphenyl
PCDDs:	Polychlorinated dibenzo-p-dioxin
PCDFs	Polychlorinated dibenzofuran
PEC:	Predicted Environmental Concentration
PFOS:	Perfluorooctane sulfonate
PG:	Phosphogypsum
PNEC:	Predicted No Effect Concentration
PNEDR:	Predicted No Effect Dose Rates
pT:	Toxic potency
QSAR:	Quantitative structure–activity relationships
QSPR:	Quantitative structure–property relationship
REACH	Registration, Evaluation and Authorisation and Restriction of Chemicals
RPF:	Relative potency factor
RQ:	Risk Quotient
SF:	Safety Factor
TD:	Toxicodynamic
TEF:	Toxicity equivalency factor
TEQ:	Toxicity Equivalent
TIE:	Toxicity Identification Evaluation
TK:	Toxicokinetic
TmoA:	Toxic mode of action
TSP:	Two-step prediction
TU:	Toxic Unit
TUS:	Toxic Unit Summation
US DOE:	United States Department of Energy's
UVCB:	Ultraviolet light of short wavelength (C) or medium wavelength (B)
WET:	Whole Effluent Testing
WHAM:	Windermere Humic Aqueous Model
YES:	Yeast estrogen screen

Explanation of Terms

For explanation of terms we refer to Van Gestel et al. (2011) Mixture Toxicity: Linking Approaches from Ecological and Human Toxicology.

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1 Objectives and Scope

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 radioecological risk 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.

This Deliverable deals with the first objective.

The issue of multiple contaminants has been addressed in a number of international projects (e.g. NoMiracle (Lokke, 2009), BEAM (Backhaus et al., 2000), PHIME (2011)) and reviews (Kortenkamp et al., 2009; Van Gestel et al., 2011).

We have built on the outcomes of these programmes and reviews to critically evaluate how readily these mixed contaminant approaches can incorporateionisingradiation or exposure to radionuclides as one of the stressors. The scope of the review is limited to multiple contaminant conditions and not to multiple stressor conditions (which would also include stressors like temperature, UV-radiation, salinity).

Following an introductory chapter (Chapter 2), the review was organised around three areas, targeting different aspects of multiple contaminants.

In Chapter 3, we reviewed approaches and tools for assessing the impact of co-contaminants on environmental availability and uptake of the contaminants of interest and theoretically evaluated their usefulness for conditions that include radionuclides.

In Chapter 4 approaches and tools to assess or predict the effect of contaminant mixtures were reviewed and their advantages and disadvantages and applicability in the context of assessing effects in relevant mixed contaminant scenarios that include radionuclides were analysed.

Chapter 5 discusses the general ecological risk assessment framework and methods for mixtures and how these methods can be applied when radionuclides are in a mixture.

The conclusions are presented in Chapter 6.

In Annex 1, scenarios are described where radionuclides occur together with conventional contaminants. Annex 2 presents the detailed geochemical speciation results. An overview on how mixture risk assessment (both human health and ecological) is dealt within a regulatory context is presented in Annex 3.

2 Introduction

2.1 General issues of multiple contaminants

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. In Europe roughly 150 000 chemicals were preregistered for a later full registration within REACH (Registration, Evaluation and Authorisation and Restriction of Chemicals, Backhaus et al., 2010). Although contaminants never occur in isolation, their legislation is largely based on studies that examined the effects caused by single contaminants, not mixtures. Kortenkamp et al. (2010) reviewed the regulations concerned with chemicals and found that only four pieces of European legislation address mixture toxicity (See also Annex 3). The task of assessing health and ecological risks from even single contaminants is overwhelming: most chemicals on the European market today have never been tested for their effects on health and the ecosystems. In the 12 years prior to the instigation of REACH only 140 chemicals have been subjected to detailed risk assessment (European Commission, 2003).

Interestingly, REACH excludes radioactive contaminants and the derivation of environmental radiation protection criteria by international organisations (e.g. IAEA, 1992; ICRP, 2008; UNSCEAR, 2008). The EURATOM projects ERICA (Larsson, 2008) and PROTECT (Howard et al., 2010) are based on studies that considered radiation as the sole contaminant, in isolation to other stressors. Thus, there is a large void in our understanding of contaminant mixtures that include radiation or radionuclides. However, there is considerable evidence from research on non-radioactive contaminants that (1) the effects of multiple contaminants are frequently additive; (2) effects induced by a combination of stressors contaminants can differ from the sum of the individual effects and (3) compounds can exert effects in mixtures at concentrations in which the single contaminants do not show effects (Kortenkamp et al., 2007; Baas et al., 2010b).

2.2 Scenarios where radionuclides occur together with other contaminants

Other contaminants commonly occur in situations where radionuclides are generally the key focus of attention. For example, routine liquid releases from nuclear power plants contain a substantial array of chemicals (e.g. Cu, Zn, boric acid, ammonium, morpholine, lithine, hydrazine, Fe) as well as radionuclides (Garnier-Laplace et al., 2008). An analysis of the ecological impact of the releases showed that there was very little potential impact of the discharges, with the contribution from chemical contaminants relatively more important than that from radioactive substances (Garnier-Laplace et al., 2008). Additionally, liquid releases from nuclear power stations constitute a complex mixture of contaminants and other stressors as they also alter the temperature and pH of receiving rivers.

High-level radioactive waste disposal involves chemical contaminants in many components. As an example, the zirconium alloys and spent fuel contain constituents that include virtually the entire periodic table, whilst the waste containers contain Cr, Ni, Zn and the over-pack contains Cr, Ni, Mn, Pd, To, Mo. All these elements may potentially be released to the environment after disposal (Harju-Autti and Volckaert, 1995).

Uranium mining and milling has resulted in an important legacy of contamination that includes ^{238}U-series radionuclides mixed with, among other components, a series of different heavy metals, residues from chemical treatments (e.g. barium). As an example, acid rock drainage in a discrete catchment area of the former uranium mining site of Ronneburg, Germany, lead to surface waters enriched in Fe, Ca, Mg, Al, Mn, SO_4, Si, Cu, Zn, Ni, Cd, Cr, U and rare earth elements (Geletneky et al., 2002). A series of other trace elements such as As, Cd, Cu, Ni, Pb, Mo in elevated levels were seen together with Al, Fe, Mn and U and Th and their progenies in waters from U mining sites in Central Asia (Salbu and Stegnar, 2011)

The NORM (Naturally Occurring Radioactive Materials) industry is also an important source of mixed contamination with U- and Th-series radionuclides and heavy metals. Tayibi et al. (2009) reported on the chemical composition of phosphogypsum (PG), a by-product of the phosphate industry. Apart from relatively high levels of U-series radionuclides, high total concentrations of Ag, Au, Cd, Se, some light earths and Y were reported in PG from different origins. Some PG additionally showed elevated levels of As, Ba, Cr, Pb and Hg. .Reservoir sediments contaminated with Cd, Ag, Bi, Sb, Pb, Zn, U, W, Mb, Cu, Tl, Cr, resulted from historical tin mining, but also from Cu, W, Mo, Bi and Ag mining in Erzgebirge, Germany (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, including nuclear weapon tests, use of depleted uranium ammunition, nuclear weapons accidents, nuclear reactor accidents (Chernobyl, and recently Fukushima) and dumping of nuclear waste at sea. Adding to the list is the use of radionuclides for medical purposes, research, or specific uses in the industry. This shows that radionuclide releases in the environment are expected to occur in extremely various situations where other contaminants are present.

Ecosystems are also clearly exposed to combinations of anthropogenic and natural stressors such as excess UV, sub-optimal temperature, pH or nutritive status and predation. Though outside the scope of this study it is worth alluding to this issue since ecotoxicological effect studies often expose test organisms under optimal environmental conditions and results obtained may differ from those in the natural environment. Organisms in their natural settings rarely experience optimal conditions, but are forced to cope with sub-optimal conditions or even with severe environmental stress due to events as flooding and drought. Whether interactive effects are common in natural settings and whether these are predominantly synergistic or antagonistic is a key unresolved question. Holmstrup et al. (2010) reviewed more than 150 studies to provide a synthesis of existing knowledge on the interactions between effects of “natural” and chemical (anthropogenic) stressors. Stressors considered in these studies included heat, cold, desiccation, oxygen depletion, pathogens and immunomodulatory factors combined with a variety of environmental pollutants. Synergistic interactions were reported in more than 50 % of the available studies. Antagonistic interactions were also detected, but in fewer cases.

2.3 Multiple contaminants and how they may affect exposure, effect and risk assessment

Although most scientists acknowledge that contaminant mixtures within the environment are the norm, rather than the exception, relatively few researchers properly study the effect of combined stressors. Instead, the vast majority of research and Ecological Risk Assessment frameworks are focused on single contaminants in isolation from all others.

In ecotoxicology, models have been developed for assessing metal availability and some of them have been tested for mixtures of metals. For example, the Biotic Ligand Model (BLM) has been proposed as a tool to quantitatively predict the manner in which water chemistry affects the biological availability of metals in aquatic systems. Because of its mechanistic fundament in dealing with the interaction of components, the BLM approach has the potential to significantly advance risk assessments of metal mixtures (Chen et al., 2010). Chen et al. (2010) evaluated the validity of the BLM for bioaccumulation assessment of Pb and Cu present as a metal mixture. Biotic ligand models in combination with geochemical speciation models (e.g. CHESS (van der Lee and De Windt, 2002); Geochemist's Workbench (GWB), 2011) could be valuable tools for assessing the influence of mixed contaminant conditions on radionuclide environmental availability. These different models and tools for availability and uptake assessment and their applicability in a mixed contaminant context where radionuclides are involved are reviewed and discussed in Chapter 3.

Effects and ecological risk assessments under multiple stressor or mixed contaminant exposure conditions are a major challenge (Eggen et al., 2004). Because there is an unlimited number of mixture combinations and organisms, it is not feasible to experimentally investigate the adverse effects caused by each combination. Instead, two main approaches exist; top-down assessment of whole mixtures and bottom-up assessments starting with individual components of the mixture.

When there is only interest in the toxic effect of the entire mixture, the top-down approach can be used during which the entire mixture is tested without identifying the type of interactions between the individual chemicals. The data from these toxicity tests can subsequently be used for risk assessment for that specific situation (Groten et al., 2001). However, this approach will not identify the chemicals responsible for interactionsor whether there are any non-additive effects.

In the bottom-up approach, the combined action of the components in the mixture is assessed. This approach is mostly used for risk assessment of simple mixtures with a known composition. Firstly, knowledge on the toxicity and mode of action of the individual components is required. Then different mathematical models can be used for predicting the combined effects based on the known individual effects (Groten et al., 2001). Based on the dose-response curves and EC_x-values of the individual toxicants, two generally accepted reference models "concentration addition" (CA) and "independent action" (IA) can be used for the prediction of the combined toxicity effects. The assumption of both models is that no interacting effects are present. Although both concepts operate differently they both require that the composition of the mixture (i.e. the exposure)is precisely known both qualitatively and quantitatively (Backhaus et al., 2000).

Interactions between pollutants in a mixture may occur in four ways: pollutants may (1) influence each other's mobility in the environmental media and hence, each other's availability to organisms; (2) block or enhance each other's uptake into the organism (toxicokinetic-absorption interactions); (3) once inside the organism, block or enhance each other's detoxification (toxicokinetic-metabolism interactions); (4)alter the nature of their toxic actions, and/or impacting on repair capacities (toxicodynamic interactions). The primary molecular and cellular effects of the many agents potentially involved in combined effects are numerous and diverse. For example, the mode of action may be genotoxic or non-genotoxic and they may interact via different pathways and through different mechanisms. Understanding the 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.

Generally, CA and IA models are used to describe and predict mixture effects based on single time-point dose-response data and evaluate if observed effects deviate from CA or IA. The aim of biology-based approaches such as the Dynamic Energy Budget theory (DEB) (Jager et al., 2010) is to use the time and concentration dependent data to extract information on the underlying mechanisms of mixture toxicity. It is therefore necessary to understand effects of single compounds in sufficient detail before attempting a biology-based analysis of contaminant mixtures. To this complex dynamic data set, CA/IA is then applied. Chapter 4 gives a comprehensive overview of effect assessment methods for mixture exposure conditions.

In the last decades, risk assessment has become a commonly used approach in examining environmental problems caused by human activities. Within an ERA perspective, the global objective is to estimate the adverse effects on the ecosystems resulting from anthropogenic activities either quantitatively or qualitatively. Ecological Risk Assessment of mixtures is still under development and in Chapter 5 different ERA approaches to mixtures are discussed, including when considering radiation. In ERA in general as well as in our specific case, there are two main challenges in the applicability of the ERA methods:

(1) Which ERA methods should be applied to perform, for instance, a Cumulative Risk Assessment including radionuclides? How can we rank the different contaminants to identify the main contributors to the risk?

(2) What is the degree of conservatism of the method applied? In the case of mixtures, there should be some degree of confidence that there are no substantial synergisms occurring that would jeopardize the assumption of addition for the different groups of contaminants. Alternatively, a conservative safety factor may be applied. Effects studies are generally performed under controlled laboratory conditions and a number of lower tier (screening) ERA steps (further explained in Chapter 5) are based on these effects data.

It should be emphasised that there is a major difference in experimentation and ERA. Experimentation must and should be the best approach possible to increase understanding of the (mixture) effects, while ERA may result in the application of methods which have been shown “to work”, while certainly not understood. In ERA, an approach may be practicable and valid to rank, while almost nothing is understood why exactly.

2.4 Radiation in a multiple contaminant context

A lack of knowledge about complex mixtures of contaminants is among the major challenges facing the environmental sciences (Eggen et al., 2004) since both the short- and long-term human and ecological risks from chronic exposures to contaminant mixtures are unknown. In the framework of radiological protection of the environment, data are needed to determine whether, and to what extent, radiation should be considered in a multiple pollution context (Bréchignac and Doi, 2009). Recent consideration has been given to the issue under the umbrella of the IUR (2011) and IAEA EMRAS II (IAEA, 2011) working groups on Multiple Stressors. Vanhoudt et al. (2012) reviewed studies in which combined effects of radiation and other stressors on non-human biota were evaluated and determined if the effects observed were generally additive, synergistic or antagonistic. The approach and findings of the review are summarized in Section 4.7.2 of this report.

Our overarching goal within Work Package 4 is to determine if radiation protection criteria for wildlife are robust, even within a mixed contaminant context.We intend to evaluate whether radiation protection criteria for wildlife need to consider contaminant mixtures by first determining if interactions exist among several plausible mixtures. If no interactions occur then consideration of mixtures may not be required. If, however, significant interactions do exist, then additional consideration may be required when evaluating protection criteria for radiation in the presence of other contamination.

Radiation (or ionising radiation) is here defined as every form of radiation capable of causing ionisations and excitations by energy transfer from the radiation field to matter or tissue.Both electromagnetic and particulate radiations act on cells to cause free radicals and subsequent molecular damage through direct as well as indirect actions.

International recommendations and guidelines on international level and a comprehensive system to protect the environment from ionising radiation are being developed. As a consequence, radiation protection criteria for wildlife have been derived using a number of approaches (IAEA, 1992, ICRP, 2003, 2007, 2008; UNSCEAR 1996, 2008; Garnier-Laplace and Gilbin, 2006; Andersson et al., 2008). These protection criteria derived by different organisations, can be considered as Predicted No Effect Dose Rates (PNEDR). The values derived by different organisations differ in protection target and the value of the PNEDR; the exact definitions and the values themselves are summarised in Andersson et al.(2008).

It may be of interest to evaluate the robustness of radiation protection criteria applied to a single, specific radionuclide. Using aradioactive isotope of a given element would hence be the focus point, thereby considering both the radiation aspect (PNEDR) and the specific characteristics of the element leading to a chemical effect (with its appropriate physico-chemical forms, chemical behaviour in environmental media, relevant organisms and potential chemical effect). This approach could be followed for uranium[1]for which an

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[1] The naturally occurring element uranium includes 3 radioactive isotopes ^{238}U, ^{235}U, ^{234}U, which may, or may not be in equilibrium. U EQS refers only to its chemical toxicity independent of the isotope considered. However, radiological vs. chemical risks may change as a function of isotopic composition of U (e.g. depleted vs natural vs enriched) (Mathews et al., 2009).

Environmental Quality Standard (EQS) of 5 µg L^{-1}has been proposed (Beaugelin-Seiller et al., 2009a). The evaluation of the robustness of radionuclide-specific EQS in a mixture toxicity environment is not considered in this review (as it is not considered for human impact assessment either) except for uranium.

3 State of the art on approaches and tools for bioavailability and exposure assessment under mixed contaminant conditions

3.1 Introduction

3.1.1 Background

Assessment of the risks of exposure of organisms to contaminants in soils and waters largely focuses on exposure to single contaminants, yet this is rarely if ever the case in the natural environment, where exposure to multiple contaminants is the norm. Other contaminants commonly occur in situations where contamination by radionuclides attracts the major focus, as explained in the introduction and further in this document (e.g. Annex 1).

The effect of exposure to multiple contaminants is an active research area, yet the prediction of the effects of multiple contaminants is poorly developed. Improving understanding of how organisms are exposed to multiple contaminants is clearly important. Research over the past 20-30 years has identified the concept of bioavailability as being a key factor determining exposure to organisms in the environment of both metallic and organic (non-ionic) contaminants. Despite this, research applying the concept of bioavailability to mixtures of contaminants remains poorly developed, in part due to the current focus on single contaminant exposure in risk assessment. The bioavailability of metal contaminants to organisms has been shown to be controlled by two key aspects of the chemistry of the medium:

- The chemical speciation of the metal in the medium (e.g. a water, or soil/sediment-water system). The speciation depends on the releasing source and is controlled by the chemical composition of the medium.
- The concentrations of elements in the medium that can compete with the contaminant for uptake by the organism. Greater competition leads to lower uptake of the contaminant and hence lower toxicity. Again, it is the chemical composition of the medium and the resulting speciation that influences the exposure to, and toxicity of, the contaminant.

Prediction of the influence of metal bioavailability on toxicity thus requires the prediction of both chemical speciation and the influence of the medium on contaminant uptake. For non-ionic contaminants, which comprise a large proportion of organic contaminants, bioavailability is generally considered to relate to the ‘freely dissolved’ fraction, while uptake is generally considered to relate to the tendency to partition into and accumulate in the fatty tissues of organisms. The latter distinction is not applied in radioecology.

Exposure to multiple contaminants adds a layer of complexity to the picture. Contaminants may affect each other’s chemical speciation, and may compete for uptake to the organism. Where exposure to mixtures of contaminants is to be considered, it is thus important to understand the extent to which the contaminants influence each other's speciation and thus their exposure.

3.1.2 Objectives

The objective of this chapter is to assess the current state of knowledge on whether, and how, the presence of multiple contaminants in a system influences the bioavailability of radionuclide contaminants and hence the exposure of organisms to these radionuclides. We will evaluate tools that can predict the effect of co-contaminants on bioavailability. We also consider whether these tools are appropriate to also assess the influence of co-contaminants on radionuclide bioavailability and hence exposure and/or to what extent these models should be adapted.

As a preliminary test we have assessed the applicability of geochemical speciation models to relevant environmental scenarios involving radionuclides, and evaluated how well such models can integrate the effects of multiple contaminants on speciation of a selection of elements including radionuclides of interest (i.e., U, Th, Pb, Ra, Po). The aim of this exercise was to specifically identify gaps in the models' capabilities with a view to identifying potential research studies to address these deficiencies.

3.1.3 Scope

The scope of this chapter is to review existing approaches for assessing the influence of co-contaminants on the exposure of organisms to radionuclides. We concentrate on contaminants and not on other stressors (such as pH, temperature, drought, predation). In terms of co-contaminants, we review approaches for assessing the bioavailability of both organic and inorganic contaminants.

3.1.4 Definition of terms

Chemical speciation. According to the IUPAC (International Union of Pure and Applied Chemistry) chemical species of elements are ‘‘defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure’’ and refers to ‘the chemical form or compound in which an element occurs in both non-living and living systems. It may also refer to the quantitative distribution of an element.’(IUPAC, 1997).

Bioavailability concerns the tendency of an element or compound to be taken up by an organism from the surrounding environment. Hamelink et al. (1994) consider three aspects of bioavailability for environmental organisms:

Environmental availability is defined as the supply of contaminant to the organism's immediate environment (i.e. from where it may potentially be taken up by the organism) and the chemical forms in which the contaminant is supplied, i.e. its speciation.

Environmental bioavailability is defined as the physiologically-driven processes of contaminant uptake by the organism.

Toxicological bioavailability is defined as the sum of the processes contributing to the actual toxic effect following uptake, e.g. internalisation, tissue redistribution and detoxification.

In this section of the review we are concerned with the first two aspects of bioavailability, i.e. those which concern the exposure of organisms to contaminants and the resulting uptake. The third aspect, being concerned with effects following uptake, is dealt with in Chapter 4.

In the broadest terms, bioavailability should refer to the tendency to be taken up by any possible route. Potential routes include ingestion of contaminated food or water and subsequent transfer of contaminants across the gut, inhalation and transfer via respiratory tissues to tissues or binding of the contaminant to external tissues such as gills or the skin/dermis, followed by transfer to tissues.

3.1.5 Classification of contaminants

Contaminant chemicals in the environment are extremely diverse in their chemical structure and behaviour, and in the modes of action by which they exert toxic effects upon organisms. It is therefore necessary to attempt a broad classification of contaminants according to their chemical behaviour and toxic mode(s) of action. It is important, however, to appreciate that the relationships between chemical structure and mode(s) of action can be complex, particularly for anthropogenically-produced chemicals designed to have specific biochemical activity, such as biocides, pharmaceutical compounds and nanoparticles.

Chemically, contaminants can be broadly classified as ionic (polar) and non-ionic (non-polar), although this classification must be used with caution since some classes of contaminant (such as ionic surfactants) may exhibit both polar and non-polar characteristics. Ionic contaminants are those that can form ionic species in the environment. This includes the majority of inorganic contaminants, including metals and metalloids. It also includes organic chemicals that can ionise under environmental conditions. Ionic contaminants are typically characterised by relatively high solubility in water, due to the stabilising effect of the polar solvent water molecules upon the ions formed. However, ionic contaminants are also characterised by the tendency to form complexes and ion pairs with other components of environmental systems such as inorganic and organic ligands (e.g. carbonate, EDTA, humic substances). This tendency can result in strong removal of ionic contaminants from the aqueous phase, in systems where strong complexing agents form part of the solid phase. A good example of this is in soils, which can strongly retain metals by their binding to soil components such as humic substances and metal oxide and clay minerals, reducing concentrations in the aqueous phase. The solubility of ionic contaminants such as metals may also be reduced by the formation of solid precipitates under chemical suitable conditions. For example, the solubility of lead in soils may be reduced by the formation of the mineral chloropyromorphite ($Pb_5(PO_4)_3Cl$). All these processes are highly dependent upon the chemical conditions of the system, in particular the pH, redox system and the ionic strength.

Numerous anthropogenically-produced organic chemicals are also able to form ions in the environment, including many pharmaceutical compounds (e.g. diclofenac), pesticides (e.g. glyphosate) and surfactants (e.g. perfluorooctane sulfonate (PFOS)). For example, the antifungal and antibacterial compound pyrithione (2-mercaptopyridine-N-oxide; C_5H_5NOS) can ionise in water to form a negative ion which can itself then complex with ions of metals including zinc, copper and cadmium. Depending upon their structure and intrinsic chemical properties, such contaminants may exhibit behaviour characteristic of ionic species (e.g. high water solubility, binding to other ionic species) and of non-ionic species.

Non-ionic contaminants are those that do not form ions under environmental conditions. They comprise many important classes of organic compound, including polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins and dioxin-like compounds. Chemically, they are characterised by low aqueous solubility (hydrophobicity) and a tendency to associate with other components of the environment (e.g. organic matter in soils) by hydrophobic mechanisms (Delle Site, 2001).

The chemical behaviour of a contaminant has important implications for its mode(s) of toxic action towards organisms.

3.2 Modelling of contaminant speciation in the environment

3.2.1 Modelling the speciation of metals and metalloids

The chemistry of metals and metalloids is controlled by their tendency to form complexes with (i) other ionic species such as H^+, CO_3^{2-}, SO_4^{2-}, (ii) ionic organic species such as $EDTA^{4-}$ and humic substances, (iii) solids possessing charged functional groups, such as iron oxide and silica, and (iv) solids possessing structural charge, such as clays. The possibility of undergoing oxidation-reduction reactions and of forming solid precipitates also exists. In this context, chemical speciation refers to the distribution of an ionic element among its possible different forms. One central aspect of the speciation of ionic elements is that competition for the formation of complexes is common. Therefore, the speciation of major elements such as Na, Mg, Al, K, Ca, Fe, Mn as well as total organic carbon (TOC) must be considered when studying the speciation of trace elements (into which category contaminants will almost always fall).

Thermodynamic equilibrium is the state at which the forward and backward rates of each chemical reaction pairs in the system are equal, and thus the composition of the system is invariant in time. Conceptually, complete thermodynamic equilibrium is only attainable in a closed system, while environmental systems are open. Nevertheless, equilibrium modelling is useful for predicting and understanding the behaviour of ionic chemicals in the environment, because the composition of some environmental systems may approach equilibrium provided that the residence time of the reacting components is large enough relative to the timescale of the reaction or reactions under consideration. This condition is most likely to be satisfied for systems of large volume with relatively small inputs and outputs, particularly the oceans, large lakes and groundwater systems. In many other systems, reactions may be sufficiently fast that the assumption of equilibrium is a reasonable approximation. For some reactions, the attainment of true thermodynamic equilibrium is sufficiently slow that for practical purposes equilibrium can be assumed to involve metastable intermediate species. The precipitation and dissolution of solids is good example of this. For some reactions, such as processes in mixing zones such as estuaries and those involving the precipitation/flooding and dissolution of solids, the thermodynamic equilibrium is too slow to be applicable. In such cases, transient intermediate species being highly toxic to fish can be produced, as well documented for Al and Fe in estuaries (Rosseland et al, 1992), although thermodynamic equilibrium modelling would predict good water quality conditions.

Speciation models for metals and metalloids are well-established tools, and there are many models available for use. Models available include MINTEQA2, MINEQL+, Visual MINTEQ, WHAM, ECOSAT, WATEQF, ALCHEMI, PHREEQC, The Geochemist's Workbench (GWB), CHESS and EQ3/6.

Inclusion in speciation models of all important ligands that can bind ions is important. Humic substances (humic and fulvic acids) form the most important class of organic ligands in soils and waters. They bind cationic metal species, in many cases significantly influencing the aquatic and soil speciation of the metal. There is also increasing evidence that they can play a role in the speciation of anionic species (e.g. molybdate, arsenate). Humic substances are chemically complex and heterogeneous and consequently require special consideration in models.

Specific submodels have been developed over the past 20 years to simulate the chemistry of humic substances. Two distinctive approaches have been taken to simulate their chemical heterogeneity:

1. The discrete site approach. This considers binding to occur at an array of specific binding site types, each type having its own distinct ion binding behaviour.
2. The continuous distribution approach. This considers binding to occur to a collection of sites having a continuous distribution of ion binding strengths.

Currently, Humic Ion-Binding Models VI and VII (Tipping et al., 2011) are the most developed discrete site models for humic substances, while NICA-Donnan (Benedetti et al., 1995) is the most developed continuous distribution model. Both models are parameterised to simulate the binding of a large number of cations to humic substances. Each model has a distinct parameter set for simulating ion binding to humic acids (important in soils) and fulvic acids (important in soils and waters). An important feature of these models is that they currently simulate only the binding of cationic species. The binding of anions (e.g. anions of arsenic, molybdenum, tungsten, technetium) has received little attention. Ions such as arsenite (As(III), AsO_3^-) and arsenate (As(V), AsO_4^{3-}) have been shown to bind to humic substances, but as yet no available model includes constants describing their binding.

Similarly, the binding of ions to charged mineral surfaces (metal oxides and clays) is important in the speciation of ionic contaminants in soils and bottom/suspended sediments in aquatic systems. For surfaces that possess functional groups that can bind ions, a closely-related family of models (the surface complexation models) have been developed over the past 30-40 years. These models all simulate the specific binding of ions to surface groups in a similar manner, but differ in the simulation of the resulting electrical charge at the surface, and how this influences the ionic binding.

Equilibrium chemical speciation models have some clear advantages over simpler methods of simulating the behaviour of ionic chemicals in the environment. For example, the partitioning of metal between the dissolved and suspended particulate forms in water could be described by a single partition coefficient (K_d). However, K_d is essentially a descriptive rather than a predictive parameter, and varies according to the chemical composition of the system. Use of a chemical speciation model instead of a K_d can take the effect of chemical composition into account but they require more data than empirical models. In response to this, some researchers have derived semi-empirical models that predict K_d as a function of key chemical properties of the system (Tipping et al., 2003; Groenenberg et al., 2010).

Depending upon the system to be simulated, equilibrium speciation models may not necessarily be able to provide a physico-chemically realistic description of the speciation, due to the existence of processes that are insufficiently rapid for a reasonable description using equilibrium modelling. Examples of such processes include the weathering of minerals in soils, and oxidation-reduction reactions. Many speciation models allow oxidation-reduction reactions to be simulated at equilibrium, but in reality they are generally slow in comparison to reactions not involving changes in oxidation state.

3.2.2 Modelling the chemistry of organic contaminants

The chemistry of organic contaminants tends to be dominated by low aqueous solubility and consequent strong sorption to solid phases such as organic matter in soils and sediments. Efforts to model their chemistry (and also their bioavailability and toxicity) are driven in part by the need to assess the behaviour of hundreds of man-made contaminants, with new compounds being continuously developed and released into the environment. There is thus an emphasis on deriving chemical properties (such as soil organic carbon-water partition coefficients, K_{OC}) from molecular structure (e.g. Kahn et al., 2005), rather than considering how the chemistry is influenced by factors such as the pH of the medium and the presence of other contaminants, and how the presence of the compound might affect the chemistry of other contaminants (particularly ionic ones). Work has also been done on how the composition of organic matter in soils affects adsorption (e.g. Kubicki and Apitz, 1999). Some work on competition between organic compounds for sorption to soil (and how this affects bioavailability) has been done (Haws et al. (2006) review the available data) and interpreted in terms of competition for binding sites on soil organic matter. Non-ionic chemicals also bind to humic substances in water (e.g. Kim and Kwon, 2010; DePaolis and Kukkonen, 1997; Durjava et al., 2007) and K_{OC} values for some compounds have been derived.

Modelling studies of non-ionic contaminants that progress beyond simple calculations of partitioning coefficients appear uncommon. Lee and Kuo (1999) presented a mechanistic sorption model to describe the influence of dissolved organic matter on the partitioning of highly hydrophobic compounds between the aqueous and particulate phases in waters. Kipke and Di Toro (2011) have developed a model for the binding of organic contaminants to dissolved organic carbon (DOC) using a linear salvation energy relationship model whereby the coefficient of partitioning to DOC is predicted as a linear function of five properties of the organic chemical. The model was able to predict partitioning largely to within an order of magnitude for a number of natural organic matter types. There is also evidence that the electrical charge on natural organic matter, which is a function of its ionic chemistry, alters its binding affinity for organic chemicals by changing the hydrophobicity of the natural organic matter (Galle et al., 2005; Gadad et al., 2007) although such knowledge has not yet been incorporated into models.

Similarly, studies looking at the combined behaviour of ionic and non-ionic contaminants in the environment are rare, and studies modelling such interactions could not be found in the literature. As an example, Xu et al. (2007) studied the effect of copper on the binding of two phthalate compounds to sediments in the presence of dissolved organic matter. The partitioning of the phthalates to the sediment was influenced by their binding to dissolved organic matter, which acted to retain them in the solution phase. Addition of copper increased the binding of the dissolved organic matter to the sediments and consequently influenced the binding of the phthalates.

3.2.3 Dynamic modelling

To quantify the spatial and temporal interactions of radionuclides and co-contaminants (both chemical, and interactions in terms of effects on organisms), chemical models need to be incorporated into models of contaminant transport. Depending upon the nature of the processes and interactions that need to be simulated, submodels for non-equilibrium processes may need to be incorporated into the code. Examples of coupled speciation-transport models for ionic contaminants include models that simulate accumulation of contaminants in soil and their leaching to surface water and groundwater on a catchment scale, and models that simulate transformations within water bodies (particularly lakes), including water-column-sediment transfers. For non-ionic contaminants, models tend to be focused on predicting concentrations across environmental compartments at different scales to enable hazard and risk assessment. Because non-ionic contaminants may be volatile and subject to long-range atmospheric transport, models need to be capable of operating at large (regional, hemispheric, global) scales.

3.2.4 Choice of chemical models for assessment

Chemical models for ionic species are well-developed in comparison with models for non-ionic species. Furthermore, multiple models are readily available for comparison. Therefore, our assessment of chemical models will focus on equilibrium models for ionic species. In selecting a model, it is also important to know whether their supporting databases are comprehensive for the radionuclides of interest. For thorium and uranium, for example, the models and associated databases are generally well-developed. For radium and polonium none of the models contain binding constants, so speciation predictions were not possible. A more in depth comparison of the models capability to deal with radionuclides was not performed in the context of this preliminary testing of speciation models.

We have elected to further focus on four speciation models: WHAM, Visual MINTEQ, CHESS, and GWB, as they are widely used for metal ions and are familiar to STAR participants.

3.2.4.1 Windermere Humic Aqueous Model

The Windermere Humic Aqueous Model (WHAM) was developed at the former Institute of Freshwater Ecology (UK) now the Centre for Ecology and Hydrology which is a STAR partner. The first version of the model was published in 1994 in two versions specifically designed for modelling (i) soils (WHAM-S) and (ii) surface waters (WHAM-W). A subsequent updated version for waters, WHAM6, was released in 2002 (http://www.ceh.ac.uk/products/software/wham).

The soils version comprised four submodels: (i) cation binding to humic and fulvic acids; (ii) solution speciation; (iii) cation exchange on a fixed-charge mineral (i.e. a clay); (iv) soil-porewater partitioning of fulvic acid. In WHAM-S and WHAM-W, the submodel for cation binding to humic and fulvic acid is Humic Ion Binding Model V (Tipping and Hurley, 1992) (usually abbreviated to Model V), a discrete-site/electrostatic binding model for humic substances. WHAM6 contains Humic Ion Binding Model VI (Tipping, 1998), a development of Model V, as well as a surface complexation model and the cation exchanger submodel. Model VII, the successor to Model VI, has recently been developed.

WHAM was designed as a model for soils and waters where natural organic matter is an important factor in controlling speciation. The precipitation of solids cannot be simulated by the model, with the exceptions of iron(III) hydroxide and aluminium hydroxide. The model does not allow redox equilibriums to be simulated; if multiple redox states of an element (e.g. Fe(II) and Fe(III), or U(IV) and U(VI)) are to be simulated, input concentrations of each redox state of the element must be input.

WHAM includes databases for the simulation of ion binding to four metal oxides -iron(III) oxide (specifically ferrihydrite), an amorphous iron(III) oxide, aluminium oxide, manganese oxide - and silica.

3.2.4.2 Visual MINTEQ

Visual MINTEQ is the most readily available model (http://www2.lwr.kth.se/English/OurSoftware/vminteq/) that contains a version of the NICA-Donnan model. The model is a Windows version of the USEPA's MINTEQA2 model, comprising a number of linked submodels:

- Solution speciation, including the formation of solid phases and redox equilibriums;
- Ion binding to organic matter (either dissolved or in the solid phase), using the NICA-Donnan model.

Visual MINTEQ also includes submodels for the binding of ions to solids: metal oxides and clays. Six different types of surface complexation submodel for simulating the binding of ions to metal oxides are included. The model comes with several databases for ion binding to different oxide surfaces: ferrihydrite, manganese oxide, goethite (a crystalline iron(III) oxide) and gibbsite (an aluminium oxide). Additionally, ion binding to fixed charge minerals can be simulated.

3.2.4.3 Geochemist's Workbench (GWB)

The GWB is a sophisticated modelling tool with a wide range of capabilities. It can simulate equilibrium in the solution phase, including oxidation-reduction and precipitation reactions. It can also simulate ion binding to mineral surfaces, including ion exchangers (clays). In principle, any type of surface can be simulated, provided suitable parameters are available. In practice, two databases for ion binding to ferrihydrite are available: the database of Dzombak and Morel (1990), and an expanded version of this database. The model does not have a component specifically for the binding of ions to humic substances. The model's capabilities extend beyond equilibrium modelling to modelling redox disequilibrium, kinetic processes, and 1D/2D transport.

3.2.4.4 CHESS

CHESS, which stands for Chemical Equilibrium with Species and Surfaces, is a speciation model with the following capabilities:

- Equilibrium speciation of ionic species in solution, including the precipitation of minerals and redox;
- Formation and dissolution of colloids. A colloid is a mineral or organic material (e.g. ferrihydrite, humic acid) that is in the aqueous phase of the system being modelled, rather than forming part of the solid phase (e.g. a soil or sediment);
- Binding of ions to minerals, using surface complexation modelling. Three types of model are available. A mineral may have multiple types of surface group;
- Cation exchange with minerals, including the possibility to define a mineral that can simultaneously bind ions through both surface complexation and ion exchange;
- Kinetic control of mineral precipitation and dissolution.

CHESS has a number of databases for ion binding to minerals and organic materials. The most comprehensive of these is for ferrihydrite (termed hydrous ferric oxide, HFO in the model documentation). There are also databases for Aldrich humic acid (AHA), quartz and silica, although the number of binding constants for these phases is limited.

3.2.5 Application of models to field situations

Application of models to field situation requires input data defining the composition of the system under study. Depending on the system to be modelled this may include data on the composition of the solid phase as well as the solution phase. For calculation of speciation in relation to exposure of organisms to contaminants, if sufficient data are available for the calculation to be done solely for the solution phase then this is the preferable option, simply because it is relatively simple.

For exposure (e.g. to apply a Biotic Ligand Model (BLM)in freshwater), then it is preferable to use the solution phase because using the solid phase is not necessary to calculate the "free" metal ion concentrations that the BLM uses to predict toxic effect. Of course this depends upon the medium – the solid phase is a more sensible option to consider in soils and sediments. But even there, confining speciation calculations to the aqueous phase is preferable because it requires fewer input parameters and the uncertainty in the result should be lower. Consideration of the solid phase becomes essential if transport through the environment is being assessed, but this is not the priority of WP4.

For the calculation of speciation in the solution phase, a number of input variables are required alongside the concentrations of the contaminant(s) of concern:

- the solution pH;

- concentrations of major and/or competing ions: Na, Mg, Al, K, Ca, Fe(III), Cl, NO_3, SO_4, CO_3, PO_4;
- concentrations of important binding ligands: e.g. humic substances
- concentration of the radionuclide in question.

Where the sorption of contaminants to a solid phase is to be modelled (e.g. for simulating sediment-solution partitioning in the water column or in soils/sediments), concentrations of the major binding phases are needed. These may include organic matter, oxides of iron(III), manganese, aluminium and silicon (i.e. silica, quartz)and clays. In the scenarios to be assessed here, we use chemical data that relate only to the dissolved (filterable) phase, so that there is no need to consider the role of the non-filterable phase in speciation. The possible presence of minerals in the filterable phase (e.g. iron(III) oxyhydroxides (Lofts et al., 2008)) and their effects on speciation will be considered if the model has the capability to do this.

3.2.6 Databases for chemical speciation of contaminants

All equilibrium speciation models require databases of binding constants to compute speciation. Databases are required for:

- equilibrium speciation of ions in solution, including mineral precipitation and redox if the model is capable of simulating these processes;
- binding of ions to organic matter, oxides and clays.

The models under consideration here are all flexible with respect to database use; so the user can specify a database to use for a particular purpose (solution speciation, binding to organic matter, etc.) if multiple databases are available, the databases themselves may be edited to add or remove specific binding constants, and new databases may be created from scratch. Considering speciation in solution, the capabilities of a specific model with respect to the contaminants it can simulate are flexible – if a binding constant for a specific reaction is not available in any of the model databases, it can be inserted if it is available in the literature or in another database. Care is needed to maintain, as far as possible, the internal consistency of the database when doing this, since many binding constants are not derived in isolation but are calculated in groups from the same set of experimental data. Where this is done, it is strongly advisable to utilise the entire group of binding constants, rather than individual values. Some modification may be required to convert binding constants to the format required by a particular model, but this is not a difficult task with chemical knowledge.

Constants for ion binding to humic substances and mineral surfaces (i.e. oxides) are ‘portable’ between models only if the specific submodel for ion binding is of the same type. So, for example, since Visual MINTEQ and CHESS both contain the surface complexation model of Dzombak and Morel (1990), for ion binding to ferrihydrite, constants relating to this submodel can be used in both Visual MINTEQ and CHESS. On the other hand, binding constants in Model VI and NICA-Donnan model cannot be transferred from one model to the other, because the formulation of the models is different, even though they simulate the same process. In this situation, binding constants need to be calculated for different types of model independently from the source data on ion binding to the oxide in question.

Turning to the issue of available databases for the models considered here, the solution speciation databases currently available for each model are:

- WHAM: a ‘default’ database, compiled in Tipping (1994).
- Visual MINTEQ: a database based on the MINTEQA2 model database, updated with new constants from the NIST thermodynamic database (NIST 46, Critical Stability Constants) versions 6.0 and 7.0.
- Geochemist’s Workbench: this model is currently supplied with a number of databases:
    - thermo.dat - The default database, based on the Lawrence Livermore National Laboratory (LLNL) thermo dataset.
    - thermo.com.v8.r6+.dat - an expanded and updated version of the LLNL database.
    - thermo_phreeqc.dat - The thermodynamic database from the PhreeqC speciation model, release 2.8.
    - thermo_hmw.dat – Data supporting the Harvie-Moller-Weare implementation of the "Pitzer equations" for calculating models of saline waters.
    - thermo_phrqpitz.dat – Data from the Phrqpitz speciation model; an extension of the Harvie-Moller-Weare database.
    - Databases in the GWB format compiled for modelling radionuclide migration by the Japan Nuclear Cycle Development Institute (JNC).
    - The Visual MINTEQ default database.
- CHESS: the model is supplied with a number of legacy databases:
    - chess.tdb: the EQ3/6 (V.8-R.6) speciation model database (Wolery 1992). This database is moderated by disregarding a number of organic redox species and their derived species.
    - eq36.tdb: the full EQ3/6 (V.8-R.6) database (Wolery 1992).
    - minteq.tdb: the MINTEQ speciation model database (Allison et al. 1991).
    - phreeqc.tdb: the PHREEQC speciation model database.
    - nea.tdb: the database compiled in the NEA thermochemical database project.
    - wateq4f.tdb: the WATEQ4F speciation model database.
    - ctdp_v3.tdb is not supplied with CHESS, but is formatted to work with the model. It is based on the merger of the irsn_lre database (Denison, 2002) and the LLNL database (chess.tdb), compiled in the framework of the Common Thermodynamic Database Project in 2006 (www.ctdp.org)
    - ctdp_v3_dong.tdb is the previous database updated with data on ternary complexation of uranyl, carbonate and alkaline earth metals (Dong and Brooks, 2006, 2008; Geipel et al., 2008)

3.2.7 Chemical speciation of multiple contaminants

All speciation models are generic frameworks, designed to simulate the speciation of any ionic elements present in a water (or water-soil, water-sediment) system. Competition among elements, for example for binding to ligands such as phosphate, carbonate, sulphate or humic substances, is automatically simulated. The key limitation on what can be simulated is the availability of binding constants relating to a particular contaminant.

3.3 Measurement of contaminant speciation in the environment

3.3.1 Metals and metallic radionuclides

Speciation analysis is defined as the analytical activity of fractionating, isolating, identifying and quantifying one or more individual species, or classes of species, in a sample, and should include *in situ*, on line, in laboratory fractionation techniques. Depending on the contaminant and the environmental compartment, there are a wide range of potential techniques. It is essential to use speciation and fractionation techniques to obtain information about real-time distributions of species in waters. In general, fractionation of species should take place in the field, *in situ* or at site, to avoid storage of samples, as sorption, aggregation and sedimentation, changes in pH or redox conditions, and bacterial growth during storage will influence the distribution of species. Surface water or soil water speciation techniques typically seek to quantify the free ionic form, the 'labile' form, or to fractionate the sample in terms of size or availability to identify the portion of contaminant that is associated with a particular class of ligand, e.g. dissolved organic matter. Examples of techniques that seek to quantify the free ion include ion-selective electrodes (e.g. Vulkan et al., 2000), competitive ligand exchange voltammetry (e.g. Xue and Sigg, 1999) and the Donnan membrane technique (e.g. Weng et al., 2001a; Kalis et al., 2007). The efficacy of these methods in application to natural systems is an area of ongoing research, and questions remain regarding applicability (e.g. van Leeuwen and Town, 2005). Size fractionation methods include size exclusion chromatography, ultrafiltration/dialysis (e.g. Singhal et al., 2005) and the family of field-flow fraction techniques. Size and charge fractionation has frequently been applied and the combination of these techniques is well suited for speciation in natural water systems. Examples of size fractionation techniques include field-flow fractionation, which continuously separates the colloids present in a water body based on their hydrodynamic size. Lyven et al. (2005) used this technique to show that colloidal-sized uranium in a river flowing into the Baltic Sea was associated with organic matter. Ranville et al. (2007) applied asymmetrical flow field-flow fractionation to the speciation of U in groundwaters and showed that at a pH of around 7.0-8.1, most U was not in the colloidal size fraction but colloidal fraction was associated with organic matter.

Separation on the basis of charge and/or availability usually aims to separate out the fraction of the element bound to organic matter, since organic colloids in natural waters usually possess a negative charge whilst the majority of small solution complexes of metals (e.g. with Cl^-, CO_3^{2-}) are neutral or positively charged. For example, a well-established separation method for Al in acidic waters involves passing the sample through a cation exchange column to separate the relatively labile, positively charged inorganic Al species from the relatively less labile, negatively charged organically-bound Al (Driscoll, 1984). Unsworth et al. (2005) used a column resin separation method to quantify the fraction of organically-bound U in surface waters. Cooper et al. (1995) used anion exchange resin to quantify anionic forms of Co, Ru, Pu and other actinides in groundwater.

Passive samplers, such as Diffusion Gradient in Thin Films (Zhang and Davison, 1995), may also be used to quantify metals or metallic radionuclides based on their availabilities. Such samplers have been applied successfully for heavy metals, such as Cu (Tusseau-Vuillemin et al., 2003). Their applicability for uranium measurement in waters (Li et al., 2006, 2007) or in soils (Vandenhove et al., 2007; Duquène et al., 2010; Mihalik et al., 2012) has been recently tested, but requires further development.

A number of speciation techniques exist that appear to have been applied mainly to radionuclide, particularly actinide, speciation. Valence speciation techniques (e.g. Nelson and Lovett, 1978) are able to separate species on the basis of their oxidation state, e.g. Pu(III) and Pu(IV). Use of advanced spectroscopy techniques has also been carried out. For example, X-ray absorption fine structure (XAFS) has been used to probe the speciation of Pu (including its oxidation state) in organic-rich groundwater (Dardenne et al., 2009). Panak et al. (2003) used time-resolved laser fluorescence spectroscopy (TRLFS) and radiometry to probe the forms of Am(III) and Cm(III) in the presence of hydroxyaluminosilicate (HAS) colloids.

To describe interactions and transfer of metals and metallic radionuclides in soil-water and sediment–water systems, impact assessments have been traditionally based on the solid – liquid distribution coefficient K_d. These constants are based on the assumption that equilibrium conditions are valid in the ecosystems, without taking radionuclide speciation into account. For most radionuclides, published K_d values vary over several orders of magnitude, thus the uncertainties associated with impact assessments based on such constants are correspondingly large. Variations in equilibrium K_d values can be attributed to the variability in the complexation strength of solid phase and solution ligands across different soil types, i.e. to the speciation of the metal/radionuclide. The role of organic matter in the porewater can be of particular importance as it is a strong ligand promoting the desorption from the soil solid into solution. The variation in partitioning can be quantified using chemical speciation models (e.g. Weng et al., 2001b), although recently more emphasis has been placed on using semi-empirical functions to calculate the free metal ion from the soil composition (e.g. Groenenberg et al., 2010). Such approaches are amenable to use for radionuclides if suitable data are available for model parameterisation/testing.

Radioactive particles have been released to the environment following a series of historic events, including nuclear weapon tests, nuclear accidents and dumping of nuclear waste at sea (IAEA, 2011). The particle composition (radionuclides, isotope ratios, metals) will reflect the emitting source, while particle characteristics such as size distribution, structure and oxidation states (i.e. variables that will influencing mobility and bioavailability) will depend on the release scenarios (Salbu, 2007). Information on the solid speciation of radionuclides within such particles, and the weathering rates at which radionuclides in labile forms are released, is important for understanding changes in the radionuclide speciation over time.

The speciation of radionuclides in soil and sediment will depend on species deposited and transformation processes, both of which will influence the distribution of species in ecosystems over time. Input of radionuclides in particulate form to soils introduces a time-dependent aspect to their subsequent behaviour. In contrast to ‘mobile’ forms, the radionuclides in particles remain inert until weathered into the mobile form. Ecosystem transfer is relatively fast for mobile species, whereas the ecosystem transfer is delayed if radionuclides are included in particulates. Weathering of particles will mobilise associated radionuclides and increases ecosystem transfer over time, i.e. the apparent K_d increases (Salbu et al., 2007). Thus, particle-contaminated soils and sediments can act as diffuse sources in the future. Due to interaction with clays or solid phase organic matter, the mobility of species may decrease over time depending on binding mechanisms, so the apparent K_d decreases. This process, sometimes termed 'aging', is well known for metals (e.g. Buekers et al., 2008). Reversibly-associated species can potentially be mobilised due to exchange reactions, while redox processes or microbial activities are needed for mobilisation of irreversibly associated species. Thus, the distribution of metals and radionuclides between solid and solution is a time-dependent process and information on binding mechanisms and kinetics is essential. Itis therefore highly desirable that the equilibrium K_d constants should rather be replaced by time-functions in the future.

Chemical extraction of metals and metallic radionuclides is a useful and frequently applied method to obtain a 'snapshot' of speciation in the solid phase. Methods include quantification of the total 'geochemically active' pool, for example by isotopic dilution, or the estimation of the pools associated with different solid phases, by sequential extraction (Young et al., 2005). The isotopic dilution method has been applied to a number of 'common' metals although its use is constrained by the availability of a suitable isotope to use as a tracer for the element considered. Wet chemical extraction methods, intended to extract the 'geochemically active' pool (e.g. Quevauviller et al., 1997) are more readily applicable although some doubts remain regarding their general applicability. Sequential extraction procedures entail the extraction of the same soil or sediment sample using a series of reagents intended to progressively extract metal from different binding phases (e.g. organic matter, oxides, carbonates) in the solid material, typically starting weakly bound metal and progressing through to residual (i.e. inert) forms. Sequential extraction procedures are most useful when reagents are chosen to differentiate among binding mechanisms. To identify reversible physical sorption, a consecutive layer model is assumed and inert non-reacting electrolytes (no change in pH) are applied. For reversible electrostatic sorption, a monolayer model is assumed and addition of competing ions (lowering the pH) is useful. For irreversible chemisorption, a monolayer model is assumed and redox reagents at elevated temperature should be appropriate. Thus, the fraction of metals/radionuclides which is readily displaced by an inert electrolyte (i.e. H_2O or NH_4OAc with pH of soils) can easily be distinguished from that being released after dissolution due to breakage of chemical bonds induced for instance by redox agents (Salbu, 2000). Therefore, the procedure includes the introduction of reagents with increasing displacement dissolution power, and results should be interpreted according to the reagents applied (e.g.pH sensitive) and not according to some presumed solid phases (e.g.carbonates).

There are no known models predicting size, structure or oxidation states of colloids and particles. Different techniques have been applied to identify the size, structure and oxidation state, as well particles inhomogeneously distributed. Localised heterogeneities (hot spots) can be observed in the field by portable alpha, beta and gamma detectors, or in lab by autoradiography using phosphorous image plates, as a substitute for X-ray and alpha radiation sensitive films. Following autoradiography, several solid state speciation techniques such as electron microscopy and synchrotron radiation X-ray microtechniques have proved most useful (Salbu et al., 2001). Alternative potential solid-state-speciation techniques include µ-PIXE and SIMS providing information on distribution of elements within particles, µ-RAMAN and electron diffraction providing structural information and Electron Energy Loss Spectroscopy (EELS) providing information on oxidation states. For most of these techniques, however, standardisation and calibration with respect to µm sized particles are still needed for proper interpretation of signals. For structure and elemental analysis of submicron particles such as colloids or nanoparticles Transmission electron microscopy (TEM) interfaced with XRMA, Scanning electron microscopy (SEM) interfaced with X-ray microanalysis (XRMA), Synchrotron based X-ray microscopic techniques using microbeams have been applied (Salbu et al., 2001, Eriksson et al., 2005).

3.3.2 Organic contaminants

Study of the speciation of organic contaminants in the environment has been largely concerned with the decline in bioavailability and toxicity of persistent organic compounds in soils and sediments over time following their input (Alexander, 2000). The causal processes leading to the decline (collectively termed ‘aging’ (similar considerations apply to metals and metalloid elements; see section 3.1.1)) are not well understood. Some of the variation in aging across different soils/sediments has been correlated with soil properties (e.g. Alexander, 2000). Solid phase microextraction (SPME) (Arthur and Pawliszyn, 1990) has been extensively used in the assessment of the ‘free’ fraction of persistent organic compounds in porewaters (e.g. Liu et al., 2011).

The binding of organic contaminants to dissolved organic matter has also been a focus of study, as DOM-binding may affect processes such as loss by volatilization, degradation, and biotic uptake. Typically, binding is assessed by measurement of the octanol-water partition coefficient of a contaminant in the presence and absence of DOM (e.g. Burkhard, 2000; Whelan et al., 2010).

3.4 Bioavailability Modelling

3.4.1 Meaning and implications of bioavailability

Bioavailability refers to the tendency of a contaminant to be able to be taken up by organisms, leading to its accumulation and/or toxicity. In broad terms, bioavailability refers to uptake by any route (e.g. ingestion or uptake through the dermis). In practice, the most-developed tools for predicting bioavailability (such as the BLM) focus on direct uptake of a contaminant from the aqueous phase in aquatic and terrestrial ecosystems (e.g. via binding to the gills, dermis, root cells) and we will focus on this type of model. Though this has not been developed and studied, in principle the BLM approach could also be applied to gut and long.

Research into the direct uptake of ionic and non-ionic contaminants suggests that, broadly speaking, ionic contaminant bioavailability can be considered in terms of contaminant binding to sites in or on the organism (the biotic ligand), while non-ionic contaminant uptake can be considered in terms of the tendency of the compound to partition into a lipid phase, representing the fatty tissues of an organism. There are of course exceptions; for example, ionic species can form neutral complexes with ligands that may have a different bioavailability than charged complexes. However, the available tools for modelling bioavailability focus on contaminants for which one or other mechanism can be considered the dominant means of uptake.

Some important issues are not dealt with in this report, but need consideration in general exposure and risk assessment. Most bioavailability studies are performed under equilibrium conditions whereas reality is different. An organism may be exposed to contaminants present in fluctuating concentrations, with high and low exposure peaks and to continuously different contaminant ratios. Further when considering the exposure assessment of especially higher organisms, like mammals, food-chain issues might additionally modify exposure of such species, next to all other aspects mentioned in this report.

3.4.2 Metal bioavailability: critical evaluation of BLM and empirical bioavailability models

The Biotic Ligand Model (BLM) is a widely recognised predictive tool, used by ecotoxicologists and proposed for use by some regulatory bodies, which accounts for variations in metal toxicity with water quality. It is fundamentally a chemical equilibrium model, based on the assumption that toxicity is determined by the strength of metal binding to the site of toxicity in organisms. As such, the model combines knowledge on chemistry, physiology and toxicology, in a simplified way.

3.4.2.1 The Gill Biotic Ligand Model

The first model developed for the interaction between trace metals in water and the gill surface was the Gill Surface Interaction Model (GSIM) introduced by Pagenkopf (1983). This was also the first model to account for differences in water chemistry (hardness, pH, trace metal complexation and gill reactivity) in relation to toxicity of metals. A similar model was developed for the critical binding sites on algae, called the Free Ion Activity Model (FIAM) (Morel 1983). Both models recognized the importance of DOC in complexing reactions, and accepted that metals other than free ions (Low Molecular Mass -LMM- ionic species) could bind to critical sites. As such the GSIM and FIAM are the ancestors of the Biotic Ligand Model (BLM) (Di Toro et al. 2001; Niyogi and Wood 2004).

The model has been developed for a number of metals and organisms, but the gill is perhaps the most commonly referred to biotic ligand, largely reflecting the fact that the first applications of the BLM concept were made to fish. The acute mechanism of toxicity for many metals (e.g. Pb and Cu) was linked to disruption of ion regulation following accumulation on fish gills. In developing the model, the concentrations of metals on gills (i.e. the biotic ligand) of exposed fish were measured and compared to observed toxic effects. The BLM uses water quality data to predict the degree of metal binding (e.g., Cu and Pb) at the site of action (e.g., gills), and the level of accumulation is in turn related to a toxicological response (Paquin et al. 2002; Niyogi and Wood 2004). The BLM model for fish is utilized within REACH for a series of metals including Al and Fe.

3.4.2.2 Application to other organisms

The BLM has later been applied to other organisms, both aquatic, e.g. *Daphnia magna* (De Schamphelaere and Janssen 2002), and terrestrial including plants, invertebrates and microbes (Steenbergen et al. 2005; Koster et al. 2006; Thakali et al. 2006a; Thakali et al. 2006b). In these examples there is no explicit identification of the site of action (or the biotic ligand), hence the model is parameterised by fitting whole organism, water or soil concentrations directly to the toxicity data. Some of the most comprehensive work has been carried out on copper, wherein effects have been compared in two plant species, two invertebrates and three microbial processes (Thakali et al. 2006a,b). Given the more complex chemical environment of soils, and the relative lack of chemical data typically available in toxicity studies for parameterisation of BLMs, empirical approaches to describing the variability in metal toxicity with soil properties have also been developed (e.g. Lofts et al., 2004; Song et al., 2006)

In the terrestrial environment, work has tended to focus more on the importance of soil chemistry and metal speciation on bioavailability and toxicity, for example in comparing metal toxicity to invertebrates in different soils, or on efficiency of metal, including radionuclide, transfer to plants. In an extensive study on earthworm toxicity, Pb (2,000 mg kg^{-1}) was added to twenty-one soils with a wide range of properties to determine the effects of soil chemistry on Pb bioavailability and toxicity to earthworms (Bradham et al., 2006). Earthworm mortality ranged from 0 to 100% acute mortality, and internal Pb concentrations in earthworms ranged from 28.7 to 782 mgkg^{-1}, with a mean of 271 mgkg^{-1}. Path analysis was used to partition correlations in an attempt to discern the relative contribution of each soil property. Results indicated that pH was the most important soil property affecting earthworm mortality ($p < 0.01$) and internal Pb ($p < 0.05$). Soil pH was related inversely to mortality and internal Pb, soil solution Pb, and Pb bioavailability. The most important soil property modifying reproduction was amorphous iron and aluminium oxides (FEAL). Because FEAL is rich in pH-dependent cation-exchange sites, several soil properties, including pH, FEAL, and cation-exchange capacity, have a causal effect on Pb adsorption and soluble Pb. Similar results of variability of toxicity with soil properties were found for Cd, As and Zn (Bradham et al., 2006). Nevertheless, while the results are of obvious interest and relevance to the BLM concept, the paper makes no reference to the BLM.

3.4.2.3 The BLM and radionuclides

There is not much literature on the application of the BLM for modelling of radionuclides, besides a few papers on BLM type models in relation to uranium. Alves et al. (2008) explained bioaccumulation of uranium in the crustacean *Hyalella azteca* satisfactorily using a BLM type saturation model. Fortin et al. (2007) found that the proton-metal competition described by the BLM was not successful in depicting algae-uranium interactions, although earlier studies for green algae showed that the accumulation of uranium was correlated to the free uranyl concentrations as predicted by the BLM and FIAM (Fortin et al. 2004). Similarly, the effect of varying solution composition on the bioavailability of uranium on excised gills of the freshwater *Corbicula fluminea* (Denison, 2004) showed relatively small change as a function of pH (factor of ca. 2), despite the extremely large changes to the solution speciation within the range of pH investigated (5.0 to 7.5). Thermodynamic equilibrium modelling, including uncertainty analysis (Denison *et al.*, 2005), did not allow to conclude on the most plausible hypothesis for uranium bioavailability (uptake of inorganic uranium complexes, competition with protons, changes to membrane transport system activity).

3.4.2.4 Application of the BLM in mixture exposures

Some work has been done on the application of the BLM to metal mixtures (Playle 2004; Birceanu et al. 2008; Borgmann et al. 2008; Hatano and Shoji 2008; Kamo and Nagai 2008; Chen et al. 2010; Jho et al. 2011). Playle (2004) postulated a theoretical model for metal mixtures (up to 6 metals simultaneously) by combining existing BLM's for single metals in MINEQL+ to generate predictions on mixture effects using the toxic unit (TU) concept. Briefly, the authors assumed an LA50 (50% lethal accumulation) when half the gill sites were occupied by a metal (Paquin et al., 2002), and one toxic unit was defined as the corresponding LC_{50}, - 50% lethal concentration (See Newman and Unger (2003) and section 4.2.1 for further discussion of the TU concept). For Pb, half the gill sites in this simulation were filled at an aqueous concentration of 6.0 µM Pb

The main conclusions from the model simulation were that multiple metal-gill modelling yields greater than strict additivity at low aqueous metal concentration, less than strict additivity at high concentrations and strict additivity when the metals sum one toxic unit. The content of natural organic matter can influence the metal interaction. A test of the model was performed for the binary exposure to Pb and Cd to rainbow trout (*Oncorhynchus mykiss*), where it was concluded that the mixture interacted in a far more complex manner than suggested by the model (Winter, 2008).

Hatano and Shoji (2008) tested the combined toxicity of Cu and Cd to duckweed (*Lemna paucicostata*), assuming Cu^{2+} and Cd^{2+} to bind to the same biotic ligand in competition with H^+. They found that the BLM clearly predicted the toxicity of mixtures of the metals, using the toxic unit approach. Kamo and Nagai (2008) modified the BLM to be able to predict the toxicity of Zn, Cu and Cd to rainbow trout (*O.mykiss*), however, the results were not tested using live animals, only a theoretical outcome. Jho (2011) proposed a version of the BLM to predict mixture toxicity of Pb and Cd that assumed that (i)both metals bind to the same biotic ligand, in competition with Ca^{2+}, and (ii) the potency of the metals is the same, i.e. that one mole of biotic ligand-bound Pb results in the same level of effect as one mole of biotic ligand-bound Cd. This model was shown to predict mixture toxicity better than a model assuming the metals to bind to different biotic ligands. However, while the paper builds on the BLM concept to include competition between both toxic metals and other ions (e.g., Ca^{2+}), it still makes a key assumption, namely that Cd^{2+} and Pb^{2+} are competing for the same biotic ligand with the same potency (i.e., one Cd ion binding to the BL has the same effect as one Pb ion). Furthermore, for mixture toxicity it is important to recognize that

a) not all metals may necessarily bind to a common biotic ligand, and so may require different BLMs when mixture effects are considered;

b) a robust and realistic BLM should be able to account for non-additive effects resulting from competition among metals at the BLM, butno matter how the bioavailability modelling is carried out, it will not be able to account for biochemical interactions between and among individual toxicants in a mixture that may modify the toxic effect (i.e. non-additive effects of mixtures).

3.4.3 Bioavailability of organic compounds

The tendency of organic compounds (both non-ionic compounds and compounds that may form ionic species) to be bioavailable has been studied. Studies have largely focused on compounds that exert effects by narcosis. Since the tendency to cause effects by narcosis is related to the lipophilicity and hydrophobicity of the chemical, the toxicity of sets of narcotic compounds to a particular organism is related to their octanol-water partition coefficients (K_{OW}). The latter can be considered to be a measure of the tendency of each compound to dissolve in water or an organic solvent (hydrophobicity)and thus is related to the tendency to partition and accumulate in fatty tissues (Di Toro et al., 2002).

3.5 Overview of exposure scenarios for radionuclides and co-contaminants

3.5.1 Introduction

As mentioned in the introduction, there are many situations where the environment is impacted by both radionuclides and conventional contaminants. For a number of exposure scenarios we have reviewed the type sand quantity of radionuclides and co-contaminants released to the environment. Additional information is provided to allow an appreciation of the environmental relevance of these releases and the resulting environmental concentrations. The different industries reviewed were: uranium mining and milling, nuclear fuel production, nuclear power plants, reprocessing plants, nuclear waste disposal and the NORM industry. For hospitals no relevant information on released co-contaminants could be found.

A further motivation for the review was to select mixtures of interest for further experiments. Four criteria were considered:

- the capacity of the co-contaminant(s) to modify the chemical speciation in the medium and bioavailability of the radionuclide(s) of interest;
- the possible toxicokinetic and toxicodynamic interactions between the co-contaminant(s) and the radionuclide(s);
- the mode of action of each co-contaminant(s) and the radionuclide(s), either similar or dissimilar;
- the environmental relevance of the co-contaminant(s) and the radionuclide(s) based on either the quantity released per year by the nuclear industry or the possibility to exceed environmental guideline values (such as PNEC).

To begin with we will focus on the quantity of radionuclides and chemicals released by the nuclear power plant industry. We also give additional information on the environmental relevance of these releases.

Important exposure scenarios for contaminant mixtures with radionuclides occur from industries that release multiple contaminants. However, man and environment may also become exposed to an increasing contaminant background through diffusive contamination. There are many published examples of Hg from coal combustion, pesticides, endocrine disruptors, etc. that are ubiquitous in developed countries and that are commonly found in humans. These now constitute a ”new background” to which ionizing radiation can interact. These elevated background contaminants are possibly as important to investigate as are the co-contaminants that come from a single (nuclear) facility. This is, however, not evaluated in the present report.

3.5.2 Comparison of contaminant releases and environmental relevance for a number of scenarios.

It is clear from the studies discussed that many co-contaminants are present and should be considered when assessing the potential impact from nuclear fuel production, nuclear power plants and reprocessing plants. However the extent to which environmental guideline values are exceeded seems to be small although a detailed survey of all EU countries is beyond the scope of this review. For the French case, only Cu and to a minor extent, hydrazine, might exceed the (French) environmental guideline values and this only for the nuclear power plants.

For low, medium and high level waste disposal, the expected environmental concentrations are much lower than the guideline values and as such all these co-contaminants released are not expected to affect the impact from radionuclides or ionising radiation. Also for radionuclides the levels are extremely low and no impact is expected.

For the uranium mining and milling industry as well as for NORM industries, where radiological impact is linked with U and Th-series radionuclides, the picture is different. It is clear from a partial review of co-contaminants in the uranium mining and milling industry, that heavy metals (Cd, Cr, Cu, Pb, Zn, …) or metalloids such as As may be present at concentrations higher than the guidelines. Additionally, other elements such as Ba, Fe, Al, F, Cl, Se may be of concern. The actual contaminants of concern will be highly site specific.

For the NORM industry, concentrations exceeding the guideline values have mostly been reported for heavy metals (e.g. Cd, Cu, Pb, Zn, …). For the oil and gas extraction industry significant releases of organic components as well as scale inhibitors have been reported.

3.5.3 Preliminary tool testing: can we use geochemical speciation models to evaluate the effects of multi-contaminants on radionuclide availability

3.5.3.1 Scenarios selected

As stated above, levels of co-contaminants present in the environment are highest for the uranium mining and milling industry. We therefore selected these sources as they represent an important case for evaluating whether co-contaminants would impact the speciation and hence environmental availability of the radionuclides of interest using the selected geochemical speciation models.

We selected two case studies for which we had relevant and adequate information on environmental characteristics to perform geochemical speciation calculations. The two test cases 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 also high levels of Fe and Al for which monitored surface water values were above French guidelines values. Therefore, Fe and Al could also be considered as contaminants. The Ritord ecosystem has a slightly acid pH. 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).

3.5.4 Detailed description of scenarios

3.5.4.1 Ritord

The Ritord basin is situated in the Limousin region of France and contains several closed uranium mines. Chemically treated mine waters are discharged to surface water at two locations within the catchment, following chemical treatment. For this scenario we chose a single water sample, taken on 18th June 2009, downstream of the uppermost mine water discharge. The chemical treatment of the mine water comprises addition of barium chloride to precipitate radium, aluminium sulphate to co-precipitate iron and uranium, and a flocculating agent to minimise suspended solids. The physical-chemical data available comprised:

> Physical-chemical: pH, temperature, conductivity, dissolved oxygen, suspended particulate matter, dissolved inorganic carbon, dissolved organic carbon (DOC).
>
> Major and trace ions: sodium, magnesium, potassium, calcium, silicon, ammonium, chloride, nitrite, nitrate, sulphate, phosphate .
>
> Contaminants: manganese, barium, aluminium and iron (as stated above).
>
> Radionuclides: uranium(and associated daughter products).

Concentrations of major and trace ions, contaminants and radionuclides refer to the dissolved phase.

The system is slightly acidic (pH 6.4) with moderate hardness (58 mg $CaCO_3$ l^{-1}) although this is elevated compared to the upstream unimpacted site (8.8 mg $CaCO_3$ l^{-1}) as a result of the mine water discharge. The dissolved organic carbon concentration is also elevated (9.6 vs. 6.3 mg C l^{-1}) as is the sulphate concentration (48 mg l^{-1} vs. 2.6 mg l^{-1}). The dissolved inorganic carbon concentration is slightly lower downstream of the discharge (2.0 mg l^{-1} vs. 2.4 mg l^{-1}). The water is also enriched in dissolved Ba (224 µg l^{-1} vs. 7.2 µg l^{-1}) and dissolved U (35 µg l^{-1} vs. 1.1 µg l^{-1}). There is slight enrichment in dissolved Al (56 µg l^{-1} vs. 48 µg l^{-1}) and dissolved Fe is lower (396 µg l^{-1} vs. 3053 µg l^{-1}). There appears to be a loss of dissolved Fe between the upstream reference site and the scenario site as the mine water discharge increases the ionic strength of the stream. The decrease in Fe could be attributed to the aggregation of Fe oxy/hydroxides colloids provoked by the increased ionic strength, and subsequent sedimentation.

3.5.4.2 Beaverlodge Lake

The location of the scenario is close to Uranium City in northern Saskatchewan, Canada. Past uranium mining operations have caused contamination of a number of local surface waters (lakes and streams). The scenario location is at the outflow of Greer Lake, upstream of its inflow into Beaverlodge Lake, the largest water body in the area. We used mean water chemistry data for the period July 1st 2003 to 30th June 2004 (Environmental Management Group, 2004), with the exception of DOC, for which a single monitored value from 2011 was used since no corresponding data were available for the earlier time period. The chemistry data comprised the following determinants:

Physical-chemical: pH, hardness, alkalinity, temperature, conductivity, total suspended solids, total dissolved solids

Major and trace ions: sodium, potassium, magnesium, calcium, iron, chloride, sulphate, carbonate, hardness, alkalinity.

Contaminants: arsenic, barium, copper, nickel, lead, selenium, zinc.

Radionuclides: lead-210, polonium-210, radium-226, thorium-230, uranium

Concentrations of major and trace ions, contaminants and radionuclides refer to the dissolved phase. Each determinant was the mean of between 2 and 11 individual samples. The water was alkaline with a mean pH of 8.0. Water hardness was moderate (84 mg l^{-1} as $CaCO_3$) and the total carbonate concentration was 171 mg l^{-1}. Mean water temperature was 1^{o}C. Concentrations of metals were 1.8, 560, 1, 1, 3, 4.8 and 5 µg l^{-1} for As, Ba, Cu, Ni, Pb, Se and Zn respectively so Ba seems to be the main co-contaminant of non-radionuclide origin in the system. The dissolved organic carbon concentration used was 14 mg C l^{-1}.

3.5.5 Evaluation of geochemical model performance

A detailed description of the results of the geochemical speciation model evaluations, including the assumptions used to make each prediction and a description of the binding constant databases used, is provided in Annex 2. Here we summarise the most important results of the speciation analysis in table form and present the main features of the results. The predicted speciation of U using different speciation models is summarised in Tables 1-5.

Generally, carbonate complexes are important contributors to U speciation, particularly in the higher pH Beaverlodge Lake scenario (Table 1). In the Ritord scenario (Table 3), hydrolysis products are also important, along with silicate and mixed carbonate-hydroxo complexes where the database used allows the formation of such species. Dissolved Organic Matter (DOM) is relatively unimportant as a complexant in the Beaverlodge Lake scenario, due to strong competition from carbonate (Table 2). In the Ritord scenario, DOM is predicted to be more important, but its predicted significance varies considerably among the models (Table 4-5).

Overall, the effect of co-contaminants on the speciation of the radionuclides of concern was not significant in the scenarios assessed, except to some extent in the Ritord scenario (Table 5) because of the extremely high Fe and Al concentrations.

- The choice of model and binding constant database has an important influence on the results. For example: in the predictions made for the Beaverlodge scenario using the GWB (Table 1), the predicted speciation is highly dependent upon which of the three binding constant databases (Thermo.com.V8.R6, Thermo.Minteq or NEA/NO3) is used to make the calculations. When Thermo.com.V8.R6 or NEA/NO3 are used, the speciation of U is dominated by two carbonate complexes ($UO_2(CO_3)_3^{4-}$and $UO_2(CO_3)_2^{2-}$). When Thermo.Minteq is used, however, the speciation is predicted to be dominated by the ternary Ca-UO_2CO_3 complex $Ca_2UO_2(CO_3)_3$, which is not present in either of the other two databases. A similar pattern is seen when comparing the results of the CHESS predictions using the Chess and Ctdp_v3_Dong databases.

- Differences in the predicted speciation may be seen when comparing predictions made using databases having similar sets of binding constants. In the Beaverlodge scenario (Table 1), the predictions made by (i) GWB using the Thermo.Minteq database, (ii) CHESS using the Ctdp_v3_dong database and (iii) Visual MINTEQ using its default database, all have the same set of possible U complexes forming. However, the prediction made using the Geochemist’s Workbench and Thermo.Minteq shows a notably different distribution of the abundances of these complexes, compared to the other two predictions.
- The implementation of dissolved organic matter differs among the models, and this has an important influence on the results (Table 2 for the Beaverlodge scenario, Tables 4 and 5 for the Ritord scenario). This influence is most obvious in the predictions of the Ritord scenario. The GWB model was run using either EDTA or citrate as an analogue for dissolved organic matter, producing very different predictions of its importance (unimportant using using EDTA, the most abundant complex when using citrate). WHAM and Visual MINTEQ both allow the simulation of dissolved organic matter as fulvic acid, which is more realistic than using a simple organic substance as an analogue. GWB gives very different predictions depending on which analogue is used (Table 4), with EDTA having little influence on the speciation, but citrate having a large influence. WHAM and Visual MINTEQ also give contrasting predictions, with DOM predicted to be less important by WHAM than by Visual MINTEQ. This is due to WHAM being able to simulate the binding of U to colloidal iron(III) hydroxide (FeOx), which Visual MINTEQ does not. Therefore, Visual MINTEQ predicts DOM complexation to be more important for U than does WHAM. In the absence of co-contaminants (which are assumed to include Fe(III)), WHAM cannot form the Ucomplex with FeOx and predicts that U-DOM complexes will be of a similar significance as Visual MINTEQ predicts.

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Table 1. Summary of predicted U speciation for the Beaverlodge Lake scenario, including co-contaminants, in the absence of DOM, by all the model/database combinations used. The numbers are the % abundance of the species. Only species predicted by at least one of the models to have an abundance of >1% total U are listed. The term 'Absent' indicates that the species in question was not present in the database used to generate the prediction. The term UO_2-FeOx refers to U predicted to be complexed at the surface of colloidal iron(III) hydroxide, $Fe(OH)_{3\,(s)}$.

Model	GWB			CHESS		WHAM	Visual MINTEQ
Database	Thermo.com.V8.R6	Thermo.Minteq	NEA/NO3	Chess	Ctdp_v3_Dong	Default	Default
$UO_2(CO_3)_3^{4-}$	58.0	3.5	84.4	52.8	1.2	Absent	4.7
$UO_2(CO_3)_2^{2-}$	40.1	1.6	15.2	44.0	1.0	96.7	(<1)
$UO_2(OH)_2$	1.3	(<1)	(<1)	1.9	(<1)	(<1)	(<1)
$CaUO_2(CO_3)_3^{2-}$	Absent	2.1	Absent	Absent	56.8	Absent	46.0
$Ca_2UO_2(CO_3)_3$	Absent	92.7	Absent	Absent	40.6	Absent	49.0
UO_2CO_3	(<1)	(<1)	(<1)	(<1)	(<1)	1.5	(<1)
UO_2-FeOx	Absent	Absent	Absent	Absent	Absent	1.8	Absent

Table 2. Summary of predicted U speciation for the Beaverlodge Lake scenario, including co-contaminants, in the presence of DOM, by all the model/database combinations for which simulations including DOM were done. The numbers are the % abundance of the species. Only species predicted by at least one of the models to have an abundance of >1% total U are listed. The term 'Absent' indicates that the species in question was not present in the database used to generate the prediction. The term UO_2-FeOx refers to U predicted to be complexed at the surface of colloidal iron(III) hydroxide, $Fe(OH)_{3\,(s)}$. The models 'GWB with EDTA' and 'GWB with citrate' refer to the GWB model using EDTA and citrate as proxies for DOM.

Model	GWB with EDTA	GWB with citrate	WHAM	Visual MINTEQ
Database	Thermo.Minteq	Thermo.Minteq	Default	Default
$UO_2(CO_3)_3^{4-}$	3.6	4.2	Absent	5.0
$UO_2(CO_3)_2^{2-}$	1.6	1.9	94.4	(<1)
$UO_2(OH)_2$	(<1)	(<1)	(<1)	(<1)
$CaUO_2(CO_3)_3^{2-}$	2.1	2.3	Absent	46.9
$Ca_2UO_2(CO_3)_3$	92.7	91.7	Absent	46.5
UO_2CO_3	(<1)	(<1)	1.5	(<1)
UO_2-FeOx	Absent	Absent	(<1)	Absent
UO_2-DOM	(<1)	(<1)	3.2	1.2

Table 3. Summary of predicted Uspeciation for the Ritord scenario, including co-contaminants, in the absence of DOM, by all the model/database combinations used. The numbers are the % abundance of the species. Only species predicted by at least one of the models to have an abundance of >1% total Uare listed. The term 'Absent' indicates that the species in question was not present in the database used to generate the prediction. The term UO_2-FeOx refers to U predicted to be complexed at the surface of colloidal iron(III) hydroxide, $Fe(OH)_{3\,(s)}$. CHESS predictions have been made with precipitation/dissolution equilibria both disabled and enabled.

Model	GWB		CHESS		CHESS		WHAM	Visual MINTEQ
Database	Thermo.com.V8.R6	Thermo.Minteq	Chess	Ctdp_v3_Dong	Chess	Ctdp_v3_Dong	Default	Default
			Precipitation disabled		Precipitation enabled			
UO_2^{2+}	(<1)	(<1)	(<1)	1.3	(<1)	1.3	(<1)	(<1)
UO_2OH^+	4.5	8.4	2.9	7.1	(<1)	7.1	2.3	9.0
$UO_2(OH)_2$	79.0	2.4	82.0	10.4	9.1	10.4	(<1)	7.3
UO_2CO_3	12.1	68.3	10.0	35.4	1.1	35.4	4.1	57.0
$UO_2(CO_3)_2^{2-}$	2.5	6.2	1.5	5.5	(<1)	5.5	(<1)	8.8
$UO_2(CO_3)_3^{4-}$	(<1)	(<1)	(<1)	(<1)	(<1)	(<1)	Absent	(<1)
$(UO_2)_2CO_3(OH)_3^-$	1.3	5.9	2.9	38.5	(<1)	38.5	Absent	Absent
$CaUO_2(CO_3)_3^{2-}$	Absent	(<1)	Absent	(<1)	Absent	(<1)	Absent	4.0
$Ca_2UO_2(CO_3)_3$	Absent	(<1)	Absent	(<1)	Absent	(<1)	Absent	3.5
$UO_2H_3SiO_4^+$	Absent	7.9	Absent	Absent	Absent	Absent	Absent	8.5
UO_2-FeOx	Absent	Absent	Absent	Absent	Absent	Absent	92.5	Absent
$(UO_2)_2SiO_4.H_2O$ (s)	Absent	Absent	Absent	Absent	89.2	Absent	Absent	Absent

Table 4. Summary of predicted U speciation for the Ritord scenario, including co-contaminants, in the presence of DOM, by all the model/database combinations for which simulations including DOM were done. The numbers are the % abundance of the species. Only species predicted by at least one of the models to have an abundance of >1% total Uare listed. The term 'Absent' indicates that the species in question was not present in the database used to generate the prediction. The term UO_2-FeOx refers to U predicted to be complexed at the surface of colloidal iron(III) hydroxide, $Fe(OH)_{3\ (s)}$. The models 'GWB with EDTA' and 'GWB with citrate' refer to the GWB model using EDTA and citrate as proxies for DOM.

Model	GWB with EDTA	GWB with citrate	WHAM	Visual MINTEQ
Database	Thermo.Minteq	Thermo.Minteq	Default	Default
UO_2^{2+}	(<1)	(<1)	(<1)	(<1)
UO_2OH^+	8.4	1.8	2.1	(<1)
$UO_2(OH)_2$	2.4	(<1)	(<1)	(<1)
UO_2CO_3	68.0	14.2	3.8	2.6
$UO_2(CO_3)_2^{2-}$	6.2	1.3	(<1)	(<1)
$UO_2(CO_3)_3^{4-}$	(<1)	(<1)	Absent	(<1)
$(UO_2)_2CO_3(OH)_3^-$	5.9	(<1)	Absent	Absent
$CaUO_2(CO_3)_3^{2-}$	(<1)	(<1)	Absent	(<1)
$Ca_2UO_2(CO_3)_3$	(<1)	(<1)	Absent	(<1)
$UO_2H_3SiO_4^+$	7.8	1.6	Absent	(<1)
UO_2-FeOx	Absent	Absent	82.2	Absent
UO_2-DOM	(<1)	80.1	10.8	95.7

Table 5. Summary of predicted Uspeciation for the Ritord scenario, in the presence of DOM, by WHAM & Visual MINTEQ. The numbers are % abundance of the species. Only species predicted by at least one of the models to have an abundance of >1% total Uare listed. The term 'Absent' indicates that the species in question was not present in the database used to generate the prediction. The term UO_2-FeOx refers to U predicted to be complexed at the surface of colloidal iron(III) hydroxide, $Fe(OH)_{3\ (s)}$.

Model	WHAM		Visual MINTEQ	
Database	Default		Default	
Co-contaminants	Present	Absent	Present	Absent
UO_2^{2+}	(<1)	(<1)	(<1)	(<1)
UO_2OH^+	2.1	(<1)	(<1)	(<1)
$UO_2(OH)_2$	(<1)	(<1)	(<1)	(<1)
UO_2CO_3	3.8	1.5	2.6	(<1)
$UO_2(CO_3)_2^{2-}$	(<1)	(<1)	(<1)	(<1)
$UO_2(CO_3)_3^{4-}$	Absent	Absent	(<1)	(<1)
$(UO_2)_2CO_3(OH)_3^-$	Absent	Absent	Absent	Absent
$CaUO_2(CO_3)_3^{2-}$	Absent	Absent	(<1)	(<1)
$Ca_2UO_2(CO_3)_3$	Absent	Absent	(<1)	(<1)
$UO_2H_3SiO_4^+$	Absent	Absent	(<1)	(<1)
UO_2-FeOx	82.2	[a]	Absent	[a]
UO_2-DOM	10.8	97.3	95.7	99.5

[a] Not formed since Fe(III) was not input to the model

3.5.6 Discussion and main conclusions

3.5.6.1 Speciation (environmental availability) models

Speciation models are well established tools for predicting the chemistry of metals and radionuclides assuming thermodynamic equilibrium conditions. A detailed comparative assessment of models is presented here. As already discussed, models vary in the scope of chemical species (solution complexes, solid phases, complexes with humic substances and mineral solids) for which speciation can be simulated. The models chosen for the assessment here provide a good example of this range of capabilities. To validate the models, however, experimental data is still needed.

Since the models should all give similar results for speciation in solution (not including DOM when modelled as humic substances in WHAM and Visual MINTEQ) if identical databases are used, the comparison of models in the absence of DOM is essentially a comparison of the databases. So, for example, the WHAM default database and Thermo.com.v8.r6+.dat have fewer carbonate complexes of U defined than do the other databases, and the predicted speciation is thus dominated by different carbonate complexes.

In the Beaverlodge Lake scenario, carbonate complexes are predicted to dominate the U speciation, although the predicted distribution of U among its carbonate complexes depends on which of these complexes are defined in the database. The predictions made by Visual MINTEQ and CHESS suggest that the ternary Ca-UO_2-CO_3 complexes (Dong and Brooks, 2006, 2008) are important, and their incorporation into the other databases should be considered. The U speciation is consistently predicted to be dominated by carbonate complexes, even when (for WHAM and Visual MINTEQ) humic substances are included. This suggests that as this pH, carbonate can compete effectively with humics for U, even though the DOC concentration in the scenario is relatively high (compared to a global median of 5.2 mg C l^{-1} estimated from the data presented by Harrison et al., 2005). This should however not be taken to mean that binding of U to DOM will generally not be significant. Goulet et al. (2011) made theoretical predictions of the binding of U to fulvic acid over a wide pH range, which suggested that the proportion of U bound to DOM will vary with pH, with a maximum at a pH of approximately 7.4 and, at a given pH, increasing with the concentration of DOM in the solution. This is borne out by the predictions of WHAM and Visual MINTEQ for the Ritord scenario (pH = 6.4) where DOM was predicted to be a more significant binding ligand than was predicted for the Beaverlodge scenario.

In the Ritord scenario, the models generally predict that hydrolysis products and carbonate complexes are important, although the relative importance of different complexes varies from model to model. Again, this is a function of the databases, not the specific model. In WHAM and Visual MINTEQ, DOM is predicted to bind some if not most of the U, but WHAM predicts that DOM is more important for U binding than does Visual MINTEQ. The reasons why this occurs need to be investigated; it may be because of differences in the databases for binding to inorganic ligands between the two models.

Using citrate as an analogue for DOM in the GWB predictions produces a prediction of extensive binding, but using EDTA does not. This suggests that if an analogue for DOM is needed it should be chosen carefully as using different organic ligands may produce very different predictions. The most realistic results will be produced using a model that can simulate humic substances, since these organic substances are known to be the most important organic ligands for metals and metallic radionuclides.

The importance of Fe(III) and Al as co-contaminants and as influences on metal and radionuclide speciation is unavoidably complex. Both occur naturally and ubiquitously in surface waters. Natural concentration ranges of Fe(III) and Al in freshwaters have important influences on the speciation of metals (and by extension, radionuclides) as their strong binding to humic substances makes them important competitors (Tipping et al., 2002). Both are rather insoluble and Fe(III) is known to form a colloidal oxyhydroxide in freshwaters, which itself can influence speciation by binding metals (e.g. Lyven et al., 2003). Although the solubility of Al in freshwaters appears to be controlled by its oxide (Tipping, 2005), evidence for the presence of this oxide in freshwaters is not currently available. Contamination of surface waters by Fe(III) can occur due to industrial discharges and also due to mining activities. Excess Fe(III) in freshwaters will be present as colloids, usually Fe-oxyhydroxide form and will influence speciation by competing with other ligands, such as DOM and carbonate, for metals and metallic radionuclides. In this assessment, WHAM predicted that Fe(III) oxide may be an important complexer of U. This is, however, not borne out by the small amount of experimental evidence available (e.g. Lyven et al., 2003). Contamination of surface waters by Al can occur via industrial discharge, mining activities and also by soil acidification. Excess Al in freshwaters is also likely to be present in colloidal form although knowledge about the exact form is poor. In both cases, assuming equilibrium in the water, the presence of an excess due to contamination should not increase the ‘truly dissolved’ (i.e. non-colloidal) concentration and thus not increase the competition of these elements for binding to dissolved ligands.

Overall, the effect of co-contaminants on the speciation of the radionuclides of concern was not significant in the scenarios assessed, although interactions due to high conc of Fe and Al cannot be excluded.. This is because competition between multiple metals for binding to a given ligand tends to become important (and to thus have effects on predicted speciation) only when the metal:ligand ratios become relatively large, which is not generally the case for trace metals and metallic radionuclides. Nonetheless, speciation modelling remains important if the influence of bioavailability on exposure to metallic species is to be addressed.

Considering the further use of speciation models within STAR, all the model/database combinations used in this assessment have advantages and disadvantages. These largely relate to (i) the comprehensiveness of the different databases, (ii) the ability to simulate complexation to humic substances (dissolved organic matter) and mineral oxides, and (iii) the ability to simulate oxidation-reduction and precipitation reactions.

The most comprehensive model used here is Visual MINTEQ, which can simulate complexation to humic substances and mineral oxides, oxidation-reduction and precipitation reactions. The other models have a lower level of capability in this area. WHAM can simulate complexation to humic substances and mineral oxides, but cannot simulate oxidation-reduction and has limited ability to simulate precipitation. GWB and CHESS have limited ability to simulate humic substances, but can both simulate oxidation-reduction and precipitation equilibria. In application to field situations, the modelling of complexation to humic substances has been shown to be of great importance (Tipping, 2002) and so modelling should include this if possible. The importance of oxidation-reduction and precipitation reactions in surface water conditions is debatable. Redox couples (of which Fe(II)-Fe(III) is generally the most important in surface waters) are frequently far from equilibrium, and so speciation modelling predictions including redox need to be interpreted with care. In practice, separate determination of the different oxidation states (e.g. Fe(II)/Fe(III), $NH_4/NO_2/NO_3$) and input as separate species is preferable to direct prediction of redox speciation. Similarly, careful consideration is needed of mineral precipitation. The most thermodynamically stable precipitates are not necessarily those that will dominate if precipitation occurs, since amorphous forms of a mineral (e.g. iron(III) oxide) may only slowly transform to more thermodynamically stable forms.

Under laboratory conditions (e.g. mixture toxicity experiments using a radionuclide and a metal) it is advantageous to simplify the chemistry by not including DOM in the exposure solutions unless the specific effect of organic matter on toxicity is to be studied. In this situation, the choice of model becomes less critical. If validation of an exposure model against field conditions is done (e.g. by exposing the test organism to the toxicant mixture in natural water) then the choice of model becomes more critical, since complexation by dissolved organic matter will have similar importance to the field conditions.

The advantages and disadvantages of each model and database outlined above are generally applicable to the radionuclides considered here, namely uranium, thorium radium and polonium. The models are generally well-developed with respect to uranium and thorium. The comprehensiveness of the different databases used varies with respect to dissolved complexes, so that different models give different predictions of solution speciation. Those databases that incorporate the most recent developments in knowledge about solution species (e.g. Dong and Brooks, 2006; 2008; Geipel et al., 2008 for UO_2^{2+}) are the most comprehensive. In applying a particular model to speciation prediction, consideration needs to be made regarding the possible updating of the database to reflect the latest knowledge. Similarly, the models that can simulate binding to humic substances are parameterised with relatively recent data for both uranium and thorium and constitute the state of the art in the field. Binding to iron oxide is also considered, although in the case of both WHAM and Visual MINTEQ the binding constants are estimated, rather than being based on measurements. Such measurements do exist in the literature (Mahoney et al., 2009 reviewed the available data) and could be used to calculate binding constants.

The situation with regard to Ra and Po differs from the other radionuclides. None of the databases contain binding constants for either element, with the exception of an Ra-acetate complex in ctdp_v3_dong.tdb database. Hence speciation prediction was not possible. Clearly, it would be advantageous to develop such knowledge. In the first instance, the importance of speciation for exposure could be assessed by considering the (radio)toxic action of the element. Since both Ra and Po have radioisotopes that are alpha emitters and deliver the greatest dose internally, then the effect of speciation on uptake (as exemplified by the BLM) would be likely to exert an influence on exposure. Thus, steps may be required to establish relevant binding constants. One potential route is to estimate constants by analogy with chemically similar elements. This is likely to be more productive for the Group II element Ra, as knowledge of the chemistry of the other Group II elements is extensive. For Po the situation is less clear. As a Group 16 element its nearest chemical analogue is the semimetallic tellurium, yet the increase in metallic character moving down the periodic table to Po implies that extrapolation of chemical behaviour would need to be done with care.

3.5.6.2 Bioavailability models

The modelling of contaminant bioavailability is most advanced for metals, where the BLM provides an established framework for understanding and predicting how environmental chemistry affects toxicity. The structure of the BLM, whereby metal ions compete with other solution ions for uptake by the organism at a specific uptake site (the ‘biotic ligand’) lends itself well to extension to the uptake and toxicity of multiple metals. The success of the BLM in describing patterns of metal toxicity suggests that the study of the effects of metal/radionuclide mixtures on organisms needs to consider the BLM as a means to rationalise observations into a coherent framework. Thus far, relatively little study has been done on the application of the BLM as a tool to understand (non-radionuclide) metal mixture effects. Nevertheless, extension of the approach to understanding the effects of mixtures of radionuclides and metals is certainly feasible. Some key points can be made regarding the suitability of the BLM as a tool for such work:

- The scope of applicability of the BLM needs to be considered. To date, BLM application has been solely to cationic metals. Exploration of the applicability of the BLM to anionic metals (e.g. Mo, As) would be essential to establish its general usefulness (or not) for such species.
- The mode of action of the radionuclide needs to be considered in deciding whether the BLM is an appropriate tool to use. If the radionuclide exerts an effect solely via an external irradiation, then the BLM is not appropriate, since the exposure will not be a function of either the environmental availability or environmental bioavailability of the radionuclide, but simply of its external concentration. On the other hand, if the effects are exerted either partly or totally following uptake of the radionuclide (internal irradiation and/or specific chemical toxicity of the element), the BLM may be an appropriate tool to describe the variability in uptake and consequent toxicity.
- Prior to applying a BLM to mixtures of metals/radionuclides, it is important to parameterise the model for the individual components of the mixture to provide a robust basis for extending the model application to the effects of the mixture itself.
- There may be a need to carefully consider the usefulness of the BLM for specific radionuclides. In the first instance the availability of speciation (i.e. environmental availability) knowledge needs to be assessed. In this review we have assessed exposure scenarios containing radionuclides (Ra, Po) for which speciation data are lacking. This would hamper the development of BLMs for such radionuclides, since prediction of the speciation in the medium is an essential part of BLM development.

Compared to metals, the bioavailability of organic compounds has been little studied, although there is evidence that the medium chemistry can influence uptake and toxicity, for example by the binding of organics to DOM. Knowledge of exposure mechanisms is best developed for non-polar compounds that exert narcotic (baseline) toxicity, and less developed for other classes of compounds (i.e. ionic organic compounds), which are likely to exhibit more complex uptake behaviour. In the first instance, study of the effects of radionuclides and organic compounds would most usefully utilise an organic compound or compounds that have the simplest chemistry, since (i) there is more likely to be information on the bioavailability of such a compound, and (ii) the mechanism of uptake is likely to be simple. A non-polar organic compound would thus be the best choice for study.

4 State of the art on approaches and tools for effects assessment under mixed contaminant conditions

4.1 General Introduction

4.1.1 Objectives and chapter content

The aim of this chapter is to give an overview on how effect assessment in multiple contaminant situations is performed nowadays and how to apply this within a radiological context.

In the introduction a short overview will be given initially describing general effects following exposure to ionising radiation. Section 4.2 will deal with the two most commonly used component based reference models namely concentration addition (CA) and independent action (IA) with their weaknesses and strengths and possible deviations from these models. The section will end with an overview of different experimental designs used to test CA/IA. The next section (4.3) will focus on whole mixture approaches including Whole Effluent Testing (WET), Effect-Directed Analysis (EDA) and Toxicity Identification Evaluation (TIE). These are so-called diagnostic approaches that include a step-by-step assessment of toxicity of an environmental sample and that have close connection to approaches used in risk assessment as described in Chapter 5. This is followed by section 4.4 which describes an overview of the use of bioassays and biomarkers in radioecology. Section 4.5 is dedicated to toxicokinetic (TK) applications that include the fate of toxic components into the organism, from the point of absorption to its internal distribution, metabolism and final excretion. This section is closely related to the review on exposure (Chapter 3)but is included here as it leads directly to the toxicodynamic (TD) approaches of a toxic mixture (4.6). TD approaches incorporate how components affect the organism over time. Dynamic Energy Budget (DEB) theory will also be discussed. DEB provides a conceptual framework which specifies how energy is taken from food and allocated to growth and reproduction. Finally, in the general discussion(4.7), we will consider issues such as quality criteria and data demands needed for the different approaches.

4.1.2 Short description of mode of action of ionising radiation

Radiation is the physical process in which energetic particles or energetic waves move through a medium. Ionising radiation is any kind of radiation that when it interacts with material can induce (directly or indirectly) ionisation in which energy is transferred from the radiation field to the material. One can distinguish different types of ionising radiation and of importance here is the difference between alpha, beta and gamma radiation. Alpha (configuration of a He nucleus) and beta (electron or positron) radiation are particles whereas gamma radiation consists of waves. The distance radiation penetrates through a medium depends on its energy and mass. As such, shielding from alpha particles, being the heaviest, can be achieved by a piece of paper, from beta particles by aluminium and for gamma rays lead is needed. This also indicates that alpha particles are not harmful to organisms as long as they are outside as they cannot penetrate trough the outer (dead) cell layer. However once taken up, alpha particles form a greater risk. Within radiology the term relative biological effectiveness (RBE) is formulated as the relative amount of damage that a fixed amount of ionizing radiation of a given type can inflict in an organism. The ICRP has defined standard RBE values independent of tissue type. For gamma and beta radiation these are set at 1 whereas for alpha it is set at 20.

Biological effects induced by ionising radiation in organisms originate from the deposition of energy from the radioactive material to biomolecules (e.g. DNA, proteins). 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. As for a great number of other biotic stressors, ROS can be formed, for instance, through the radiolysis of water. Hence, ionising radiation can lead to DNA lesions, including oxidised and methylated bases, DNA adducts, and single- and double stranded breaks (Streffer et al., 2004). Indirect effects of oxidative stress can alter protein and lipid structure and/or function. Organisms respond to this interaction by inducing DNA repair mechanisms, but if DNA damage remains unrepaired or is misrepaired DNA mutations are sustained as single base substitutions, small deletions, recombinations or chromosomal aberrations. Depending on the nature and location of these mutations, this can lead to the cell death (apoptosis or necrotic), hereditary effects or stochastic effects. Radionuclides can exert an effect either via external irradiation and/or internal irradiation following the uptake and accumulation of radionuclides (especially important for alpha and beta emitters). In the second case, in addition to the understanding of the radiation mode of action, knowledge on bioavailability and toxicokinetics will be necessary for an appropriate description of the overall toxicity.

For protection of wildlife and ecosystems population linked individual endpoints (morbidity, reproductive capacity, mortality) are required and not, for instance, DNA damage, which can be rather considered as an early marker of a potential effect. Chapter 4.4. describes several endpoints and bioassays/biosensors.

4.1.3 Short description of mode of action of toxic compounds

Modes of toxic action fall into two classes,: non-specific and specific. Non-specific toxicity results from the accumulation of a compound in the lipophilic layer of cell membranes, resulting in a disruption of the membrane integrity (Maier et al., 2009). The non-specificity of this toxicity mechanism describes the general tendency of a compound to disrupt the cell membrane integrity due to its general lipophilicity, rather than to any specific chemical properties that it possesses. This mode of action is usually described as narcotic, or baseline. As would be expected, non-specific action is an important toxicity mechanism for non-polar compounds, which have high hydrophobicity and lipophilicity. Specific toxicity, on the other hand, results from the binding of a contaminant to a specific target or target(s) within the cell, such as proteins and nucleic acids. The specific mode(s) of action of a particular contaminant are highly dependent upon its chemistry, which dictates the specific molecular targets within the cell to which it might bind. Classification of organic contaminants on the basis of mode of action generally utilises four categories (Verhaar et al., 1992):

- Non-polar narcotics act non-specifically by accumulating in the lipid phase of cell membranes.

- Polar narcotics display greater toxicity than expected on the basis of simple narcosis, yet there is no evidence of reaction with specific receptors. Exact mechanisms of toxicity are unknown. Polar narcotics have greater bipolarity and/or hydrogen bond donor acidity (Penttinen, 1995) than non-polar narcotics.
- Non-polar reactive compounds react non-selectively with chemical structures within cells (Vaal et al., 1997);
- Specific reactive compounds react specifically with certain receptor molecules within cells.

Trace elements are typically considered separately from organic compounds with respect to their mode of action. They may have a number of modes of action and multiple trace elements may share similar modes of action, such as the induction free radicals, production of ROS and subsequently of oxidative stress (Shanker, 2008).

4.2 Component-based modelling concepts and deviations of these reference models

4.2.1 Concentration addition (CA) and Independent Action (IA) as reference models

One of the key goals of mixture toxicology has always been to be able to predict quantitatively the effects of a mixture from knowledge about the toxicity of the individual components. Two concepts that have been used for this purpose are Concentration Addition (CA) and Independent Action (IA). These models allow the calculation of expected effects based on the dose-response curves of the individual compounds. CA is sometimes called "dose additivity", "Loewe additivity", "additive joint action" or "similar joint action", whereas IA is also referred to as "Bliss independence", "effect multiplication", "Abotts formula" or "response addition". These concepts describe a quantitative relationship between single substance effects and the toxicity of the mixture composed of these chemicals. Both are so-called component-based approaches since they need toxicity information of the individual components in order to enable predictions on mixture effects,. The main assumption made for both CA and IA is that the toxic components in the mixture do not show interacting effects, *i.e.* they exert their toxic effects without enhancing or diminishing each other's toxicity. In addition, the mode of action of each compound is considered the same at all doses. A major difference between the two concepts is that CA assumes similar modes of action for the different toxicants, while IA assumes dissimilarity in the mode of action of the different toxicants in the mixture.

In Concentration Addition the toxicants are assumed to have the same mode of action or act on similar physiological processes or systems within an organism. Thus, all components in the mixture behave as if they were simple dilutions of each other. The joint effect is equal to the sum of the concentrations of each chemical expressed as a fraction of their own individual toxicity (Greco et al., 1992; Warne, 2003; Backhaus et al., 2004). It is written mathematically as follows:

$$\sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1$$

with c_i the exposure concentration of chemical *i* in the mixture which elicits x % effect, EC_{xi} the concentration of chemical *i* alone which would elicit x % effect (e.g. EC_{50} when x = 50 %).The ratio c_i/ECx_i is called a toxic unit (TU) and was introduced by Sprague in 1970, when he measured water pollutants. One toxic unit (1 TU) is the concentration of a chemical that corresponds to the selected toxic effect (e.g. x = 50 %).

Hence, the joint load or joint concentration of the mixture given in a common unit can be rewritten as follows:

$$\sum_{i=1}^{n} TU_i = TU_{mix}$$

Knowing or estimating the shape of a typical dose-response curve on the organism or system from which the EC values have been derived, an effect estimate of the ΣTU can be made.

The concentration of a mixture giving x % effect can be found by rewriting the overall equation of TU as follows with p_i the relative fraction of chemical i in the mixture:

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1}$$

The concept of Independent Action, on the other hand, is based on the assumption of each compound acting on a different system/receptor (dissimilar mode of action) independently, while contributing to a common response. The concept was developed for binomial responses and is based on the probability of a chemical having an effect on an individual or target. For a binary mixture this would mean that the mixture effect of chemical A and B is the sum of the individual effects (E) of A and B minus the portion of the population in which toxicities overlap:

$$E_{A,B} = E_A + E_B - (E_A * E_B)$$

This means, if chemical A causes 20 % mortality and chemical B 70 % mortality, the mixture effect of these two chemicals is not 90 % but 76 %. For a multicomponent mixture this relationship can be expressed as follows:

(1) $E_{mix} = 1 - (1 - E(c_1))\,(1 - E(c_2))\ldots(1 - E(c_n))$

(2) $E_{mix} = 1 - \prod_{i=1}^{n}[1 - E(c_i)]$

with E_{mix} being the expected effect of the mixture, *n* the number of mixture components, and $E(c_i)$ the effect of the i^{th} mixture component if applied alone in the concentration (Backhaus et al., 2004; Altenburger and Greco, 2009).

In addition to CA and IA, the concept of effect summation can also be found in the literature. Effect summation is based on the idea that the total effect of a given mixture equals the sum of the effects of the individual components. However, this concept lacks a sound scientific background. It is actually based on the idea that dose-response curves are linear and do not follow a sigmoidal curve. It would, for example, mean that a mixture composed of 10 compounds, each present at a concentration causing 50 % effect if applied singly, would provoke 500 % effect if applied together, which is clearly impossible (Backhaus et al., 2004).

4.2.2 Requirements for data and knowledge on Mode of Action

To calculate a mixture toxicity expectation according to CA or IA, one needs to know the concentration (or dose) of each of the toxicants in the mixture as well as their toxicity – so concentration-response (or dose-response) curves are needed for the individual components. It is important that the endpoints as well as the test species are the same for each toxicant (Warne, 2003). In addition, specifically for IA the effects of each single compound at the specific concentration in the mixture need to be known .

To calculate expected mixture toxicity according to CA or IA the dose-response curves of the single compounds are normally fitted with a sigmoidal regression curve like logit-, loglogistic, Weibull or other models. For the actual prediction of the mixture toxicity only the fitted curve-parameters are used and not the original data. A good and meaningful fit of the data is therefore essential for a good prediction. Scholze and co-workers described a general-best-fit method for the estimation of dose-response curve using a pool of 10 different regression functions (Scholze et al., 2001). Hence, for the use of this kind of models it is highly recommended to obtain the best set of parameters for the sigmoidal regression curve. STAR Deliverable 5.1 (Garnier-Laplace et al., 2011) describes in detail the methodology to derive dose-response curves.

Knowledge on the mode of action of the components of the mixture is required for the CA or IA approaches to be representative as a tool to assess the toxicity of the mixture. The term mode of action describes the key events and processes starting with interaction of a compound with a cell via operational and anatomical changes, resulting in the toxic effect. Mechanism of action implies a more detailed understanding and denotes the molecular sequence of events starting from the absorption of the toxicant to the production of the biological response. In other words, it includes the understanding of the causal and temporal relationships between the different steps leading to a certain biological response (US EPA, 2000, Borgert et al., 2004). If the mechanism of action is known, the mode of action is also known but not the other way around. Although in theory this is simple, in reality the mode of action is often not known or the observed effect is a sum of responses induced by the toxicant in the organism. As such a clear mechanisms-mode of action relationship rarely exists.

An expert group convened by the International Life Sciences Institute (ILSI) has defined that chemicals act via a common mode of action if they (i) cause the same critical effect, (ii) act on the same molecular target issue, (iii) act by the same biochemical mechanism of action, or (iv) share a common toxic intermediate (Botham et al., 1999; Mileson et al., 1998; Botham et al., 1999). If the CA approach is applied for a mixture in which the toxicants act via different biological mechanisms and interact with one another, then the predicted toxicity may not be realistic (William, 2005; Mason et al., 2007).

A so-called Two-step prediction (TSP) model can be applied to deal with mixtures containing components that have similar and dissimilar mode of action (Ra et al., 2006). This model implies that the CA model and the IA model are applied in a stepwise manner. Firstly, the CA model is applied to all the chemical groups within the mixture that have similar modes of action. The concentration-response curves predicted by the CA model are subsequently imported in the IA model as if they came from a single chemical.

It is important to bear in mind that a contaminant (or radiation stress) may have multiple Modes of Action, and these may shift over time, especially when primary lesions over time elicit series of secondary lesions. ince results from CA/IA do not differ too much, often the more conservative CA is used in risk assessment purposes.

4.2.3 Use of Concentration Addition and Independent Action approaches

The CA and IA approaches provide a more environmentally realistic alternative for assessing possible environmental and health effects than do single-substance toxicity tests by reducing uncertainties (Warne, 2003). The main reason for using these models is to make predictions about the combined effect of chemicals when only effect data for the individual chemicals are available, which is often the case (Greco et al., 1992; William, 2005). The CA approach has, for example, been useful in predicting pesticide mixtures, the contribution of identified but untested components in sediment contaminations, the combined effects of mixtures of components having similar endocrine-disrupting potencies, and effects at the level of functional community properties (Altenburger and Greco, 2009).

The basic principles of CA are used for risk assessment purposes as described in Chapter 5.2.3 in methods like hazard index (HI), relative potency factor (RPF) and toxicity equivalency factor (TEF) (U.S. EPA, 2000).

Comparing the use of both models, it appears that CA is the more dominantly used model. Independent action is applied when the mixture is relatively simple, say < 10 components, and the compounds are very different in their properties. However, Porsbring (2009) found that IA was better than CA for dissimilarly acting pharmaceuticals and personal care products. In complex mixtures with many compounds in low concentrations it sometimes seems that the assumption of independently acting chemicals loses its meaning. This phenomenon in which many compounds at low concentrations seem to cause a non-specific toxic effect (base-line toxicity) and that IA can no longer be applied was called the Funnel Hypothesis by Warne and colleagues (2003).

The principle of strict independent effects may only rarely hold true due to converging stress signalling pathways. In addition, when predicting possible mixture effects using both IA and CA models, in most cases CA provides the more conservative mixture toxicity estimate, although predictions were generally similar or even identical. Therefore, in mixture Ecological Risk Assessments CA has mostly been indicated as the more broadly applicable option (see Chapter 5.2.3). Theoretically (assuming infinitely steep dose-response curves for the single components) the EC_{50} value predicted by CA and IA will maximally differ with a factor that equals the number of toxicants in the mixture with CA being the more conservative (for reference see Backhaus et al 2010). However, in reality dose-response curves are not infinitely steep and CA and IA predictions are often close together. In a study base on a large number of binary mixtures for fish, algae and daphnids the difference in predictions by both concepts did not exceed a factor of four (Backhaus et al., 2010). For chemicals inducing dose-response curves with a log-logistic slope around one, the predictions of IA and CA are even the same (Dreschner et al., 1995, Cedergreen et al., 2005, 2008).

4.2.4 When co-contaminants in a mixture interact

The main assumption of CA and IA is that toxicants do not interact, in other words, the presence of compound A does not influence the presence or toxicity of compound B. Hence, these models cannot explain observed interactions and they do not incorporate the fact that mixture effects can differ in time (Baas et al., 2007) and endpoint considered (Cedergreen et al., 2007) or that there may be dose-dependent variation in interactions. Compounds may, however, influence each other’s uptake, distribution, metabolism or excretion (kinetics) or they might affect their effect on receptor, cellular target or organ. The net result of an interaction between co-contaminants can be that the toxicants act synergistically meaning that the toxicity of the mixture is greater than expected according to the reference model or antagonistically when the toxicity is lower than expected according to the reference model. An additional difficulty is that whereas toxicity data on individual compounds are often available, relevant and reliable data on interactions are mostly lacking (Borgert, 2007). Approaches to predict the interacting effects are in need of mechanistic information about the toxicity of the individual components. In terms of mechanistic understanding, interactions between different toxicants may occur at different levels.

For CA, the easiest way to identify interactions is shown by the sum of the TU defined as in section 4.2.1 not equallingone. Similarly, the fraction (p_i) of each chemical can be defined by the ratio of the dose in the mixture (c_x) and the effect dose of each chemical used alone which causes the same magnitude of effect as the mixture (EC_x). As such if in the studied mixture the sum of TU observed deviates from the theoretical one it is said that there is an interactive mixture effect. As such the mixture is additive when the sum of TU equals the predicted one, synergistic when it is smaller than 1 and antagonistic when it exceeds one (Groten et al., 2001). Jonker and co-workers (2005) presented a MIXTOX model based on CA and IA able to characterize mixture interaction effects by quantifying the degree of deviations of the data from either reference model(Jonker et al., 2005). This model allows for characterizing dose-dependent and dose-ratio dependent interactions in addition to synergism or antagonism. A drawback of this model is that it is heavily data-demanding as it builds upon data obtained through a surface design as described in 4.2.7 and in practice it is hard to reproduce dose-ratio or dose-level dependent interactions (Cedergreen et al., 2007).Alternatively, a biology-based approach can be used to describe the toxic effects of mixtures on growth, reproduction and survival over the life cycle such as the dynamic energy budget (DEB) theory that is further explained in 4.7(Baas et al., 2009b; Jager et al., 2010).

4.2.5 Limitations of Concentration Addition and Independent Action

One limitation intrinsic to the bottom-up approach used in the CA and IA concepts is that all the chemicals in the mixture need to be characterised with respect to their concentrations and toxicities in order to calculate the mixture toxicity. It may not be possible to obtain all this information when dealing with mixtures that are not created in the laboratory and with components for which toxicity is not characterized (Warne, 2003). Besides, it is not always easy to determine the mode of action of the different compounds. Also contaminants can be present in different physico-chemical forms such as ionic and particles, the form will depend the amount of the contaminant taken up and can influence the effects under mixture conditions. Sometimes the observed toxicity may not match the predicted effect because the concentrations used in a toxicity test do not always reflect the actual bioavailable concentrations of the chemicals (Warne, 2003).

As indicated above, CA and IA approaches assume no interaction. This implies that when a mixture effect is measured an interaction can be defined as a deviation of the predicted value but chemical-chemical interactions cannot be predicted by CA/IA. Therefore CA/IA have only limited predictive power to describe interactions. In a real environmental mixture situation the concentrationsor the speciation of different compounds are not always stable nor are all compounds present simultaneously. This complexity of sequential exposure scenarios and assaying time-dependent effects cannot be considered within a CA or IA model based on simple (one endpoint and one exposure point in time) concentration effect dose-response curves as described above. For example, possible recovery during exposure-free times is not considered (Altenburger and Greco, 2009). The dynamic DEBtox model as described in 4.6 is specifically developed to address changes in time and to integrate different endpoints.

CA and IA are concepts based on pharmacological assumptions about sites and modes of actions of substances (similar mode of action for CA and dissimilar for IA). However, in toxicology and ecotoxicology such knowledge is often missing for most chemicals. Hence, assumptions on specific types of combined action are often difficult to draw. For example, an antagonistic combination effect, assessed on the basis of CA, might, at the same time, be quoted as synergism with respect to IA. The minimum requirements are that if reporting on synergistic/antagonistic interactions, reference should always be made to the reference model with which it is compared. To validate experimental results and to allow for precautious assessments, Drescher and Boedeker (2003) suggested that the relationships between CA and IA should be considered. They have shown that the relationships between CA and IA depend on the distribution functions, the corresponding slope parameters, and on the concentration of the mixtures..

Finally, when measuring end-points at organism level such as mortality, reproduction or growth rate, only the net effect of the toxicity is assessed which does not always account for all processes at sub-organism level (e.g. nervous system, cardiovascular system). These systems can each show a different toxicity response or sensitivity and the effect in one subsystem might influence that in another (de Zwart and Posthuma, 2005).

4.2.6 Effects of low doses in mixtures

From an environmental toxicological as well as risk assessment perspective, it is necessary to know whether or not toxicants each present at a concentration indicated as an individual threshold or No Observed Effect Concentration (NOEC)might still have a contribution to the overall toxicity of the mixture.

As indicated above, CA builds on the idea that the mixture components act as if they were dilutions of the same compound. Hence, according to CA all components contribute in proportion to their own potency to the total effect and it does not matter if the concentration is below the threshold or not. As stated by Backhaus and co-workers (2010) "*it doesn't matter for the overall toxicity if only one compound is present at a concentration c – or whether 100* *compounds are present each at a concentration c/100."* Experimental evidence for the contribution of components present in low, individually not significant, effective concentrations to the overall mixture toxicity has been gathered by different authors and is reviewed by Kortenkamp et al.(2007; 2009). This has been called "Something from Nothing" by Silva et al. (2002).

In contrast, for dissimilarly acting compounds the theoretical concept of IA states that the resulting combined effect is calculated from the effects caused by the individual toxicants. However, although compounds might be present at a very low concentration determined as a NOEC this does not exclude that there is a small effect of the compounds at that concentration, only that it is not significantly distinguishable from the control (Backhaus et al., 2010). ANOEC is an experimentally derived concentration of a compound at which, in a given experimental design and for a given endpoint, no significant effect compared to the control could be detected. And this has its shortcomings. To give an example, take 100 chemicals each of them inducing a 1% effect instead of 0% at their NOEC, then the combined effect (found by filling in the IA equation) would mount up to 63% of a maximally inducible effect. Similarly, if each of the 100 toxicants is provoking only 0.1% effect, still 9.5% can be expected (Kortenkaemp et al., 2009; Backhaus et al., 2010).Kortenkamp and coworkers (2007, 2009) summarized a number of studies on mixtures of dissimilarly acting compounds present at threshold values and showed that clear joint mixture effects sometimes above 50%, were detected in different studies.

In summary, possible mixture effects can only be excluded *a priori*, if all components in the mixture are acting completely independently and if all of them are present at concentrations that definitely produce "absolutely no effect"(Backhaus et al., 2010), these concentrations might however only exist in theory.

4.2.7 Experimental designs for component based approaches

One of the objectives of component based approaches is to analyse whether the toxicity of a mixture composed from single known toxicants, deviates from the predicted mixture effect by CA/IA. Several specific designs have been described to analyse deviation from expected mixture effects. The final choice of the experimental set up is limited amongst other things by the number of experimental units that can be handled. An overview of common used experimental designs (full factorial, surface, isobolic, fixed ratio, "A in the presence of B" and point design) is given here.

"Full factorial design" permits the investigation of both the effects of individual chemicals and their interactions. To describe the mixture concentration-response curves, the number of concentrations of each component is defined and then all the possible combinations are tested. The experimental effort required for providing enough data increases exponentially with the number of components in the mixture. Even if only 2 concentrations per component are assayed, the number of test groups needed is still 2^n (e.g. for a mixture with 6 components, $2^6 = 64$ test groups are needed). To reliably estimate the slope of a concentration-response curve for a single chemical, usually 5 or 6 concentrations are assayed. In addition, the experimental design must consider the concentration range and distribution of concentrations to ensure that relevant concentrations are tested. The application of this design is, therefore, restricted to combinations of just few chemicals.

A more suitable design for multi-component mixtures analysis is the fractionated factorial design (also known as screening design), since only a fraction of the possible treatment combinations of the components is tested. The resolution of the experiment will decrease as the number of fractions of tested combinations is reduced. A key point of the design is to identify the most important combinations to be tested. This design is particularly appropriate for screening studies or experiments with more complex mixtures.

"Isobolic designs" do not determine the full concentration-response curves, but select concentration combinations on the bases of isoboles. An isobole is an isoeffective line through the concentration-response surface, defined by all the concentration combinations of the components of the mixture that produce an identical mixture effect.

In classical isobolic designs, one or several points on the isobole are experimentally described and then compared with the predictions obtained from the concentration addition (CA) reference model. The number of test group required is calculated with the formula $k (n + j)$, where k is the number of concentrations tested per concentration-response curve, n the number of components in the mixture and j the number of points on the isobole to be investigated.

The major advantage of isobole designs is their ability to detect interactions between the mixture components, i.e. mixture ratio-dependent deviations between the observations and the predictions made with the reference model. For binary mixtures visual representation is easy.

"Fixed ratio designs" require less experimental effort than factorial designs, and are applied when the interest is restricted to a specific ratio between the toxicants. In this design, the mixture of interest is analysed at a constant ratio of its components, while the total dose of the mixture is systematically varied. Hence, a concentration-response curve of the mixture is recorded, which can then be analysed in the same way that a concentration-response curve for a single chemical, comparing the observed data with the prediction made using the reference models (CA or IA). The number of test group needed is defined by the formula $k (n+1)$, where k is the number of concentrations tested and n the number of components.

The main advantage of this design is that the experimental results can be conveniently visualized and interpreted, even for mixtures of many components. However, if only one ratio is tested, no statement on mixture-ratio-dependent deviations from the conceptual expectations can be made.

The design “A in the presence of B” can only be used for binary mixtures. The aim of the study is to analyse the shift of the concentration-response curve of one compound, caused by the presence of a fixed background concentration of a second chemical. To compare the experimental observations with the predictions made using CA model, the number of test groups needed is $k.3$; while for comparing with the predictions made using IA model, $k.2+1$ test groups are needed.

Finally in a "point design" only one mixture concentration is tested and its effects are compared to the effects that the individual components provoke when applied singly at the concentration at which they are present in the mixture. This design requires $n+1$ test groups, not counting any control. In some circumstances, the visible deviations between observed and predicted effects may not be relevant. For example, in concentration-response curves with steep slopes, small shifts in the concentrations applied due to experimental manipulation, might lead to significant changes in the observed effects. The point design allows comparison of the observed effects with those predicted by the IA model. One particular application of the point design is to analyse a situation in which all the components are present in a concentration that is presumably below a pre-defined threshold and to see whether the mixture still provoke clear effects.

Of the above mentioned designs, the more frequently used are isoboles, point and fixed-ratio design, while full or fractionated factorial designs are rarely applied due to their high data demand.

4.2.8 Use of CA and IA approaches in mixtures including radiation or radionuclides as one of the components

In the framework of the IAEA EMRAS II programme and the IUR mixture toxicity workgroup, a review was made specifically focussing on studies that included radiation or radionuclides in the mixture (Vanhoudt et al., 2012). The review concluded that CA or IA have hardly ever been used to calculate mixture effects or as basis to identify possible interacting effects between radiation or radionuclides and other contaminants or environmental factors. Moreover, in most studies the erroneous concept of effect summation was used as the basis to indicate if synergistic or antagonistic interactions were present in the mixture. Clearly, within radioecology the concepts of CA/IA are currently not as well established as in (eco)toxicology.

4.3 Whole mixture studies: top down approach

Whole mixture approaches are used when only fragmented knowledge of the mixture components is available, or when the identification of the component(s) that mainly contribute(s) to the mixture toxic effect is not of concern. The studies can be done to assess which adverse effects are induced by the mixture and to quantify their magnitudes, without trying to determine the components of the mixture responsible of this toxicity, or the interactions between the components of the mixture (synergism, antagonism, etc.). The results obtained in these studies are exclusively applicable to the actually investigated mixture, and cannot be extrapolated to other mixtures. Moreover, since the exposure situation in the environment is highly dynamic, frequent re-testing of the mixture of concern is needed. This approach is often used for site-specific and retrospective studies.

Bioassays or biosensors can be used to reliably estimate the toxicity and potential risk of complex mixtures, when information is lacking on the mechanisms of their components (see section 4.4).These methods do not provide information on the nature of the components in the mixture responsible for its toxicity, nor on the interactions between the components of the mixture.

Another possibility to determine the toxicity of a whole mixture is to use data available for sufficiently similar mixtures. This approach is not frequently used in ecotoxicology, although in human toxicology it is often applied. A key point of this approach is to determine the degree of similarity between the mixtures, based either on their components and the proportions of them within the mixtures, or on the origin of the mixtures (source, process of production). Since there is no specific guidance to determine the similarities between mixtures, expert judgment and statistical tools have to be applied.

4.3.1 Whole mixture tests

The simplest whole mixture studies test the effect of a whole mixture, regardless of its physical or chemical composition, on an organism or biological endpoint to assess whether or not it is toxic. These types of mixture tests have mostly been used for testing the toxicity of effluents, for example, toxic effects of a facility's waste water on different aquatic organisms, and are formally called Whole Effluent Toxicity testing (WET) (US EPA, 1991/505/2-90-001). WET tests are effect based approaches that are simple, holistic, cost efficient and conducted under controlled (laboratory) conditions. In addition, this approach does not require mixture specific methodologies. However, it also has several limitations such as that the mixture itself needs to be available for testing and as such, the obtained results are only applicable to that specific mixture. It does allow testing for unknown toxicants, but it does not provide any identification of the toxicants inducing the effect or to identification of interacting effects. This also implies that this approach is largely unsuitable for prospective approaches. The usefulness of WET testing and its correspondence to field conditions in has been reviewed by (Chapman, 2000) and Sarakinos et al. (2000).

Without any identification of the components of the mixture, the toxicity found in WET testing approaches is hard to interpret further. Some whole mixture studies, besides quantifying the toxicity of the mixture, aim to identify the compounds, or group of compounds, within the mixture that are responsible for the observed toxicity, and quantify their contribution to the overall toxicity of the mixture. To do so, biological and chemical analyses are combined with physico-chemical manipulation and fractionation techniques. In all cases, conclusions about causality are reached using either recombination of specific compounds in the mixture, calculations, or field methods (or a combination of these). The assumption is usually that Concentration Addition applies to the mixture toxicity observed.

The concentrations of mixtures and the ratios of their components in an environmental sample can vary on a small spatial scale. Thus, a pooled sample can be used to represent an 'average' concentration. Alternatively, a single sample is taken and assumed to be 'representative' of the area.

There are two main types of whole mixture studies that go beyond the mere quantification of the toxicity of a complex environmental sample and aim at identifying the key toxic compounds causing the effect. Both approaches have been developed for effect and risk assessments of environmental samples; TIE (Toxicity Identification and Evaluation) and EDA (Effect Directed Analysis**)**. They are quite similar, but use slightly different paths in reaching the same targets of characterizing, identifying and confirming the cause of detrimental biological effects (Bakker et al., 2007). TIE procedures sequentially extract components from the mixture, and test the toxicity of the remainder to determine the cause of toxicity in the removed fraction of the sample. EDA procedures test the toxicity of the extractions to determine the toxic components of the mixture. Another difference between the two is that TIE usually only employs *in vivo* tests with whole organisms, whereas EDA uses a broader range of test systems also including *in vitro* receptor activation assays (Kortenkamp et al., 2009). In general, EDA is considered analytically better, while TIE is more ecologically relevant. Neither method takes into account potential changes in mixture ratios that might be seen in the field at different places, times, season, or in different organisms. Further details of each approach are given below.

TIE (Toxicity Identification and Evaluation)

This method was first developed for the characterization of waste water effluent and is used by organisations such as the US EPA and OSPAR. There are, therefore, quite specific method descriptions available. TIE-type methods usually use mostly *in vivo* bioassays and or simple bioassay/biomarker tests (e.g, Microtox). The sequentially simplified fraction is used in the toxicity testing. The procedure is as follows:

1. A very rough assessment of the toxicity of the mixture is performed, using the whole mixture on a bioassay or several bioassays (see Section 4.4). Ideally, a range of different organisms covering a range of trophic levels should be used (e.g. an alga, a crustacean and a fish), and a range of acute and chronic tests should be done.
2. A sequence of chemical extractions and biotests is performed until the most toxic (groups of) chemicals are identified by the toxic response disappearing from the remaining fraction:
    - Chemical extraction/fractionation methods are used to selectively remove different groups of potential toxicants (e.g. metals, dioxins, PAHs) or single compounds. Those that have an effect on the overall toxicity are identified/screened using e.g. GC-MS, LC-MS, HPLC.
    - The remaining fraction is used for bioassays that are as ‘relevant’ as possible to the environment under consideration (in practice these are usually standard *in vivo* bioassays).
3. Confirmation of the mixture effect is attempted by comparing the effects of the components with the effects of the mixture in one or several of following ways:
    - Recombining the different fractions of the original mixture and testing again.
    - Creating an artificial mixture of the components in the same combination as the original mix.
    - Calculating the predicted effect of the mixture from the effects of the components, usually with the assumption of CA.

EDA (Effect Directed Analysis)

In EDA, the total extracts, fractions, and individual chemicals identified are all used in the toxicity tests. The focus is more on the chemical characterisation and extraction. The principle of EDA is to use the response in a biological (test) system to direct the analytical pathway towards identifying the chemical compounds causing this response (Bakker et al., 2007). EDA is less widely used in risk assessment and the method is less standardised than TIE. The procedure is as follows:

1. In some cases, a toxicity test on whole mixtures is first done using bioassays.
2. A sequence of chemical extractions and biotests is performed to determine the components that are most toxic in the mixture:
    - Toxicity tests are performed, usually using cell based *in vitro* bioassays and biochemical tests with biomarkers, biosensors and immunoassays, though *in vivo* tests may also be used. These are done with the total extracts, fractions of the

mixture and individual chemicals identified. A wide range of different tests is used, preferably those that are sensitive to a narrow range of toxicants. In this way, a response in a biotest can be linked to the analytically identified chemicals. This step can also include the use of quantitative structure–activity relationships (QSAR).

- The chemical composition of the mixture is determined using extractions and analytical chemistry, with the focus on potentially toxic components. The chemicals of potential concern may be first indicated through bioassays.

3. A ‘copy-mixture’ of the identified toxic components is biotested to confirm the toxicity of the determined mixture. This is compared to the results from the single component tests and mixture toxicity evaluated, usually with the assumption of CA and effect summation.

4.3.2 Drawbacks of TIE and EDA approaches and their relevance for studies of mixtures including radiation

Extraction can chemically alter thespeciation and bioavailability of the substances in the remaining test mixture. It can also be difficult to find a suitable ‘control’ against which to test the mixture (e.g. a matrix that is uncontaminated, but otherwise chemically/structurally similar). In addition, EDA is rather an artificial system with great analytical power, but limited ecological relevance. Thus, it is challenging to confirm hazards resulting from key toxicants identified by EDA under realistic exposure conditions and for higher biological levels, such as whole organisms, populations and communities (Brack et al., 2008). It also requires sophisticated preparative and analytical tools to identify the pertinent compounds (Bakker et al., 2007).

Extraction usually focuses on organic compounds and excludes polar metals since metals are difficult to separate from a mixture. Most radionuclide species are charged, and polar reagents (e.g., acids) are needed for extractions. Most radionuclides (like metals) are not in an organic form and will therefore probably also not be suitable for extraction with non-polar agents.In all extractions, the yield must be determined and the fractions or the remaining solution defined, however, the interpretation of the extracted fraction is often complex. This is more of a problem in EDA where the extracted fractions are tested than in TIE where the remaining mixture (including metals, radionuclides etc.) is tested. Separately extracting radionuclides isotopes from their stable isotopes is a huge challenge. Lastly, as mentioned in Section 4.4, bioassays specific to radioactivity do not exist and thus can at this point not be used to narrow down the toxicant/biotest combinations.

4.4 Biological testing of mixtures

Within toxicology, biological testing indicates testing the effect of a toxicant on a specific endpoint and organism or biological agent. Biological testing is also used to test single chemicals, and as such many standardised methods have already been developed. However, the focus in this section is their use in radioecology and in testing mixtures. Most of the biological tests have been developed using aquatic test systems. Tests may be acute (endpoint often mortality, LC_{50}) or chronic exposures (growth, fecundity, fertility) and cover a wide range of species and *in vitro* bioassays. Effect concentrations are usually expressed as % dilution of the mixture. More details of whole mixture approaches are given above. It is generally known thatbiological species differ from each other in their sensitivity towards a toxicant. Hence, there is no such thing as the ideal biotest or the most sensitive test-species. In the case of radionuclides this can certainly be an issue as radiosensitivity varies extensively between species (see deliverable 5.1 of STAR WP5, Garnier-Laplace et al., 2011).

Many terms can be found in the literature to describe different categories of biological testing (e.g., biotests, bioassays, biomarkers, biosensors), but there is often overlap in the use of these terms, particularly the word ‘bioassay’. In addition, some biological reactions can be used as bioassays, biomarkers or biosensors, depending on the application/method. The terms mentioned are defined below.

Bioassays: Bioassays are tests that attempt to determine the relative strength/potency/biological activity or the nature of a substance by comparing its effect on a test organism / living cells with that of a standard preparation. When testing an unknown mixture, a variety of tests is usually performed, to cover a wide range of taxonomic groups and biological reactions and thus increase the chances of detecting toxicity. A distinction is made between *in vivo* bioassays that have a whole organism as the test subject and *in vitro* ones that include cell-lines subcellular responses, etc.. An overview of some common used bioassays is given in Table 6. In contrast to *in vivo* bioassays, the methodology for *in vitro* tests is less well standardised.

Table 6. Examples of in vivo and in vitro bioassays

in vivo	***in vitro***
Invertebrates e.g. *Daphnia*, *Hyalella*, *Artemia*, *Mysidopsis*, nematode	inhibition of bacteria *Vibrio fischeri* (Microtox)
Fish e.g., trout, minnow, zebrafish, medaka	enzyme induction e.g. EROD, cytochrome P450, CYP1A
Single-celled algae e.g., *Scenedesmus*, *Selanestrum*	aryl hydrocarbon receptor (AhR) agonists using the DRCALUX assay
Algae and plants e.g., *Ceramium*, *Champia parvula*, *Lemna*	mutagenic activity using the Mutatox assay
Embryo tests e.g., sea urchin, *Crassostrea* (oyster), FETAX (*Xenopus* embryo), fish embryo toxicity test (FET)	endocrine disruption assays, e.g. oestrogen receptor (ER) agonists using the yeast oestrogen screen (YES) and androgen receptor (AR) binding assays
	fish or mammal cell-based cytotoxicity assays
	genomic microarrays (toxicogenomics)

Biomarkers: A biomarker can be defined as a biological parameter that can be measured in a given subject and is in some way related to a biological effect (Durante, 2007). Their abundance or level of expression can in some cases be quantitatively related to the level of exposure, and can either indicate exposure levels (e.g., chemical metabolites) or effects (e.g., CYP1A enzyme levels). Biomarkers are used in field studies, laboratory effect studies (as bioassays) and have been incorporated into biosensors. Some can be quite difficult to couple to a specific chemical (e.g. in a field or multiple stressor environment) (see Forbes et al., 2006) and are more indicators of general stress in an organism/biological system. Brooks (1999) distinguished three different classes of biomarkers: exposure, sensitivity and disease. For exposure biomarkers a dose-response relationship can be established. Biomarkers of sensitivity are genetic markers associated with an increase in individual susceptibility towards e.g. radiation. Finally, biomarkers of disease are those biological events that can be used to anticipate the diagnosis of a specific illness. The latter class of biomarkers is in our objective not relevant.

Biosensors: Finally biosensors are analytical devices that both assess toxicity of a mixture and extract quantitative analytical information of single compounds in the mixture. They include biological material (e.g. tissue, microorganisms, cell receptors, enzymes) (or a mimic, e.g. of a membrane) with a physico-chemical detector component (transducer). Specific compounds (e.g. dioxins) trigger a biological or biochemical response (e.g. production of a protein, switching on/off a gene, enzyme action) that creates a signal (e.g. luminescence, electron production or consumption) that is then transformed by the transducer using e.g. optical or electrochemical methods into a measurable signal (e.g. change in light, colour, numbers etc).

Biosensors thus differ from bioassays in that the transducer is an integral part of the analytical system, and that they can extract quantitative chemical information. They are thus a useful analytical tool, but their ecological relevance is difficult to determine. Examples of biosensors include microarrays (e.g. DNA microarrays, protein microarrays, cellular microarrays etc.) that are 2-D surfaces coated with a range of different biologically reactive molecules (e.g. proteins, DNA sequences) that respond to an external signal/stressor and produce a measurable response such as fluorescence. These can be used for screening a range of potential biochemical responses simultaneously. Other biosensors identify more specific biochemical reactions, such as cytochrome P450 production.

4.4.1 Applicability to radioactive mixtures

The mode of action of radiation is described in the introduction of this part of the review. Typically radiation will induce DNA damage as well as oxidative stress responses. As these are rather general toxic responses there is to date no such thing as a specific biomarker for radiation stress. However, here we have tried to make an overview of different studies that aimed at identifying radiation specific biomarkers or markers that distinguish between radiosensitive and radioresistant species. Examples of biomarkers of both exposure and sensitivity that can be utilized within both human and ecological toxicology to identify the response to ionising radiation, ranging from molecular, cellular and organism levels are given in Table 8. We have classified the biomarkers according to whether they test for DNA damage and repair, oxidative stress or general stress responses. This table was compiled after a literature study, but is not complete.

Table7. Overview of biomarkers of both exposure and sensitivity that can be utilized within human and ecological toxicology to identify the response to ionising radiation. This table was built from the data gathered in deliverable 5.1 of the STAR project (Garnier-Laplace et al. 2011)

Biomarker	Method/assay	Tested endpoint	Correlation with dose or radiation sensitivity	Species tested	Reference
DNA damage and repair mechanisms					
Antibody against Gamma-H2AX	fluorescence imaging, Western blot, 2-D gel electrophoresis, flow cytometry ELISA High-throughput	DNA damage (Double strand breaks)	Sensitive to and correlating with degree of damage	Human but gamma-H2AX phosphorylation site has been shown to be highly conserved throughout eucaryotes	Redon et al., 2011; Kuo and Yang, 2008 Pereira et al., 2011
Cytogenic biomarker	Chromosomal aberrations	Genotoxicity	Validated correlation with long-term morbidity endpoints like risk to induce cancer	Human blood Turtle Deer	Durante, 2007 Ulsh et al., 2003 Ulsh, et al. 2004
Mitochondrial DNA mutation frequency	Sequence analysis	DNA mutation	Not sensitive enough for environmental relevant concentrations	Compost worm (*Eisenia fetida*)	Wilding et al. 2006
DNA strand breaks and alkali labile sites	Alkaline comet assay	DNA damage	Significantly higher levels of DNA damage in all γ-exposed embryos, but no dose(rate)-response relationship was observed.. No relation between Comet assay parameters and radiation dose observed with plants	Eggs/larval of Zebrafish (*Danio rerio*) Shoots and roots of *Phaseolus vulgaris*	Simon et al., 2011 Vandenhove et al., 2006
Oxidation of DNA and DNA	8-oxo-guanine (HPLC-analysis, GC-MS, Fpg-	DNA damage and repair	Not clear whether good biomarker due to high background (Collins et al.,	Numerous, including Compost worm (*Eisenia	Collins et al., 1996 Hertel-Aas et al., 2011

strand breaks	modified Comet-assay)		1996) and variable response in different worm generations (Hertel-aas et al., 2011)	*fetida*)	
Oxidation of DNA and DNA strand breaks	8-oxo-guanine detection with antibodies	DNA damage and repair	Linear relationship with gamma-irradiation dose and sensitive (Bruskov et al., 1999)	Species?	Bruskov et al., 1999
Repair capacity of blood cells	Comet assay	DNA damage and repair	Correlate with chronic exposure	Numerous Including blood cells of chronic (Chernobyl) exposed people	Plappert et al., 1997 Hertel-Aas et al., 2011
Cytogenic endpoints		DNA repair		human	Abdel-Rahman and El-Zein, 2011
Methylation status of DNA	Bisulphite sequencing, RT-PCR Methylation specific-PCR Western blotting	Reduced transcription through gene silencing of protein involved in DNA-repair	Relation between radiosensitivity and methylation status of ERCC1 (excision repair cross complementing protein 1) promotor	Human gliomas	Liu et al., 2009
Mutations at tandem repeat loci of DNA	minisatellites and expanded simple tandem repeats	expanded simple tandem repeats, microsatellites	Untargeted effects associated with radiation exposure including genomic instability, bystander effects, and transgenerational effects	Humans Fish	Dubrova, 2003 Tsyusko et al. 2006
Oxidative stress					
Antioxidants and antioxidant enzymes	Spectrophotometric assays of enzyme activities (POD, SOD, catalase)	Oxidative stress	No correlation between oxidative stress tolerance and gamma radiation resistance	Bacteria	Shashidhar et al., 2011

	Carotenoids levels				
Fe/Mn ratio	Atomeric Absorption Spectrometry	Protection of proteins and DNA for oxidative damage	Inverse correlation between [Mn]/[Fe] ratio and level of protein oxidation (Confalonieri and Sommer, 2011) No direct correlation with radiation resistance (Shashidhar et al., 2011)	Bacteria: *Deinococcus*, *Thermophyllus*	Confalonieri and Sommer, 2011; Shashidhar et al., 2011
General stress responses					
Heat Shock Proteins	Antibody detection: Western blot	Stress induced proteins			Lewis et al. 1999
Transcriptomic changes	Microarray etc,..	Changed gene expression	Acute exposure was comparable to other abiotic stressors whereas chronic exposure revealed a complete distinct gene expression profile Down regulation of growth/rhythm responses and up-regulation of defence/stress regulation in post irradiation reproduction state (Kim et al., 2007) Acute gamma exposure, Gamma + Al + Cd exposure of fish	*Arabidopsis* Vegetative or during flowering Atlantic salmon (*Salmo salar)*	Kim et al., 2007; Kovalchuk et al., 2007 Olsvik et al, 2010
Radiation metabolomics	GS-MS QTOFMS	Changed metabolite abundance Some could be linked to food deprivation and starvation (Johnson et al., 2011)	Dose and time dependent, cross-species (Johnson et al)	Rat, cell and mouse	Coy, 2011; Johnson et al., 2011; Lanz et al., 2009
Proteomics	Proteomic signal	Proteomic changes in gills	Proteomic changes correlated to direct irradiation and to bystander signals	fish	Smith et al. 2011.

4.5 Toxicokinetics

Toxicokinetic (TK) models aim to predict the time course of chemical concentrations in organisms, taking into account the way chemicals are absorbed, distributed, metabolized and excreted. This includes knowledge of many of the physiological and biochemical pathways involved in these processes. TK models have been used for human toxicological studies, where it is ethically not feasible to test compounds on humans and hence there is a need for informed extrapolation from data obtained on surrogate species (e.g. rats). For ecotoxicity studies, the same problem applies for protected species, as it is impossible to test them. Furthermore, several non-human species may be studied to take into account biodiversity in ecosystems and TK models may be useful to extrapolate from one species to another.

In the case of mixture studies, compounds may interfere with each other’s uptake (see Chapter 3.4) or, in the case of organic chemicals, transformation which may affect several target sites of action. With respect to uptake, metals and polar organic compounds occur as charged entities and they require mediated transport, such as ion channels or specific carrier proteins or enzymes. When present in a mixture, they can compete for the routes of mediated uptake. Neutral organic substances diffuse across the lipid bilayer of biological membranes and are therefore assumed to have less potential to interact during uptake.

Once inside the organism, chemicals may end up in metabolically inactive parts of the body, such as fatty tissues for organic chemicals or granules for metals. For the fraction of compounds that is not stored in an inactive form, the rate of overall accumulation in specific tissues depends on processes such as biotransformation or excretion. Compounds in mixtures may affect the biochemical reaction of another compound, e.g. enzymatic transformation for organic chemicals or binding to proteins for metals. For organic chemicals, the biotransformation to metabolites adds more complexity, as such metabolites may have a different toxicological profile than their parents. The same complexity may be expected from radioactive decay products leading to mixtures of radionuclides.

Interactions between metals have been commonly observed in organisms and several of them involve metallothionein, a protein which plays an important role in the sequestration of heavy metals. For example, Martin-Diaz et al. (2005) have shown that the amount of metallothioneins induced in the shore crab by heavy metals can lead to a synergistic or an antagonistic response to binary mixtures of these metals.

Two toxicokinetic modelling approaches are commonly used: data-based toxicokinetic (DBTK) modelling and physiologically based toxicokinetic (PBTK) modelling. DBTK models simply describe the experimental kinetic data (e.g. tissue concentrations in function of time) whereas PBTK equations describe the mechanistic processes involved in uptake, distribution, metabolization and excretion. For ecotoxicity studies, DBTK models have been widely used. PBTK models have been developed to a lesser extent but only in vertebrates where physiological parameters are available or at least, can be inferred. For invertebrates, the metabolic and physiological information is often not available and furthermore, it is difficult to measure chemical concentrations at the tissue level which limits the fitting of these models.

PBTK models have been developed for trout (Law et al., 1991; Nichols et al., 2004a, 2004b), starry flounder (Namdari, 1998), salmon (Brocklebank et al., 1997), channel catfish (Albers and Dixon, 2002) and beluga (Hickie et al., 1999). When the physiological parameter values are not available, allometric scaling techniques can also be applied or measured. To our knowledge, PBTK models have never been applied to mixture studies in the context of ecotoxicology or radioecology.

One-compartment DBTK models were used to study metal-radionuclide interactions (Fraysse et al., 2002). Asiatic clams and zebra mussels were exposed to ^{57}Co, ^{110m}Ag and ^{134}Cs, in mixtures with Zn, Cd or Cd+Zn. Zn and the Cd+Zn treatment increased the ^{110m}Ag uptake in mussels and clams and also increased the ^{110m}Ag depuration in mussels, but not in clams. Hence, species specificities may occur in terms of metallothionein regulation that may explain these differences.

Uranium-selenium mixture toxicity experiments were also performed on daphnids and revealed an antagonistic effect, most probably due to toxicokinetic interactions between uranium and selenium uptake (Zeman, 2008). Baas et al. (2007) also used a one-compartment model for the analysis of time-series survival data for the springtail *Folsomia candida*, but without taking into account the toxicokinetic interactions.

TK interactions between metals and organic compounds have also been shown. For example, in the amphipod *Hyalella azteca*, chlorpyrifos enhances the accumulation of methylmercury, but as methylmercury presumably forms a chlorpyrifos-MeHg complex, the toxic effect (acetylcholinesterase inhibition) is reduced (Steevens and Benson, 1999).

4.6 Toxicodynamics including Dynamic Energy Budget

4.6.1 Physiology Based Toxicokinetics and Toxicodynamics (PBTK/TD)

Toxicodynamics is the study of the toxic actions on living systems, including the reactions with, and binding to, cell constituents, and the biochemical and physiological consequences of these actions (IUPAC, 1997b).

The ecotoxicological approaches to toxicodynamics rely on the basic concept of individual tolerance, where an adverse effect is assumed to occur in an organism when its internal concentration exceeds a certain critical level. This concept is closely linked to the critical body residue (CBR) approach. This approach leads to classical S-shape dose-response curves, from which values such as LC_{50} or EC_{50} can be obtained.

The CBR approach has been applied to mixture toxicity of narcotic chemicals at a single time-point (e.g. Van Wezel et al., 1996; Leslie et al., 2004). For multiple time points, the CBR concept has been applied to the effect of mixtures on survival, by using a one-compartment TK model linked to a fixed CBR to describe LC_{50} (McCarthy et al., 1992).

The stochastic approach of Bedaux and Kooijman (1994) has been extended to mixtures by Baas et al. (2007). They analysed survival data for 6 binary mixtures of heavy metals using the springtail *Folsomia candida*, over a period of 21 days. The approach used is a combined TK/TD approach, allowing the fit of the survival data for all time steps simultaneously. For sub-lethal endpoints, the studies of Van Gestel and Hensbergen (1997) and Jonker (2003) showed that the apparent mixture interactions change with time. Different interactions were also observed by Zeeman (2008) for the toxicity of a mixture of U and Se on the daphnids, depending on the endpoint studied. The statistical analysis method of Jonker (2003) was applied to fecundity measurement, concluding to an antagonistic effect, whereas no interaction was observed on growth. These conclusions emphasize the need for more mechanistic models to understand this behaviour.

Recently, to support a better mechanistic understanding of interactions in mixture toxicology, a framework to support experimental studies to investigate the basis of observed interactions was proposed by Spurgeon et al. (2010). In this paper, in addition to classical TK/TD modelling approaches, omics (toxicogenomics, proteomics, metabolomics) are proposed to identify similarly and dissimilarly acting chemicals in support of mixture assessment. Another promising approach is the use of energetic metabolism, as described in the Dynamic Energy Budget (DEB) theory below.

4.6.2 Dynamic Energy budget model including the effect of toxicants (DEBtox)

Authors have suggested the use of DEBtox models to mechanistically interpret effects of mixtures of compounds within the framework of the Dynamic Energy Budget theory (Baas et al., 2010a, 2010b). The DEB theory describes how organisms acquire energy from food and allocate it to somatic growth, maintenance, maturation and reproduction. DEBtox models examine how contaminants accumulate in organisms, causing perturbations in one or several DEB-related processes (STAR Work Package 5 – Task 3). How toxicants accumulate in organisms over time is described assuming a simple two-compartment model (with intake and elimination kinetics and dilution process due to somatic growth).Effect intensity is expressed through a stress function "*s*" proportional to the (scaled) internal concentration *"c"* above a threshold level known as the "*NEC*" (for No-Effect Concentration)[2].

$$\begin{cases} s = 0 & \text{if } c \le NEC \\ s = \dfrac{1}{c_T}\left(c - NEC\right) & \text{if } c > NEC \end{cases}$$

Possible perturbations (e.g. increase of 1+*s*, decrease of 1–*s*) in DEB-related processes (referred to as "Modes of Actions") include decrease in energy intake through nutrition, increase in somatic maintenance, in maturity maintenance, in costs for growth, increase in costs for egg production etc. causing observed reductions in body size, reproduction or survival (Jager et al., 2004).

The approach has already been successfully applied to a range of chemicals andbiological species to understand effects of mixtures on growth, reproduction and survival (Baas et al., 2007, 2009a, 2009b, 2010b; Jager and Kooijman, 2009; Jager et al., 2010). In a mixture context effects on organisms result from the combined individual actions of each single compound composing the mixture. Figure 1 shows the structure of the DEB approach for mixture toxicity.

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[2] Note that other mechanisms of toxicity induction specifically designed for radiation emitters, need to be explored, assuming that effect intensity is correlated to either instantaneous dose rate, cumulated dose or a level of cumulated damage subjected to repairing processes.

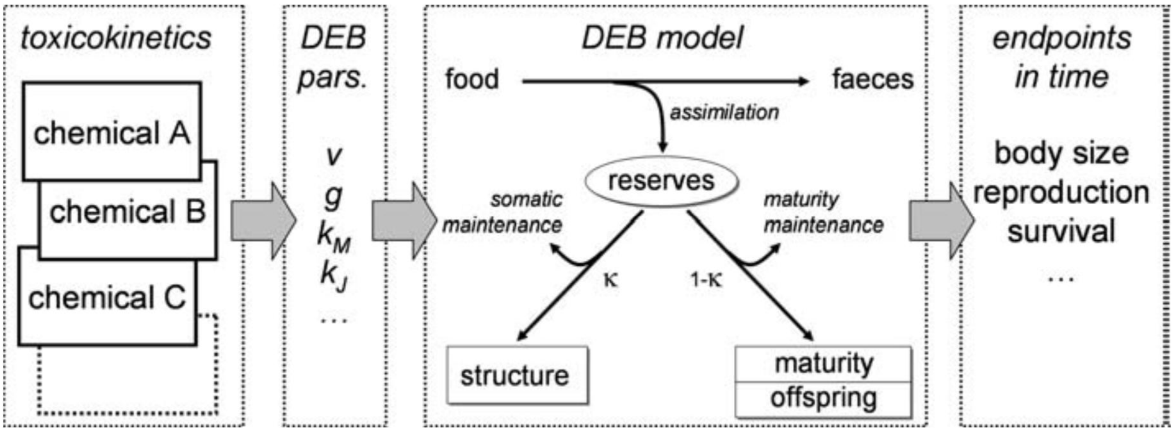

Figure 1. Modelling approach, including a toxicokinetic module as a first step, followed by a description of how processes are affected by each toxicant and a feedback on the kinetics as a result of observed effect on growth (from Jager et al., 2010).

Each component of the mixture has its own toxicokinetics module, which implies that exposure to a constant mixture composition will generally lead to a time-varying mixture inside the organism. For predicting possible mixture effects, DEBtox uses the principles of CA and IA (Jager et al., 2010), although the DEB theory implies a certain degree of interaction among the different metabolic processes. Thus, although different toxic components may have independent toxicokinetics for example, any effect on growth induced by one component will influence the toxicokinetics of all components indirectly. One strength of the approach is to distinguish toxicants which interact at the toxicokinetics level from those which interact at the toxicodynamics level. Mixture components may interact within an organism through one or several modes of action and one or several target sites:

- two components A and B of a mixture may act through different modes of action (necessarily through different target sites), each of them affecting their specific target DEB parameters through independent stress functions s_A and s_B (with their own parameters);
- two components A and B of a mixture may act through a same mode of action and may still affect the common DEB parameters independently through different target sites and independent stress functions s_A and s_B, with an effect intensity of

  $$(1 - s_A) \times (1 - s_B) \text{or} (1 + s_A) \times (1 + s_B)$$

  The underlying idea is similar to the concept of IA for single dose-response curves;
- two components A and B of a mixture may act through a same mode of action and a same target site. In such case, the common stress function s_+ affecting DEB parameters is proportional to the concentration c_+ calculated as:

  $$c_+ = c_A + W_B \cdot c_B$$

  where c_A and c_B are the (scaled) internal concentrations of A and B and W_B is the weight factor for compound B relative to the (arbitrary) reference compound A

(Jager et al. 2010). This in its turn is similar to the additivity principle that is also behind the concept of CA for single dose-response curves.

Table 8. List of studies using DEB-tox for the description of combined exposure of toxicants

Tested species	Type of mixture	Endpoints	Conclusion	Reference
flour beetles (*Triboliumcastaneum*)	mixture of poly aromatic hydrocarbons (PAHs), same mode of action	Survival	Good predictions of the observed effects of a mixture of four PAH sharing of the NEC for various PAHs	Baas et al., 2010b
fathead minnows (*Pimephalespromelas*)	14 PAH mixture with known Kow values	Survival	Same conclusions as above	Baas et al.,2009b and references therein
fathead minnows (*Pimephalespromelas*)		Survival	Application of the hazard model from DEBtox to survival data. Different modes of action resulted in different patterns in the parameter estimates.	Jager and Kooijman, 2009 and references therein
collembolan (*Folsomia candida*)	cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn)	Survival	Agreement between measured and calculated survival data. Slight antagonistic effect for Cu/Pb. No interaction for others.	Baas et al., 2007
Waterflea *(Daphnia magna*) - 10 day old	in situ exposure (PAH, metals, pesticides, salts, pH, oxygen)	Survival	Correct prediction for 34 out of 37 cases: predict the effect of a complex mixture given the chemical composition of the water, and identify which chemical or group of chemicals was responsible for the observed mortality	Baas et al.,2009a
waterflea *(Daphnia magna*)	pyrene and fluoranthene = narcotic mode of action, with negligible metabolization	partial life-cycle experiments (growth, reproduction, survival)	assumption of additivity provides an excellent description for the mixture effects on growth and reproduction, and do not suggest any form of interaction. Model predictions are less convincing for survival data.	Jager et al., 2010

The DEB allocation rules specify the consequences of these changing parameter values over the life cycle, resulting in predictions for survival, growth and reproduction. DEB theory also provides a way to analyse effects on other endpoints such as respiration or product formation (see Baas et al. 2009b, 2010a). A mixture analysis in DEB context is therefore conceptually quite straightforward. The DEB framework was successfully applied to assess effects of complex mixtures on survival and binary mixtures on survival in species such as the springtail (*Folsomia candida*), fathead minnows (*Pimephalespromelas*), the flour beetle (*Triboliumcastaneum)*, the nematode (*Caenorhabditis elegans*)and the microcrustacean *Daphnia magna* (Table 8). The recent study from Jager et al. (2010) was the first to apply a biology-based approach for mixture toxicity of multiple endpoints over the life cycle on daphnids for two PAHs.

DEB theory offers an approach which integrates both toxicokinetics and toxicodynamics within a single consistent framework for analysing mixture effects. As stated above, effects of a mixture are predicted based on the same underlying theory of additivity as used for CA/IA for simple dose-response curves.CA and IA are classically applied to descriptive dose-response curves (dealing with one single endpoint and one single time point), DEBtox will integrate interacting or independently acting effects as dynamic processes affecting growth and reproduction over time. As such mixture-DEBtox has the ability to elucidate in which major processes possible interactions take place. This information can help to further target investigations in causes of interactions.

DEBtox integrates organism biology and makes the link between sub-lethal effects on different endpoints, such as feeding, maintenance, growth, maturity and reproduction, analysing interactions independent of exposure time. Key biological mechanisms underlying observed interactions can be identified, improving our understanding and description of mixture toxicity both at the sub-individual level (identification of metabolic modes of action), the individual level (effects on life history traits) and higher levels of biological organisation (coupling of DEBtox outcomes with population dynamics using Leslie matrices).

The key strengths of DEBtox approaches can be summarised as follows (i) DEBtox provides a single framework to interpret different endpoints independent of exposure time, (ii) sub-lethal effects can be studied, (iii) the focus of the study is the individual and not the toxic compound, and (iv) DEBtox opens possibilities to extrapolate to different species and to population effects. Its greatest drawback is the high data-requirement necessary to parameterise the model both for the organism as for the toxicant.

4.7 General discussion

4.7.1 Comparing different approaches: Challenges and knowledge gaps

For the different approaches described above, Table 9gives a comparative overview of the different data requirements, applicability and capacity to predict mixture effects.

Table 9. Overview of the different approaches reviewed

	Component-based approach		**Whole mixture approach**
	CA/IA for single endpoint dose-response curves	**mixDEBtox**	**WET, TIE, EDA**
Specific data requirements	- Dose-response curve of individual toxicants (if not CA/IA lose their capacity to predict) - Concentrations of all toxicants in the mixture - Monotonic dose-response curves	-Parameters describing growth, survival, reproduction of individual species -Parameters describing toxic effects of individual toxicant - Concentration of all toxicants in the mixture	Toxicity measurements on entire mixture
Applicability (species and toxicants)	No specific assumption on biotest or toxicant needed	Only applicable for those species for which DEBtox is parameterised and toxicants for which a toxicokinetic module has been developed	- Whole mixture is tested; as such results only applicable to that specific mixture - Identification of different toxicants and of effect contributing toxicants by TIE or EDA depends on available fractionation techniques
Predictability	Can predict effect concentrations or effects of mixtures but limited to tested exposure situation (time, endpoint, ecotox test)	Can predict effect for mixtures and generalize for unknown exposure situations (e.g. time varying or food limitation) at individual level	Normally only testing effect of a known mixture without prediction towards unknown mixtures. Aim of these tests it to find toxicant contributing mostly to effect. No predictive power.
Measuring interactions	Conceptually CA/IA assume non-interacting compounds; as such interactions can be defined as statistical deviations from predicted effects according to CA or IA	Interactions are here also defined as deviations from what is expected according to additivity of similar or dissimilar acting compounds. In addition as interaction will change one or more parameters, mixDEBtox gives the possibility to analyse the observed interactions based on the biological mechanisms or pinpoint interactions, that can be readily explained by, e.g. the toxicokinetics.	Indications of interactions are given by comparing effect of fractions with effect of total mixture
Mode of action	Do not give any indication on MoA	Gives indication on which individual endpoint is affected (physiological MoA e.g. reproduction,…)	Indication to which component in mixture contributes to effect

As described by Kortenkamp et al. (2009) and Backhaus et al. (2010), a number of empirical and conceptual knowledge gaps of mixture toxicity approaches can be defined. For all component based approaches detailed information on the composition of the mixture of interest is required. In practice, this is almost never available to the extent required and criteria are therefore needed to identify the relevant components and their chemical speciation in a mixture.

The general concepts of CA and IA start from distinguishing the mode of action of the different compounds. Experimental evidence indicates that the similarity or dissimilarity of the toxic mode of action of a compound is a valid criterion for selection of the appropriate concept for a given mixture (Backhaus et al., 2010). However, for many environmental relevant mixtures knowledge about the mode of action is scarce and the mode of action can be species specific as well as endpoint specific. Moreover, as already mentioned, many contaminants have several modes of actions or mechanisms of action. Hence, criteria to select either CA or IA to use are not evident and generally both concepts are applied. Since results of CA and IA are generally not too different, the more conservative CA approach is applied for risk assessment purposes.

For most approaches information on the dose-response curves of the single toxicants is required. Again, for some toxicants like pharmaceuticals, extensive data are available. For others, in particular radiation and many radionuclides, this information is scarce (see also 4.7.2). In addition, the general concepts can only handle monotonic response with a typical sigmoidal shape and log-scaled concentration or dose axis. For IA the concept implies a response scaled from 0% to 100% but CA also assumes a similar shape of the dose-response curve due to the premise that all components act as if they were dilutions of each other. As such, compounds that are stimulating in low concentrations but toxic at higher concentrations, bell-shaped curves typically for environmental factors like (e.g., temperature, light) and finally specific biomarkers like gene expression that can be induced or inhibited depending on the time and compound, can, currently, not easily be considered. Finding an answer to this will require adaptations to existing approaches or development of new models. Hormesis, *i.e.* stimulatory response at low concentrations of a stressor, also falls in the category of giving a non-monotonic response. Recently improved statistical models are already available for coping with hormesis (see Garnier-Laplace et al., 2011).

Whole mixture approaches are normally not conducted with the aim of elucidating interacting effects or be able to predict mixture effects(Table 9). However, whole mixture approaches such as TIE and EDA can give an initial indication of the contribution of a toxicant to the overall effect. As such they have been able to identify new chemical toxic effects (e.g. organophosphate insecticides, surfactants and treatment polymers in industrial effluents) (see references in Chapman, 2000). In contrast to whole mixture approaches, component-based ones can and have been used to predict mixture effects based on data for the individual compounds as well as to identify interacting effects as deviations of the general concepts. However, CA/IA do not give any information on the mechanisms that drive these interactions. The mechanisms of toxicity and of possible interactions between different compounds require additional, separate testing.

The strength of models like CA/IA to identify interacting effects as deviations from the predictions relies on the reproducibility of the (binary) mixture toxicity experiments. Reproducibility depends on the variance of the endpoint and the tested species, and this both within and between experiments (Cedergreen et al., 2007). The degree of reproducibility of deviations from CA predictions of different herbicide binary mixtures on two different plant species formed subject of a study by Cedergreen and colleagues (Cedergreen et al., 2007). The main conclusion of that work was that it is not always that easy to reproduce deviations of the general concepts. The authors warned for sufficient replication and careful interpretation of the results. A preference for test systems with low variability was also given (e.g. *Lemna* was superior to a more complex terrestrial plant (*Tripleurospermum*)) keeping in mind, however, that the relevance and resemblance to the natural conditions is more prevalent in more complex systems).

DEBtox will provide an indication of the possible physiological mode of action of a toxicant or a mixture. For example with DEBtox one is able to tell whether toxicants mainly induce changes in different life history traits like reproduction or growth. In a recent study it was investigated for three different toxicants (Cd, fluoranthene (a PAH) and atrazine (herbicide)) whether or not these physiological mode of actions could be associated with specific changed gene expression profiles for the different toxicants (Swain et al., 2010). The authors indicated the possibility of linking information of DEBtox to that of a mechanistic approach like transcriptomics to identify the mode of action of toxicants and finally to help in the categorisation of chemicals for risk assessment purposes. It needs to be emphasized, however, that this study only looked at individual compounds that were specifically chosen to greatly differ in their mode of action and hence further work is still needed to generalize these results.

Organisms are not only exposed to mixtures of chemicals simultaneously and constantly over time. The general models of CA/IA cannot handle sequential or pulsed exposure profiles. DEBtox, on the other hand, is one of the approaches that aims at including time-variable exposures and as such has a major additional value. However together with other approaches that deal with this the development of DEBtox models is still relatively new. Parameterization has only been done for a limited number of organisms and even applied to a more limited number of toxicants, as data demand is high to enable parameterization of the effects of the different toxicants on the growth, maintenance and reproduction endpoints.

4.7.2 From ecotoxicology to radioecology

A major objective of this review was to look at the possible applicability of the different approaches for mixtures having radiation or radionuclides as one of the stressors. Within the IUR working group multiple stressors and the IAEA-EMRAS II programme a considerable effort was made to review the approaches and outcome (interacting effects or not) of the different studies performed to date that included radiation or radionuclides in the mixture (Vanhoudt et al.,2012). For this review a meta-analysis of literature on mixture experiments that included radiation or radionuclides as one of the stressors was performed. Data were collected for plants and animals within terrestrial, freshwater and marine ecosystems from 35 references. Information was collected on ecosystem type, species, stressors applied and effects evaluated. All but one study was laboratory based. Most of the studies investigated two-component mixtures. Exposure conditions were mostly gamma or X-ray irradiation combined with heat shock or heavy metals for terrestrial animals; metals, temperature or starvation for freshwater animals; temperature and salinity for marine/estuarine species. For terrestrial and aquatic plants, experiments involved one radionuclide or one radiation type in combination with metals, other radionuclides or radiation types, pro-mutagens and herbicides. About three-quarters of the papers reviewed suggested some form of interaction of effects existed among the stressors. From the review it was concluded that although often statements about additivity, synergism or antagonism were made, these 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 designed specifically to investigate mixture or interacting effects. For example rarely dose-response curves for the single stressors were developed. However, as indicated above, these form, however, the basic data input for a CA/IA approach. In addition, many studies included environmental factors such as temperature as one of the stressors. These further complicate calculations as well as these will not give a monotonic response. However, if suitable dose-response curves had been established for the endpoints of interest, the effects of the mixture could have been predicted using CA or IA and statistical analysis could then have revealed if observed effects were significantly higher (synergism) or lower (antagonism) than predicted. In conclusion, the review of Vanhoudt et al. (2012) pointed towards a lack of systematic mechanistic understanding and quantitative assessments of combined exposures and the resulting possible interacting effects. A clear need was indicated for further research in the interdisciplinary field of multiple stressors (including radiation) to allow predictions of the potential presence of combined effects of low exposure levels on biota.

In the current review an overview was given on the available approaches that can be used to assess mixtures that contain radiation or radionuclides as one of the contaminants. As summarised in Table 9 three different groups of approaches were distinguished: two component based approaches were described one applying on the general concepts of CA/IA on single time and endpoint dose-response curves, and one applying them in a toxicodynamic manner (namely DEBtox) and whole mixture approaches (WET/TIE/EDA). From a radioecological perspective, all three concepts have advantages but also specific limitations. The whole mixture approaches do not, as outlined above, have predictive value, but can be useful to identify different (groups of) toxicants contributing to the toxic effect. Looking at the expected composition of the different mixtures that arecontaining radionuclides (for an overview see Annex 1), it will be a challenge to distinguish the possible contribution to the effects observed of the co-contaminants from that of the radionuclides with these techniques. This is because the co-contaminants are often metals or other water soluble elements that will be difficult to separate from the radionuclides by chemical extraction.

The general concepts of CA/IA can easily be applied on mixtures containing radiation or radionuclides both to assess possible interacting effects as well as to make predictions on mixture effects if dose-response curves of the different components in the mixture are available. However, again some points must be made. For radiation and some radionuclides it has been shown that very high radiation doses are needed to derive full dose-response relationships (e.g. Garnier-Laplace et al., 2006; Vandenhove et al., 2009). From an experimental point of view this may be challenging to achieve as radiation facilities in which such chronic radiation exposure experiments can be performed are scarce. In addition, for general endpoints like growth it has been shown that different organisms respond to low doses of radiation by increasing the growth rate before they show adverse effects (hormesis-like effect) (Upton, 2001; Vanhoudt et al., 2011a, 2011b) and as such do not deliver monotonic dose-response curves. Belz and colleagues (2007) studied the effect of hormesis in binary mixtures to see whether or not mixture effects could still be predicted if an hormetic response was present and on the other hand whether the size and range of the hormetic effect could also be predicted (Belz et al., 2008). From this work it was concluded that hormetic effects appear to be mostly additive (following CA) and that predicting the hormetic effect within a mixture seems possible starting from the individual dose-response curves. It was further shown by Spurgeon and colleagues (personal communication) that the outcome of the dose-response curve modelled either with the standard or the hormetic models is rarely qualitatively different. As such the standard dose-response curves can in most cases also be used.

Finally, the toxicodynamic approaches like DEBtox have been recently successfully applied to describe the toxicity of chronic uranium exposure over several generations of daphnids (Massarin et al., 2010). Recent studies have shown the possibility of applying DEBtox for combined exposures (see Table 8). However, as outlined above the data-demand for DEBtox is high, especially if parameterization of the organism nor the toxicants has not yet been obtained yet. For radionuclides, up to date parameterization has only been done for uranium on daphnids (Massarin et al., 2011) and fish (Augustine, S, personal communication). Within WP5 of the STAR project an experimental effort will be done to apply the DEB approach to external gamma for a freshwater plant (*Lemna minor*) and a nematode (*C. elegans*) (see Garnier-Laplace et al., 2011). As such the success to apply DEBtox to mixtures containing radiation or radionuclides depends largely on obtaining the necessary data for parameterising the different toxicants and species. However, the possibility to obtain indications on the possible mode of action and to derive NEC concentrations from it makes this an approach of great promise for future effects research as well as risk assessment.

5 State of the art on regulation and ecological risk assessment of mixtures

In the last decades, risk assessment has become a commonly used approach in examining environmental problems caused by human activities. Risk assessment is a scientific process, carried out to identify and quantify a risk to enable Risk management decisions to be made. Within an Ecological Risk Assessment (ERA) perspective, the global objective is to estimate either quantitatively or qualitatively the adverse effects on the ecosystems resulting from anthropogenic activities.

Risk assessment process is facing the lack of information that makes difficult to obtain a precise prediction. This can be due to several factors, e.g. low statistical power of data, insufficient number of observations, imprecision of measurements, spatio-temporal variability, differences between natural and laboratory conditions, among others. The reduction of all those uncertainties is generally achievable if sufficient effort is made to enhance the knowledge, e.g. on exposure pathways and/or effects on ecosystems in a more realistic manner. However, a more problematic issue is the inadequacy of conceptual models used to perform the Risk Assessment. One of those potential failures to consider is the problematic of multiple contaminants: most ERA frameworks are focused on single type of contaminant (e.g. ERA framework for radioactive substances recently developed). Such frameworks need to be questioned for their robustness within the context of mixture, and adapted tools need to be developed and tested.

Regarding multiple contaminants, science evolved in the past years and has developed potentially useful tools and data. However, transfer of scientific knowledge into appropriate regulatory approaches is not a trivial task. For instance, the USEPA spent many years on the development of its guidelines for the health risk assessment of chemical mixtures. Without the legal mandates laid down in the US American CERCLA and FQPA, cumulative risk assessment would not have been implemented in the USA. Conversely, there are no consistent and clear mandates in Europe for taking mixture toxicity into account, and the numerous pieces of legislation that contribute to the protection of the environment from chemical risk do not help for the emergence of an integrated Ecological Risk Assessment framework that allows to take into account all types of contaminants (see Annex 3for details).

The objective of this section is to give an overview of the state of the art on Ecological Risk Assessment of mixtures, including radioactive substances. It first recapitulates Ecological Risk Assessment principles for single contaminants(both chemicals and radioactive substances). Then, a general overview of different approaches to deal with mixtures is presented, with their application in ERA assessments and regulation, illustrated by some examples of application to mixtures, and finally their potential application to mixtures with radioactive substances.

5.1 Introduction: the current practices for Ecological Risk Assessment

5.1.1 Ecological Risk Assessment: general framework

Ecological Risk Assessment is defined as a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (US EPA, 1992). Initially, the development of risk assessment frameworks was mainly focused on human health protection, and was expanded to Ecological Risk Assessment with considerable development during the last 3 or 4 decades. Almost all the effort focused on single groups of contaminants (e.g. specific types of chemicals such as pesticides, biocides… or specific types of releases such as industrial releases, waste waters…). The main difference between human health protection and ecological risk assessment is the level of protection goals: for human risk assessment, protection of individuals/population is the objective, whereas assemblage / community level issues are generally of concern regarding the protection of ecosystems (with the exception of the protection of rare and endangered species).

For radionuclides, an ERA framework development was initiated during the last decade: the ICRP formulated some thoughts concerning protection of the environment from ionising radiation and initial considerations with respect to a framework for environmental protection has been included in its Basic Recommendations (ICRP, 2007). Some countries in the meantime had also taken steps in response to environmental protection legislation by providing guidance on environmental impact assessments for ionising radiation (Copplestone et al., 2001; US DOE, 2002). At a regional level, methodologies to assess the impact of exposure to ionising radiation on flora and fauna in European temperate and Arctic environments have been developed in two European collaborative projects “FASSET - Framework for Assessment of Environmental Impact” (Larsson et al., 2004) and “EPIC - Environmental Protection from Ionizing Contaminants in the Arctic” (Brown et al., 2003) respectively. These studies have been superseded by the project “ERICA - Environmental Risk from Ionising Contaminants: Assessment and Management” wherein ecological risk assessment methodologies have been developed and issues relevant to decision making in the context of the management of environmental impacts of radioactivity have been addressed (Larsson, 2008).

There is currently a general agreement that risk assessment is best addressed in four steps, (Environment Canada, 1997; US EPA, 1998; EC, 2003b; Suter, 2007; EC, 2003b), where Risk Characterization represents the final integration of the first three steps in the risk assessment process, namely Problem Formulation, Exposure analysis and Effects analysis (see Björk and Gilek(2005) for a comprehensive overview of Ecological Risk Characterization Methodologies). This framework was initially proposed almost twenty years ago (US EPA, 1992). Figure 2 shows how this general Ecological Risk Assessment framework links with Risk Management and Communication. It is conceptually similar to the approach used for human health risk assessment of chemicals, offers a simple, flexible structure for conducting and evaluating Ecological Risk Assessment. Whether for prospective or retrospective purposes, it was used and developed worldwide for the derivation of many specific guidelines. For radionuclides, the most widely applied Ecological Risk Assessment approaches, namely the ERICA integrated approach (Larsson, 2008) and the United States Department of Energy's RESRAD-BIOTA graded approach (US DOE, 2002), are both largely adapted from this framework.

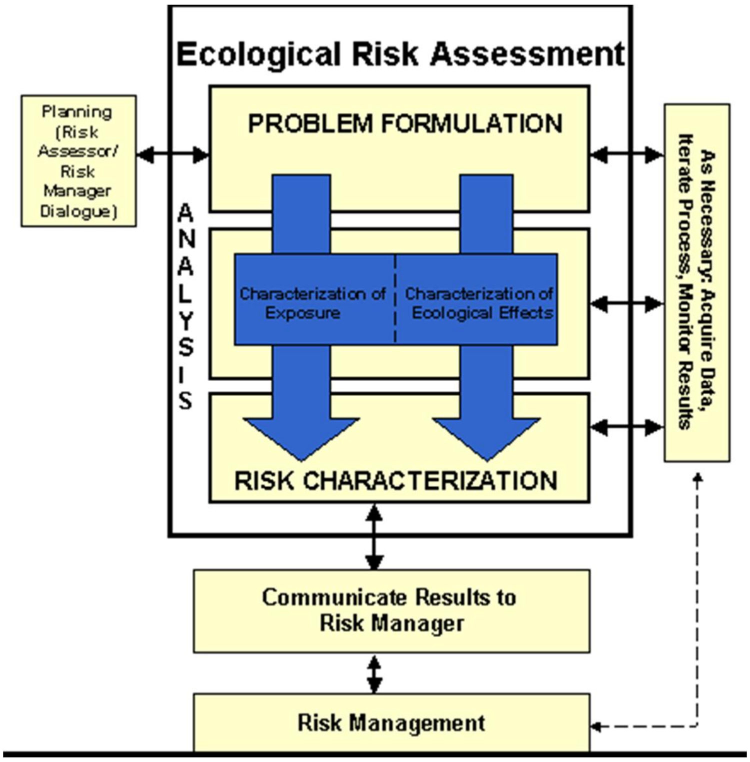

Figure 2. Framework for Ecological Risk Assessment (USEPA, 1998)

The probability of overlap between exposure and effect distributions are not very useful as quantitative predictors of risk in themselves, but rather provide Criteria for the risk assessor and manager on relative risk and ranking (between stressors, between scenarios…).Another application of ERA is the quantitative ecological risk analysis (QERA) defined as the quantitative evaluation of the frequency and consequences of undesired events, together with a weighting concerning the significance of these events.

The quantitative estimation of the risk to an ecological assemblage is basically given by the Risk Quotient (RQ), which is the ratio of the Predicted Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC). If the PEC exceeds the PNEC (*i.e.* RQ>1), there is considered to be risk of environmental damage in proportion to the ratio of PEC to PNEC. The calculation of the RQ value is a stepwise, iterative procedure, generally developed within a tiered approach.

For ionizing radiation, all effects data existing are expressed in terms of radiation absorbed dose (rate) to which the organism has been exposed (Gy or Gy/time) rather than the exposure concentration. Radiation dosimetry is therefore essential to convert activity concentration in a given medium or biota into the quantity of energy absorbed by an organism from both internal and external sources. The quantitative estimation of the risk is thus based on dose (rate) units (D(R)) whether than concentrations (C), and PEC/PNEC ratio is replaced by PED(R)/PNED(R).

5.1.2 Tiered Approach for an appropriate and efficient use of resources

The use of the ERA framework is generally performed within a tiered approach (Cowan et al., 1995; VanLeeuwen and Hermens, 1992; Solomon et al., 1996; USEPA, 1998; Solomon et al., 1996). The early stages of a tiered risk assessment typically use conservative estimates for exposure and effects. When a risk has been identified, or cannot be isolated, subsequent tiers use additional data and tools to address the uncertainties and fill the gaps of knowledge that were incorporated into the initial assessment(s). Typical PEC refinement options are based on use of real emissions, dynamic dispersion, bioavailability,... instead of default, steady-state or equilibrium transfer values.

Regarding the determination of the PNEC value at the ecosystem level (i.e. for assemblage/community level issues),one fundamental concept, now widely scientifically accepted, is the ”species sensitivity distribution” (SSD; Posthuma et al., 2002), which, for a specific chemical, is a distribution modelling the interspecies variability of sensitivity in an assemblage of different biological species with respect to certain observable toxicological endpoints. SSDs thus provide a way, separate from any use of assessment factors for other purposes, to formally relate the tolerances of tested species to those of other untested species.

The tiered approach provides a systematic way of determining what level of investigation is appropriate for a given scenario, minimizing unnecessary investigations and allowing an efficient use of resources. It requires defining triggers to pass from a lower to a higher tier (*i.e.* situations where the assessment needs refinements), compared to situations where no further action is required (*i.e.* the assessment gives a sufficiently accurate risk characterization).

Such an approach was proposed within the ERICA Integrated Approach to assess the radiological risk to biota, enabling the early screening out of situations of negligible radiological concern, leaving only those of potential or real concern for more in-depth assessment or to consult external expertise (Brown et al., 2008 - Figure 3):

- Tier 1 assessments are media concentration based: environmental concentrations are compared with a conservative pre-calculated concentration limit to estimate risk quotients;
- Tier 2 calculates dose rates, but allows the user to examine and edit most of the parameters used in the calculation (concentration ratios, distribution coefficients, apparent density of soil or sediment, dose conversion coefficients, radiation weighting factors and occupancy factors);
- Tier 3 offers the same flexibility as Tier 2, but allows the option to run the assessment probabilistically if the underlying parameter probability distribution functions are defined.

5.1.3 Radioactive substances: Adaptation of the ERA framework and Tiered approach

Most countries use the recommendations of the International Commission on Radiological Protection (ICRP, 2007) and the various safety standards, safety and technical reports of the International Atomic Energy Agency (e.g. IAEA, 1996, 2002) to guide the development and formulation of national regulations. In addition to the health protection of people, the ICRP radiation protection system now addresses specifically the protection of biota against exposure to ionising radiation (ICRP, 2008).

In Europe this is elaborated at a regional level by a European Basic Safety Standard (EC, 1996). On 29 September 2011, the European Commission adopted the proposal for a Council Directive laying down the basic safety standards for protection against the dangers arising from exposure to ionising radiation[3]. This includes a chapter on environmental protection. In line with the new ICRP Recommendations, the objective is to complement the Revision of the Basic Safety Standards Directive with specific consideration of the exposure of biota in the environment as a whole. The aims are to provide a means to demonstrate compliance with environmental criteria and to require Member States to consider suitable protection of non-human species in their radiation protection legislation.

Several tools to conduct Ecological Risk Assessment were developed in Europe and USA, as mentioned above (ERICA integrated approach; RESRAD-BIOTA graded approach). As mentioned by Garnier-Laplace et al. (2008b), the ERICA Integrated Approach requires that an assessment screening dose rate is defined for the risk characterisation within Tiers 1 and 2 (Figure 3). One of the major outputs from the ERICA integrated approach development was the derivation of such an assessment screening dose rate for its use in lower-Tier ERA. Species sensitivity distribution has been used to derive a predicted no-effect dose rate (PNEDR) following EC recommendations for the estimation of PNEC for chemicals (TGD - EC, 2003b). The method used was based on the mathematical processing of data (external γ irradiation effect data on 19 marine, freshwater and terrestrial species were used) and resulted in a PNEDR of 10 μGy.h^{-1}. This dose rate was assumed to ascribe sufficient protection of all ecosystems from detrimental effects on structure and function under chronic exposure. The value was weighted against a number of points of comparison: (i) PNEDR values obtained by application of the safety factor method, (ii) background levels, (iii) dose rates triggering effects on radioactively contaminated sites, and (iv) former guidelines from literature reviews.

The screening value derived within the ERICA integrated approach, as for the methodological frameworks in the TGD (EC, 2003b), does not explicitly account for a possible combined action of pollutant mixtures. Nonetheless, it is assumed that the safety factors applied in the effects assessment do cover the possible occurrence of combined action of pollutants in most instances to a great extent. From a conceptual viewpoint these factors are equivalent to the “margins of safety” employed in the human

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[3] Updated information is available at :
http://ec.europa.eu/energy/nuclear/radiation_protection/radiation_protection_en.htm

health risk assessment; however it is generally considered that the factors are expressions of risk, not expressions of safety (Forbes and Calow, 2002).

In the sense of looking at the combined action of radioactivity with other contaminants/stressors the EU-EURATOM funded project PROTECT (Howard et al., 2010) did not consider mixture toxicity explicitly. The project, however, provided some useful observations on approaches to consider environmental impacts of both chemical and radionuclides within a regulatory context.

Part of the work in PROTECT involved the collation of information, through circulation of a bespoke questionnaire and interviews within a community of national authorities, industry and Non-Governmental-Organisations, in relation to application of approaches to protect the environment from chemical and radioactive stressors (Hingston et al., 2007). For chemicals, key European legislation is covered in REACH and the Water Framework Directive (EC, 2000). Whilst the Water Framework Directive makes passing reference to radionuclides as a possible pressure on water quality there is limited work being done in this area. Radionuclides are not covered by REACH.

Of further interest from PROTECT was the observation that most respondents considered optimisation to be important when regulating discharging industries and that cost-benefit criteria were integral to this (Hingston et al., 2007). Therefore, the optimisation principle As Low As Reasonable Achievable (ALARA) is often implemented in this process through studies of the Best Available Technology/Techniques. The important point is that this view is held for both radioactive and non-radioactive substances in the licencing of industrial discharges in some countries. A good example is provided by the ECs Integrated Pollution Prevention and Control (IPPC) Directive (2008/1/EC), as discussed above, where the Best Available Technology/Techniques approach is strongly promoted and that is the process of being implemented in countries like the UK. This integrated approach will definitely be under application considering the impacts of contaminants, both considering mixtures of stable and radioactive substances. In the next section, based on the very comprehensive review of Ragas et al. (2011) on the subject, and its subsequent presentation during the STAR WP4 meeting (Mol, Belgium, 24-27 may 2011) we will give an overview of the recent research, guidelines and regulation already proposed for the implementation of ERA of mixtures. This will be address in the logic of the ERA framework ((i) problem formulation, (ii) exposure analysis (iii) effects analysis, and (iv) risk assessment) and will also rank the different methods in the perspective of a tiered approach.

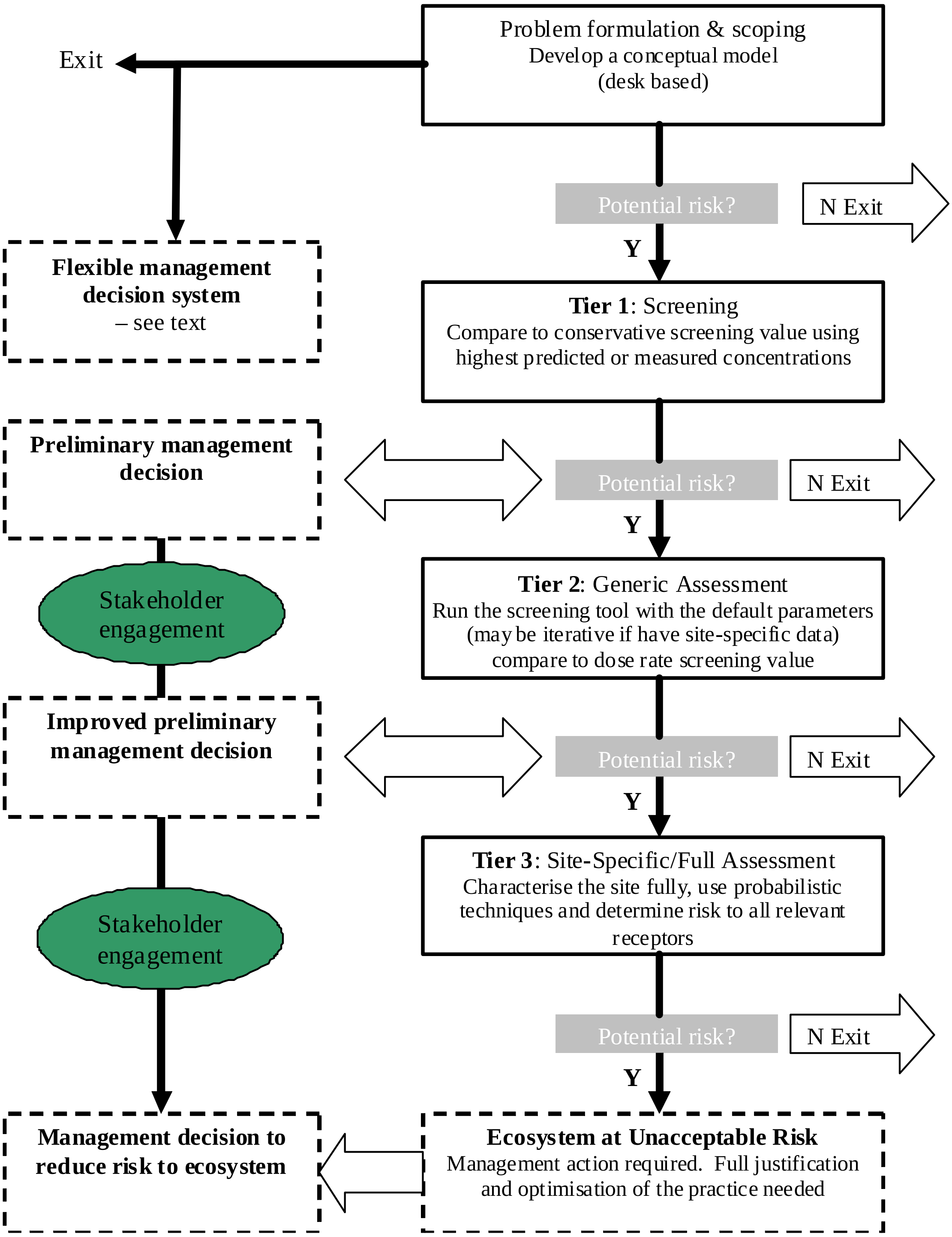

Figure 3. Schematic representation of the Integrated Tiered approach of the ERICA tool, showing how the assessment process is organized within a tiered approach (from J. Brown, Introduction to the ERICA Tool, 2nd WG EMRAS Meeting 23-25/11/2009, IAEA, Vienna - adapted from Brown et al., 2008).

5.2 Overview of methods proposed for the Ecological Risk Assessment of mixtures

Frameworks for human risk assessment for mixtures are developed (see Ragas et al., 2011;Kortenkamp et al., 2009 and Annex 3 of this Deliverable for this aspect), yet proposed frameworks for Ecological Risk Assessment of mixtures are very limited. Various regulations and proposed methodologies address the issue of mixture ERA in one way or another, but few generalized frameworks and explicit guidance were proposed. The European Union has mixture provisions in several directives: Kortenkamp et al. (2009) recently analysed 21 pieces of EU legislation with respect to their scope in dealing with multiple chemicals, and found that four out of these appeared to be particularly noteworthy from a mixture toxicity perspective. EU legislation generally provides the opportunity to account for mixture effects, but explicit guidance is often lacking. Most of the Directives and Regulations are substance or product-oriented. Typically, hazards and risks of these substances and products are treated as if they were present in isolation. With the exception of the recent changes in European pesticides regulations, where mixture risk assessment is mandated, comparative legal frameworks that clearly address cumulative risk assessment do currently not exist in Europe.

Only the REACH regulation address explicitly Ecological Risk Assessment, whereas others are more human health oriented, even though they give objectives for the prevention of pollution in the environment. Regulation (EC) No 1907/2006 concerning registration, evaluation, authorization and restriction of chemicals (REACH) covers the obligations of a manufacturer/importer of a substance (on its own and in a mixture) with respect to a chemical safety assessment (CSA) before it is placed on the market. One purpose of the CSA is to determine the intrinsic hazard of a compound or mixture by estimating Predicted No-Effect Concentrations (PNEC) for environmental assessments and to assess substance properties relating to persistence, bioaccumulation and toxicity properties. This information is then used to derive hazard threshold levels for the environment. Three categories of chemicals are considered: (1) Preparations/isomeric mixtures (e.g. paints); (2) multi-constituent substances; and (3) substances of unknown or variable composition, such as petroleum products.

The limited amount of EU guidance presently available regarding mixture ERA does not imply that the issue of mixture toxicity is not addressed by individual member states/agencies. Many environmental authorities and collaborating research institution in EU member states have extensive experience with two main approaches, namely (i) whole-mixture testing approaches (a ‘top-down’ approach in which the type of chemicals and their interactions are not relevant or unknown – rather the total toxicity of the mixture is measured, especially useful for retrospective assessments), and (ii) various types of component-based approaches (a ‘bottom-up’ approach where the chemicals in the mixture are quite well known, allowing a prediction of the toxicity of the mixture – this approach applies to prospective assessments). The choice between these two approaches has to be defined in the preliminary step of the ERA framework, which is Problem formulation.

5.2.1 Problem formulation: Whole Mixture or Component-based approaches?

The problem formulation phase focuses on scoping and planning, and is best described as the scientific definition of the problem under consideration. "Problem Formulation" synonymous with "Hazard identification" is used to define the nature of initial activities that should occur as part of the risk assessment process. The aim of problem formulation is to establish the goals, scope and focus of the assessment. This includes the identification of receptors that are (actually or potentially) exposed to given environmental stressors and the selection of assessment endpoints. Selection of the assessment endpoint is the definition of the environmental component(s) that is to be protected. Data gaps that must be filled to complete the environmental assessment are identified during problem formulation. This stage may also involve stakeholders, augmenting democracy and transparency associated with the decision-making process. Problem formulation may also need to take into account relevant policy or regulations that direct the formulation of the assessment.

In the case of mixtures, problem definition was suggested by Ragas et al. (2011) as an iterative process that strongly depends on factors such as resources, methods, data availability, desired level of accuracy, and results of previous studies. Problem formulation is also dependent on the objective (e.g. need for safe exposure levels in a prospective regulatory context; another objective can be specific to a retrospective assessment of a site where mixture is an identified problem).

Figure 4 below illustrates the different alternative options to assess the risk of mixtures. The first option, Unique Whole Mixture assessment, corresponds to an actual toxicity testing in the field or the laboratory. This is particularly adapted to completely unknown mixtures: a direct toxicity assessment is performed with no attempt to identify the composition of the mixture. On the other hand, mixtures of which the components are well known (for both exposure and effect assessment) can be evaluated using Mixture component based assessments through the use of mixture algorithms. Alternatively, an intermediate option addresses Component Whole Mixture assessments with Partially characterized or Sufficiently similar mixtures. Details of these options are given below for Unique and Component Whole Mixture assessments (Section 5.2.2) and Mixture Component-based assessments (Section 5.2.3).

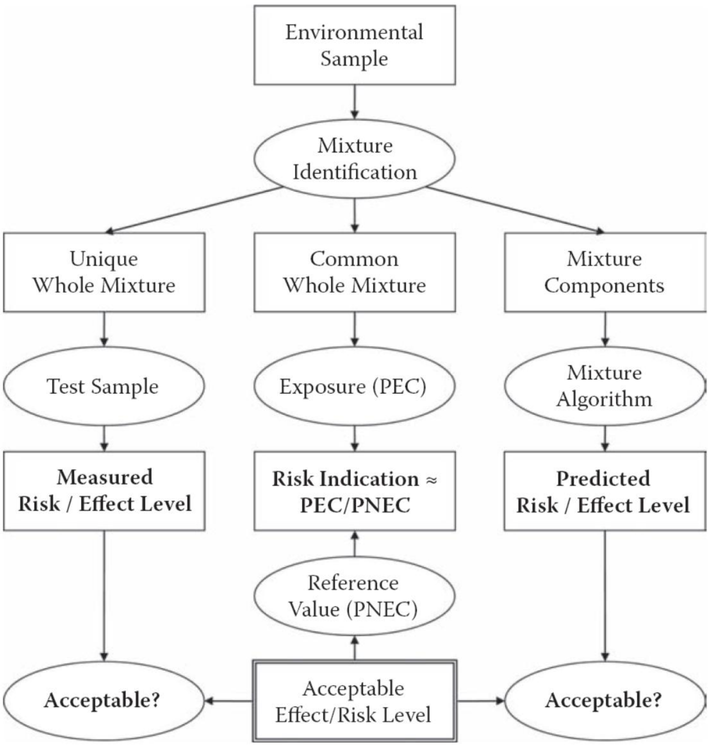

Figure 4. Three alternative options to assess the risk of mixtures - Figure from Ragas et al. (2011).

(left) mixtures can be tested in the field or the laboratory, particularly completely unknown mixtures; (centre) if toxicity data on (sufficient) similar mixtures are available, the mixture can be evaluated using a reference value, for example, in a PEC/PNEC ratio; and (right) mixtures of which the components are known can be evaluated using component-based approaches (mixture algorithms). PEC =Predicted Environmental Concentration, PNEC = Predicted No Effect Concentration.

Different aspects are driving the choice of one alternative option to assess the risk of mixtures. First, the level of knowledge on the composition of the mixture of concern (known, partially known, or unknown) will drive the methodology to be used and the objective. If the frequency of occurrence of an unknown mixture is rare, a direct Whole Mixture approach should be chosen. Conversely, a mixture with known composition allows its assessment through a Component-based approach.

Another aspect is the relative concentration of the different components within a mixture. If concentration ratios between the mixture components are fixed, a Common Whole Mixture approach can be used if toxicity data on (sufficient) similar mixtures are available**.**

5.2.2 Whole mixture approaches

Whole Mixture approaches are applied to assess the overall risk of a mixture based on its direct testing as a whole or partially. This is manly useful when a full chemical characterization of the mixture may be prohibitive or analytically difficult. For those reasons, Whole Mixture approaches were mainly used for toxicity assessments of waste water effluents for the control of emission permits under Integrated Pollution Prevention and Control Directive (Directive 2008/1/EC). OSPAR has developed a Whole Effluent

Assessment, which besides toxic effects of the mixtures, includes the determination of persistence and bioaccumulation of the mixture (OSPAR, 2005).

General overview of Whole Mixture approaches

Such approaches are applied to practically all types of environmental samples for the purpose of general environmental monitoring, ecological risk assessment of contaminated sites, priority setting for risk reduction measures, and the control of remedial work (SETAC, 2004; Kortenkamp et al., 2009). The complexity of the Whole Mixture evaluation depends on the goal of the assessment and on the degree of characterization of the mixture of concern. Taking into account the degree of characterization of the mixture, four assessment situations can be distinguished:

- Completely uncharacterized mixtures: no information on mixture composition, toxicity and origin is available;
- Partially characterized mixtures: one or more components of the mixture are known, so that they can be used to estimate the Whole Mixture toxicity. In these circumstances, it is assumed that the toxicity of those compounds is representative of the toxicity of the Whole Mixture. In ecological risk assessment, partially characterized samples are often treated as completely uncharacterized samples;
- Sufficiently similar mixtures: mixtures are considered sufficiently similar either if their chemical composition is of the same class, or if they are emitted by a common source or produced by similar processes. Exposure and toxicity data of a sufficiently similar mixture can be used as a substitute to evaluate the detrimental effects of the mixture of concern. The accuracy of the results obtained depends on the certainty that the samples used as surrogate are sufficiently similar to the mixture of concern and on the model used. In ecological risk assessment this approach has not been widely applied (e.g. QSAR[4] models);
- Well-characterized mixtures: more information about the mixture or its fractions is available (origin, chemical composition, toxic effects), although the exact composition of the mixture is not necessarily known. In ecological risk assessment, methods for well-characterized mixtures are rarely applied, mainly because it is often more practical to test the mixture of concern directly in the laboratory or the field (the approach used for completely uncharacterized mixtures).

Thus, a variety of methods for direct testing of Whole Mixtures have been developed and implemented in ecotoxicology, like whole effluent toxicity (WET) testing, toxic identification and evaluation (TIE) procedures, bioassay-directed fractionation (BDF) techniques or the toxic potency (pT) approach.

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[4] QSAR : Quantitative Structure-Activity Relationship (sometimes QSPR: quantitative structure–property relationship). The QSARs have been used to predict concentrations of components in mixtures from joint effects and defined mixture ratios. See Altenburger et al. *(*2003) for a comprehensive review and Tong et al. (2005) for limitations.

Application of Whole Mixture approaches in ERA assessments and regulation

Except for Completely uncharacterized Unique Whole Mixtures assessments, examples for other Component Whole Mixture assessments (partially/sufficiently/well-characterized mixtures) are scarce. A rare example is the hydrocarbon block method. In this case, mixture effects are predicted on the basis of partial characterization of hydrocarbon mixtures. The hydrocarbon block method is used to determine the risks of a total hydrocarbon mixture on the basis of discriminating different chain length fractions of hydrocarbons, for each of which toxicities are known (King et al., 1996).

Conversely, while direct toxicity studies of uncharacterized Whole Mixtures are rare in human health assessments, in ecological risk assessments these studies are conducted on a regular basis. In fact, in ecological risk assessments these approaches are feasible, practical, and often more accurate than the modelling techniques which require many assumptions. This approach is particularly adapted for situations where it is not possible, or inefficient to determine the mixture composition. In this case, direct toxicity testing of the environmental sample is sometimes easier and cost-effective.

Many European countries use acute and chronic toxicity tests, as well as tests of mutagenicity, biodegradation, or bioaccumulation, as a part of a whole effluent assessment. According to the cited review, the longest tradition and most developed system in mandatory effluent toxicity assessment in licensing and/or compliance monitoring can be found not only in the USA, Canada and Germany but also in France, Northern Ireland, Norway and Sweden, while many other countries have already adopted guidelines for whole effluent toxicity (WET) approach or have intentions to introduce WET as mandatory requirement under various regulations. The advantages of this approach were also recognized by the European Oslo and Paris (OSPAR) Commission (OSPAR, 2005), which includes bioassays in its recent proposal for effluent monitoring.

The outcomes of the direct toxicity test are relatively certain and representative of the problem, but reproducibility can significantly vary depending on the sample collection conditions (sampling locations, weather conditions or sampling dates). It is also important to note that the outcomes of the analysis cannot be used for the assessment of other mixtures.

Even though the results of the assessment are relatively certain, many uncertainties still exist, for example related with the behaviour of the sample in the environment or the long-term detrimental effects of the mixture. However, these uncertainties are not necessarily larger than those for partially characterized or similar mixtures assessments.

Example of Whole Mixture approach: the toxic potency (pT)

An example is the so-called “toxic potency” (*pT*) approach (Sloof and de Zwart, 1991; De Zwart and Sloof, 1993) where the toxic pressure of an environmental sample (e.g. sediment, soil) is directly tested for its toxicity. The pT value is defined as:

$$pT = Log\left(\frac{T}{100}\right)$$

where,

T : number of times (x) the sample has to be diluted (1/x) or concentrated (x) to produce an EC_x (e.g. EC_{20} in the MicroTox test).

The scaling of *pT* is chosen to produce a *p*T value of 0 at the maximum tolerable level, and a *p*T value of 1 at the desirable level. These levels are analogous to those used in the procedure for setting standards for individual compounds. Direct toxicity assessments (DTAs), also known as whole effluent toxicity (WET) tests, are equivalent to the pT approach when applied to specific effluents or environmental samples. Within this approach, a series of dilutions of the effluent samples are tested using a bioassay, to estimate the effluent concentration above which detrimental impact from the effluent would be predicted to occur in the receiving stream. The advantage of this approach is that the Risk is directly assessed, including potential interactions of the substances present in the environment. However, this approach does not give any information on degradation and transformation and long-term effects. This is quite costly, as specific biological testing need to be performed, whereas the results remains very specific and cannot be used for the ecological risk assessment of other mixtures or samples.

Whole Mixture approaches extension (TIE, BDF…)

An extension of the Whole Mixture approaches are toxicity identification evaluation (TIE) or biological directed fractionation (BDF). The basic concept in those approaches is to use physical/chemical manipulation of a sample to isolate or change the potency of different groups of toxicants potentially present in a sample. Rather than using a chemical detector to determine whether a change occurred, a biological test, in a similar manner than for pT or WET, is used as the “indicator” to determine whether the manipulation changed toxicity. By simultaneously conducting tests using multiple manipulations targeted at different physico-/chemical properties (e.g.liquid/liquid extraction, adsorption/desorption on active carbon, ion exchange resin, XAD resin, C18…), one can build a physico-/chemical characterization of the toxicant(s), which in turn becomes the basis for additional studies to isolate and ultimately identify the specific chemicals causing toxicity. The USEPA has published a series of toxicity identification evaluation (TIE) methods that can be used to identify the causes of toxicity in aqueous samples using chemical characterization, identification, and confirmation procedures (USEPA, 1991, 1993a, b).

When applied to a site-specific case study (high-tier ERA), this type of direct toxicity testing of samples/effluents can be included in more comprehensive approaches, e.g. as one of the lines of evidence of a TRIAD approach (Chapman, 1996; Rutgers et al., 2000). The TRIAD approach is based on the simultaneous and integrated deployment of site-specific chemical, ecotoxicological and ecological information in the ecological risk assessment (Jensen and Mesman, 2006). The major assumption is that a Weight of Evidence approach in three independent disciplines will lead to a more precise answer than an approach, which is solely based on, for example, the concentrations of pollutants at the site. Such integrated assessment of mixtures could benefit from media-, site-, or population-oriented elements of legislation, such as the Water Framework Directive (EC, 2000), the Marine Strategy Directive (2008/56/EC), or the proposed Soil Directive (Kortenkamp et al., 2009).

Whole Mixture approaches: application to mixtures with radioactive substances

In the literature, we found no example of Whole Mixture approaches including radioactive substances. However, there is no conceptual problem for the application of Whole Mixture approaches to mixtures containing radioactive substances. As the principle of the approach is based on a direct toxicity testing of the effluent of sample, those methods could apply for all mixtures. The main domains where research and development would be needed for the application of such approaches on radioactive substances in mixture would be:

- To standardize alternative methods and endpoints, including the application of specific biosensors-based tools in WET approach, which would be more sensitive to priority pollutants and emerging substances (e.g. Barata et al.,2008; Kwon et al.,2008), but also potentially radioactive substances. This would also be potentially useful for the development of a BDF approach, through the identification of sensitive and specific to ionising radiation.
- To develop an adapted TIE approach for radioactive substances. This adaptation could gain from the knowledge in radiochemical separation of radionuclides, including recent development of flow techniques to environmental samples (e.g. Fajardo et al., 2010), while a priori very difficult to implement.

On the other hand, one can recall that current ERA developments for radioactive substances is mainly based on radiation dosimetric calculations for internal and external exposure. The total absorbed dose(rate) calculation is usually performed, adding internal and external exposure to ionising radiation. This is an implicit consideration of radioactive substances as a more or less “Sufficiently similar mixture”: all types of ionising radiation are considered sufficiently similar, with an eventual radiation weighting factor to account for the relative biological effectiveness of the radiation type (Chambers et al., 2006). Within STAR, experimental developments will give knowledge on radiation toxicity (both gamma and alpha – WP5). Those results will potentially comfort the consideration of radiation as a common/similar mixture, and use external gamma irradiation as a relevant substitute to evaluate the detrimental effects of all radionuclides.

Whole mixture approaches also have several limitations. The first one is that the toxic mixture has to be available for a direct experimentation, and is thus inappropriate for prospective assessments (e.g. for setting of environmental standards). Moreover, as a function of the test used, the measured toxicity does not take into account fully the fate and accumulation kinetics in the organisms that are critical for chronic ecotoxicological assessment. Finally, Whole mixture testing appears more adapted to the assessment of acute (short-term) toxicities.

There is an extremely large number of potentially relevant mixtures, with respect to the number of compounds that are of proven or potential environmental relevance, including radioactive substances. In this context, there is a need for a pragmatic, economic and ethically sustainable methodology that does not necessarily need new experimental data, at least for its use under screening Tiers of an Ecological Risk Assessment. Component-based approaches were mainly developed for this purpose.

5.2.3 Component-based approaches

As reviewed by Ragas et al. (2011), several methods based on predicting mixture toxicities from a known or assumed chemical composition and knowledge on the toxicities of the mixture components have been developed. These approaches are termed “Component-based” and are more or less all based on the classical mixture toxicity concepts of Concentration Addition (CA) and Independent Action (IA) to perform a Cumulative Risk Assessment (CRA), *i.e.* the combination of risks from aggregate exposures to multiple stressors.

General overview of Component-Based approaches

All published guidelines and recommendations on mixture toxicity assessment in regulatory settings focus on Independent Action (IA) and Concentration Addition (CA) as the central mixture toxicity concepts (see Chapter 4 for details) for the estimation of the risk under the assumption of zero interactions between substances. As environmentally realistic mixtures cannot be expected to be composed of either only similarly or of only dissimilarly acting compounds, two basic options exist for the predictive assessment of pollutant mixtures in a regulatory context: (a) a case by case selection of the most appropriate modelling approach or (b) the *a priori* choice of one of the concepts as a pragmatic default approach. For implementing mixture toxicity assessments into regulation, it is important, to analyse whether and how these options are applicable. As both concepts come to fundamentally opposite conclusions with respect to the contribution of low, individually non-toxic concentrations, this issue requires special attention.

However, for many, if not most, environmentally relevant mixtures, knowledge about the (dis)similarity of the modes of toxic action of most components is scarce, or even absent. Filling in these gaps for all potentially relevant exposure scenarios requires a substantial effort, especially because the modes of toxic action might be specific for each potentially exposed species and considered biological endpoint. Although some detailed knowledge exists for certain groups of environmental chemicals, such as pesticides, PAH… this information is usually restricted to a very few species (target organisms) and/or biological endpoints. With the (eco)toxicological data that are generated or compiled during, e.g. the registration of industrial chemicals with REACH, such a case-by-case approach is certainly not possible.

The *a priori* choice of one concept as a pragmatic default approach is only justifiable if on average the concept is conservative or only minor errors occur. Also, when having the precautionary principle in mind, that concept should be *a priori* selected, which in the case of an error does not lead to an underestimation of the mixture toxicity. Empirical evidence strongly argues for CA from this perspective. Mathematical analyses showed that considerable errors (> one order of magnitude) may occur only with large number of individual mixture components (>10) and extremely steep concentration-response relationships (Faust, 2000). IA, in contrast, can only be applied if full concentration-response curves are available for each compound in the mixture – which is rarely the case. Thus, in summary it may be concluded, that for the *a priori* selection of concept empirical evidence and mathematical considerations as well as the precautionary principle point to CA as a pragmatic and defendable default approach.

Application of Component-Based approaches in ERA assessments and regulation

As mentioned above, EU legislation generally provides the opportunity to account for mixture effects, but explicit guidance is often lacking. Most of the Directives and Regulations examined by Kortenkamp et al. (2009) are component-based approaches. In REACH, for example, CRA for multiple chemicals from multiple sources, routes and pathways is only addressed to a very limited extent in the current guidance. Other relevant European legislation does not contain a mandate for CRA for multiple chemicals from multiple sources, routes and pathways (Kortenkamp and Hass, 2009).Process-oriented pieces of environmental legislation that control emissions from production, transportation, and recycle processes, such as the IPPC (Directive 2008/1/EC), provide a basis for assessing mixtures of chemicals released from a definite source.

Many environmental authorities and collaborating research institutions in EU member states have extensive experience with various types of component based approaches (see review from Kortenkamp et al., 2009). Most member states use one or more approaches of concentration addition (CA), whereas only a few apply independent action (IA) (*i.e.* Denmark, Spain and the Netherlands) or mixed models – combining CA and IA (*i.e.* Spain and the Netherlands). Generally, CA is used for substances with an assumed similar mechanism/mode of action (MOA), such as dioxins, furanes, dioxin like PCBs, substances with estrogenic activity, PAHs, phenols, some metals, pharmaceuticals, and pesticides (Kortenkamp et al., 2009).

The more detailed example regarding use of Component-based approaches can be provided using the Netherlands case. There are no legal requirements in the Netherlands to perform complete mixture toxicity tests and no plans to introduce such legal requirements. Nevertheless, there is extensive experience in dealing with mixtures. Generally this work has been people-driven – focused on gaining scientific insights; or considered from a precautionary principle perspective (Kortenkamp et al., 2009).

Example of Component-Based approaches

Many different component-based techniques have been developed, varying from very simple and rough to highly sophisticated and accurate. Posthuma et al. (2008) proposed a tiered system for component-based methods in ecological risk assessment, from simplest/conservative approaches to more detailed characterization and sophisticated models. Following this classification, the most used component based approaches are summarized in Table 10. This synthesis is derived from the review of Kortenkamp et al. (2009), Ragas et al. (2011) and some detailed examples are given below. Most of the described methods were built under the assumption of zero interactions between substances in mixture, except for Sophisticated Mechanistic Models used in higher tiers and for a modification of Hazard indexes method (see below).

Table 10. Synthesis of Component-based approaches used for Ecological Risk Assessment, ranked within a tiered approach

	Method	Assumption/model	Information required
Tier 0: conservative assessment			
	SF (Safety Factor)	A. Does not cover mixture B. Partial information C. Interaction effects	None Assess information extent Assess likelihood of interactions
Tier 1: summation of PEC/PNEC			
	HI(Hazard indexes). Optional: modify HI based on binary interactions data.	Point estimates on concentration–effect curves(optional: assume binary interaction data represent higher-order interactions)	Toxicological reference values for the mixture components, for example, EC_{50}, NOEC(optional: binary interaction data)
Tier 2: CA or IA models			
	CA (Direct application)	Full-curve-based approaches, modes of action assumed fully similar or fully dissimilar - Safety factors can be used	Concentration-response relationships for the components expressed incomparable units
	TEF (Toxic Equivalence Factor), **TEQ** (Toxicity Equivalent)		
	RPF (Relative Potency Factor)		
	TUS (Toxic Unit Summation)		
	msPAF (multi-substance Probably Affected Fraction).		
Tier 3: Mixed CA/IA models			
	TSP (two-step prediction method)	1) CA is used for quantifying the net effects within a subgroup of compounds in the mixture for which similar MoAs are assumed (e.g. all narcotic acting compounds or all organophosphorus insecticides) 2) IA is used to aggregate to the net effect of the whole mixture. In the latter action, the toxic pressures posed by the subgroups of compounds with similar MoAs are aggregated over the different MoAs.	Concentration-response relationships for the components, mode of action information
	msPAF (multi-substance Probably Affected Fraction).		
Tier 4: Sophisticated Mechanistic Models			
	PBPK = Physiologically Based Pharmacokinetic	Sophisticated mechanistic models, including interaction data and data on different characteristics in the set of receptor species(assemblage-level assessments only)	Similar and dissimilar action, full-curve-based approach, kinetics and dynamics of mixture components and toxicological interactions…
	BRN = Biochemical Reaction Network		

Tier 0: Safety Factors

In tier 0, mixture effects are considered potentially relevant for the assessment, but detailed data on mixture effects are lacking. In such cases, a non-mixture-data or theory-driven safety factor is used, whereby the mere presence of this factor in the assessment reflects uncertainty on various issues, including mixture impacts. Three different situations can be distinguished as a function of the degree of knowledge: *(i)* where mixture assessment is not possible, an arbitrary safety factors can be applied (e.g. application of an extra safety factor when setting standards for single compounds to account for potential mixture effects); *(ii)* where only partial information about the mixture composition is available, the safety factor is supposed to cover for the components for which information is lacking. The value of the safety factor should reflect the extent of the missing information; *(iii)* when there are known interaction, but quantitatively poorly defined, safety factor will depends on the nature of the suspected effects (e.g. synergistic or antagonistic) and the quality of the available information.

Tier 1: Hazard Indexes

Hazard Indexes involve the application of a simplified form of CA, by calculating the quotient between the exposure concentration of each component and a point estimate from its concentration–effect curve (e.g. the EC_{10}, EC_{50}, or NOEC), and then the summation of quotients is calculated using the following equation:

$$HI = \frac{C_1}{NOEC_1} + \frac{C_2}{NOEC_2} + \ldots + \frac{C_n}{NOEC_n}$$

where :

C_i	Concentration of the substance i
$NOEC_i$	No Observed Effect Concentration for the substance i

These methods are often applied in cases where the assessment problem is quite simple. For human risk assessment, a tiered-approach is sometimes followed using Hazard Index: first, the ratios of all substances in the mixture are summed, irrespective of the effect they cause. If this sum exceeds 1, target-organ-specific HIs are calculated. One step further, separate HIs for specific molecular receptors can be calculated if detailed information about these receptors is available.

Finally, qualitative information about potential interaction effects can be included, resulting in an interaction-based HI. The United States Environmental Protection Agency (US EPA, 2000) developed such an interaction-based Hazard Index (HI_{int}-EPA) that can be used to quantify interaction effects in a mixture. This method was applied by Ragas et al.(2011) to perform a Cumulative Risk Assessment of chemical exposures in urban environments. The authors mentioned that key assumptions are that the interactions in a mixture can be represented as departures from concentration addition and that the interactions in a complex mixture are a function of the interactions of all possible binary combinations of the individual mixture components. Starting point is the calculation of a concentration additive HI for each endpoint considered, and this HI is then modified to reflect the possible interactions. The HI_{int}-EPA can include substances that do not directly contribute to the concentration additive HI, but do influence the toxicity of the other substances that contribute to the endpoint considered. HI_{int}-EPA is calculated using the following equation:

$$HI_{\text{int}} = \sum_{i=1}^{n} \left(\frac{C_i}{Standard_i} \cdot IF_i \right)$$

where,

IF_i represents the interaction factor that accounts for the influence of the other substances on substance i. This interaction factor was calculated as follows:

$$IF_i = \sum_{j \neq i}^{n} f_{ij} M_{ij}^{B_{ij}\theta_{ij}}$$

where:

B_{ij}	Evidence factor that reflects the strength of evidence that chemical j will influence the toxicity of chemical i and that this influence will be relevant to risk assessment ($-1<B_{ij}< 1$)
M_{ij}	The expected maximum interaction effect of substance j on chemical I(default = 5)
f_{ij}	Scaling factor that scales the contribution of chemical j by its importance relative to all the other chemicals that can interact with chemical I($0 <f_{ij}< 1$)
θ_{ij}	Factor that represents the degree to which substances i and j are present in equitoxic amounts. It is assumed that the greatest deviation from additivity will occur when both components are present in equitoxic amounts ($0 \leq \theta_{ij} \leq 1$)

<u>Tier 2</u>: Concentration Addition or Independent Action

Tier 2 assumes generally similarly acting compounds (*i.e.* concentration addition), hardly ever a complete non-uniform set of modes of action (*i.e.* independent action – also called response addition). CA in Tier 2 differs from that in Tier 1 by using the full-dose–response curve. First, the concentration of the components is expressed in comparable units. Subsequently, these units are summed and a dose–response model is applied to predict the response. Examples include the application of RPFs, TEFs, and toxic units. These techniques are commonly used in human as well as in ecological risk assessment of mixtures.

The method of Toxic Unit Summation (TUS) (Sprague, 1970) is a direct application of the CA concept and defined by the formula,

$$TUS = \sum_{i=1}^{n} TU = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

where,

c_i	actual concentrations (or doses) of the individual substances in a mixture

ECx_i equieffective concentrations (or doses) of these substances if present singly

The quotients $\frac{c_i}{ECx_i}$ are termed Toxic Units (TU). Toxic Units rescale absolute concentrations (or doses) of substances to their different individual toxic potencies. They express the concentrations (or doses) of mixture components as fractions of equi-effective individual concentrations (or doses) ECx_i. Typically, x = 50 % (EC_{50i}) is chosen as the reference level, but TUS can also be calculated for any other effect level x. If TUS = 1, the mixture is expected to elicit the total effect x. If the sum of Toxic Units is smaller or larger than 1, the mixture is expected to elicit effects smaller or larger than x, respectively.

For example, the Toxic Equivalency Factors (TEFs) are commonly used for polychlorinated dibenzo-p-dioxins (PCDDs),dibenzofirans (PCDFs) and dioxine-like polychlorinated biphenyls (PCBs) for both human and ecological risk assessment. Chemical congeners have differing toxicities to organisms, so each congener is “normalized” to the toxicity level of the most toxic congener (Van den Berg et al., 1998; USEPA, 2001). On this basis, Toxic Equivalent (TEQ) is calculated by multiplying the exposure value of each component by its corresponding TEF. The contribution of that congener to the equivalent toxicity for the sample is calculated as follows:

$$TEQ = \sum_{i=1}^{n}[PCDD_i \times TEF_i] + \sum_{i=1}^{n}[PCDF_i \times TEF_i] + \sum_{i=1}^{n}[PCB_i \times TEF_i]$$

In a similar way, RPF (Relative Potency Factors) are sometimes used for carcinogenic PAHs (Budinsky et al., 2006). RPF is defined as a factor that express the toxic potency of a mixture component relative to an index compound.

Tier 3: Mixed Models

At this tier, the use of both CA and IA models together (mixed-model approaches) is performed. Detailed information on the MOA for the different mixture components as well as full-curve-based modelling approaches are used (De Zwart and Posthuma, 2005; Ra et al., 2006)

An example is given by Ra et al.(2006) to estimate the combined toxicity by two-step prediction model on the complicated chemical mixtures from wastewater treatment plant effluents. Because toxicity models (IA and CA) are applicable only to one mode of action, the authors proposed to overcome this limitation, using a two-step prediction (TSP) method developed by Junghans (2004) with some modification (Figure 5). The TSP model can predict the toxicity of a mixture with a combination of binary modes of action, both similar and dissimilar, based on the chemical modes of action of 10 target chemicals, divided into three groups: Acetylcholinesterase (AChE) inhibitors, narcosis inhibitors, and seedling root inhibitors.

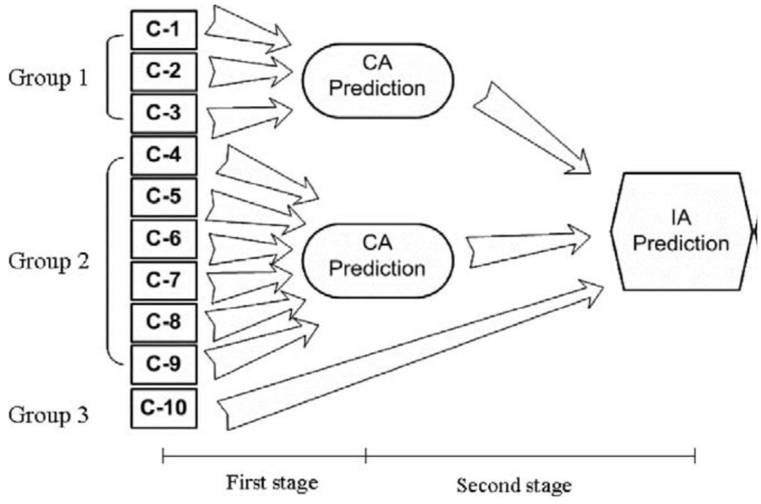

Figure 5. The stepwise approach of the two-step prediction model - from Ra et al. (**2006).**

C-i indicates the chemical components comprising the mixture. CA concentration addition model; IA independent action model.

In a similar way, to calculate the toxic pressure of multiple chemicals on an ecosystem (multisubstance PAF[5] [msPAF]), Henning-De Jong et al.(2008) used a combination of concentration-addition and response-addition calculations for the ranking of agricultural pesticides in the Rhine-Meuse-Scheldt basin based on toxic pressure in marine ecosystems. Concentration-addition rules were applied for chemicals with the same toxic mode of action (TMoA) to calculate the PAF_{TMoA}, further aggregated to an overall msPAF by means of Independent Action, assuming absence of correlation of sensitivities of species for different pesticides and pesticide groups:

$$msPAF = 1 - \prod_{TMoA} (1 - PAF_{TMoA})$$

<u>Tier 4</u>: Sophisticated Mechanistic Models

This tier includes all methods that go beyond CA or RA for a mechanistic explanation of interactions. It requires detailed information on the toxicokinetic and toxicodynamic processes involved, and only applies to problems that are defined in a very specific wayand where an accurate result is preferred over a conservative one.

Risk assessment methodology for chemical mixtures that accounts for toxicokinetic interactions among components was rarely developed. An example is given by Haddad et al. (2001) to assess the health risk associated with occupational inhalation exposure to airborne mixtures of dichloromethane, benzene, toluene, ethylbenzene, and m-xylene. The basis of the proposed risk assessment methodology relates to the characterization of the change in tissue dose during mixed exposures using PBPK models. In this study, an interaction-based hazard index was calculated using data on tissue dose of mixture

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[5] PAF is a general notation to identify that the probably affected fraction (PAF) of species by a toxicant is based on Species Sensitivity Distribution with a specific type of endpoint, e.g. NOECs.

constituents, obtained using a mixture PBPK model that accounted for the binary and higher order interactions occurring within the mixture.

In this study, the presence of competitive inhibitors such as toluene, ethylbenzene, benzene and m-xylene was shown to reduce the rate of dichloromethane metabolism by P450, resulting in a diminution of the formation of carboxyhemoglobin. The approaches developed in this study permit, for the first time, the consideration of the impact of multichemical toxicokinetic interactions at a quantitative level in mixture risk assessments.

Component-Based approaches: application to mixtures with radioactive substances

Few attempts were made to implement a mixed assessment of the chemical and radiological risks in Ecosystems. The first example reported below concern the combined Ecological Risk Assessments for radiological and chemical toxicity of uranium.

Ecological Risk Assessments for uranium (U) are confronted with a unique challenge in that U induces both chemical and radiological toxicity, and the relative importance of these toxicities depends on the contribution of the different isotopes considered. Diverse nuclear applications (nuclear fuel cycle, military use, etc.) take advantage of the properties of different isotopic compositions of U, and consequently discharges from these applications represent different radiological and chemical risks for the environment. A number of studies suggest that there are greater risks due to the chemical rather than radiological toxicity of U. Beaugelin-Seiller et al. (2009) and Mathews et al. (2009) were the first to consider both the chemical and radiological toxicity of U, through the calculation of Risk quotients. Results showed that the percentage of species radiologically protected by the chemical benchmark decreases with increasing degrees of U enrichment (relatively more ^{235}U compared to ^{238}U) and with increasing periods of radioactive decay. In contrast, the freshwater ecosystem was almost never chemically protected by the radiological benchmark, regardless of the source term or decay period considered, confirming that the risks to the environment from uranium's chemical toxicity generally outweigh those of its radiological toxicity. These results highlighted the need for (i) further research on the speciation, bioavailability, and toxicity of U-series radionuclides under different environmental conditions, and (ii) the adoption of both chemical and radiological benchmarks for coherent ERAs to be conducted in U-contaminated freshwater ecosystems.

Another example is given by Garnier-Laplace et al. (2009). The authors used a Screening Level Ecological Risk Assessment and ranking method for liquid radioactive and chemical mixtures released by nuclear facilities under normal operating conditions. The method to estimate the toxic pressure under the assumption of zero interactions between substances when they are in mixture (similar to concentration addition), was similar to msPAF and calculated as follow:

$$\Delta PAF_i = 0.5 \times \sum_i \frac{\Delta C_i}{HC_{50i}}$$

Where:

HC_{50i}	Hazardous Concentration of the substance i affecting 50% of species at their 50% effect (e.g. determined from an SSD)
ΔPAF_i	Change in the potentially affected fraction of species (PAF)
ΔC_i	Change in concentration of the substance i

The method allowed to rank the potentially released substances on the basis of the associated ecotoxicological hazard for the environment and therefore to identify high-risk chemicals and/or radioactive substances for ecosystems. The method enables to compare the relative importance of released chemicals and radioactive substances in terms of ecological risks. On a yearly based emission scenario, the authors showed that total percentage of potentially affected species for freshwaters remains lower than 1%. Moreover, the PAF variation in one year was shown to be 10^4 to 10^6 times higher for chemicals (Cu > Zn > H_3BO_3) than for radioactive substances ($^{14}C > ^{110m}Ag > ^{60}Co$) according to the site.

Those two examples show that there is no conceptual limit for the application of Component-based approaches on mixtures including radioactive substances, as far as an estimate of the predicted no-effect dose rate (PNEDR) is derived in a consistent way similar to that of other chemicals. As for other above examples on chemical mixtures, the two main advantages using Component-based approaches for Risk Assessment are, (1) the use of existing ecotoxicity data on single substance without needs for specific toxicity experimentation on the mixtures, and (2) the empirical support of approach by CA and IA models. However, the use of such approaches is faced with several uncertainties, mainly in the case of complex mixtures where the composition is often partially unknown (a risk would be to forget one or several significant toxic contributors). Moreover, even though some empirical methods allows interactions to be calculated (e.g. interaction-based Hazard Index), scientific basis is too weak for its generalization. On the other hand, the development of real mechanistic models would be very laborious and costly for an extended application in Ecological Risk Assessments (such approaches mainly remains within the scope of scientific research).

5.3 Conclusions: what are the needs for an integrated Ecological Risk Assessment of mixtures, including radioactive substances?

The above overview of current development for the Ecological Risk Assessment of mixtures showed that several methodologies were proposed in the scientific literature, from very basic and conservative ones to fully mechanistic approaches. However, no comprehensive guideline for the ecotoxicological assessment of chemical mixtures has been developed yet, although approaches have been developed and discussed in the scientific literature. Below are listed the main conclusions highlighted by this overview; each of them requires specific developments and research, to develop a robust ERA framework for mixtures including radioactive substances:

(1) The developments of an Ecological Risk Assessment framework for mixtures remains consistent with the general ERA framework, but integration in regulation is needed.

Guidance is still lacking for the use of different tools recently proposed, from conservative and simple tools (e.g. Safety factors) to the more realistic, accurate but complex and data rich methods (e.g. Based on mode of action, toxicokinetics, taxonomy…). An important issue is to define the trigger to go from a basic, simplistic and conservative tier to a higher tier. Regarding the integration of such approaches in the current legislation, one option should be to consider a Tiered Mixture Assessment Approach, as proposed by Posthuma et al.(2008). This would also need an integration of the different elements of legislation dealing with the different types of toxicants to evolve to an integrated framework.

In this sense, media-, site-, or population-oriented elements of legislation, such as the Water Framework Directive (EC, 2000) or Integrated pollution prevention and control (EC, 2008), will help for the emergence of more general, trans-sectorial strategies, especially through the use of Whole Mixture approaches in combination with (i) a chemical characterization-driven Component-Based approach, and (ii) field ecological status characterizations (weight-of-evidence approaches, e.g. TRIAD; eco-epidemiological analysis…). This type of comprehensive approach would in addition balance the assessments based only on the knowledge of isolated compounds (e.g. with chemical monitoring data, and toxicity evaluated in simplified systems), where exhaustiveness is hardly proven (both in regard to exposure and effect analysis), and to ensure a sufficient representativeness with respect to protection of the structure and the function of the ecosystems.

Regarding radioactive substances, the on-going revision of the European Basic Safety Standards Directive (EC, 1996) to consider the protection of non-human will probably help the specific consideration of radiation exposure of biota in the environment as a whole.

(2) CA and IA models are consensual scientific support for component-based Cumulative Risk Assessments under the assumption of zero interactions, and provide a basis for the consideration of mixtures with radioactive substances.

Research is still in progress, so there is quite a long way forward to develop novel methods and to refine approaches. At least, the scientific basis underpinning the development of a pragmatic Ecological Risk Assessment of mixtures for prospective and screening purposes is now sufficiently mature: there is a clear consensus to conform the different component-based approaches on CA and IA under the assumption of zero interactions between substances, and CA statement appears a pragmatic and defendable default conservative approach.

For the generalization of this framework, there is still a clear need for a comprehensive classification scheme for stressors on a “mechanisms/modes of action” basis, to support application of CA/IA models. Grouping substances through a receptor-oriented approach would lead to a more adequate effect assessment approach, and would allow focusing on the receptor rather than on a single chemical or specific cocktail.

For radioactive substances, the radiotoxic effects seem non-specific (e.g. DNA and protein alteration, oxidative stress) and should theoretically best rely to Independent Action, more than Concentration Addition. On the other hand, chemical toxicity and specific tissue/cellular distribution (and internal dose) of some alpha or beta emitters would need a specific consideration(e.g. uranium), in the same way than trace metals (e.g. Cu, Cd, Pb…) where bioavailability, bioaccumulation and bioconcentration in specific tissues or organs are key processes to link exposure and effect. These two aspects are clear research needs for the future to support CA/IA models developments including radioactive substances, e.g. mixed models based for the estimation of toxic pressure (e.g. msPAF).

(3) And what about interactions within mixtures?

The challenge remains to identify the exceptions from the rule, *i.e.* cases where interactions (especially synergistic interactions) occur. In those cases, a need for mechanistic framework that accounts for mixture interactions at different process levels, e.g. absorption, metabolism, target site, physiological process… is needed. For radioactive substances, mechanistic models are an option if radiation or specific radionuclides are proven to affect bioavailability, toxicokinetics or detoxification of specific chemicals, or inversely if some chemicals affect bioavailability, toxicokinetics or detoxification of radionuclides. As for other toxicants, toxicokinetic rather than toxicodynamic mechanisms would seem to be more likely sources of toxicologically significant interactions. This is also a domain where experimental research, confrontation with data from real case-studies, and modelling development would be valuable to explore the possibilities for interactions with various relevant chemicals and radionuclides.

In the meantime, on-going developments for an integrated Ecological Risk Assessment of mixtures, including radioactive substances, will need to address at least the two following questions:

(4) While waiting for the perfect ERA framework for mixtures: identification of uncertainties and assessment factors.

The uncertainty/assessment factors implemented for the estimation of PNEC and PNED(R) are intended to deal with statistical uncertainties and a number of extrapolations (species – species extrapolations, inter-individual variations, extrapolations from acute to chronic effects…). Such uncertainty factors do not cover properly the possibility of combined effects. How large would an additional assessment factor have to be to take account of mixture effects remains a key issue.

In the case of mixtures that contain chemicals with unknown modes of action or different modes of action, Warne (2003) proposed to assume that the toxicity is mildly synergistic (*i.e.* all mixtures are 2.5 times more toxic than concentration addition). This was recommended, as only 5% of mixtures are more synergistic than 2.5 times additivity. Within REACH, such a default mixture assessment factor (MAF) is under discussion (Backhaus et al., 2010).

(5) How to validate the approach? Needs for confrontation with real mixture scenarios.

A major knowledge gap that hampers the actual validation of options for the Ecological Risk Assessment of mixtures is the currently limited knowledge on expected or typical exposure situations with respect to representative cases of industrial releases. There is a need for information to support the identification of relevant mixture exposure situations (analysis of more probable mixture exposure scenarios, occurrence of common mixture) and to identify unexpectedly high risks compared to the standard null models (*i.e.* synergistic effects). REACH regulation (Backhaus et al., 2010) or OECD guidance (OECD, 2007) already provides a series of classifications that may provide a basis for the analysis of exposure scenarios from different perspectives: sectors of use, product categories and article categories. Such an approach on radionuclides, as already done partially within this review (see Annex 1) is indispensable for identification of relevant mixture exposure situations (e.g. NPP normal operation releases, radiopharmaceuticals, uranium milling and tailing, oil spills, accidental releases…).

6 Conclusions

The aim of the state of the art review performed was to give an overview of mixed contaminant approaches and 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 (Chapter 3); (ii) the assessment of effects and possible interactions between contaminants (Chapter 4); (iii) the risk assessment framework and methods (Chapter 5).

Four different geochemical **speciation** models were compared to predict the speciation of selected radionuclides in the presence or absence of a number of (metal) co-contaminants, using two real life scenario's (Beaverlodge lake and Ritord scenario). Within each of the models different databases were further compared. It was concluded that:

- The speciation models are generally well-developed for some radionuclides of interest such as U and Th, but did not contain binding constants for other radionuclides like Ra and Po. The models are based on the assumption that thermodynamic equilibrium is valid
- As could be expected, all the model/database combinations considered have advantages and disadvantages. These largely relate to (i) the comprehensiveness of the different databases, (ii) the ability to simulate complex formation to humic substances (dissolved organic matter) and mineral oxides, and (iii) the ability to simulate oxidation-reduction and precipitation reactions. The most comprehensive model used was Visual MINTEQ.
- Overall, no significant effect of co-contaminants on the speciation of the studied radionuclides, U, Th and Pb was found, although the role of high concentrations of Fe and Al was unclear.. However, the importance of speciation modelling to understand the influence of bioavailability on exposure to ionic moieties is stressed.

The use of BLM as an established framework to study the **bioavailability** and uptake of different cations by organisms was considered. An important issue for radionuclides is the mode of action. If the radionuclide exerts its effects solely via external radiation without any uptake by the organism, the application of BLM model is not appropriate. On the other hand, if the effects are exerted either partly or totally following uptake of the radionuclide, as would be the case for alpha emitters, the BLM could be a valuable tool to describe the variability in uptake and consequent toxicity. However, to apply a BLM to mixtures of metals and radionuclides, a model would need to be parameterised for the individual components, and validated with experimental data.

The overview on tools for **effects related** research focused on CA/IA for single dose-response curves and thermodynamic DEBtox as two component based approaches and WET, TIE and EDA as whole mixture approaches. A comparative summary of the different tools is provided. As for different speciation models, each of these approaches has advantages and disadvantages. The main conclusions towards their use in radioecology can be summarised as follows:

- TIE and EDA can give an idea on which toxicant contributes most to the observed effect, but these approaches lack predictive power. A particular problem for mixtures containing radionuclides might be the difficulties in separation of radionuclides from other (metal) co-contaminants by chemical extraction;
- If a dose-response curve can be derived for each of the single components in the mixture, the general concepts of CA/IA can easily be applied on mixtures including ionising radiation or radionuclides, both to make predictions on mixture effects addition as well as to assess deviations from addition due to interacting effects in experimental data-sets. Derivation of a sufficient number of experimental mixture data-sets for comparison against CA/IA predictions can allow a systematic assessment of the variation in data fit to these models;
- DEBtox gives the possibility to explain and pinpoint observed interactions by the underlying biological mechanisms, and to generalize the observed effects independently of the tested exposure conditions (time, dose…). It is a dynamic approach that can give further insight in possible physiological mode of action of the toxicants and has the additional possibility of being able to derive NEC for further use e.g. in risk assessment. However, the parameterization of a DEB model for one organism is data-demanding, and will be a prerequisite for the derivation of a DEBtox for each contaminant (radionuclides and stable contaminants).At the present time, the number of DEBtox approaches applied to mixtures is very limited.

Ecological **risk assessment** for mixtures is a relatively new issue and the proposed methods are still under development. Different methods have been proposed within an Ecological Risk Assessment framework. Cumulative Risk Assessment methods are supported by the existing consensus on the validity of CA and IA concepts, with CA being operationally more conservative compared to IA. In addition, a comprehensive guideline for the ecotoxicological assessment of chemical mixtures and an integration of regulation is urgently needed. These include the proposition of a Tiered Approach from conservative and simple methods (e.g. Safety factors) up to the recent more realistic, accurate but complex and data rich methods (e.g. based on mode of action, toxicokinetics, taxonomy…). The main conclusions of the review of different approaches and their applicability within a radioecological context are as follows;

- To date the application of a whole mixture ERA approach for mixtures including radiation risks has not been documented in the literature and additional research and development are needed for its application. This should include the standardization of alternative methods and endpoints including the application of specific biosensors-based tools in WET/BDF approaches and the development of an adapted TIE approach specific for radioactive substances.
- From a component based perspective, some attempts have been made to implement a mixed assessment of the chemical and radiological risks in ecosystems, but also here specific development and research is required to develop a robust ERA framework.
- An integrated and validated ERA of mixtures, including radioactive substances, will need to address, at the very minimum, how to identify:

- The relevant mixture exposure situations (analysis of more probable mixture exposure scenarios, occurrence of common mixtures);
- Unexpectedly high risks compared to the standard null models (*i.e.* in which cases synergistic effects occurs).
- Uncertainty and variability in relation to interactive toxicity within mixture data-sets (e.g. through use of assessment factors) and how to deal with this issue.

A combination of practical and fundamental research, aimed at a better understanding of mixture effects, as well as adapted ecological risk assessment methods, aimed at predicting or analysing mixture effects at the community level based on the best of current knowledge, will make it possible to evaluate if environmental radiation protection criteria are robust in a multiple contaminant context.

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Annexes

Annex 1: Review of exposure scenarios where radionuclides and co-contaminants are involved

1. Introduction

To select the mixture of interest for further experiments, 4 major criteria may be considered:

- the capacity of the co-contaminant(s) to modify the chemical speciation in the medium and bioavailability of the radionuclide(s) of interest,
- the possible toxicokinetic and toxicodynamic interactions between the co-contaminant(s) and the radionuclide(s),
- the mode of action of each co-contaminant(s) and the radionuclide(s), either similar or dissimilar,
- the environmental relevance of the co-contaminant(s) and the radionuclide(s) based on either the quantity released per year by the nuclear industry or the possible exceeding of environmental guideline values (such as PNEC for example).

We here review for a number of industries the type and quantity of radionuclides and co-contaminants impacting the environment and additional information is provided to allow to appreciate the environmental relevance of these releases and resulting environmental concentrations. The different industries reviewed were: uranium mining and milling, nuclear fuel production, nuclear power plants, reprocessing plants, nuclear waste disposal and the NORM industry. For hospitals no information on co-contaminants released could be found.

2. Uranium mining and milling

2.1 Introduction

Uranium mining and milling, the first two steps in the nuclear fuel cycle, is mainly done in Australia, Canada, China, Kazakhstan, Namibia, Niger, the Russian Federation, South Africa, the USA and Uzbekistan. Canada produces the largest share of uranium (29 % of the worldwide supply) followed by Australia (21 %) (Abdelouas, 2006). Uranium mining was extensive in former German Democratic Republic during the period 1946-1990 with a total production of 200 kt and was in that period the world's third largest producer of U after Canada and the USA (Vandenhove et al., 2000).

Uranium mine debris and mill tailings (residue after U extraction from the ore) are hazardous due to radioactive and toxic elements that are originally present in the ore deposits. The mobility of radionuclides and toxic metals is enhanced in the milling process and by acidification of tailings due to sulphide oxidation catalysed by microbial activity.

For the sake of this review, we concentrate on the toxic elements associated with uranium mining and milling. After an overall review of the chemical composition of uranium mill tailings from different locations, we here present a few example cases for Canada, Germany, France and Brazil. These examples will rather give an appreciation than a holistic overview of the problematic.

Abdelouas (2006) collected information on the chemical composition of uranium mill tailings from different locations (Table 1). This highlights that, apart from the potential acidity (SO$_4$

content), As, Pb, V, Ba, Fe and Al and to a lesser extent Mo and Cr are potentially hazardous when leached from these tailings.

Table 1. Chemical composition of uranium mill tailings from different locations

Analyte	Canada	France	Utah[a]	Utah[b]
SO_4 (µg/g)	101,000	110,522	61,100	6760
Ca (µg/g)	51,800	52,500	63,100	17,700
Al (µg/g)	34,100	69,527	-	-
Fe (µg/g)	19,200	27,00	18,200	7640
As (µg/g)	5640	120,6	74	48
Pb (µg/g)	749	150,5	158	72
V (µg/g)	205	-	517	2820
U (µg/g)	152	126	531	350
Mo (µg/g)	91	-	35	10
Ba (µg/g)	77	195,4	1010	777
Cr (µg/g)	25	48,5	-	-
SiO_2 (µg/g)	28	52,3	-	-
^{226}Ra (Bq/g)	74	28,4	26,3	21,2
^{210}Pb (Bq/g)	18	-	-	-
^{210}Po (Bq/g)	14	-	-	-
^{230}Th (Bq/g)	19,5	-	32,3	26,1

(a) acid-leached ore, (b) carbonate-leached ore

2.2 Canada

Canada North Environmental Services (CanNorth, 2006) conducted aquatic investigations in areas of Lake Athabasca in 2004 and 2005 as part of the Saskatchewan Research Council (SRC) site characterization and remedial options review of the orphan Gunnar uranium mine site, located near Uranium City, Saskatchewan. The Gunnar uranium mine is one of a number of orphan mines in the Beaverlodge area, located on the north shore of Lake Athabasca, northern Saskatchewan. During mine operations, between 1955 and 1964, a total of 5.5 x 10^6 tonnes of ore were removed from the open pit and underground mine. The narrow rock ridge separating the pit from St. Mary's Channel was breached after the mine was closed in 1964. The open pit mine filled with water from Lake Athabasca and the breach was subsequently closed with crushed rock in 1966. During operations of the Gunnar uranium mine, mill tailings, consisting of 32% solids, were discharged into a small lake located 500 m north of the mill, referred to as Gunnar Main Tailings. Eventually this basin filled and a small rock outcrop was blasted to allow the tailings to flow from the Main Tailings area to a small depression, referred to as Gunnar Central Tailings. Once this small depression filled, the tailings flowed downhill and eventually entered Langley Bay of Lake Athabasca. The volume of tailings discharged into Langley Bay was sufficient to eventually fill in a large area of the bay, dividing Langley Bay into two distinct portions: Langley Bay, connected directly to Lake Athabasca, and a smaller, isolated area now referred to as Back Bay. Back Bay is connected to Langley Bay by a narrow channel (Figure 1) (CanNorth, 2006).

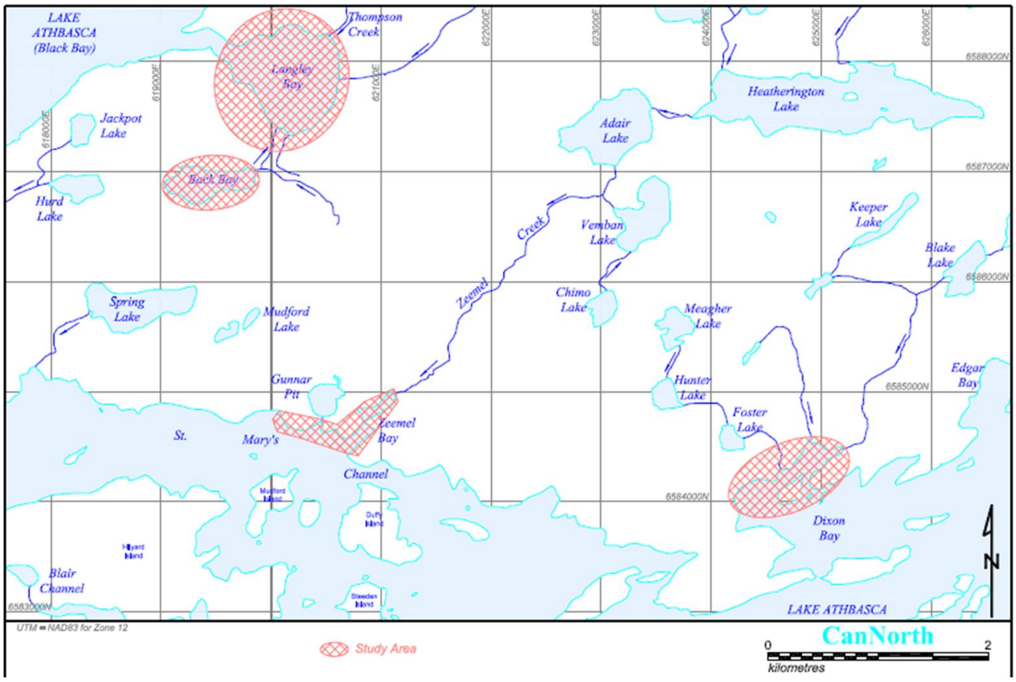
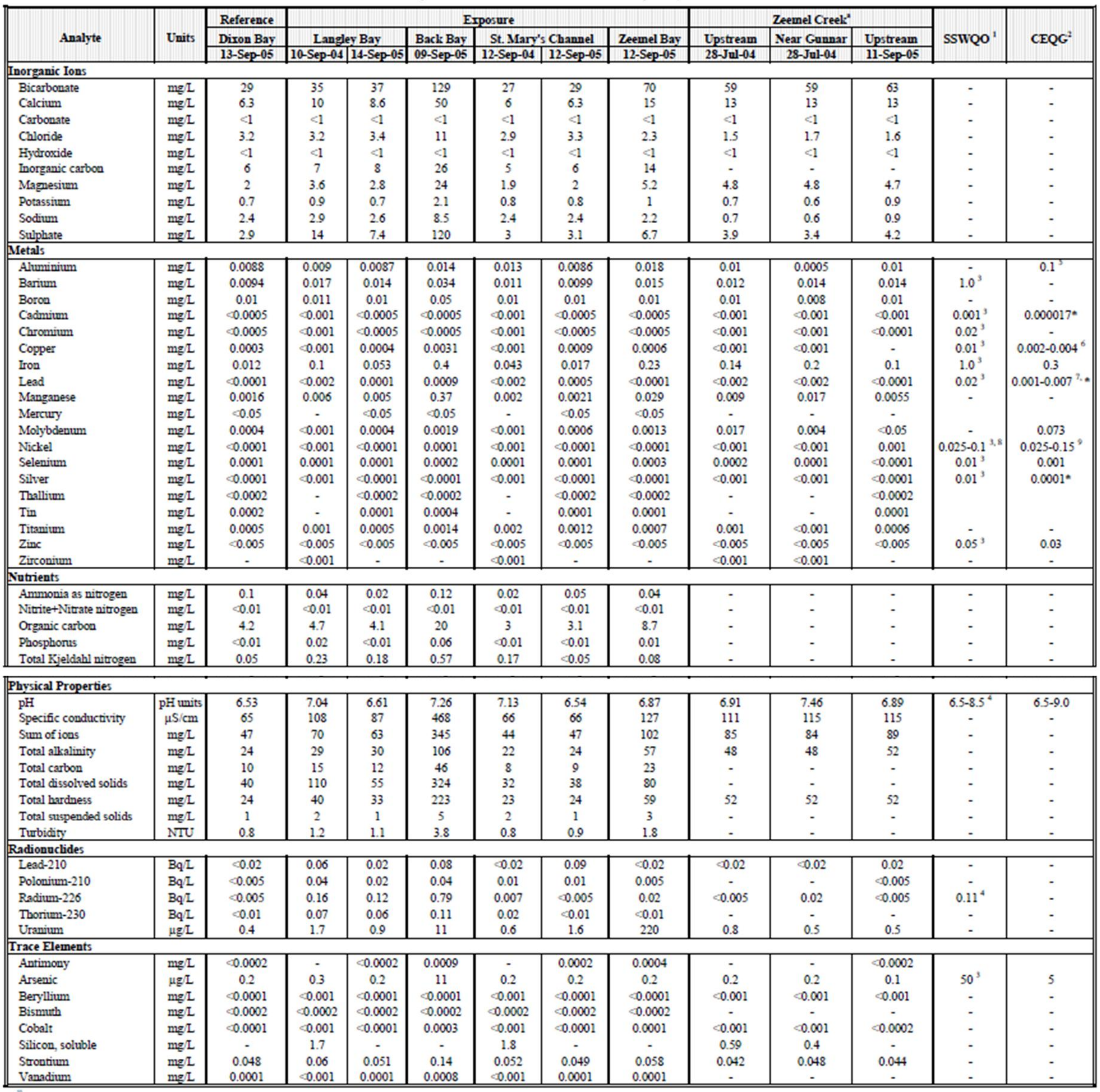


Figure 1. Location of the study area at the Gunnar uranium mining and milling area (CanNorth, 2006).

The objective of the aquatic investigations was to gather site-specific information to use in assessing remedial activities in these areas and in the risk assessment. These studies collected information on a.o. water, sediment, plant, and fish chemistry and plankton, benthic macroinvertebrate, and fish communities from the following study areas in Lake Athabasca: St. Mary’s Channel, Zeemel Bay, Langley Bay, Back Bay, and Dixon Bay. We here only discuss the results for the water and sediment chemistry, concentrating on the non-radioactive contaminants.

Water samples were collected in St. Mary’s Channel and Langley Bay in 2004, and from all study areas in 2005 (Table 2). Water chemistry in St. Mary’s Channel was comparable to the reference station and metal concentrations were lower than applicable provincial and federal guidelines. The uranium concentration in Zeemel Bay (220 μgL^{-1}) was much higher than all other study areas, where levels ranged between 0.4 and 11 μgL^{-1}. The Zeemel Bay sample was collected near the seep from the waste rock and this may be a localized effect. Langley Bay contained levels of metals, trace elements, ions, and nutrients that were similar to the reference station in Dixon Bay. In Back Bay, water concentrations of ions, nutrients, and some metals, such as arsenic, iron, manganese, and strontium, were higher than at the reference station. The only metals above guidelines were iron and arsenic in Back Bay, which were above the federal guidelines, but were well below the SSWQO (Saskatchewan Surface Water Quality Objectives).

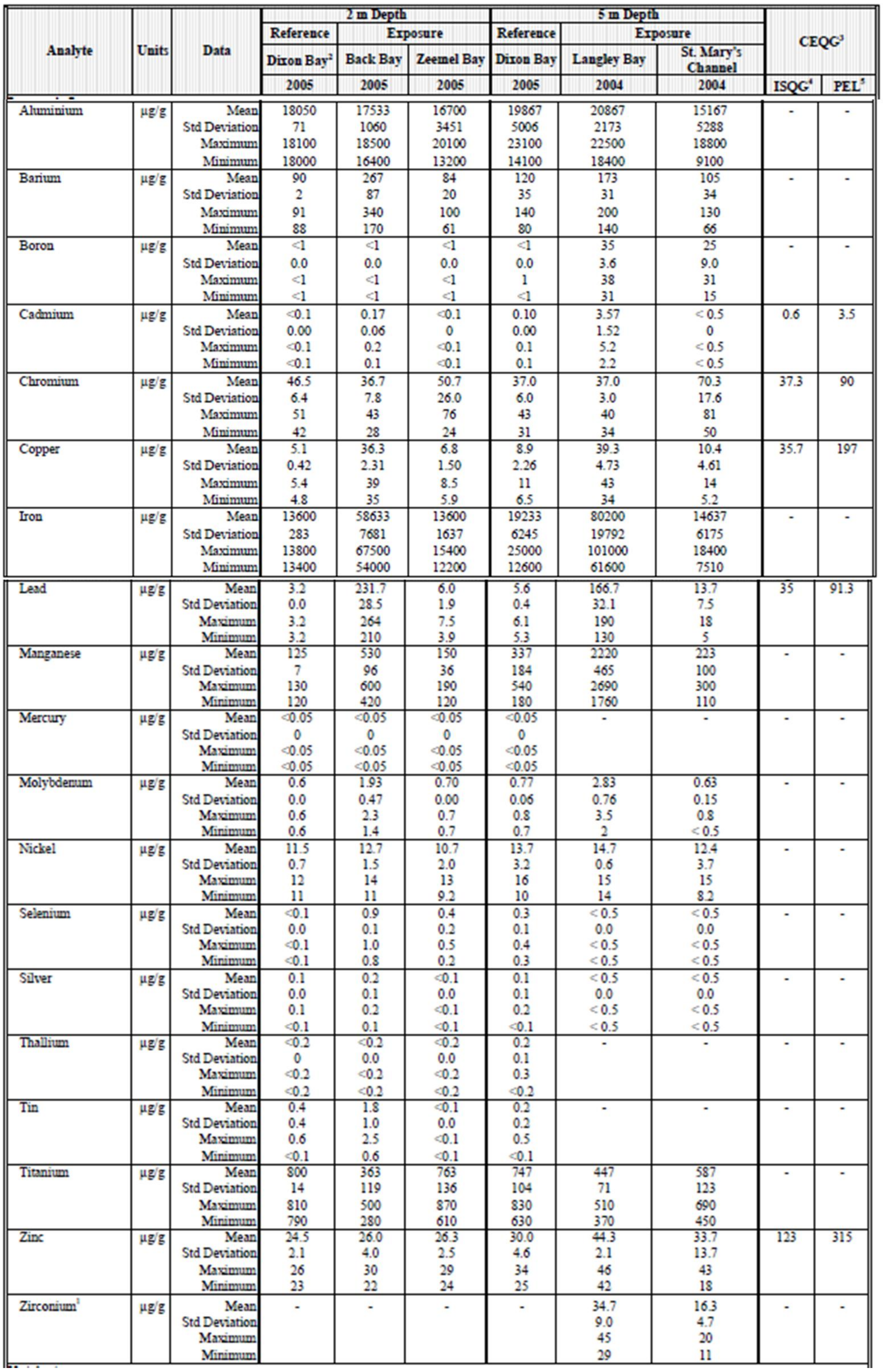
Table 2. Water chemistry results from the Gunner mine site study area, 2004 and 2005 (from CanNorth, 2006).



Analyte	Units	Reference	Exposure						Zeemel Creek[a]			SSWQO[1]	CEQG[2]
		Dixon Bay	Langley Bay		Back Bay	St. Mary's Channel		Zeemel Bay	Upstream	Near Gunnar	Upstream		
		13-Sep-05	10-Sep-04	14-Sep-05	09-Sep-05	12-Sep-04	12-Sep-05	12-Sep-05	28-Jul-04	28-Jul-04	11-Sep-05		
Inorganic Ions													
Bicarbonate	mg/L	29	35	37	129	27	29	70	59	59	63	-	-
Calcium	mg/L	6.3	10	8.6	50	6	6.3	15	13	13	13	-	-
Carbonate	mg/L	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	-
Chloride	mg/L	3.2	3.2	3.4	11	2.9	3.3	2.3	1.5	1.7	1.6	-	-
Hydroxide	mg/L	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	-
Inorganic carbon	mg/L	6	7	8	26	5	6	14	-	-	-	-	-
Magnesium	mg/L	2	3.6	2.8	24	1.9	2	5.2	4.8	4.8	4.7	-	-
Potassium	mg/L	0.7	0.9	0.7	2.1	0.8	0.8	1	0.7	0.6	0.9	-	-
Sodium	mg/L	2.4	2.9	2.6	8.5	2.4	2.4	2.2	0.7	0.6	0.9	-	-
Sulphate	mg/L	2.9	14	7.4	120	3	3.1	6.7	3.9	3.4	4.2	-	-
Metals													
Aluminium	mg/L	0.0088	0.009	0.0087	0.014	0.013	0.0086	0.018	0.01	0.0005	0.01	-	0.1[5]
Barium	mg/L	0.0094	0.017	0.014	0.034	0.011	0.0099	0.015	0.012	0.014	0.014	1.0[3]	-
Boron	mg/L	0.01	0.011	0.01	0.05	0.01	0.01	0.01	0.01	0.008	0.01	-	-
Cadmium	mg/L	<0.0005	<0.001	<0.0005	<0.0005	<0.001	<0.0005	<0.0005	<0.001	<0.001	<0.001	0.001[3]	0.000017*
Chromium	mg/L	<0.0005	<0.001	<0.0005	<0.0005	<0.001	<0.0005	<0.0005	<0.001	<0.001	<0.0001	0.02[3]	-
Copper	mg/L	0.0003	<0.001	0.0004	0.0031	<0.001	0.0009	0.0006	<0.001	<0.001	-	0.01[3]	0.002-0.004[6]
Iron	mg/L	0.012	0.1	0.053	0.4	0.043	0.017	0.23	0.14	0.2	0.1	1.0[3]	0.3
Lead	mg/L	<0.0001	<0.002	0.0001	0.0009	<0.002	0.0005	<0.0001	<0.002	<0.002	<0.0001	0.02[3]	0.001-0.007[7,*]
Manganese	mg/L	0.0016	0.006	0.005	0.37	0.002	0.0021	0.029	0.009	0.017	0.0055	-	-
Mercury	mg/L	<0.05	-	<0.05	<0.05	-	<0.05	<0.05	-	-	-		
Molybdenum	mg/L	0.0004	<0.001	0.0004	0.0019	<0.001	0.0006	0.0013	0.017	0.004	<0.05	-	0.073
Nickel	mg/L	<0.0001	<0.001	<0.0001	0.0001	<0.001	<0.0001	<0.0001	<0.001	<0.001	0.001	0.025-0.1[3,8]	0.025-0.15[9]
Selenium	mg/L	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001	0.0003	0.0002	0.0001	<0.0001	0.01[3]	0.001
Silver	mg/L	<0.0001	<0.001	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	<0.001	<0.001	<0.0001	0.01[3]	0.0001*
Thallium	mg/L	<0.0002	-	<0.0002	<0.0002	-	<0.0002	<0.0002	-	-	<0.0002		
Tin	mg/L	0.0002	-	0.0001	0.0004	-	0.0001	0.0001	-	-	0.0001		
Titanium	mg/L	0.0005	0.001	0.0005	0.0014	0.002	0.0012	0.0007	0.001	<0.001	0.0006	-	-
Zinc	mg/L	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.05[3]	0.03
Zirconium	mg/L	-	<0.001	-	-	<0.001	-	-	<0.001	<0.001	-	-	-
Nutrients													
Ammonia as nitrogen	mg/L	0.1	0.04	0.02	0.12	0.02	0.05	0.04	-	-	-	-	-
Nitrite+Nitrate nitrogen	mg/L	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	-	-	-	-
Organic carbon	mg/L	4.2	4.7	4.1	20	3	3.1	8.7	-	-	-	-	-
Phosphorus	mg/L	<0.01	0.02	<0.01	0.06	<0.01	<0.01	0.01	-	-	-	-	-
Total Kjeldahl nitrogen	mg/L	0.05	0.23	0.18	0.57	0.17	<0.05	0.08	-	-	-	-	-
Physical Properties													
pH	pH units	6.53	7.04	6.61	7.26	7.13	6.54	6.87	6.91	7.46	6.89	6.5-8.5[4]	6.5-9.0
Specific conductivity	µS/cm	65	108	87	468	66	66	127	111	115	115	-	-
Sum of ions	mg/L	47	70	63	345	44	47	102	85	84	89	-	-
Total alkalinity	mg/L	24	29	30	106	22	24	57	48	48	52	-	-
Total carbon	mg/L	10	15	12	46	8	9	23	-	-	-	-	-
Total dissolved solids	mg/L	40	110	55	324	32	38	80	-	-	-	-	-
Total hardness	mg/L	24	40	33	223	23	24	59	52	52	52	-	-
Total suspended solids	mg/L	1	2	1	5	2	1	3	-	-	-	-	-
Turbidity	NTU	0.8	1.2	1.1	3.8	0.8	0.9	1.8	-	-	-	-	-
Radionuclides													
Lead-210	Bq/L	<0.02	0.06	0.02	0.08	<0.02	0.09	<0.02	<0.02	<0.02	0.02	-	-
Polonium-210	Bq/L	<0.005	0.04	0.02	0.04	0.01	0.01	0.005	-	-	<0.005	-	-
Radium-226	Bq/L	<0.005	0.16	0.12	0.79	0.007	<0.005	0.02	<0.005	0.02	<0.005	0.11[4]	-
Thorium-230	Bq/L	<0.01	0.07	0.06	0.11	0.02	<0.01	<0.01	-	-	-	-	-
Uranium	µg/L	0.4	1.7	0.9	11	0.6	1.6	220	0.8	0.5	0.5	-	-
Trace Elements													
Antimony	mg/L	<0.0002	-	<0.0002	0.0009	-	0.0002	0.0004	-	-	<0.0002	-	-
Arsenic	µg/L	0.2	0.3	0.2	11	0.2	0.2	0.2	0.2	0.2	0.1	50[3]	5
Beryllium	mg/L	<0.0001	<0.001	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	<0.001	<0.001	<0.001	-	-
Bismuth	mg/L	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	-	-	-	-	-
Cobalt	mg/L	<0.0001	<0.001	<0.0001	0.0003	<0.001	<0.0001	0.0001	<0.001	<0.001	<0.0002	-	-
Silicon, soluble	mg/L	-	1.7	-	-	1.8	-	-	0.59	0.4	-	-	-
Strontium	mg/L	0.048	0.06	0.051	0.14	0.052	0.049	0.058	0.042	0.048	0.044	-	-
Vanadium	mg/L	0.0001	<0.001	0.0001	0.0008	<0.001	0.0001	0.0001	-	-	-	-	-

[a]Water chemistry data collected from Zeemel Creek by SRC was included for comparison purposes. Upstream means upstream of the Gunnar site.; [*]Guideline values are below analytical detection limits; [1]SSWQO - Saskatchewan Surface Water Quality Objectives for the protection of aquatic life; [2]CEQG - Canadian Environmental Quality Guidelines for the protection of aquatic life; [3]Specific Saskatchewan Surface Water Quality Objectives for the Protection of Aquatic Life and Wildlife; [4]General Surface Water Quality Objective 1997).; [5]Aluminium: 0.005 at pH < 6.5; 0.1 at pH > 6.5; [6]Copper: 0.002 at total hardness = 0-120 mg L^{-1}; 0.004 at total hardness >180 mg L^{-1}; [7]Lead: 0.001 at total hardness = 0-60 mg L^{-1}; 0.007 at total hardness >180 mg L^{-1}; [8]Nickel: 0.025 at total hardness <100 mg L^{-1}; 0.1 at total hardness >100 mg L^{-1}; [9]Nickel: 0.025 at total hardness = 0-60 mg L^{-1}; 0.15 at total hardness >180 mg L^{-1}.

Table 3. Summary chemistry results (only metals) from sediment samples collected in the Gunnar mine site study area, September 2004 and 2005 (from CanNorth, 2006).



Analyte	Units	Data	2 m Depth			5 m Depth			CEQG[3]	
			Reference	Exposure		Reference	Exposure			
			Dixon Bay[2]	Back Bay	Zeemel Bay	Dixon Bay	Langley Bay	St. Mary's Channel		
			2005	2005	2005	2005	2004	2004	ISQG[4]	PEL[5]
Aluminium	µg/g	Mean	18050	17533	16700	19867	20867	15167	-	-
		Std Deviation	71	1060	3451	5006	2173	5288		
		Maximum	18100	18500	20100	23100	22500	18800		
		Minimum	18000	16400	13200	14100	18400	9100		
Barium	µg/g	Mean	90	267	84	120	173	105	-	-
		Std Deviation	2	87	20	35	31	34		
		Maximum	91	340	100	140	200	130		
		Minimum	88	170	61	80	140	66		
Boron	µg/g	Mean	<1	<1	<1	<1	35	25	-	-
		Std Deviation	0.0	0.0	0.0	0.0	3.6	9.0		
		Maximum	<1	<1	<1	1	38	31		
		Minimum	<1	<1	<1	<1	31	15		
Cadmium	µg/g	Mean	<0.1	0.17	<0.1	0.10	3.57	< 0.5	0.6	3.5
		Std Deviation	0.00	0.06	0	0.00	1.52	0		
		Maximum	<0.1	0.2	<0.1	0.1	5.2	< 0.5		
		Minimum	<0.1	0.1	<0.1	0.1	2.2	< 0.5		
Chromium	µg/g	Mean	46.5	36.7	50.7	37.0	37.0	70.3	37.3	90
		Std Deviation	6.4	7.8	26.0	6.0	3.0	17.6		
		Maximum	51	43	76	43	40	81		
		Minimum	42	28	24	31	34	50		
Copper	µg/g	Mean	5.1	36.3	6.8	8.9	39.3	10.4	35.7	197
		Std Deviation	0.42	2.31	1.50	2.26	4.73	4.61		
		Maximum	5.4	39	8.5	11	43	14		
		Minimum	4.8	35	5.9	6.5	34	5.2		
Iron	µg/g	Mean	13600	58633	13600	19233	80200	14637	-	-
		Std Deviation	283	7681	1637	6245	19792	6175		
		Maximum	13800	67500	15400	25000	101000	18400		
		Minimum	13400	54000	12200	12600	61600	7510		
Lead	µg/g	Mean	3.2	231.7	6.0	5.6	166.7	13.7	35	91.3
		Std Deviation	0.0	28.5	1.9	0.4	32.1	7.5		
		Maximum	3.2	264	7.5	6.1	190	18		
		Minimum	3.2	210	3.9	5.3	130	5		
Manganese	µg/g	Mean	125	530	150	337	2220	223	-	-
		Std Deviation	7	96	36	184	465	100		
		Maximum	130	600	190	540	2690	300		
		Minimum	120	420	120	180	1760	110		
Mercury	µg/g	Mean	<0.05	<0.05	<0.05	<0.05	-	-	-	-
		Std Deviation	0	0	0	0				
		Maximum	<0.05	<0.05	<0.05	<0.05				
		Minimum	<0.05	<0.05	<0.05	<0.05				
Molybdenum	µg/g	Mean	0.6	1.93	0.70	0.77	2.83	0.63	-	-
		Std Deviation	0.0	0.47	0.00	0.06	0.76	0.15		
		Maximum	0.6	2.3	0.7	0.8	3.5	0.8		
		Minimum	0.6	1.4	0.7	0.7	2	< 0.5		
Nickel	µg/g	Mean	11.5	12.7	10.7	13.7	14.7	12.4	-	-
		Std Deviation	0.7	1.5	2.0	3.2	0.6	3.7		
		Maximum	12	14	13	16	15	15		
		Minimum	11	11	9.2	10	14	8.2		
Selenium	µg/g	Mean	<0.1	0.9	0.4	0.3	< 0.5	< 0.5	-	-
		Std Deviation	0.0	0.1	0.2	0.1	0.0	0.0		
		Maximum	<0.1	1.0	0.5	0.4	< 0.5	< 0.5		
		Minimum	<0.1	0.8	0.2	0.3	< 0.5	< 0.5		
Silver	µg/g	Mean	0.1	0.2	<0.1	0.1	< 0.5	< 0.5	-	-
		Std Deviation	0.0	0.1	0.0	0.1	0.0	0.0		
		Maximum	0.1	0.2	<0.1	0.2	< 0.5	< 0.5		
		Minimum	<0.1	0.1	<0.1	<0.1	< 0.5	< 0.5		
Thallium	µg/g	Mean	<0.2	<0.2	<0.2	0.2	-	-	-	-
		Std Deviation	0	0.0	0.0	0.1				
		Maximum	<0.2	<0.2	<0.2	0.3				
		Minimum	<0.2	<0.2	<0.2	<0.2				
Tin	µg/g	Mean	0.4	1.8	<0.1	0.2	-	-	-	-
		Std Deviation	0.4	1.0	0.0	0.2				
		Maximum	0.6	2.5	<0.1	0.5				
		Minimum	<0.1	0.6	<0.1	<0.1				
Titanium	µg/g	Mean	800	363	763	747	447	587	-	-
		Std Deviation	14	119	136	104	71	123		
		Maximum	810	500	870	830	510	690		
		Minimum	790	280	610	630	370	450		
Zinc	µg/g	Mean	24.5	26.0	26.3	30.0	44.3	33.7	123	315
		Std Deviation	2.1	4.0	2.5	4.6	2.1	13.7		
		Maximum	26	30	29	34	46	43		
		Minimum	23	22	24	25	42	18		
Zirconium[1]	µg/g	Mean	-	-	-	-	34.7	16.3	-	-
		Std Deviation					9.0	4.7		
		Maximum					45	20		
		Minimum					29	11		

[3] CEQG - Canadian Environmental Quality Guidelines; [4] ISQG - interim freshwater sediment quality guidelines; [5] PEL - probable effects level (CCME 2005).Values less than detection limit were set equal to detection limit for calculation of means and standard deviations.All results are presented on dry weight basis.

Sediment concentrations of sulphate, numerous metals and trace elements, and all radionuclides were substantially higher in Back Bay compared to Dixon Bay (Table 3). Mean concentrations of copper, lead, and arsenic in Back Bay exceeded federal sediment quality guidelines. Mean chromium concentrations were higher than the guideline in both Dixon and Zeemel bays, indicating that high chromium levels are found naturally in parts of Lake Athabasca. In Zeemel Bay, sediment uranium concentrations were elevated at the stations located near the seep, measuring 291 and 316 $\mu g\ g^{-1}$, while the station located closer to the mouth of the bay contained a uranium level that was similar to reference (7 $\mu g\ g^{-1}$). In St. Mary's Channel, mean sediment concentrations of boron, chromium, lead, and radionuclides were higher than in the reference area. Langley Bay sediment contained mean concentrations of several metals, trace elements, and all radionuclides that were higher than reference. Federal sediment quality guidelines were exceeded by cadmium, copper, lead, and arsenic in Langley Bay, and chromium in St. Mary's Channel.

2.3 Germany

Uranium mining was extensive in the former German Democratic Republic during the period 1946-1990 with a total production of 200 kt. About half of the uranium production originated from the Ronnebrug operations and heap leaching at the Gessenhalde site, where low-grade black shale uranium ores were leached during the period 1970-1989. The area is remediated since the mid-nineties but residual contamination is still seen. Carlsson and Büchel (2005) provide general information about residual contamination in soil and water. Soil concentrations of soils sampled in the valley at Gessenhalde were found to be elevated for V, Cr, Ni, Cu, Zn, As, Mo, W, Th and U (Table 4).

Table 4. Concentrations of heavy metals in soils sampled in the valley of Gessenhalde (after Carlsson and Büchel, 2005).

N = 26	Median	Average	SD[a]	Min	Max	HBS[b]	HBS[c]
V	151	171.3	53.6	111	334	18 - 28	30 - 61
Cr	77	82.7	15.8	65	121	17 - 21	37 - 55
Ni	76	83.8	28.5	48	170	5 - 10	36 - 48
Cu	41	49.2	27.6	24	149	10 - 21	14 - 23
Zn	93.5	100.7	21.1	80	164	29 - 40	48 - 72
As	20.5	27.7	24.3	9	112	4,5 - 9	12 - 16
Mo	4	5.3	4.7	b.d	23	0.2 - 0.38	0.4 - 0.5
W	6	6.5	2	3	12	0.72 - 0.94	1.2 - 1.5
Th	12	12.1	2	8	17	5.5 – 6.1	9.6 - 11
U	8.5	12.7	10.4	2	40	1.1 - 1.2	1.7 - 2.4

(a) SD= Standard deviation. (b,c) Hintergrundwerte fur Böden des Fraistaates Sachsen (Stand 10/2001) (Background values for soils in Sachsen): (b) Interval indicates average values range for farmland and meadow from periglacial sediments (Sand-loam, (c) Interval indicates 90th percentile interval for farmland and meadow from periglacial sediments (Sand-loam).

In the groundwater in the vicinity of the Gessenhalde (Table 5) concentrations for many elements (e.g. F, Cl, Fe, Mn, Ni, Cd, U) were above limits for drinking water).

Table 5. Groundwater concentrations at Gessenhalde (after Carlsson and Büchel, 2005).

Parameter (n=28)		Median	Average	SD[a]	Min	Max	TVO[b] (c)
pH	-	4.04	4.14	0.49	3.46	5.66	
F	mg/l	18	25	23.5	2.2	101	1.5
Cl	mg/l	123	198	188	9	584	250 (5)[c]
SO_4^{-2}	mg/l	3725	4549	4076	1475	20.570	240
Fe	µg/l	161	3281	7308	>20	29370	2000
Al	µg/l	36	71	97	58	381	200
Mn	µg/l	103400	183067	209251	3767	808000	400[c]
Co	µg/l	3043	5070	5576	48	20550	
Ni	µg/l	14	21	21	541	70	20
Cu	µg/l	291	669	924	2	3433	2000
Zn	µg/l	3734	4808	5334	48	20	
Cd	µg/l	63	115	131	1.6	513	3[c]
Ba	µg/l	18	20	10	6	49	700[c]
U	µg/l	59	338	667		3263	15[c]

(a) SDD = standard deviation; (b) Trinkwasserverordnung" German Drinking Water Ordinance (drinking water limiting value), available at www.dvgw.de; (c) WHO drinking water guidelines (WH0, 2008)

2.4 Brazil

Fernandes et al. (1996) provide information on the inventory of metals and radionuclides at the tailing dam of the uranium mining and milling facility of Poços de Caldas, Brazil (Table 6). These data allow comparing the potential importance of hazard from heavy metals compared to the radiological hazard.

Table 6. Inventories of metals and radionuclides at the tailings dam at Poços de Caldas (Mobile and Total Amount) (after Fernandes et al. 1996)

Element	*Total inventory (t)*	*Mobile inventory*	
		(t)	*(%)*
Al	2.5 x 10^5	600	0.24
Fe	5.6 x 10^4	2100	3.68
Mn	4.1 x 10^2	410	100
Zn	1.8 x 10^2	56	26.2
Ca	3.5 x 10^3	2100	61.2
U	3.70 x 10^2	26	7.1
Th	8.20 x 10^1	17	21
^{226}Ra	5.1 x 10^{12} (Bq)	3.0 x 10^{12} (Bq)	59.4
^{228}Ra	2.9 x 10^{12} (Bq)	4.5 x 10^{11} (Bq)	15.6
^{210}Pb	6.9 x 10^{12} (Bq)	4.5 x 10^{12} (Bq)	65

2.5 France

Herlory et al. (submitted) aimed to assess the impact from U-pit water from the Ritord area, in the Limoges uranium mining district. They did not only evaluate the impact from U but also from a range of other contaminants thereby considering also the importance of other relevant environmental parameters. For the sake of this study, we here only provide information on the concentrations in the Ritord catchment system.

More than a third of the total 73 000 tons of uranium extracted in France came from the Crouzille mining division (Limousin region). Seven watersheds drain this area and among them, the Ritord basin was selected to illustrate the impact of mining effluents on aquatic ecosystems. The Ritord basin corresponds to a small watercourse (12 km long for a 17 km² watershed) connecting two lakes and receiving pit waters from seven former mine sites. They collected river water at 4 locations: S0 as a reference station was situated upstream from mining effluent discharges and was considered by mines; S1 immediately downstream the discharge of Fanay-Augères; S2 further downstream between the two main discharge points; S3 downstream the Silord pit-water treatment plant ($BaCl_2$ to precipitate radium, $Al_2(SO_4)_3$ to precipitate iron and to fix uranium on hydroxides).

Figure 2 presents the metal concentration (dissolved fraction) in the water samples at these 4 sampling points.

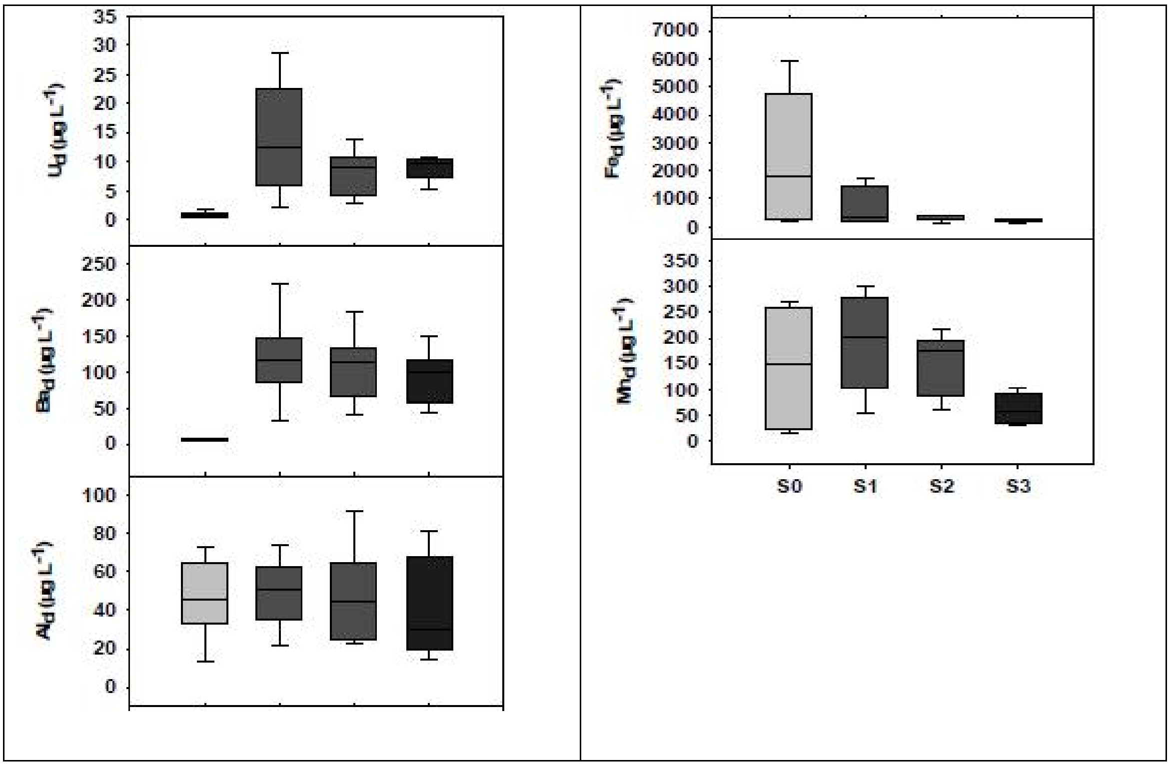


Figure 2. Metal concentration in water samples. Dissolved fractions.- Box-plot by stations calculated over the 7 month survey. (After Herlory et al., submitted)

2.6 Conclusions

We here performed a partial review of the potential co-contaminants at uranium mining and milling sites by presenting a few example cases. It is clear from this partial review that heavy metals or metalloids like As may be present at concentrations higher that the guidelines but also other elements like Ba, Fe, Al, F, Cl may be of concern. Which other contaminants that do matter is very much site specific. Though not the intention of this review, other site characteristics like pH, carbonate content, organic matter load, etc. may be much more important in determining the behaviour of the radionuclides of concern than are the concentrations of co-contaminants.

3. Nuclear fuel production plants

3.1 Description of the French plants involved in the nuclear fuel production

Nuclear fuel production industry begins after uranium's extraction from the mining sites and its further purification and concentrations into "yellow cake" (U_3O_8). A series of uranium transformation, including uranium enrichment, takes place to obtain the final nuclear fuel.
Yellow cake must be first converted to uranium hexafluoride (UF_6), which is the form required by most commercial uranium enrichment facilities currently in use. UF_6 changes from a solid state into a liquid or gaseous state with only slight changes in temperature. Thus, UF_6, which is a gas at 65 ºC, is in the right form to be enriched using gaseous diffusion or centrifugation. The UF_6 conversion product contains only natural, not enriched, uranium (U-235 content is about 0.7%). In France, the fabrication of this raw material for enrichment is carried out by COMURHEX in Malvési and Pierrelatte (at the Tricastin site).

The COMURHEX Malvési site purifies the "yellow cake" coming from all over the world to a very high level and converts it into uranium tetrafluoride (UF_4). The COMURHEX Malvési plant is the only reception point for natural uranium in France. The transformation of ore concentrate into UF_4 involves the following steps:

- dissolutionof the ore concentrate in nitric acid to obtain uranyl nitrate,
- purification of the uranyl nitrate solution
- precipitation of "purified" uranyl nitrate solution into ammonium diuranate, obtained by the injection of gaseous ammonia
- calcination of this ammonium diuranate in ultra-high temperature furnaces, to obtain uranium trioxide (UO_3),
- reduction of the UO_3 to uranium dioxide (UO_2) by passing through a furnace
- and fluorination of the UO_2 into UF_4 upon contact with hydrofluoric acid. This is the final chemical transformation step carried out at the Malvési site. The UF_4 is packed in a special container and transported to the Pierrelatte site.

The COMURHEX Pierrelatte converts the UF_4, originating from COMURHEX Malvési, into UF_6. The conversion process involves 2 steps: electrolysis of hydrofluoric acid to produce fluorine and fluorination of UF_4 into UF_6 by bringing fluorine gas into contact with UF_4 powder. This chemical reaction takes place at ultra-high temperatures in a flame reactor. This plant is now on the verge of closure and will be replaced by a new plant (COMURHEX II) leading to reduced releases in the environment. COMURHEX Pierrelatte also recycles the uranium contained in various materials produced during the conversion process. The plant also converts (recycled) processed uranium originating from the uranium hexafluoride (UF_6) treatment.

Then natural UF_6 must be enriched in U-235 to be used as nuclear fuel. The different levels of enrichment required for a particular nuclear fuel application are specified by the customer: light-water reactor fuel normally is enriched to 3.5% U-235, but uranium enriched to lower concentrations is also required. Enrichment is accomplished using one or more methods of isotope separation. Gaseous diffusion and gas centrifuge are the commonly used uranium enrichment technologies, but new enrichment technologies are currently being developed. The French pressurized water reactors require a U-235 content between 3 and 5 %. Raising the isotopic content of U-235 from 0.7 to 3-5% is the role of the EURODIF plant in Tricastin. This plant performs uranium enrichment by gaseous diffusion. It separates the UF_6 by means of a twin-stream gaseous diffusion process, with one stream becoming enriched in U-235 while the other becomes depleted during the course of the process. Note that here again, the process will evolve soon and EURODIF plant will stop its activity at the end of 2012. In the future, U will be enriched thanks to a centrifugation technique, which will change the nature and the quantity of releases as well.

On the Tricastin site, U is mainly present in the form of uranium hexafluoride (UF_6), uranium tetrafluoride (UF_4), uranium oxifluoride (UO_2F_2), uranyl nitrate and uranyl oxide (U_3O_8). The different workshops use important quantity of chemicals, such as fluor, chlorine, ammonia, sulphuric acid, nickel, chrome, bore or potassium hydrogen fluoride that are recovered in the gaseous or liquid effluents. All liquids are released in the Donzère-Mondragon canal.

For use as nuclear fuel, enriched UF_6 must be converted into uranium dioxide (UO_2) powder that is then processed into pellet form. The pellets are then fired in a high temperature sintering furnace to create hard, ceramic pellets of enriched uranium. The cylindrical pellets then undergo a grinding process to achieve a uniform pellet size. The pellets are stacked, according to each nuclear reactor core's design specifications, into tubes of corrosion-resistant metal alloy. The tubes are sealed to contain the fuel pellets: these tubes are called fuel rods. The finished fuel rods are grouped in special fuel assemblies that are then used to build up the nuclear fuel core of a power reactor. The metal used for the tubes depends on the design of the reactor. Stainless steel was used in the past, but most reactors now use zirconium. For the most common types of reactors, boiling water reactors (BWR) and pressurized water reactors (PWR), the tubes are assembled into bundles with the tubes spaced precise distances apart. FBFC, a subsidiary of AREVA, is the leading global producer of fuel assemblies for nuclear reactors of the PWR type. FBFC comprises three sites, one of which is at Romans (Isère, France). This facility produces UO_2 powder, as well as pellets, nozzles, fuel rods, and fuel assemblies for pressurized water reactors (PWRs).

3.2 Radiological and chemical releases of 2 French plants involved in the nuclear fuel production

Data on the annual releases of the French plants involved in the nuclear fuel production are most often aggregated data, not easily usable in the framework of our scenario compilation. Therefore, we will focus here on the releases of only two of these nuclear plants: COMURHEX Tricastin and EURODIF (Figure 3). As stated before, the processes used in these two plants are currently under change, with some of them being abandoned or notably improved. Therefore, releases reported below are only representative for the year 2010 but tell nothing about the releases of the future nuclear fuel production plants.

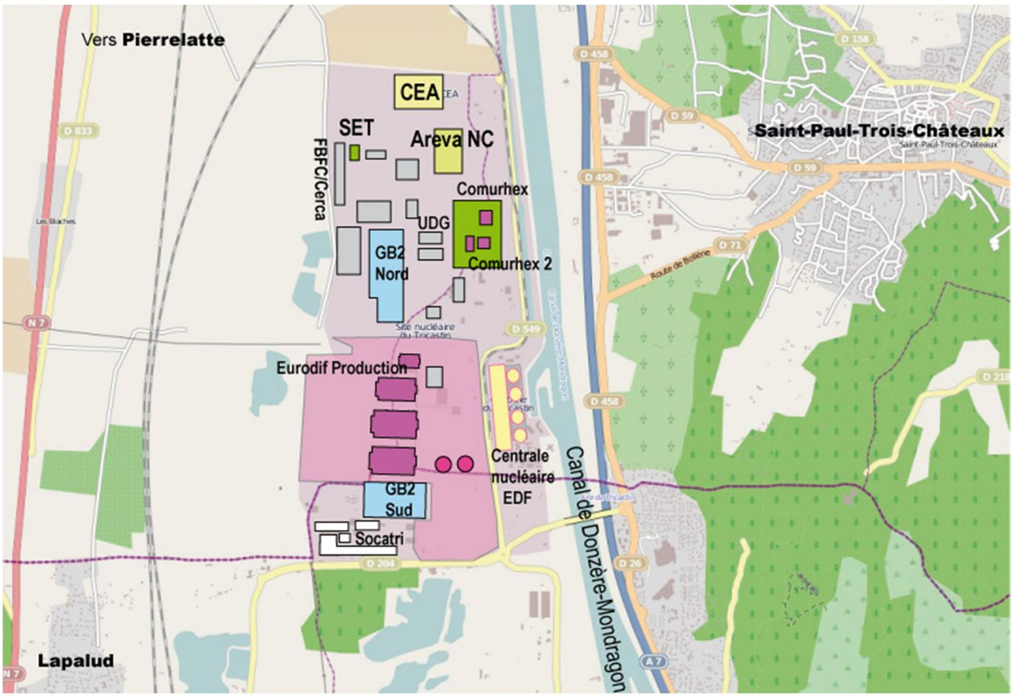


Figure 3. Map of the Tricastin nuclear site(from fr.wikipedia.org/wiki/Site_nucléaire_du_Tricastin)
The annual radiological and radioactive releases of these two plants are given in Table 7 below.

Table 7. Main characteristics of chemical and radioactive liquid releases in 2010 from COMURHEX and EURODIF plants on the Tricastin site (AREVA, 2010a)

		COMURHEX	EURODIF PRODUCTION
Radioactive substances (Bq year^{-1})			
	C-14	9.6E+8	
	H-3	9E+7	
	Tc-99	3.9E+8	
	α emitters	9E+7	1.3E+6
	β emitters (without H-3 and C-14)	2.2E+9	
Chemical substances (kg/year)			
	Ammoniac (NH_4)	38	
	Arsenic (As)	4.6	
	Chloride (Cl)	351	618 778
	Chromate (CrVI)	0.21	
	Chrome total (Cr)	1.06	

	Copper (Cu)	0.61	
	Fluoride (F-)	97	1 728
	Hydrocarbon	2.8	
	Iron (Fe)	0.96	879
	Nickel (Ni)	0.71	
	Nitrate (NO_3)	1020	
	Potassium (K)	139 788	
	Sulphate (SO_4)	106 837	
	Uranium (U)	0.41	
	Zinc (Zn)	0.67	1 685

As stated before, these releases are aggregated values, coming from the whole plant whatever its activity. Here, beta emitters (without H-3 and C-14) are the main substances released by the COMURHEX plant. H-3, C-14 and Tc-99 releases are not attributable to the transformation of UF_4 to UF_6 but to the use of uranium originated from used fuel. Therefore, these radionuclides were not further considered for our scenario compilation.

Table 8. Concentrations of radionuclides released by the COMURHEX and EURODIF plants at Tricastin, based on the mean annual stream flow of the Rhône at Tricastin

		COMURHEX	EURODIF PRODUCTION	Environmental Guideline values
Radioactive substances (Bq/m3)				
	α emitters	1.90E-03	2.75E-05	
	β emitters (without H-3 and C-14)	4.65E-02		
Chemical substances (µg/l)				
	Ammoniac (NH4)	8.03E-04		61 to 112 µgN/l (pH 8 and 25 °C)
	Arsenic (As)	9.72E-05		4.4
	Chloride (Cl)	7.42E-03	13.1	25 200 µg as NaCl
	Chromate (CrVI)	4.44E-06		4.1
	Chrome total (Cr)	2.24E-05		4.1
	Copper (Cu)	1.29E-05		0.3
	Fluoride (F-)	2.05E-03	3.65E-02	370
	Hydrocarbon	5.92E-05		
	Iron (Fe)	2.03E-05	1.86E-02	32
	Nickel (Ni)	1.50E-05		0.5
	Nitrate (NO_3)	2.16E-02		2000
	Potassium (K)	2.96		5245
	Sulfate (SO_4)	2.26		62 500
	Uranium (U)	8.67E-06		
	Zinc (Zn)	1.42E-05	3.56E-02	8.6

Table 9. Concentrations of radionuclides released by the COMURHEX and EURODIF plants at Tricastin, based on the lowest annual stream flow of the Rhône at Tricastin

		COMURHEX	EURODIF PRODUCTION	Environmental Guideline values
Radioactive substances (Bq/m3)				
	α emitters	5.10E-03	7.36E-05	
	β emitters (without H-3 and C-14)	1.25E-01		
Chemical substances (µg/l)				
	Ammoniac (NH4)	2.15E-03		61 to 112 µgN/l (pH 8 and 25 °C)
	Arsenic (As)	2.60E-04		4.4
	Chloride (Cl)	1.99E-02	35	25 200 µg as NaCl
	Chromate (CrVI)	1.19E-05		4.1
	Chrome total (Cr)	6.00E-05		4.1
	Copper (Cu)	3.45E-05		0.3
	Fluoride (F-)	5.49E-03	9.78E-02	370
	Hydrocarbon	1.59E-04		
	Iron (Fe)	5.44E-05	4.98E-02	32
	Nickel (Ni)	4.02E-05		0.5
	Nitrate (NO_3)	5.78E-02		2000
	Potassium (K)	7.92		5245
	Sulfate (SO_4)	6.05		62 500
	Uranium (U)	2.32E-05		
	Zinc (Zn)	3.79E-05	9.54E-02	8.6

The chemical releases are more diverse. EURODIF plant release only 4 substances but in very high quantity (Cl, F-, Zn and Fe). For COMURHEX plant, the main releases are constituted by potassium (K) and sulphate.

Uranium is considered in this monitoring as a chemical substance. Its release is mainly due to COMURHEX plant and reaches 0.41 kg /year.

The emission point of the Tricastin plants is the Donzère-Mondragon Canal. However, this canal joins the Rhône River downstream the site. Therefore, the Rhone flow rate downstream Tricastin was used to calculate concentrations of releases in the Rhône River. Results are presented below (Tables 8 and 9), together with environmental guideline values for chemical as reference. The latter are either chronic PNEC (Beaugelin-Seiller and Garnier-Laplace, 2006) or French water quality guidelines (Agence de l'Eau, 2003).

Even when we consider the lowest stream flow, environmental concentrations of contaminants do not exceed the environmental guideline values.

4. Nuclear power plants

4.1 Chemical and Radiological releases of French Nuclear Power plants

78% of the French electricity is produced by nuclear power plants (NPP). 19 NPPs, comprising in total 58 nuclear reactors are distributed over the French territory (Figure 4). All French reactors are pressurized waters reactors, developing three capacities (900 MW (34), 1300 MW(20); 1450 MW (4)). Among the 19 NPP, 15 of them have their emission release points in surface waters, either in large streams (such as the Rhône or the Loire river) or in lower streams (such as the Meuse and the Moselle rivers).

Figure 4. Nuclear Power plant on the French territory (http://www.econologie.com/photo/carte_centrales_nucleraires_France-fr.gif)

Liquid releases from nuclear power plants under normal operating conditions are characterized by chemical and radioactive mixtures, which are quantified by their yearly emission rates.

If radiological releases are similar for almost all French NPP, site-specific differences in the process for operation and maintenance might lead to differences mainly in the released chemical substances.

Table 10 reports the chemical and radiological composition of liquid effluents produced by 4 French NPP along the Rhone river in France in 2005and already reported by Garnier-Laplace et al. (2009).

Table 10. Main characteristics of chemical and radioactive releases in 2005 from 4 French nuclear power plants (from Garnier-Laplace et al., 2009)

	Bugey	**St Alban**	**Cruas**	**Tricastin**
	4 x 900 MW	2 x 1300 MW	4 x 900 MW	4 x 900 MW
Radioactive subtances (Bq/year)				
Ag-110m	7.77E+08	4.60E+07	5.14E+08	1.18E+08
C-14	4.54E+10	3.06E+10	4.49E+10	4.44E+10
Co-58	4.06E+08	4.27E+08	3.46E+08	4.80E+07
Co-60	2.07E+08	4.56E+08	1.31E+08	1.45E+08
Cs-134	2.10E+07	1.70E+07	4.80E+07	3.30E+07
Cs-137	8.80E+07	8.70E+07	5.10E+07	4.30E+07
H-3	4.91E+13	4.74E+13	4.58E+13	4.60E+13
I-131	2.00E+07	1.20E+07	3.70E+07	3.20E+07
Mn-54	2.00E+07	2.60E+07	4.30E+07	3.70E+07
Ni-63	2.45E+08	2.08E+08	1.30E+08	1.01E+08
Sb-124	1.55E+08	3.30E+07	8.30E+07	9.60E+07
Sb-125	1.93E+08	4.40E+07	2.27E+08	1.00E+08
Te-123m	3.50E+07	1.10E+07	4.20E+07	3.00E+07
Chemical substances (kg year^{-1})				
Ammonium (NH_4)	1006	183	312	4024
Boric acid (H_3BO_3)	25000	8250	24000	10000
Copper (Cu)	0	32980	11200	0
Hydrazine (N_2H_4)	1	0	32	11
Lithine (LiOH)	5.7	0.29	0.66	0
Morpholine (C_4H_9NO)	210	155	98	910
Zinc (Zn)	0	11250	2970	0

For radioactive releases, the main substance released is tritium, followed by C-14 for all NPP. Other radionuclides released in relatively high amounts are mainly Co-60, Co-58, Ni-63, Sb-125. Ag-110m is also released in high quantity from these plants, except for St Alban plant.

Concerning the chemical releases, a wide diversity of substances are released including mineral salt, heavy metals and organic molecules. In terms of annual quantity, the main releases concerned copper (for St Alban), boric acid (all NPP), zinc (St Alban and Cruas) and iron (Bugey and Tricastin). Among organic substances, morpholine is the main contributor to organic releases.

4.2 Environmental relevance of releases

Garnier-Laplace et al. (2009) used these data to rank the potentially released substances on the basis of the associated ecotoxicological hazard for the environment and to identify high-risk chemicals and/or radioactive substances for ecosystems. Briefly, these annual releases were transformed to concentrations in water downstream the emission point of the NPP, based on the Rhône river mean annual flow rate at the emission point. Resulting concentrations were very low: around 10^{-6}-10^{-5} Bq/l for all radionuclides, except C-14 (around mBq/l) and H-3 (around 1-10 Bq/l). They are in agreement with the environmental monitoring associated to the NPP releases. Among radionuclides, only some of them are qualitatively detected in the surface waters downstream from the NPP (H-3, Co-60, Co-58, Sb-124, Cs-137, Mn-54 and Cs-134). From a quantitative point of view, they all fall below the analytical detection limit, except H-3 (http://sws.irsn.fr).

In terms of ecotoxicological impact, Garnier-Laplace et al. (2009) showed that the total percentage of potentially affected species for freshwaters by these NPP remained lower than 1%. The major contributor in terms of ecotoxicological impact were chemicals (Cu > Zn > H_3BO_3) rather than radioactive substances (C-14 > Ag-110m > Co-60).

To take account of the possible lower dilution of the release in the river during summer, the conversion of annual releases into concentrations in water can also be done with the lowest flow rate of the Rhone River at the emission point. In that case, Cu and to a minor extent hydrazine might exceed the chronic PNEC derived by Beaugelin-Seiller and Garnier-Laplace (2006).

However, aquatic contamination by NPP effluents can be significant locally. A field study at the Temelin NPP (Bohemia) showed that H-3 was the only radionuclide which impacted the hydrosphere at an activity of 0.013 Bq/ml (Hanslik et al., 2009). Another study showed that environmental H-3 activities reached 0.053-0.43 Bq/ml downstream from the Arrocampo NPP reservoir (Spain, Baeza et al., 2009).

5. Reprocessing plants

Nuclear reprocessing technology was developed to chemically separate and recover plutonium and uranium from irradiated nuclear fuel. Originally reprocessing was used solely to extract plutonium for producing nuclear weapons. With the commercialization of nuclear power, the reprocessed plutonium was recycled back into MOX nuclear fuel for thermal reactors and the reprocessed uranium, which constitutes the bulk of the spent fuel material, can in principle also be re-used as fuel. The fission products and minor actinides which cannot be re-used anymore are stabilized by vitrification and treated as high level radioactive wastes.

Reprocessing of civilian fuel has long been employed in Europe, at the AREVA NC La Hague site in France, the Sellafield site in the United Kingdom, the Mayak Chemical Combine in Russia, and at sites such as the Tokai plant in Japan, the Tarapur plant in India, and briefly at the West Valley Reprocessing Plant in the United States.

The AREVA-NC La Hague site is located on the French Cotentin Peninsula (Figure 5). It has been in operation since 1967 and has now a capacity of about 1 700 tonnes per year (annual processing of used fuel from 80 to 100 nuclear reactors). It treats spent nuclear fuel from a lot of different countries, including France, Japan, Germany, Belgium, Switzerland, Italy, Spain, Australia and the Netherlands. It processed 1 049 tons in 2010. The 3 major steps in processing are

- the separation of the various components of used fuels and radioactive materials,
- the recovery of energy materials (uranium and plutonium) with a view to recycling them in the form of new fuels for the production of electricity,
- and the waste conditioning: integrated in glass for safe, stable conditioning over the very long term, or compacted to reduce their volume.

The procedure used for uranium and plutonium extraction at La Hague is based on the PUREX (Plutonium and Uranium Recovery by Extraction) process, which is a liquid-liquid extraction of uranium and plutonium. U and Pu are extracted from the fission products of the spent nuclear fuel independently of each other.

Figure 5. Site location of the AREVA-NC La Hague plant

5.1 Radioactive and chemical atmospheric effluents

Radioactive atmospheric effluents come from the dissolution of irradiated fuel, from the calcination of high level liquids during vitrification, from the air released by aeration installations and possibly also from the evaporation units. They are released into the environment after partial decontamination in the workshops they are produced (Davis et al., 2009).

Radioactive gaseous effluents in 2010 are given below in Table 11.

Table 11. Main characteristics of chemical and radioactive releases in 2010 from the AREVA-NC La Hague reprocessing plant (AREVA, 2010b)

TBq year^{-1}	
H-3	56.8
C-14	16
Radioactive iodines	0.00495
Rare gases, among which Kr-85	226 000
Other β and γ emitters	0.000114
Other α emitters	0.0000019

The only chemical gaseous effluent followed in the same installation is nitrous oxides (NOx). Its higher concentration released is 37 mg/Nm^3 of dry gas, with a flow rate of 4 kg/h.

Other gaseous chemicals are released by the plants (from the thermal production plant), but not in mixture with radioactive effluents.

5.2 Radioactive liquid effluents

Liquid effluents may be very radioactive, depending on where in the process they are generated. So part of them are concentrated and vitrified. Other effluents, less radioactive, come from the treatment of gaseous effluents, fuel storage pools, various cleaning operations and laboratories (Davis, 2009). After treatment, they are filtered and monitored and released into the English Channel by means of a pipe, the end of which is located in the Raz Blanchard current. 3 types of liquid effluents are released, depending on their activity:

- first type is called effluent "V", if the activity apart from tritium is less than 1.85 MBq l^{-1} and alpha activity less than 3.7 kBq l^{-1}: 101 007 m^3 released in 2010.
- other radioactive effluent is called effluent "A": 0 m^3 released in 2010.
- the third type of effluent is called "GR" and is constituted by some of the stormwater and drainage water of the site that may contain radioactivity from the site plus that from the radioactive waste storage facility of Beaumont-Hague, situated just near the AREVA-NC plant: 476 628 m^3 released in 2010.

These releases for radioactive and chemical substances are presented in Tables 15 and 16 for the year 2010.

Table 12. Quantity of radionuclides released by the AREVA-NC La Hague reprocessing plant (AREVA, 2010b)

Radioactive releases (Bq/year)	**2010**
C-14	7.34E+12
Co-60	6E+10
Cs-134	7.5E+10
Cs-137	1.1E+12
H-3	9.95E+15
Radioactive Iodines	1.38E+12
Ru-106	1.03E+12
Sr-90	1.3E+11
Other β and γ emitters*	2.56E+12
Other α emitters*	2.6E+10

Table 13. Main other radionuclides released by the AREVA-NC La Hague reprocessing plant (AREVA, 2010b)

Other β and γ emitters releases (Bqyear^{-1})	**2010**
Ni-63	3.5E+10
Pu-241	1.6E+11
Rh-106	1.03E+12
Sb-125	1.2E+11
Y-90	1.3E+11
Other α emitters releases (Bq year^{-1})	
Am-241	3.2E+9
Cm-244	1.9E+9
Pu-238	7.3E+9
Pu-239+240	1.9E+9
U	1.3E+9

The main substance released is tritium, followed by C-14, radioactive iodines, Cs-137, Ru-106 and Rh-106.

5.3 Chemical liquid effluents

22 chemical substances are monitored. They have different origins according to their use in the process or not. Chemical substances or products used during the reprocessing of spent fuel are:

- TBP (tributylphosphate), used as solvent for the extraction
- nitrates and nitrite coming from the acid nitric treatment
- hydrazine, used to reduce nitrite
- ammonium

Other substances are used for the effluent's treatment. They include:

- cobalt: used to co-precipitate Ru
- barium: used to co-precipitate Sr
- sulphate: coming from sulphuric acid
- iron, nickel and potassium: used to co-precipitate Cs

Other heavy metals released include: aluminium, chromium, lead, mercury, zinc, manganese, zirconium and cadmium. Other substances may also be released such as sulphur, phosphorous, fluoride and hydrocarbons.

The quantity released for all of them is given in Table 14 for the year 2010.

Table 14. Quantity of chemical substances released by the AREVA-NC La Hague reprocessing plant (AREVA, 2010b)

Chemical releases (kgyear^{-1})	**2010**
Al	200
Ba	5.05
Cd	2.23
Co	7.21
Cr	5.05
F^-	11.1
Fe	145
Hg	1.31
Hydrazine (N_2H_4)	10.1
Mn	12.7
NH_4^+	55.8
Ni	20.2
NO_2^-	4.12E+4
NO_3^-	2.14E+6
P total	523
Pb	5.05
S total	6 810
TBP	1 890
Zr	5.05

The main substances released are nitrate and nitrite, followed by sulphur and TBP.

5.4 Environmental relevance of liquid releases

To evaluate the environmental relevance of these releases, the annual releases were transformed to concentrations that can be compared to environmental guideline values. As the emission point is in the English Channel, a dilution factor specific to the sampling sites must be applied to obtain local concentrations in the sea water. The dilution factor values were derived from data collected during offshore campaigns and hydrodynamic models which make it possible to calculate dispersion of any substances in the English Channel (Bailly du Bois and Guéguéniat, 1999; Bailly du Bois and Dumas, 2005). Dilution factor values, expressed in Bq m^{-3}/TBq y^{-1} varied between 0 (La Rochelle, Concarneau and Roscoff) to 0.76 (Diélette, Herquemoulin and Goury) according to the sampling sites (Figure 6).

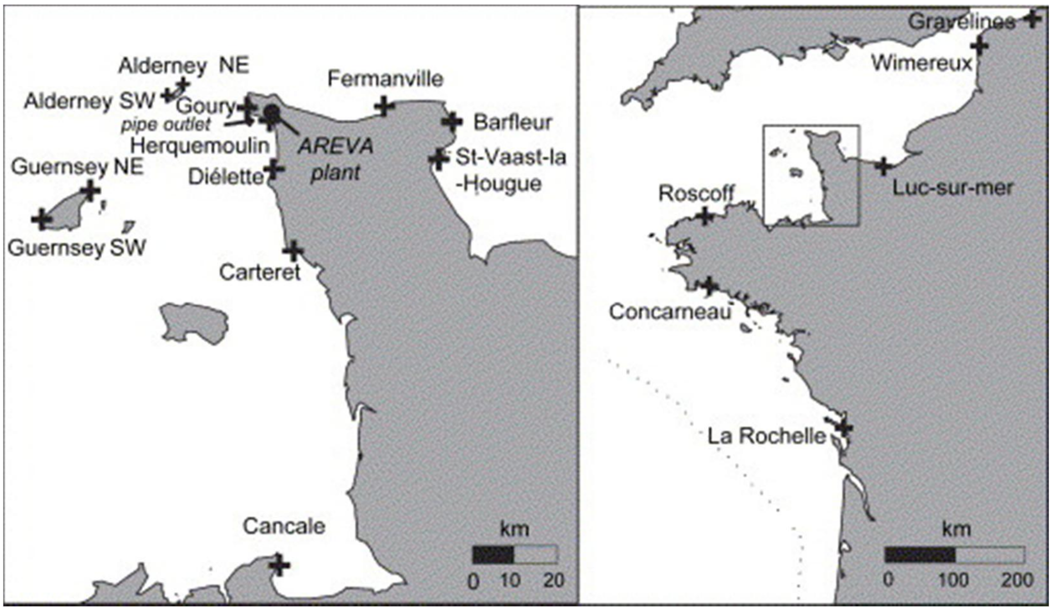


Figure 6. Map of French Atlantic and English Channel shorelines showing sampling locations with the Cotentin peninsula being highlighted. Dilution factors for the different sampling sites: La Rochelle (0); Concarneau (0); Roscoff (0); Cancale (0.16); Carteret (0.31); Guernsey SW (0.30); Guernsey NE (0.30); Alderney SW (0.40); Alderney NE (0.40); Diélette (0.76); Herquemoulin (0.76); Goury (0.76); Fermanville (0.53); Saint-Vaast-La-Hougue (050); Barfleur (0.50); Luc-sur-mer (0.38); Gravelines (0.30) and Wimereux (0.30) (from Fiévet et al., 2006).

Only calculations with the highest dilution factor (0.76) were considered. For chemical substances the same dilution factor expressed in kg.m^{-3}/Tg.y^{-1} was applied. Results are given in Table 15.

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Table 15. Concentrations of radionuclides and chemicals released by the AREVA-NC La Hague reprocessing plant

Radioactive releases (Bq m^{-3})		**2010**
	C-14	5.58
	Co-60	0.05
	Cs-134	0.06
	Cs-137	0.84
	H-3	7562
	Radioactive Iodines	1.05
	Ru-106	0.78
	Sr-90	0.10
Other β and γ emitters		1.95
	Ni-63	0.027
	Pu-241	0.122
	Rh-106	0.783
	Sb-125	0.091
	Y-90	0.099
Other α emitters		0.02
	Am-241	0.002
	Cm-244	0.001
	Pu-238	0.006
	Pu-239+240	0.001
	U	0.001
Chemical releases (µg/l)		
	Al	1.52E-04
	Ba	3.84E-06
	Cd	1.69E-06
	Co	5.48E-06
	Cr	3.84E-06
	F^-	8.44E-06
	Fe	1.10E-04
	Hg	9.96E-07
	Hydrazine (N_2H_4)	7.68E-06
	Mn	9.65E-06
	NH_4^+	4.24E-05
	Ni	1.54E-05
	NO_2^-	3.13E-02
	NO_3^-	1.63
	P total	3.97E-04
	Pb	3.84E-06
	S total	5.18E-03
	TBP	1.44E-03
	Zr	3.84E-06

This theoretical calculation falls well within the concentrations of radionuclides measured by AREVA in the framework of its monitoring. AREVA reported concentrations in sea water at Goury of 6.5 and 0.0018 Bq l^{-1} of H-3 and Sr-90 respectively. Other radionuclides measured at Goury are under the analytical detection limit.

All chemicals concentrations are well under any environmental guideline values.

6. Scenario: Waste and disposal

Historically, most effort was dedicated to the radiological consequences in the performance assessment of radioactive waste disposal systems. Nevertheless toxic chemical substances, such as heavy metals, occur in radioactive waste. In contrast with radionuclides, the amount of chemical substances does not decrease with time as a consequence of radioactive decay.

In what follows, we will discuss a few case studies where the chemical-toxic consequences of radioactive waste was evaluated for high level waste and low-level waste disposal. This screening will allow to obtain an idea of the more important co-contaminants and their potential level in the environment.

6.1 High level waste disposal

Harju-Autti and Volckaert (1995) carried out an environmental impact assessment of the more important toxic elements of a high level waste repository for the Belgian case.

The elements considered were derived from 3 sources: used nuclear fuel including the zircaloy cladding and construction elements of assemblies, waste from reprocessing of spent engineered barriers (containers, backfilling material). The elements ubiquitous in man's natural environment are not considered (Al, Ca, C, H, Fe, Mg, N, O, P, K, Si, Na, V). Be was not evaluated since considered more hazardous via inhalation than via ingestion and the dose by inhalation was not of concern in the study mentioned.

Within the spent fuel almost all elements of the periodic table are present and only those with an initial quantity of 10 ppb in the spent fuel were considered.

For the waste containers, Cr, Ni, Zn are the toxicants of interest and for the overpack Cr, Ni, Mn (for Inox), Cr, Ni and Mo (for Hastelloy) and Ti and Pd (for Ti/Pd 0.2 %).

Table 16. Maximum calculated concentrations in the aquifer of the most penalizing toxicants and the maximum allowed concentration in groundwater according to Flemish regulation.

Element	Maximum calculated concentration in aquifer (kg m^{-3})	Maximum allowed concentration in water (kg m^{-3}) (VLAREM titel II, 1992)
B	1.5E-6	1
Ba	1.5E-9	0.1
Cr	6.0E-10	0.05
Cr-Inox	6.0E-10	0.05
Cr-Hast	6.0E-10	0.05
Mn	1.0E-9	0.05
Mn-Inox	7.0E-9	0.05
Mo	1.2E-8	0.05 (Cr)
Mo-Hast	1.5E-7	0.05 (Cr)
Ni	1.4E-8	0.02 (Cu)
Ni-Inox	1.4E-8	0.02 (Cu)
Ni-Hast	1.4E-8	0.02 (Cu)
Sm	1.7E-9	No data
U	2.5E-8	No data
Zn	7.5E-8	0.5

Elements present in the ILW and decommissioning waste, which for the Belgian scenario may arise from reprocessing at FBFC, the former MOX production plant at Belgonucléaire, the research reactor from SCK•CEN Mol and the medical isotope production at IRE, are Ag, B, Be, Cd, Co, Cr, Hg, Ni, Pb, Sb and Zn.

Table 16 gives the maximum calculated concentrations in the aquifer of the most penalizing toxicants and the maximum allowed concentration in groundwater according to Flemish regulation.

It was found that the most important elements in the case of geological disposal of radioactive waste are, from a chemical-toxic point of view U, B, Mo and Ni. However, under the prevailing conservative assumptions, it does not seem likely that the geological disposal of nuclear waste should give rise to serious chemical impacts on man and environment as even for the most penalizing toxicants predicted environmental concentrations are a factor 10^6 below the limits for drinking water.

With these low concentrations, very little impact from heavy metals as co-contaminants is to be expected.

Hart and Lush (2004) performed a screening evaluation of the chemical toxicity of CANDU spent fuel. They developed a rationale for such assessment to focus on elements of greatest potential concern.The fuel used by CANDU reactors is natural uranium in the form of uraniumdioxide. The likely fuel impurities are Al, B, Cd, Ca, C, Cr, Cu, Dy, Gd, Fe, Mg, Mn, Mo, Ni; Si, Ag, Th.

The UO_2 fuel is packaged for use in bundles, which are encased in a zircaloy cladding. Zircaloy is an alloy of zirconium and tin, with a variety of other elements present as impurities. For every kg of initial uranium in fuel, there is 0.12 kg of zircaloy cladding. Considering all the sources of elements in the spent fuel and zircaloy cladding, most of the periodic table is represented, at least in trace quantities, at the time of fuel discharge from the reactor.

Table 17. Estimated environmental concentrations compared to guideline values for elments that exceed geological background in spent fuel

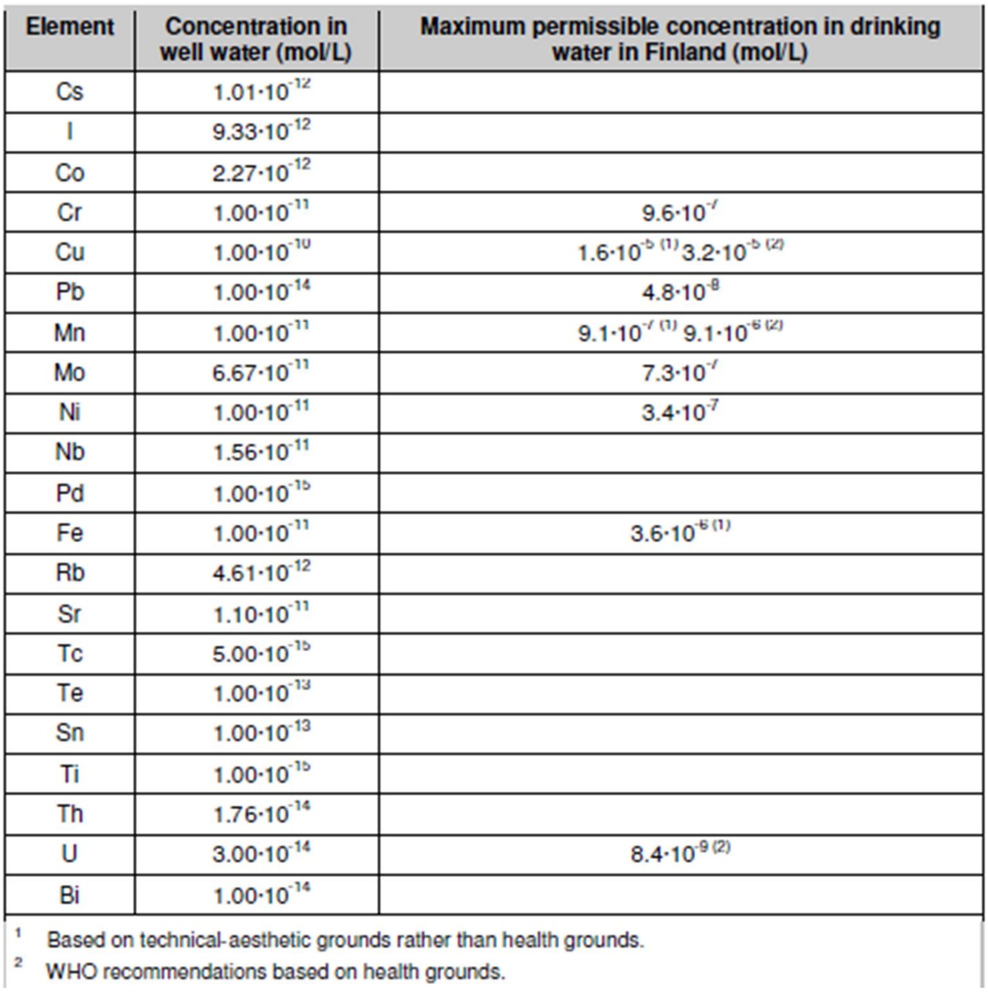
		Estimated environmental concentrations					Guideline values			
Element	Origin	Ground Water mgL^{-1}	Surface Water mgL^{-1}	Sediment Conc. $mg\ kg^{-1}$	Soil Conc. $mg\ kg^{-1}$	Air Conc. mgm^{-3}	Drinking Water mgL^{-1}	Sediment $mg\ kg^{-1}$	Soil $mg\ kg^{-1}$	Air mgm^{-3}
Cd	**FP**,FI,Z	7.87E-01	7.87E-04	4.41E+00	4.41E+02	2.60E-05	0.005	0.6	1.4	0.002
Co	FI**,Z**	1.48E+02*	1.48E-01	1.93E+03	1.93E+05	1.14E-02	0.73^+	50^+	40	0.0001
Cr	FI,**Z**	5.20E-01	5.20E-04	7.80E+00	7.80E+02	4.60E-05	0.05	37.3	64	0.0015
Mo	**FP**,Z	5.35E+04	5.35E+01	6.69E+04	6.69E+06	3.95E-01	0.18^+		5	0.018^+
Ni	**FI**,Z	1.17E+03	1.17E+00	7.63E+03	7.63E+05	4.50E-02	0.73^+	16^+	50	0.002
Pb	AP,**Z**	2.07E+02	2.07E-01	3.32E+04	3.32E+06	1.96E-01	0.01	35	70	0.0007
Sb	**FP**,Z	7.90E+00	7.90E-03	1.90E+01	1.90E+03	1.12E-04	0.006		20	0.025
Sn	FP,Fi,**Z**	1.19E-04	6.24E-05	4.18E-01	4.18E+01	2.47E-06	22^+		5	2.2^+
Tc	**FP**,Fi	4.85E+00	4.85E-03	5.82E-02	5.82E+00	3.43E-07			$0.2^{\times}$	
U	**F**,AP	2.38E-05	1.49E-01	2.23E+03	2.23E+05	1.32E-02	0.02		10	0.00048
Zr	FP,FI,**Z**	4.56E-06	2.85E-02	9.41E+02	9.41E+04	5.55E-03			11^+	

Fields marked grey exceed related limites. FP: Fission product; FI: fuel impurity; Z: zircalloy; bold is main source

The waste is disposed of in vaults that are backfilled with bentonite that fill with water after the underground work is completed. The elements identified as of potential concern based on screening-level (very conservative) estimates of concentration in water (groundwater and/or surface water) included lead (Pb), nickel (Ni), uranium (U), molybdenum (Mo), antimony (Sb), cobalt (Co), cadmium (Cd), chromium (Cr) and zirconium (Zr). All of these elements were also identified based on estimates of concentration in soil, as well as sediment or air in some cases. Elements identified based only on estimates of concentration in soil included lanthanum (La), iodine (I), bromine (Br), tungsten (W), technetium (Tc), tin (Sn) and niobium (Nb). The soil and air estimates, by direct partitioning from undiluted groundwater, were considered to be particularly conservative. Table 17 shows that for the conservative impact assessment, estimated environmental concentrations may exceed the relevant environmental guidelines for an important number of element/environmental compartment combinations.

Neall et al. (2007) documented the environmental risk assessment for the high level waste repository in Olkiluoto, Finland. The assessment was done for the KBS-3H design for the geological disposal of spent nuclear fuel in Finland and Sweden. In the KBS-3H design, multiple assemblies containing spent fuel are emplaced horizontally in parallel, approximately 300 m long, slightly inclined deposition drifts. The copper canisters, each with a surrounding layer of bentonite clay, are placed in perforated steel shells prior to deposition in the drifts. The radiological hazard associated with the spent fuel in the KBS-3H repository was studied in depth. The risks associated with chemical toxicity of the variety of metals such as copper, antimony, cadmium, lead, uranium and plutonium and with other problematic elements like selenium and arsenic which are present in the repository and may be released into the groundwater were also assessed.The elements expected to be of interest were identified based onthe spent fuel composition. However, the repository also contains large amounts of Feand Cu in the canisters which are not usually considered as part of the waste, per se. Theannual releases from the repository were assumed to be diluted in 100000 m³ of wellwater, as in the calculation of radiological dose. With this dilution factor, if there is less than 100 g of theelement in the spent fuel canister, the concentration in the water will not exceed thelowest limit, mercury in this case, set at 0.001 mgL^{-1} (assuming the failure ofa single canister) and that element can be discounted. The results are compared with drinking water standards set by WHO. The concentrationlimit for those elements for which there is no standard is assumed to be the same as thatof mercury (0.001 mgL^{-1}).Maximum releases of elements in terms of concentration and the corresponding limits for drinking water results are shown in Table 18. The values in Table 18 indicate thatchemical toxicity is unlikely to be a significant issue since concentrations in groundwater following releases from spent fuel elements are at least four to six orders ofmagnitude lower than the limits.

Table 18. Maximum concentrations of elements released from a single TILA-99 spent fuel canister and their permissible concentrations in drinking water. The limit for drinking water for those elements that do not have a concentration limit is assumed to be equal to that of mercury (0.001 mg L^{-1} or 5 x 10^{-9} mol L^{-1}).



Element	Concentration in well water (mol/L)	Maximum permissible concentration in drinking water in Finland (mol/L)
Cs	$1.01 \cdot 10^{-12}$	
I	$9.33 \cdot 10^{-12}$	
Co	$2.27 \cdot 10^{-12}$	
Cr	$1.00 \cdot 10^{-11}$	$9.6 \cdot 10^{-7}$
Cu	$1.00 \cdot 10^{-10}$	$1.6 \cdot 10^{-5\,(1)}$ $3.2 \cdot 10^{-5\,(2)}$
Pb	$1.00 \cdot 10^{-14}$	$4.8 \cdot 10^{-8}$
Mn	$1.00 \cdot 10^{-11}$	$9.1 \cdot 10^{-7\,(1)}$ $9.1 \cdot 10^{-6\,(2)}$
Mo	$6.67 \cdot 10^{-11}$	$7.3 \cdot 10^{-7}$
Ni	$1.00 \cdot 10^{-11}$	$3.4 \cdot 10^{-7}$
Nb	$1.56 \cdot 10^{-11}$	
Pd	$1.00 \cdot 10^{-15}$	
Fe	$1.00 \cdot 10^{-11}$	$3.6 \cdot 10^{-6\,(1)}$
Rb	$4.61 \cdot 10^{-12}$	
Sr	$1.10 \cdot 10^{-11}$	
Tc	$5.00 \cdot 10^{-15}$	
Te	$1.00 \cdot 10^{-13}$	
Sn	$1.00 \cdot 10^{-13}$	
Ti	$1.00 \cdot 10^{-15}$	
Th	$1.76 \cdot 10^{-14}$	
U	$3.00 \cdot 10^{-14}$	$8.4 \cdot 10^{-9\,(2)}$
Bi	$1.00 \cdot 10^{-14}$	

[1] Based on technical-aesthetic grounds rather than health grounds.

[2] WHO recommendations based on health grounds.

6.2 Low level waste disposal

The Port Granby Project is a Government of Canada project that involves the clean-up of the current low-level radioactive waste management facility at Port Granby. For the Port Granby project a screening environmental impact assessment was performed by Natural Resources Canada and the Canadian Nuclear Safety Commission (2009). The waste would be transferred to a new facility and the environmental assessment of the Port Granby Project considered the environmental effects predicted to occur as a result of the project. We here present the conclusions for the predicted environmental concentrations for the non-radioactive contaminants in groundwater and surface water.

The steady state contaminant mass loadings to groundwater would not be reached within the several hundred-year design life of the long-term waste management facility (LTWMF). The estimated time for steady state mass loading rate to groundwater to be reached varies from approximately 10,000 years to 50,000 years (arsenic and uranium) and to 5,000,000 years (copper). The predicted steady state concentrations of contaminants of potential concern (COPC) are less than 5% of the applicable PWQO (Ontario Provincial Water Quality Objectives) and ODWS (Ontario Drinking Water Standards) guidelines, except for arsenic, cadmium, cobalt and uranium. Arsenic concentrations are predicted to exceed the PWQO criteria under two of the modelled scenarios while cadmium, uranium and cobalt concentrations would remain below them for all scenarios. There are no predicted exceedances of ODWS guidelines for any COPC. The predicted COPC steady state mass loading rates would only be reached far into the future. Over the 500 year design life of the LTWMF, any release from the facility should be much less than the steady state mass loading rates. The steady state rates are not considered likely to result in adverse environmental effects.

Concentrations of surface water contaminants in the near-shore zone of Lake Ontario are generally typical of the region. However, because of uncertainties in the assessment of surface water quality caused by site groundwater seeps, verification of the concentrations of such releases has been identified for inclusion in the follow-up program. Increased levels of arsenic and uranium during the Construction and Development Phase in the treated leachate plume, may exceed interim PWQO levels (as they do currently). Levels are not expected to exceed chronic effect levels for fishes or aquatic invertebrates. In the event of a fuel oil spill to a tributary of Port Granby Creek, concentrations of aromatic hydrocarbons in the main creek may reach toxic levels for sensitive larval and juvenile organisms for a short period of time. Some fish, benthic invertebrate and aquatic plant mortality may be observed.

Mallants et al. (2005) carried out long-term performance assessment calculations for a generic design of a surface disposal system for the inorganic non-radioactive components that are present in low-level waste (category A waste). Only chemical elements were considered, whereas chemical compounds (e.g. nitrate) and organic molecules were not included. Screening calculations were performed first to decide which non-radioactive components could potentially increase concentrations in groundwater to levels above the natural background. On the basis of very conservative calculations, only 6 out of 41 chemical elements could not be classified as having a negligible impact on man and environment. Those 6 elements, notably B, Be, Cd, Pb, Sn, and Zn, were treated separately in a detailed safety assessment using a two-dimensional transport model for calculating their release from the repository to groundwater. Transport simulations with those six elements were done considering a calculation period in excess of 10 000 years. Transport calculations for B, Be, Cd, Pb, Sn, and Zn showed that highest fluxes to groundwater were due to B. The latter element is mainly present as a soluble boron salt. During active institutional control, no leaching to groundwater will occur. When active control of drainage water is stopped, leaching to groundwater occurs. As a result, calculated maximum concentration of boron in groundwater could increase up to 0.01 mg l^{-1} 300 years after repository closure. Thereafter, boron concentrations still increase and reach a maximum of 0.07 mg l^{-1}, 10 000 years after closure. Comparison with background concentrations of boron in groundwater demonstrates that both are of a similar magnitude. Hence, the release of boron does not lead to a noticeable increase of natural boron levels. Other elements will not be detectable in the drainage water the first 300 years after closure.

Considering long-term safety, i.e. the period from 300 to 10,000 years after closure, zinc (mainly present in the waste as metallic zinc) and antimony (mainly present in the waste as antimony oxide) concentrations in groundwater are more than two orders of magnitude smaller than the maximum boron concentration. Calculated zinc concentrations are considerably smaller than the natural background. The antimony concentrations are of the same magnitude as the detection limit, i.e. negligible. Concentrations in groundwater due to lead (mainly present in the waste as metallic lead) and cadmium (mainly present in the waste as metallic cadmium) are again two orders of magnitude smaller. Such low concentrations are hardly detectable, and thus negligible. The same is true for beryllium. The results illustrate that the impact on groundwater quality of inorganic chemo toxic elements present in low- and intermediate level short-lived radioactive waste disposed of at the surface is negligible.

6.3 Conclusions

Though it is clear from the studies discussed that many co-contaminants should be considered when assessing the potential impact from low, medium and high level waste disposal, the expected environmental concentrations are much lower than the guideline values and as such these co-contaminants are hence not expected to affect the impact from radionuclides. Also for radionuclides the levels are extremely low and no impact is expected.

7. NORM Industry

7.1 Introduction

Naturally Occurring Radioactive Materials (NORM) can be defined as those materials where human activities haveincreased the potential for exposure in comparison with the unaltered situation.Concentrations of radionuclides may (i.e. TE-NORM) or may not have beenincreased (IAEA, 2003). These activities have been listed several times by different international organizations, as UNSCEAR (1988) or IAEA (2003), and the related radioactive issues have been clearly detailed.

The new draft of the European Basic Safety Standards (EC, 2010), includes a comprehensive list of those activities, for which the activity concentrations and radioactive impacts are well known.

A complete compilation of non-radioactive contaminants generated by these NORM activities was not carried out up to date. It seems clear that those industries can affect the potential exposure to not only radionuclides, but also to other non-radioactive compounds.

The NORM industries for which we have found quantitative data on radioactive and non-radioactive contaminants are:

- Electricity Production Plants from Coal Combustion.
- Oil and Gas.
- Processing and Uses of Phosphate Mineral.
- Mining (other than uranium mining).
- Production of Titanium Dioxide Pigment.

- Geothermal Energy.
- Facilities Filtering Groundwater (Drinking Water Treatment And Irrigation).

The NORM industries for which we have found quantitative data only for radioactive contaminants are:

- Zircon and Zirconia Industries.
- Cement Production.
- Metal Production.
- Extraction of Rare Earths from Monazite.
- Processing of Ore for Niobium and Tantalum.
- Metal Processing.

Only the NORM industries for which information was found on non-radioactive components apart from radioactive components were discussed.

7.2 Electricity Production Plants from Coal Combustion.

The fuel used in coal combustion plants have variable concentrations of natural radionuclides from the ^{238}U, ^{235}U, ^{232}Th and ^{40}K series, which are increased during the industrial processes. The concentrations of the main radionuclides present in coal vary depending on the origin (Table 19) and typical annual releases from a plant in Table 20 (IAEA, 2003).

Table 19. Ranges and/or mean values of the concentrations of the main radionuclides present in coals of different origin (Bq g^{-1}) (IAEA, 2003).

Country	**^{238}U**	**^{230}Th**	**^{226}Ra**	**^{210}Pb**	**^{210}Po**	**^{232}Th**	**^{228}Ra**	**^{40}K**
Australia	0.005-0.009	0.02-0.07	0.019-0.024	0.02-0.03	0.016-0.03	0.011-0.07	0.011-0.06	0.023-0.14
Brazil	0.02	-	0.07	0.072	-	0.06	0.06	-
Egypt	0.06	-	0.026	-	-	0.008	0.008	-
Germany	-	-	0.01-0.15	-	-	0.01-0.06	-	0.01-0.7
Germany		-	0.001-0.06	-	-	0.001-0.06	-	0.004-0.22
Germany	-	-	0.01	-	-	0.008	-	0.022
Greece	<0.4	-	0.04-0.21	0.06-0.2	-		0.009-0.04	-
Hungary	<0.5	-	-	-	-	0.012-0.1	-	0.03-0.4
Poland	<0.16	-	-	-	-	<0.12	-	<0.8
Italy	0.023	-	-	-	-	0.018	-	0.2
Romania	<0.4	-	<0.6	<0.5	<0.58	<0.17	-	-
United Kingdom	0.007-0.019	0.008-0.025	0.007-0.022	-	-	0.007-0.019	-	0.06-0.3
USA	0.006-0.007	-	0.009-0.06	0.012-0.08	0.003-0.05	0.003-0.021	-	-

The document EUR 16956 (Scholten, 1996) indicates that the activity concentrations of coals and fly ashes are below the exemption levels given in the European BSS (Scholten, 1996; Euratom, 1996). However, the presence of some materials that could go beyond such levels makes recommendable to establish certain control on the coals used. It is also recommended that since the fly ashes cannot be reused without restrictions according to the European BSS dose limits, the coal-fired power plants should be controlled, as well as the factories using the fly ash (Table 21).

Table 20. Annual releases (GBq a^{-1}) of a typical coal combustion plant compared with the releases of a typical gas combustion plant.

	^{238}U	^{232}Th	^{226}Ra	^{222}Rn	^{210}Pb	^{210}Po
Coal combustion plant (600 MWe)	0.16	0.08	0.11	34	0.4	0.8
Gas combustion plant (400 MWe)	-	-	-	230	-	-

In the combustion process part of the coal mineral component smelts forming a vitrified ash. The heaviest part of these ashes goes to the bottom of the boiler in form of bottom ashes. In big electric power plants the proportion of bottom and fly ashes is usually between 10-20 % for bottom ashes, and 80-90% for fly ashes.

Table 21. Arithmetic mean of the radionuclide concentration in certain ashes and waste of coal combustion (Bq g^{-1}).

	^{238}U	^{232}Th	^{228}Th	^{228}Ra	^{226}Ra	^{210}Pb	^{210}Po	^{40}K
Fly ash	0.2	0.07	0.11	0.13	0.24	0.93	1.7	0.365
Slag	0.24-0.20	0.24-0.20			0.24-0.20	0.15-0.22	0.14-0.22	0.65-0.67
Fly ash (turf)	0.27-1.05				<0.215			<1.48

The activity concentrations found in 4 coal-fired power plants in Spain, both in fuels and in the sub-products, are shown in Table 22 (Robles et al., 2008; Mora et al., 2008).

Table 22. Activity concentration ranges in the fuels and sub-products of Spanish coal power plants (Bq g^{-1} dry).

	^{40}K	^{226}Ra	^{232}Th	^{210}Po
Local coal	0.09 – 0.3	0.05 – 0.07	0.02 – 0.03	NM
Imported coal	< 0.1	0.02 – 0.03	0.015 – 0.03	0.014 – 0.09
Consumption mix	< 0.028 – 0.3	< 0.005 – 0.06	< 0.005 – 0.03	0.014 – 0.11
Fly ashes	0.25 – 1.1	0.05 – 0.19	0.04 – 0.15	0.26 – 1
Bottom ashes	0.14 – 1.1	0.023 – 0.15	0.02 – 0.14	0.009 – 0.06
Coal world average	0.14 – 0.8	0.017 – 0.06	0.011 – 0.06	-
Fly ashes world average	0.016 - 0.758	0.014 – 1.5	0.05 – 0.15	-

Coal-fired power plants also produce non-radioactive contaminants. In Tables 26-28, data on the concentration of different non-radioactive contaminants produced by coal power plants in the UK are described.

Table 23. Leachates found using the DIN 38414-S4 method: typical ranges (mg l^{-1}) from UK sources of fly ash (Lindon and Sear, 2001).

Element	Typical range of leachable elements	Element	Typical range of leachable elements
Aluminium	<0.1*-9.8	Magnesium	<0.1*-3.9
Arsenic	<0.1*	Manganese	<0.1*
Boron	<0.1*-6	Molybdenum	<0.1*-0.6
Barium	0.2-0.4	Sodium	12-33
Calcium	15-216	Nickel	<0.1*
Cadmium	<0.1*	Phosphorus	<0.1*-0.4
Chloride	1.6-17.5	Lead	<0.2*
Cobalt	<0.1*	Sulfur	24-510
Chromium	<0.1*	Antimony	<0.01*
Chromium VI	<0.1*-1	Selenium	<0.01*-0.15
Copper	<0.1*	Silicon	0.5-1.5
Cyanide	<0.01*	Tin	<0.1*
Fluoride	0.2-2.3	Titanium	<0.1*
Iron	<0.1*	Vanadium	<0.1-0.5
Mercury	<0.01*	Zinc	<0.1*
Potassium	1-19	pH	7-11.7

The data include a seawater-conditioned sample, hence the high chloride values.
*Value below detection limit.

Table 24. Leachates found using the Harwell method: typical range (mg l^{-1}) from 10 samples from a single UK source of fly ash (Lindon and Sear, 2001).

Element	Typical range of leachable elements	Element	Typical range of leachable elements
Bed volume	1	Molybdenum	0.15-0.88
pH	8.1-8.8	Sodium	5-44
Aluminium	<0.1*-0.5	Nickel	<0.01*
Arsenic	0.06-0.16	Lead	<0.01*
Boron	1.8-4.3	Tin	<0.01*
Calcium	33-250	Titanium	<0.01*
Cadmium	<0.005*	Vanadium	0.22-0.55
Cobalt	<0.01*	Zinc	<0.01*
Chromium	0.02-0.06	Nitrogen	0.2-1
Copper	<0.01*	Phosphorus	<0.1*
Iron	<0.01*	Sulfur	15-70
Mercury	<0.001*	Chloride	5-9
Potassium	5-29	Fluorine	<0.1*
Magnesium	16-100	Selenium	0.04-0.16
Manganese	<0.01*	Antimony	0.01-0.02

*Value below detection limit.

Table 25. Levels of polycyclic aromatic hydrocarbons in emissions from coal-fired power stations (Lindon and Sear, 2001).

Compound	Concentration range(ng/g)		Concentration range(ng/m^3)
	Respirable particles	Non respirable particles	Vapour phase
Naphthalene	ND-18	0.5-23	10-1800
Phenanthrene	-	-	26-640
Anthracene	-	-	0.4-100
Fluoranthene	0.2-0.3	0.05-1.5	0.5-240
Pyrene	0.2-7	0.08-1.1	0.2-2850
Chrysene	ND	ND-4	0.1-28
Benz[a]pyrene	ND	ND	0.1-120
Benz[a]anthracene	ND	ND-0.3	NM
Benz[ghi]perylene	NM	NM	3-22

ND not detected at the limit of 0.05 ng g^{-1}; NM: not measured.

7.3 Oil and Gas

Among the processes carried out in the oil and gas industry, there are certain conditions under which NORM wastes are produced. In the water that is extracted along with oil and gas there are relatively high concentrations of ^{226}Ra, a descendant of ^{238}U, and ^{224}Ra and ^{228}Ra, both daughters of ^{232}Th. Due to variations in pressure and temperature during the extraction a saturation of dissolved sulphates and carbonates occurs, which causes the precipitation in the form of scales of these sulphates and carbonates on the inner walls of the tubes, at the extraction points ("wellheads"), as well as in valves, pumps, separators, water treatment tanks, gas treatment tanks and oil storage tanks. These deposits, mainly occurring in the points where turbulent flow occurs and in the condensation points, can contain relatively high concentrations of radium sulphates and carbonates.

When sea water, mixed with production water, is used to enhance oil recovery, the concentration of sulphates in the produced water is also increased, thus increasing scale production.

The mixture of gas, oil and water also carries^{222}Rn (gaseous radioisotope descendant of ^{226}Ra). This ^{222}Rn disintegrates during its course, producing ^{210}Pb which is deposited as a film on the inner walls of the gas treatment equipment and transport facilities, as well as in the regasification plants. There is also mobilization of stable lead which contains relatively large concentrations of ^{210}Pb, resulting in thin layers on the inner surfaces of the production equipments and its appearance in the sludge produced.

The appearance and mobilization of the radionuclides mentioned widely varies among facilities and even between each extraction well, and even between different production conditions. Therefore, we cannot speak of "typical concentrations" of radionuclides, even annual or over the lifetime of the installation. However, data on ranges of activities are available. The most important are highlighted in Tables 29 to 31, which present data from a study done by the International Association of Oil and Gas (OGP, 2008).

The radioisotopes discharges from the offshore oil and gas industry to the North-Atlantic Sea are presented in Table 29.

Table 26. NORM concentrations in oil, gas and by-products (OGP, 2008).

Radionuclide	**Crude oil (Bq g^{-1})**	**Natural gas (Bq m^{-3})**	**Produced water (Bq l^{-1})**	**Hard scales (Bq g^{-1})**	**Sludge (Bq g^{-1})**
^{238}U	10^{-7} – 0.01		$3\cdot10^{-4}$ – 0.1	0.001 – 0.5	0.005 – 0.01
^{226}Ra	10^{-4} – 0.04		0.02 – 1200	0.1 – 15000	0.05 – 800
^{210}Po	0 – 0.01	0.002 - 0.08		0.02 – 1.5	0.004 – 160
^{210}Pb		0.005 - 0.02	0.05 – 190	0.02 – 75	0.1 – 1300
^{222}Rn		$5 – 2\cdot10^{5}$			
^{232}Th	$3\cdot10^{-5}$ – 0.002		$3\cdot10^{-4}$ – 0.001	0.001 – 0.002	0.002 – 0.01
^{228}Ra			0.3 – 180	0.05 - 2800	0.5 - 50
^{224}Ra			0.5 - 40		

Table 27. Range of activity concentrations in scales and sludge samples (OGP, 2008) .

	Activity concentration (Bq g^{-1}) (dry)			
	^{228}Th	**^{228}Ra**	**^{226}Ra**	**^{210}Pb**
Scales of the Norwegian O&G platforms in the sea	-	5 – 30	8 – 100	0 – 6
Sludge of the Dutch O&G facilities in the sea and terrestrials.	0 - 60	0 – 500	1 – 800	0 – 300
Scales of the Dutch O&G facilities in the sea and terrestrials.	0 – 200	0 – 400	0 – 900	6 – 2,500
Scales of the terrestrial German O&G facilities.	40 - 200	40 – 200	100 – 500	20 – 600
Scales of the United Kingdom O&G platforms in the sea	-	20 - 300	20 - 400	-

Table 28. Estimated order of magnitude in the NORM discharges in the sea from oil and gas platforms (OGP, 2008).

	Assumed annual oil production (10^3 m^3)	**Annual water discharge (10^3 m^3)**	**^{228}Ra and ^{226}Ra (Bq l^{-1})**	**^{228}Ra and ^{226}Ra (GBq a^{-1})**		
Oil	1000	3000	10	30		
	Assumed annual gas production (10^3 m^3)	Annual water discharge (10^3 m^3)	^{226}Ra (Bq l^{-1})	^{226}Ra (GBq a^{-1})	^{228}Ra and ^{210}Pb (Bq l^{-1})	^{228}Ra and ^{210}Pb (GBq a^{-1})
Gas	3,000	150	10	1.5	5	0.75

Table 29. Discharges from the offshore oil and gas industry in 2008 (TBq) (OSPAR, 2010).

		CP	**OSPAR Region 1**	**^{210}Pb**	**^{226}Ra**	**^{228}Ra**	**^{228}Th**	**^{3}H**	**Other β/ γ emitters**
Produced water TBq (NO1) (UK1) (UK2) (UK3) (UK9)		DK	II	9.42E-04	1.37E-02	9.22E-03			
		DE	II	1.60E-06	2.90E-05	2.50E-06			
		IE	III	2.90E-06	4.40E-06	8.90E-07			
		NL	II	8.20E-03	1.30E-01	1.40E-01			
		NO	I	5.00E-03	4.50E-02	4.10E-02			
		NO	II	3.70E-02	4.16E-01	3.31E-01			
		UK II		5.10E·02	2.14E-01	1.51E-01			
		UK	III	1.17E-04	2.15E-03	1.69E-03			
Descaling operations, both offshore and onshore, from normal production that leads to discharges (NO2) (UK4) (UK10)	Radioactivity in suspended solids arising from water-jet descaling (TBq)	NO	I	4.40E-08	2.00E-08	9.30E-08			
		NO	II	3.60E-07	5.40E-07	1.50E-07			
		UK	II	Offshore: 60.8 GBq discharge of scale from undefined processes [UK6] Onshore:4.83GBq Total alpha and 2.70 GBq Total beta/gamma (excluding tritium) discharge [UK6]					
	Radioactivity in solution as result of descaling using acids or scale dissolvers (TBq) (UK5)	DK	II	9.23E-09	1.42E-04	7.47E-05	5.97E-05		
Descaling operations, both offshore and onshore, from decommissioning of oil and gas installations that leads to discharges (UK7) (UK10)	Radioactivity in suspended solids arising from water-jet descaling (TBq)								
	Radioactivity in solution as result of descaling using acids or scale dissolvers (TBq)								
Radioactivity discharged as a result of tracer experiments, TBq (UK8) (UK11)		DK	II					7.61E-02	
		NO	I					9.85E-01	
		NO	II					2.49E-01	1.10E-01

DK (Denmark), DE (Deutchland), IE (Ireland), NL (Netherlands), NO (Norway), UK (United Kingdom).

In practice, waste streams contain not only naturally occurring radionuclides but other constituents as well. These other constituents include the compounds from chemical mixtures used for decontamination, solid or liquid organic residues from oil and gas purification and heavy metals. In particular, Hg, Pb and Zn are encountered frequently in combination with NORM from oil and gas production.

Oil and gas industries also produce solid wastes. These wastes include sludge, mud, sand and hard porous deposits and scales from the decontamination of tubes and different types of topside equipment. The activity concentrations of ^{226}Ra, ^{228}Ra, ^{224}Ra and their decay products in deposits and sludge may vary over a wide range, from less than 1 Bq g^{-1} to more than 1,000 Bq g^{-1} (Harvey, 1994). The average concentration of radionuclides in the ^{238}U decay series (including ^{226}Ra) in soils is about 0.03 Bq g^{-1}. A production facility may generate quantities of scales and sludge ranging from less than 1 t a^{-1}, to more than 10 t a^{-1}, depending on its size and other characteristics. Decontamination of equipment will produce solid and/or liquid waste, the latter also being contaminated with non-radioactive substances if chemical methods have been used.

There have been several field studies on the accumulation of heavy metals in the sediments around offshore oil and gas production facilities. A study of the East Flower Garden Bank off Texas, funded by Mobil Oil Corporation, found a three-fold increase in the concentration of Pb near the drill site. Another study of the offshore Texas area found about three-fold increases in the concentrations of Zn, Cd and Pb. A study of the Tanner Bank oil field off California, funded by Shell Oil Company, found a three-fold increase in the concentration of Cr, and a forty-fold increase in the concentration of Pb. The exact reasons for these increased concentrations of heavy metals are not obvious, however one possible source of heavy metals is drilling muds. Petroleum, with its heavy metal components of V and Cr, is another possible source of heavy metals (Newbury, 1979).

The Norwegian Oil Industry Association (OLF) publishes annual reports where the discharges of different contaminants are detailed. In Tables 33 to 37 data for the discharges that can affectliquid or solid materials are included for the years 2000 to 2010 (OLF, 2011).

Table 30. Discharge of BTX compounds in produced water in oil extraction in Norway (kg) (OLF, 2011).

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Benzene	512,037	524,008	552,074	446,233	682,490	683,080	771,347	871,200	862,411	868,175	832,031
Etylbenzene			24,705	19,074	35,533	32,648	34,271	34,565	34,675	46,135	41,758
Toluene	359,862	354,209	358,637	272,080	554,030	571,545	628,213	674,719	672,398	722,851	700,550
Xylene	143,550	156,121	154,469	123,772	213,160	192,364	210,830	246,189	234,513	265,764	243,835

Table 31. Discharge of heavy metals and other compounds in produced water in oil extraction in Norway (kg) (OLF, 2011).

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Arsenic	966	1,095	370	471	360	267	380	660	614	483	895
Barium				1,925,471	7,124,440	7,015,319	6137,119	6939,336	7,762,350	7,008,907	7,071,530
Lead	520	317	875	527	273	173	348	255	386	290	239
Iron				714,214	888,912	1,108,015	1,370,415	1,008,440	1,058,121	797,369	825,822
Cadmium	115	113	46	32	20	11	30	28	41	28	22
Copper	412	431	2,212	3,991	3,639	312	730	103	102	102	89
Chrome	492	249	119	117	231	4,018	192	175	213	154	225
Mercury	5	5	6	7	9	8	7	6	11	9	9
Nickel	751	748	209	407	452	1,073	735	299	299	142	200
Zinc	14,914	9,138	13,576	11,211	7,130	2,253	9,129	9,847	16,651	7,100	6,948

Table 32. Discharge of phenols in produced water in oil extraction in Norway (kg) (OLF, 2011).

	2002	2003	2004	2005	2006	2007	2008	2009	2010
C1-alkyl phenols	126,233	182,012	167,582	161,542	214,511	226,609	207,855	203,376	199,007
C2-alkyl phenols	51,089	76,922	79,333	70,094	92,631	82,571	87,634	80,707	83,860
C3-alkyl phenols	19,143	22,181	31,258	26,032	28,794	32,074	29,137	26,108	27,350
C4-alkyl phenols	5,963	7,827	11,013	11,115	12,524	10,438	10,451	11,624	8,707
C5-alkyl phenols	1,972	2,277	1,796	2,157	3,047	2,076	2,022	1,325	1,551
C6-alkyl phenols	95	125	95	66	51	86	84	78	125
C7-alkyl phenols	59	77	51	62	20	26	61	22	55
C8-alkyl phenols	36	123	50	81	37	33	39	20	71
C9-alkyl phenols	75	76	28	92	23	28	13	64	44
Phenols	243,552	184,168	206,962	170,118	179,405	212,822	207,560	185,041	166,660

Table 33. Discharge of organic acids in produced water in oil extraction in Norway (kg) (OLF, 2011).

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Butyric acid			644,737	755,601	709,758	752,861	671,218	77,200	714,602	627,237	519,296
Acetic acid			24,589,094	28,685,218	28,272,473	29,820,022	29,837,132	30,327,152	26,381,307	22,509,255	20,693,558
Carbonylic acid	28,070,433	27,624,780									
Fomic acid			66,731	152,368	209,953	159,966	501,911	449,707	314,221	563,669	493,913
Naphtenic acid						259,322	262,712	283,637	250,405	264,051	179,185
Valeric acid			256,215	298,361	312,267	336,195	344,439	374,276	341,590	338,214	241,354
Propionic acid			3,499,928	3,685,331	3,249,683	3,382,933	3,220,793	3,606,091	3,261,575	2,902,484	2,624,969

Table 34. Discharge of PAH compounds in produced water in oil extraction in Norway (kg) (OLF 2011).

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Acenaftene*	135	147	226	252	264	276	238	200	164	198	196
Acenafylene*	30	28	32	36	38	155	185	45	174	93	82
Anthracene*	26	30	41	113	94	118	36	36	60	10	7
Benzo(a)anthracene*	15	19	30	42	26	32	29	13	18	9	8
Benzo(a)pyrene*	6	6	10	12	10	11	14	6	5	4	3
Benzo((b)fluorantene*	12	14	16	24	16	25	132	13	16	9	9
Benzo(g,h,i)perylene*	7	8	12	17	9	21	17	5	7	6	6
Benzo(k)fluorantene**	4	4	15	4	4	5	13	2	4	2	1
C1-dibenzotiophene			1,230	1,106	1,576	1,953	1,521	690	761	667	601
C1-Phenantrene			1,980	3,483	2,935	3,238	1,345	1,886	1,589	2438	2,222
C1-aphtalene			51,647	44,188	57,796	59,929	50,250	43,939	44,155	47,410	45,000
C2-dibenzothiophene			1,282	1,404	1,476	2,096	1,453	663	634	929	878
C2-Phenantrene			2,177	3,785	2,603	3,344	1,982	1,823	1,976	2,706	2598
C2-naphtalene			20,667	26,021	25,248	27,251	21,143	16,086	19,636	24,669	21,880
C3-dibenzotiophene			9,191	119	263	474	342	71	92	20	22
C3-Phenantrene			737	517	635	466	187	375	306	662	694
C3-naphtalene			11,453	18,227	17,359	21,957	11,226	7,813	11,614	21,719	17,219
Dibenzo(a,h)anthracene*	4	4	8	10	7	9	12	3	4	3	2
Dibenzothiophene			482	615	619	748	449	429	394	435	407
Phenantrene											1,576
Phenantrene*	1,679	1,822	1,821	2,217	2,332	2,553	1,723	1,518	1,565	1,712	
Fluorantene*	32	35	47	56	39	88	53	38	28	25	27
Fluorene*	1,207	1,302	1,200	1,683	1,620	1,769	1,308	1,132	1,166	1,175	1,126
Indeno(1,2,3,-c,d)pyrene*	3	3	6	6	4	5	12	2	3	2	1
Chrysene*	82	98	68	43	57	74	61	40	61	42	30
Naphtalene											47,770
Naphtalene*	39,744	38,687	43,622	40,545	57,243	39,133	63,073	49,450	44,963	48,175	
Pyrene*	52	59	52	116	97	117	64	64	74	49	43

7.4 Processing and Uses of Phosphate Mineral

The major producers of phosphate ore are Morocco, former USSR countries and China, while the production in the EU is negligible. However, the EU imports the ore for processing it. The concentration of activity in the mineral widely varies depending on its origin (Table 35).

Table 35. Production of phosphoric rock and activity concentrations in different countries (EUR, 1997).

Region	**Ore production**		**Activity concentration (Bq g^{-1})**		
	Mt	**(%) Worldwide**	**^{238}U**	**^{226}Ra**	**^{232}Th**
China	4.1	3.3	0.15	0.15	0.025
Christmas Islands	1.3	1.0	0.33	0.3	0.007
Israel	1.2	1.0	1.5 – 1.7	n/a	n/a
Jordan	1.8	1.4	1.3 – 1.85	n/a	n/a
Morocco	17.6	14.0	1.5 – 1.7	1.5 – 1.7	0.01 – 0.2
Nauru	1.1	0.9	0.81	0.85	-
Senegal	1.8	1.4	1.3	1.4	0.07
Togo	2.9	2.3	1.3	1.2	0.1
Tunisia	3.6	2.9	0.59	0.52	0.09
USSR	24.2	19.3	0.04 – 0.09	0.03 – 0.39	0.04 – 0.23
USA – Florida – others	47.3	37.6	1.5 – 1.9 0.15 – 4.8	1 – 2.1 0.15 – 4.8	0.02 – 0.06 0.01 – 0.08
Vietnam	1.5	1.2	n/a	n/a	n/a
WORLD	126	100			

To produce 1 t of phosphate, the extraction of 3 tons of ore is required; as a result of the production process 4 to 5 tons of phosphogypsum are produced. Of all the produced phosphogypsum in the world approximately 14% is recycled, 28% is discharged via aquatic and 58% are disposed on ground (data from 1977). Four different processes for phosphate rocks are used: wet process using sulphuric acid; treatment with hydrochloric acid; treatment with nitric acid and the thermal processing. All of them produce different wastes with variable activity concentrations in them.

In 1999, in Europe, around 15% of the material is recycled as building materials. The wastes generated in the hydrochloric acid process, mainly calcium fluoride, are stored in surface. That sludge has a radium concentration among 8 and 10 Bq g^{-1}.

Depending on the method used, the wastes generated also vary. In Europe 90% of phosphate rock was treated with sulphuric acid. The phosphogypsum generated, were previously discharged to the environment, however, nowadays they are recycled or stored on the surface.

At the date of publication of RP 135 (Chen et al., 2003) there was only one plant in Europe that used the thermal processing which was located in the Netherlands. The main solid NORM waste in this case is the dust from the roasting, with a ^{210}Pb content of about 1,000 Bq g^{-1}. The sludge generated is reused, mainly as road-fill material. Only in this case remarkable atmospheric releases occur, since 85% of the ^{210}Po will be released during the calcinations' process (Table 36). Table 37 gives information on liquid discharges from a fertilizer plant.

Table 369. Indicative activity concentrations in the ore and the waste products of the phosphate industry in the EU (Chen et al., 2003).

Material	Activity concentration (Bq g^{-1})	Radionuclide
Ore	1.4	^{238}U+
	0.16	^{232}Th
	1.4	^{226}Ra+
	1.4	^{210}Pb+
Process with sulphuric acid		
Phosphogypsum	0.2	^{238}U+
	0.017	^{232}Th
	0.85	^{226}Ra+
	0.2	^{210}Pb+
Process with hydrochloric acid		
Calcium Fluoride (solid) with radium sulphate precipitation (from the precipitation step with $BaCl_2$)	8 - 10	^{226}Ra
Calcium chloride (release)	2 Bq l^{-1}	^{226}Ra
Thermal process		
Calcium silicate sludge	2.7	^{238}U+
	0.31	^{232}Th
	2.3	^{226}Ra+
	0.27	^{210}Pb+
Claimed dust	1600	^{210}Pb

Table 37: Typical liquid discharges of a fertilizer plant (Chen et al., 2003).

Year	Phosphogypsum (kt)	^{226}Ra (GBq)	^{210}Pb (GBq)	^{210}Po (GBq)
1993	563	257	279	235
1994	649	377	328	363
1995	682	361	336	299
1996	671	315	314	288
1997	670	313	315	293
1998	660	283	242	284

Regarding the fertilizers produced, their radioactive content is very variable, depending on the mineral and the method used to produce them. UNSCEAR gives values of 4 Bq g^{-1} of ^{238}U and 1 Bq g^{-1} for ^{226}Ra (UNSCEAR, 1988). The European Union gives values of 0.33-2.3 Bq g^{-1} for ^{238}U and 0.063-0.96 Bq g^{-1} for ^{226}Ra (Hofmann et al., 2000, Table 38). In all cases, the radioactive equilibrium of the U chain radioisotopes relevant from the radiological point of view is changed due to the chemical processes uses for the production of fertilizers. Although radiological activity in fertilizers is between 5 and 50 times higher than in normal soil, the annual contribution to them provides a low increase in the concentrations present in food. Regarding the phosphogypsum generated, they are used as substitutes for natural gypsum in the manufacture of building panels.

Table 38. Activity concentration in fertilizers (Hofmann et al., 2000).

Fertilizer	**Concentration (%)**			**Activity concentration**	
	N	**P_2O_5**	**K_2O**	**^{238}U chain (Bq g^{-1})**	**^{232}Th chain (Bq g^{-1})**
Single Super Phosphate, SSP	20				
Germany				0.3-0.5	0.015-0.04
Belgium				0.9-1.1	0.02
Single Super Phosphate, TSP	40-50				
Germany				0.2-0.8	0.015
Belgium				0.8-2	0.015
Fertilizer PK (1)	0	15	20	0.4	0.01
Fertilizer NPK (1)	15	15	15	0.6	0.01-0.02

(1)N: nitrogen, ammonia P: phosphorus, phosphate K: potassium

In processing and use of phosphate mineral, non-radioactive contaminants are also produced. In Tables 42-44 the concentration for different non-radioactive contaminants in products and wastes in Spain are described.

Table 39. Metal content in Dicalcium Phosphate (DCP) and Tricalcium Phosphate (TCP) samples for anima and human consumption commercialized in Catalonia (Spain) in 2006 (Casacuberta et al., 2009)..

Sample code	Mg (mg g^{-1})	Al (mg g^{-1})	Fe (mg g^{-1})	Zn (mg g^{-1})	Cr (µg g^{-1})	As (µg g^{-1})	Cd (µg g^{-1})	Hg (µg g^{-1})	Pb (µg g^{-1})
DCP for domestic animals									
1	0.18	0.19	0.41	0.16	49	2.4	0.62	<0.025	4.9
2	0.2	0.2	0.43	0.057	43	1.6	0.53	<0.025	4.7
3	0.16	0.18	0.39	0.26	57	3.9	0.55	<0.025	4.9
4	0.26	0.24	0.46	0.12	47	2.4	0.44	<0.025	4.5
5	2	2.8	1.8	0.12	156	0.22	4.1	<0.025	1.2
6	2.1	2.7	1.9	0.11	166	0.21	2.8	<0.025	0.76
7	5.3	2	1.9	0.41	67	0.99	2.8	<0.025	0.39
8	10.2	3.2	2.4	1.05	127	0.57	6.5	<0.025	0.29
9	0.27	0.26	0.44	0.07	43	2.4	1.3	<0.025	3.9
10	0.23	0.34	0.48	0.1	46	2.3	0.48	0.045	4.4
11	4.2	0.65	1.9	0.28	166	3.6	5.2	<0.025	1.5
12	3.6	0.74	1.9	0.19	163	1.4	4.2	<0.025	0.9
TCP for humans									
13	0.91	0.2	0.32	<0.01	2.7	0.058	<0.05	<0.025	0.22
14	4.6	0.44	0.55	0.014	22	0.49	0.15	<0.025	0.39

Table 40. Mass concentration (%) of several contaminants (S, Ca, P, Al and Ti) in marsh and soil samples in Spain (Martin et al., 1995). (1-4: salt marshes opposite the piles; 5,8,11: soil samples around the piles; 13,18-21 salt marshes bordering the piles).

	S	Ca	P	Al	Ti	Si/Al
1	0.69(3)	0.687(11)	0.28(13)	2.0(5)	0.529(9)	15(6)
2	0.239(12)	0.605(13)	ND	8.9(4)	0.505(8)	2.43(8)
3	0.172(13)	4.45(3)	0.07(5)	8.3(3)	0.339(7)	2.00(7)
4	2.67(3)	11.81(3)	0.43(7)	4.3(3)	0.287(8)	3.23(24)
5	1.33(2)	1.54(2)	0.08(8)	8.3(4)	0.509(8)	2.60(12)
8	0.92(3)	9.09(3)	ND	6.3(4)	0.385(9)	3.04(21)
11	0.72(3)	6.8(3)	0.15(8)	3.6(4)	0.344(8)	6.0(7)
13	1.63(3)	4.25(3)	1.79(8)	7.2(4)	0.368(8)	2.35(13)
18	0.64(3)	0.29(11)	4.76(7)	8.7(3)	0.348(8)	1.65(7)
19	0.73(3)	0.209(11)	6.31(7)	6.6(3)	0.331(8)	1.97(10)
20	0.79(3)	0.135(15)	8.37(7)	8.7(3)	0.325(8)	1.52(6)
21	0.85(3)	1.7(2)	5.7(7)	10.4(4)	0.376(8)	1.39(5)

Table 41. Mass concentration (%) of several contaminants (Fe, Cu, Zn, As and Pb) in marsh and soil samples (Martin et al., 1995). (1-4: salt marshes opposite the piles; 5,8,11: soil samples around the piles; 13,18-21 salt marshes bordering the phosphogypsum piles).

	Fe[%]	Cu[ppm]	Zn[ppm]	As[ppm]	Pb[ppm]
1	3.31(3)	940(20)	387(12)	116(8)	100(30)
2	5.87(3)	472(13)	620(15)	151(9)	140(30)
3	4.7(3)	108(9)	140(9)	51(8)	90(30)
4	2.8(3)	183(8)	487(9)	13(5)	200(30)
5	5.44(3)	492(13)	439(13)	184(11)	190(40)
8	2.79(3)	39(5)	75(5)	23(4)	ND
11	1.89(3)	71(5)	131(5)	60(30)	31(20)
13	7.83(4)	3040(150)	4200(200)	1070(30)	4400(700)
18	16.93(5)	520(20)	356(13)	2700(300)	700(200)
19	24.13(7)	320(11)	289(9)	5900(300)	3000(300)
20	20.36(7)	1970(130)	1080(0)	3600(300)	800(200)
21	16.29(5)	2930(150)	2080(150)	2500(300)	1500(200)

Heavy metal concentrations in soil and marshes surrounding the waste piles are much higher than the Canadian guidelines for agricultural soil (Table 66). Cd concentrations were 2.0 µg g^{-1} (Abril et al., 2008) which is also above the Canadian agricultural soil guideline.

7.5 Mining (other than uranium mining)

In report RP 135 the specific activity of mining is not detailed, but some typical values of the mineral used in the EU are given (Table 42) (Chen et al., 2003).

Table 42. Typical values ofnaturalradioactivityin minerals(Bq g^{-1})(Chen et al., 2003).

	$^{238}U_{sec}$	$^{232}Th_{sec}$
Bauxite (Aluminium)	0.037 – 0.53	0.041 – 0.527
Iron mineral	< 0.05	< 0.05
Pirochloride (ferroniobium)	6 - 10	7 – 80
Tin mineral	0.001	0.3

Solid wastes generated through the mining and processing of mineral ores often contain residuals and other potentially toxic substances. Lead-zinc mine tailings from the 'old lead-belt' in Missouri were chemically characterized using total chemical analysis and sequential extraction. These tailings have been considered as an inert and safe material for years. However, the levels of heavy metals in these tailings were found elevated with Pb values as high as 5000 ppm. The sequential extraction results indicated that the Pb was primarily found in the residual fraction and probably is in the PbS form. Some Pb was found in the oxide fraction. Cu, Zn, and Cd were found in several different fractions, but primarily in the residual fraction. Some Cu was found in the organic fraction. The Zn showed a wide variation between samples with high levels in the carbonate, oxide and residual fractions. Very little Pb, Cd, Cu, and Zn were leached by water (Table 43, 47)(Clevenger, 1990).

Table 43. Sieve analysis and average heavy metal concentration in Desloge tailings (Clevenger, 1990).

Sieve size	Surface area m^2g^{-1}	Metal conc. µg g^{-1}			
		Pb	**Zn**	**Cd**	**Cu**
400	2.49	3867	1329	33.9	88.6
400	1.52	1578	899	23.2	82.3
200	0.81	1120	1361	39.9	71.2
100	0.59	930	1586	33.7	54.0

Table 44. Comparison of the elemental concentrations (ppm) from three different tailings piles near Desloge (Clevenger, 1990).

Element	Desloge	Park	National
Ag	1.3	2	2
Al	1060	600	850
As	<10	< 10	< 10
B	6.6	4	7.6
Ba	11	4	4
Be	0.9	1	1.2
Bi	< 3	< 3	< 3
Ca	180200	181000	181500
Cd	29	8	4
Cr	2.3	3	3
Cu	58	100	252
Fe	30360	29500	32900
K	700	373	545
Li	2.7	1.8	2.2
Mg	98000	99800	98100
Mn	3800	3450	3734
Mo	< 3	< 3	<3
Na	< 200	246	241
Ni	18.8	18	40
P	197	147	170
Pb	1680	767	4485
Sb	28	29	28
Se	< 20	< 20	< 20
Si	650	320	480
Sn	< 4	<4	< 4
Sr	42	39	40
Ti	4.6	9.5	6.5
Tl	< 5	< 5	< 5
V	5	4.6	5
Zn	1250	300	110

7.6 Production of Titanium Dioxide Pigment

In the summary within RP 135 of the processes that are carried out in this industry, it is mentioned that titanium dioxide is produced from rutile and ilmenite ores (Chen et al., 2003). The use of the sludge from the tin production is also possible. There are two possible chemical processes: one with sulphuric acid and another one with hydrochloric acid. Both processes carry warming processes up to 1000 ºC. Around 70% of the European production is carried out with the second method.

n ilmenite processing to produce synthetic rutile and titanium dioxidepigment, large amounts of hydrated iron oxide (30,000 t a^{-1} is produced inMalaysia) and gypsum residues are produced and stored in ponds or dumpedabove ground on the plant premises.The dumping at sea of the waste from TiO_2 production, diluted sulphuricacid, caused serious environmental concern and has been stopped, at least inEurope. Per tonne of TiO_2 produced, 6–8 t of a diluted (20–22%) acid aregenerated.The acid is too diluted for further commercial use and often contaminatedby heavy metals and, indeed, NORM. In a process with a relatively highenergy consumption the acid is concentrated and accessory salts, mainly ferricsulphates and co-precipitates, are removed. The acid is fed back into theproduction process, while the ferric sulphate is used commercially as aflocculant for wastewater treatment, mainly for removal of phosphates assparingly soluble ferric phosphates. Any ferric oxides produced are land filled.

Fewer environmental pollution problems are encountered when pigmentis produced from rutile rather than ilmenite. The chloride process, using a rutilefeed, generates about 0.2 t of waste per tonne of TiO_2 product; the sulphateprocess, using ilmenite, generates about 3.5 t of waste per tonne of product.

Producing synthetic rutile from ilmenite results in about 0.7 t of waste, mainlyiron oxide, per tonne of product. Direct chlorination of ilmenite generatesabout 1.2 t of waste, mainly ferric chloride, per tonne of TiO_2.

In the chemical processes described above, the titanium is removed fromthe other metals present. All radionuclides present in the feedstock (and inother process inputs, for example, petroleum coke) go to the chemicalprocessing waste stream.The final commercial products therefore seldom haveradiation issues associated with them. A range of by-product mineralsproduced in smaller quantities are either sold, stored or disposed of.

The waste arising from the chemical processing of titanium feedstockcan be considered as ‘mixed industrial waste’, as it contains metals such as Pb, As, Zn, Mn, Mg, V and Nb, in addition to the radionuclides and neutralized acid effluent. This waste is kept in designated waste disposal areas, many of which.
A typical plant of titanium dioxide pigment of medium size processes around 90,000 t of rutile or ilmenite minerals annually. In Table 45 the values of the radionuclides activity concentration in liquid discharges that are produced in a typical plant using hydrochloric acidare presented.

Table 45. Liquid discharges of the main radionuclides from a plant of TiO_2 pigments that uses the hydrochloric acid to produce 90,000 annual ton (Chen et al., 2003).

Nuclide	GBq a^{-1}
^{228}Ra	38
^{226}Ra	22
^{210}Pb	9
^{210}Po	3

As the process seeks to remove impurities from minerals, it also removes certain radioisotopes mainly from the chains of ^{238}U and ^{232}Th.

Based on the amount of mineral used and the average concentrations of each decay chain, an average plant would process some 40 GBq a^{-1} of each of the radioisotopes in the ^{238}U chain and 60 GBq a^{-1} from that of ^{232}Th.Table 46 gives typical activity concentrations of uranium and thorium in samples of rutile.

Table 46. Activity concentrations of uranium and thorium in samples of rutile (Bq g^{-1}) (RP 135, 2003).

^{238}U	^{232}Th
0.1 – 1.1	0.02.3

In the pigment produced, virtually 100% of the mineral is almost entirely free of radioactivity. The atmospheric releases are almost negligible, so almost all the inlet radioisotopes are concentrated in solid wastes and liquid discharges.

In the process, waste materials are in an acid mixture of chlorides, and partitioning between the two forms of waste treatment will depend on the treatment of the flow of liquid wastes.Solid wastes obtained in the process using hydrochloric acid are only 5.5% of the incoming rutile mineral. If we consider, conservatively, that all the radioactivity is concentrated in these solid wastes, about 5,000 t a^{-1}, would produce a maximum activity of dry residue of 7 Bq g^{-1} for ^{238}U and 11 Bq g^{-1} for ^{232}Th, both in secular equilibrium, but of course these concentrations depend strongly on the activity concentrations of the ore used as input to the process.

For the process using sulphuric acid the document mentions that there are no data on concentrations in the waste. However the process generates sulphate deposits in pipes. The document assumes that the concentrations are similar to those obtained in the process using hydrochloric acid. If all the sulphate is converted into gypsum, the amount of solid waste will be higher, and if the entire radioactivity contained in the ilmenite ends in the waste, the concentrations that would result would be 0.06 and 0.1 Bq g^{-1} for ^{238}U and ^{232}Th chains respectively, assuming equal activity concentrations for ilmenite and rutile. With regard to the liquid discharges, after the OSPAR agreement, should be zero or negligible.
With regard to the Spanish situation, the study performed in the plant sited in Huelva is worth mentioning. Therein the production process is based upon a sulphuric acid attack of the ilmenite ore. The subsequent separation of Ti in solution is done through a process of hydrolysis. In that process the by-products and wastes generated are recycled and / or managed. In the Huelva plant about 150 000 tons of ilmenite are processed annually for the production of about seventy thousand tons of titanium dioxide pigments. The ilmenite used as feedstock in the titanium dioxide production in Huelva is imported and can come from several origins (Australia, Malaysia ...). The activity concentrations of ilmenite is of about 0.1 Bq g^{-1} for ^{238}U and 0.3 - 0.4 Bq g^{-1} for ^{232}Th, both in secular equilibrium with their daughters (Gázquez et al., 2009) (Table 47).

Table 47. Activity concentrations of uranium and thorium in rutile samples (Bq g^{-1}) (Gázquez et al., 2009).

	^{238}U	^{234}U	^{226}Ra	^{228}Ra	^{228}Th	^{230}Th	^{232}Th
Ilmenite	0.11-0.12	0.12-0.15	0.07-0.1	0.26-0.37	0.25-0.38	_	_
Unattacked sludge	0.14-0.29	0.17-0.3	0.7-0.9	2.4-2.7	0.7-0.8	_	0.3-0.4
Monohydrate sulphate	0.045-0.058	0.07-0.08	0.008-0.011	0.043-0.05	0.3-0.5	0.1-0.13	0.3-0.4
Copperas	0.0007-0.001	0.0009-0.002	-	0.001-0.003	0.007-0.01	0.004-0.005	_
Red gypsum	0.017-0.021	0.024-0.027	0.012-0.021	0.08-0.11	0.1-0.15	0.036-0.05	0.12-0.16

In industries producing titanium dioxide pigment also other non-radioactive contaminants are produced (Tables 48-50) (Gazquez et al., 2009).

Table 48. Activity Concentration (%) of major elements in samples of ilmenite and slag (Gázquez et al. 2009).

Samples	SiO_2	Al_2O_3	FeO	ZrO_2	MnO	MgO	V_2O_5	CaO	TiO_2
ILM 1	0.99	0.94	41.81	0.28	1.23	0.55	0.23	0.09	50.41
ILM 2	0.48	0.60	43.54	0.13	1.36	0.21	0.27	0.02	49.73
ILM 3	0.89	0.79	42.99	0.27	1.21	0.32	<0.01	0.06	49.75
ILM 4	0.90	0.83	46.63	0.92	1.25	0.38	<0.01	0.06	49.42
ILM 5	0.31	0.41	46.44	0.12	1.46	0.15	0.25	0.01	48.76
Average	0.71±0.30	0.71±0.21	44±2	0.34±0.33	1.3±0.1	0.33±0.16	-	0.05±0.03	50±1
SLAG 1	2.34	2.33	10.71	0.05	0.24	4.98	0.71	0.54	74.94
SLAG 2	2.61	2.36	10.46	0.06	0.24	5.11	0.71	0.57	75.77
SLAG 3	2.53	2.32	10.34	0.05	0.26	5.09	0.68	0.55	75.61
SLAG 4	2.54	2.39	10.76	0.05	0.25	5.10	0.70	0.59	75.03
SLAG 5	2.50	2.31	11.23	0.06	0.29	5.02	0.68	0.56	75.34
Average	2.5±0.1	2.34±0.03	10.7±0.3	0.05±0.1	0.26±0.02	5.06±0.06	0.69±0.02	0.56±0.02	75.3±0.4
Slag/ilmenite	3.5	3.3	0.24	0.15	0.20	15.3	2.8	11	1.52
Soil	66.6	15.4	5.04	-	0.10	2.48	-	3.5	0.64

Table 49. Trace element concentrations (ppm) in ilmenite samples (Gázquez et al., 2009).

Elements	ILM 1	ILM 2	ILM 3	ILM 4	ILM 5	Average
V	1060	840	977	994	831	940 ± 101
Cr	375	210	503	430	200	344 ± 134
Co	56	46	79	65	48	59 ± 14
Ni	14	11	22	18	11	15 ± 5
Cu	37	31	52	57	29	41 ± 13
Zn	281	252	401	303	243	296 ± 63
As	22	16	28	23	18	22 ± 5
Zr	375	210	503	430	200	251 ± 41
Nb	777	754	1094	811	684	824 ± 158
Cd	2.9	2.2	3.4	3.1	1.8	2.7 ± 0.7
Sn	25	20	36	28	17	25 ± 7
La	89	6.4	122	95	51	84 ± 28
Ce	181	144	246	201	124	179 ± 48
Pb	130	114	169	146	118	135 ± 23
Th	82	76	114	95	119	97 ± 19
U	5.6	5.8	8.7	7.4	5.1	6.5 ± 1.5

Table 50. Composition of trace elements (ppm) in raw materials and coproducts (Gázquez et al., 2009).

Elements	Ilmenite	Slag	CAP	MON	RG	Soil
V	940 ± 101	3131 ±58	25 ± 7	1105 ± 172	277 ± 20	97
Cr	344 ± 134	1126 ±212	10 ± 3	467 ± 55	133 ± 8	92
Co	59 ± 14	12 ± 1	38 ± 4	31 ± 3	12 ± 1	17.3
Ni	15 ± 5	6.6 ± 1.4	11 ± 2	28 ± 3	31 ± 8	47
Cu	41 ± 13	32 ± 7	<0.01	<0.01	12 ± 1	28
Zn	296 ± 63	35 ± 12	299 ± 72	749 ± 115	225 ± 30	67
As	22 ± 5	0.41 ± 0.23	0.25 ± 0.06	1.32 ± 0.17	12 ± 1	4.8
Zr	251 ± 41	324 ± 59	1.8 ± 0.6	62 ± 6	33 ± 5	193
Nb	824 ± 158	76 ± 14	1.5 ± 0.5	10 ± 2	111 ± 17	12
Cd	2.7 ± 0.7	2.3 ± 0.5	3.3 ± 0.7	0.87 ± 0.09	1.04 ± 0.09	0.09
Sn	25 ± 7	0.53± 0.33	1.5 ± 0.5	106 ± 8	10 ± 2	2.1
La	84 ± 28	5.2 ± 1.4	2.9 ± 0.9	94 ± 11	53 ± 12	31
Ce	179 ± 48	13 ± 4	5.9 ± 1.9	182 ± 18	102 ± 29	63
Pb	135 ± 23	36 ± 13	46 ± 14	45 ± 5	35 ± 5	17
Th	97 ±1 9	4.2 ± 1.2	3.1 ± 1.1	92 ± 11	30 ± 2	10.5
U	6.5 ± 1.5	0.77± 0.21	0.11± 0.03	5.0 ± 0.4	1.70 ± 0.29	2.7

CAP=cooperas, MON=ferrous sulphate monohydrate, RG=red gypsum.

7.7 Geothermal Energy

NORM is found in the solid residues generated by the exploration anddevelopment of geothermal systems and in the extraction of the earth'sgeothermal energy for use in either producing electric power or supplyingdirect heat.Changes in the thermodynamic properties, such as pressure,temperature and volume, can cause precipitation and gas evolution because ofthe varying solubilities of some of the constituents present in fluids.Theresulting fluids have different physicochemical properties than the original, and are these properties which are passed on to subsequent processing stages. Scales can be formed by precipitation within the equipment used for extraction and distributionto power systems. Sludge is formed by precipitation and sedimentationduring the cooling process. Gas evolution can occur at any stage underappropriate conditions. These NORM containing residues include mineralsthat precipitate out of solution and form scale or sludge on the inside surfacesof the drilling and production equipment (e.g. steam turbines, heat exchangers,process lines, valves, turbines and fluid handling equipment) used to extractgeothermal heat. Such residues contain Ba, Ca and Sr salts(carbonates, sulphates and silicates), and silica, as well as significant concentrationsof Ra and its decay products. Radium is slightly soluble and,consequently, can be brought to the surface and co-precipitated with Ba and Ca salts onto the inside surfaces of drilling and production equipment,similar to the case of oil and gas production. The principal residues of concernare the scales in pipework and production equipment and the filter cakeproduced from treatment of spent geothermal fluids prior to their reinjection. Scales are hard, insoluble sulphate deposits that form on the inside of pipes, tubulars, filters, pumps, well heads and other water handling equipment (IAEA, 2003).The only available estimate for annual waste generation is about 45,500 t for Imperial Valley, California, based on 1991 data.The total annual waste generation can be subdivided into five waste streams, including: filter cake, 18,318 t; sulphur, 16,240 t; drilling, 9,571 t; scale, 946 t; and miscellaneous, 113 t. For filter cake and scale, these amounts translate to generation rates of 87 t per MW(net) and 0.58 t per MW(net), respectively. Power plants operating in ImperialCounty (California) are estimated to produce approximately 19,200 t of filte cake and scale waste annually. On the basis of the average density of 1.3 g cm^{-3}, obtained from the final environmental impact statement for a disposal site receiving waste from liquid dominated geothermal facilities in Imperial Valley, this is equivalent to a waste volume of about 14,800 m^3. There are indications that up to an additional 40,000 t of silica scale may be produced annually from Imperial Valley (IAEA, 2003).

Similarly to oil and gas production, U and Th and their radioactive decay products may be present in formations from whichgeothermal fluids are extracted. However, the available information indicatesthat no significant quantities of U and Th are dissolved or entrained in geothermal fluids. The primary radionuclides present ingeothermal fluids are ^{224}Ra, ^{226}Ra, ^{228}Ra, ^{210}Pb, ^{212}Pb and ^{222}Rn from the ^{238}Uand ^{232}Th decay chains. In addition, ^{40}K is often the only detectableradionuclide in water produced from Californian formations exploited for bothgeothermal energy and oil and gas, which suggests that ^{40}K may also be asignificant contributor to NORM in geothermal waste, but there are no data to confirm this (IAEA, 2003).

Table 51. Radionuclide concentrations in geothermal energy production waste (IAEA, 2003).

Nuclide	Residue concentration (Bq kg^{-1})
^{228}Th	930
^{228}Ra	3440
^{226}Ra	4880
^{210}Po	3550
^{210}Pb	3550
Total*	16350

* Exclude contribution from other (short lived) radionuclides in decay chains.

An environmental impact report on four geothermal power plantslocated in the Imperial Valley of California, provides the results of analysesof filter cake and clarifier sediment samples. The data are summarized in Tables 51 and 52.

Table 52. Radionuclide concentration ranges in geothermal energy production waste from geothermal power plants in Imperial Valley, California (USA) (Sabadell and Axtmann, 1975).

Nuclide	Residue concentration range (Bq kg^{-1})
^{228}Th	Below detection level to 1628
^{228}Ra	Below detection level to 6771
^{226}Ra	370 - 9398
^{212}Po	Below detection level to 999
^{212}Pb	Below detection level to 1554
^{214}Pb	0.037 - 7622
^{214}Bi	0.037 - 6401

Data for ^{222}Rn indicate that an emission rate of 1 mBqd^{-1} per MW of power occurs at the Geysers field in northern California. Radon fluxrates were estimated to be 5.92 Bq m^{-2}s^{-1} from uncovered waste disposed of ina regulated landfill.The radon emanation coefficient was estimated to be 0.25.Radon concentrations in dry steam and ejector gas have been reported to varywidely, ranging from about 0.37 Bq l^{-1} to as low as 0.74 nBql^{-1}. Average radonconcentrations were tabulated and compared for different geothermal systems. The results indicate that concentrations are significantlyhigher in dry steam fields(Table 53). In addition, wide variations were noted, even withinindividual geothermal fields.Radon gas emissions from the power plant stacks and facilities, as well as solid waste materials, provide the potential for human and environmentalexposures.

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Table 53. Average radon concentrations by type of geothermal system (Sabadell and Axtmann, 1975).

Type of geothermal system	Location	Average radon concentration (10^{-9} Bq l^{-1})
Dry steam	Larderello, Italy	1.28
Dry steam	The Geysers, USA	0.62
Flashing brine	Salton Sea KGRA, USA	0.11
Hot water	East Mesa, USA	0.01

The origin of chemical components in hydrothermal fluids remains controversial, but in general it is accepted that the solutes present in hot natural waters (with temperatures ranging from 100 to 3400 °C) are temperature- and pressure-dependent and relate to the composition of rocks of the geological system. The salt content of such waters ranges from 30 to 300,000 ppm, and the geochemical conditions determine the relative acidity. The Mn, Fe, and As contents of thermal waters from springs and drillholes at different locations are given in Table 547.

Table 54. Composition of thermal waters from springs and drillholes (Sabadell and Axtmann, 1975).

Source	**Approx. temp. °C**	**pH (20°C)**	**Concentration in discharge, ppm**		
			Mn	**Fe**	**As**
Iceland	216	9.6	0.0	0.1	--
Ngawha, N.Z.	230	7.4	0.02	0.1	--
Broadlands, N.Z.	260	8.3	0.009	0.25	8.1
Wairakei, N.Z.	250	8.3	0.015	0.05	4.5
Taiwan	200	3.2	--	1368	--
Japan	300	4.9	--	508	--
Mexico	340	--	--	0.2	--
California	340	4.7	1400	2290	12

Table 55 presents some of the analyses made in 135 samples from thermal waters in Iceland. Ga, Ge, Fe^{2+}, Mo, Ti, and V were found in most samples, whereas Cr, Co, Ni, and Zn only appeared in few samples; Pb was almost absent, and Bi, Cd, and Cu were not found at all.

Table 55. Trace elements in thermal waters in Iceland (Sabadell and Axtmann, 1975).

Element	**Element concentration in thermal waters ppm**			
	Sample 300	**Sample 351**	**Sample 223**	**Sample 317**
Cr	<2.0	<2.0	<2.0	<2.0
Co	<1.0	<1.0	<1.0	<1.0
Ga	3.6	11.8	1.5	3.0
Ge	3.0	38.0	23.6	6.0

Fe	6.5	3240.0	12.5	194.0
Mo	21.0	9.1	47.5	11.5
Ni	<1.0	<1.0	<1.0	<1.0
Ti	0.7	3.0	1.0	8.8
V	1.4	<0.5	15.1	?
Zn	<2.0	<2.0	<2.0	<2.0

In Table 56 the compositions of brines from drillholes in the Imperial Valley, California, and Cerro Prieto, Mexico, are given. The total dissolved salt content in the Mexican well is approximately that of the sea water, but the brines in the Salton Sea area are extremely abundant in dissolved minerals, presenting formidable corrosion problems(Sabadell and Axtmann, 1975).

Table 56. Composition of brines from drillholes in the Imperial Valley (California), Cerro Prieto (Mexico) and sea water (Sabadell and Axtmann, 1975).

Constituent	Constituent concentration (ppm)		
	Sea water	CaliforniaImperial Valley.IID No. 2	Mexico CerroPrieto
Sodium	10,710	53,000	5,610
Potassium	390	16,500	1,040
Lithium	--	210	13.6
Barium	--	250	57
Calcium	419	28,800	320.4
Aluminum	--	--	--
Strontium	--	440	27.4
Magnesium	1,300	10	--
Boron	--	390	12.4
Silica	--	400	--
Iron	--	2,000	--
Manganese	--	1,370	--
Lead	--	80	--
Zinc	--	500	--
Copper	--	3	0.09
Rubidium	--	70	--
Sulfur	--	30	10
Cesium	--	20	--
Chloride	19,350	1555,000	9,694
Fluorine	--	--	0.88
CO_2	--	500	--
HCO_2	150	--	--
SO_2	2,690	--	--
Total dissolved salts	35,000	259,000	17,000

The content metals in New Zealand hot spring and drillhole discharge precipitates are shown in Table 57. Table 58 presents the data on geothermal mercury pollution in the Waikato hydrosystem and in Lake Rotorua in New Zealand.

Table 57. Metals in New Zealand hot spring and drillhole discharge precipitate (Sabadell and Axtmann, 1975).

Element	Metal concn, ppm			
	Champagne Pool	Rotokawa Hole #2	Ohaki Pool, Broadlands	Broadlands Hole #2
Au	80	70	85	55
Ag	175	30	500	200
As	2%	0.4%	400	250
Sb	2%	~ 30%	~10%	~ 8%
Hg	170	15	2000	~ 200
Tl	320	0.5%	630	~ 1000
Pb	15	50	25	50
Zn	50	100	70	200
W	--	< 10	--	--

Table 58. Geothermal mercury pollution in the Waikato hydrosystem and in Lake Rotorua in New Zealand (Sabadell and Axtmann, 1975).

Mercury concentration in top 30 cm of sediments, mg Hg/kg of sediment[b]		**Hg concentration in top 30 cm of sediment (mg/kg)**[b]				
		Lake Taupo	**Upper Waikato**	**Lake Maraetai**	**Lower Walkato**	**Lake Rotorua**
Arithmetic mean	x	<0.05	0.62	0.89	1.13	1.18
Range	*r*		0.05-213	0.45-1.43	0.30-1.97	0.57-2.40
Standard deviation	σ		0.38	0.26	0.49	0.57
No. in sample	*n*		69	13	9	20

The data available show that As, Sb, Hg, Tl, Ag, and Au are present in substantial concentrations, possibly because silica acts as an ion exchanger. Comparing the Au and Ag contents of discharged water from Broadlands with that of sea water, a factor of 10 and 2, respectively, can be found. The enrichment factor seems to be the coprecipitation of Au, Ag, and Tl with SbS, which acts as a collecting agent.

The data in Tables 59 and 60 may or may not be indicative of ambient air Hg levels at geothermal locations. The only known Hg analysis for a geothermal fluid is that for the Wairakei field (New Zealand) showing values of 0.15 ppb.

Table 59. Mercury in air and gases in volcanic regions (Sabadell and Axtmann, 1975).

Sample	**Hg concentration(x 10^3)**	
	µg m^{-3}	**(ppb)**
Air, Honolulu, Hawaii	40-910	(0.03-1.4)
Air, Kilauea Volcano	21 400-23 300	(16.5-18.0)
Gases, volcanoes,U.S.S.R.	300-4000	
Gases, hot springs,U.S.S.R.	10 000-18 000	

Table 60. Mercury in air at Icelandic and Hawaiian thermal and volcanic sites (Sabadell and Axtmann, 1975).

Location	No. of areas sampled	No. of sampled	Mercury level, µg m^{-3} Range	Mean ± S.E.
Iceland				
Fumarolic	5	16	1.3 – 37.0	10.0 ± 5.2
Magmatic	1	3	4.8 – 7.6	6.1 ± 0.6
Nonthermal	2	3	0.62 – 1.0	0.8 ± 0.2
Hawaii				
Fumarolic	1	21	1.0 – 40.7	17.6 ± 6.1
Magmatic	2	13	0.7 – 40.5	17.6 ± 6.8
Nonthermal	3	10	0.04 – 0.3	1.1 ± 0.5
New York	-	-	<0.014	<0.014
Palo Alto	-	-	<0.01	<0.01
General	-	-	0.003 – 0.030	<0.03

A comparison of the total Hg effluent rate from the Wairakei Plant (New Zealand) with that from a coal plant with the same power output, shows that this geothermal plant emits slightly more than 1% as much (Tables 61 -63) (Sabadell and Axtmann, 1975 and EPA, 1980).

Table 61. Comparison of the mercury production in the Wairakei plant and a coal plant (Sabadell and Axtmann, 1975).

Wairakei plant	
Mercury production rate, tons/yr (g/yr)[a]	0.006 (6x10^{3})
Total power output, kWh/yr	1.1 x 10^{9}
Mercury production vs. power production, g/k Wh	5.4 x 10-^{6}
Coal plant with an equivalent power output	
Total power output, kWh/yr	1.1 x 10^{9}
Power output at 40% efficiency	
(burning 10 tons/day), kWh/day	24 x 10^{4}
Total carbon consumption tons/yr	4.56 x 10^{5}
Estimated mercury content in coal, ppm (g/ton)[b]	1 (1)
Total mercury production g/yr	4.52 x 10^{5}
Mercury production vs. power production, g/k Wh	4.14 x 10^{-4}

Table 62. Geothermal fluid concentrations of various constituents that exceed the standards in geothermal water (mg l^{-1}). Data from U.S., Mexico and New Zealand (EPA, 1980).

Constituent	Worst case. Salton Sea	Worst case at any site	Typical case, East Mesa	Lowest at any site
Aluminum	450	450	0.03	0.0
Ammonia	570	570	12.6	0.1
Arsenic	15	40	0.15	0.025
Barium	1,100	1,100	2.9	0.15
Boron	745	745	4.3	0.0
Cadmium*	<0.02	<0.02	<0.02	<0.02
Chloride	210,700	210,700	2,760	0.0
Chromium	No data	<0.05	No data	<0.5
Copper	10	10	<0.1	0.0
Fluoride	18	24	1.9	0.0
H_2S	No data	30	No data	No data
Iron	3,416	3,416	1.2	0.0
Lead	200	200	5	0.0
Lithium	400	400	9.2	0.0
Magnesium	2,225	2,225	4.2	0.0
Manganese	4,000	4000	0.28	0.02
Nitrate	1,050	1,050	0.19	0.0
Selenium	1.8	1.8	0.5	0.0
Silver	1.0	1.0	0.01	0.01
Zinc	970	970	0.02	0.006
Mercury	0.014	0.014	0.006	0.002
Molybdenum	0.005	0.005	0.005	0.005
Sodium	78,000	78,000	1,619	0.4
TDS	387,500	387,500	4,422	10
Temperature, ºC	188-332	-	309-399	-
pH	3.9-7.5	-	5.4-7.1	-
Pressure, psig	220-445	-	~60	-

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Table 63. Geothermal fluid concentrations of additional constituents in geothermal water (mg l^{-1}). Data from U.S., Mexico and New Zealand (EPA, 1980).

Constituent	Worst case. Salton Sea	Worst case at any site	Typical case, East Mesa	Lowest at any site
Bicarbonate	6,900	6,900	389	0.0
Bromide	146	720	0.31	0.0
Calcium	40,000	40,000	165	0.1
Carbonate		175	4	0.0
Cesium		340	0.75	0.14
Chromium	<0.01		<0.01	
Iodine	22	22	No data	0.0
Nickel	0.16†	0.16	0.11	0.0
Phosphate			<0.1	
Potassium	29,900	29,900	177	0.0
Rubidium	168	174	40	40
Silica*	625	625	207	0.1
Strontium†††	740	740	83	0.1
Sulfate*	621	5,190	125	0.0
Sulfide	30	1,052	1.5	0.3
Tin	23 (only 1value)	180	<0.01	<0.01
Uranium	<4	<4	0.02	0.02
Tungsten	150	150	<0.1	<0.1

7.8 Facilities Filtering Groundwater (Drinking Water Treatment and Irrigation)

Public water supply systems derive their water from surface water bodies such as rivers, streams and reservoirs, or underground sources through drilled wells. Vast amounts of drinking water are obtained underground each year.

Groundwater is found almost everywhere on the earth. The depth to the groundwater and the quality of different ground waters vary greatly. Likewise, the concentrations of radionuclides in groundwater will vary depending on the local geology. For example, higher concentrations can be found in granite and U bearing formations. Naturally occurring radionuclides are leached into waters when they come in contact with U and Th bearing geological media. Hence, the predominant radionuclides found in water include those of U, Ra, Rn and their decay products.

In general, the radionuclides of concern in groundwater are ^{226}Ra and its progeny (including ^{222}Rn). Concentrations of ^{226}Ra in the USA are known to vary from 0.02 to 0.93 Bq l^{-1} ingroundwater. Reports of ^{226}Ra concentrations as high as 7.4 Bq l^{-1} have been documented, but in most instances concentrations rarely exceed 1.85 Bq l^{-1}(Elliot et al. 1990).

The ^{228}Ra to ^{226}Ra concentration ratio has been reported to vary from 0.2:1 to 5:1. However, a ratio of 1.2:1 is most likely representative of average conditions in water extracted for human or agricultural use. The higher ^{228}Ra concentration is mostly due to the average crustal ^{232}Th and ^{238}U activity ratio of about 1.2:1 to 1.5:1.

Although radium tends to be more soluble in typical water compositions, U and some ^{232}Th progeny may be found as well, and U activity levels as high as 24.1 Bq l^{-1} have been observed in both surface and groundwater samples. Concentrations of total uranium, ^{238}U plus ^{234}U, as high as 1.48 Bq l^{-1} have been described in groundwater samples. The isotopic ratio of ^{234}U to ^{238}U is known to vary, with higher concentrations of ^{234}U in both surface and groundwater. Ratios up to 28 have been reported, but more commonly a range of 1–3 has been found. Higher ^{234}U concentrations are due to the alpha recoil process, which enhances the mobilization and solubility of the decay product (^{234}U) relative to the parent (^{238}U). After the alpha particle has been emitted, the U is often stabilized in solution as a very mobile uranyl carbonate complex(Elliot et al., 1990).

The isotopes ^{230}Th and ^{234}Th are usually not detected in groundwater samples and are estimated to have upper mean concentrations of only 0.001 and 4×10^{-4} Bq l^{-1}, respectively.

The concentration of Rn in groundwater is known to vary significantly. In the USA again, ^{222}Rn analysis of 2,457 groundwater samples, gave statewide average concentrations ranging from 29.6 Bq l^{-1} in Mississippi to 513.6 Bq l^{-1} in New Hampshire. Radon concentrations have been reported to be as high as 588.3 Bq l^{-1}.

Given the amounts of water that are treated in a typical water treatment plant, the amounts of residues can be substantial roughly 50,000 t a^{-1} in the USA (Table 64)

Table 64. Characteristics of selected water treatment wastes (computed levels) (waste amounts based on an average thoughput of 1 million gallons (≈380 000 l) water per day) (Elliot et al., 1990).

Treatment method	NORM concentration In water (Bq l^{-1})		Waste volume (kg d^{-1})	Approximate Radioactivity (Bq l^{-1})
	Raw	Finished		
Coagulation/filtration (U)	1.85	0.37	10^{a}	148
Lime softening (Ra)	0.93	0.46	22^{a}	20.6
Ion exchange (Ra)^{b}	0.93	0.19	97^{c}	7.7
Reverse osmosis (U)	1.85	0.56	130^{c}	11.8

a Sludges
b Does not include ion exchange resins, which have much higher radioactivity levels.
c Waste

The US EPA indicates that NORM residues from drinking water treatment may be disposed of in landfills or lagoons or can also be used as agricultural conditioners. In the case of lagoons, evaporation is used as the means to reduce the volume of the waste. However, this results in a lower volume of residues with a higher activity concentration of radionuclides. In cases where the concentrations of radionuclides are higher, it may be necessary to dispose of the residues in a licensed radioactive waste disposal facility. Given that NORM is long lived, it may be a problem to dispose of these wastes in a near surface repository (EPA, 1980).

The vast majority of residues from water treatment are in the form of sludges. However, ion exchange resins and filter media can be a significant radiological concern given that very high concentrations of Ra are possible in those residues. During screening exercises sewage sludges and ashes from incinerated sludges sometimes have elevated levels of radionuclides. The sources are either elevated levels of NORM in groundwater or (authorized) discharges into the sewerage system by various industries. The organic solids in the sewage concentrate radionuclides, and the incineration of the sludge further concentrates them.

The common practice of dispersing sewage sludges or incinerator ashes onto agricultural land can lead to the accumulation of radionuclides and, in particular, heavy metals, in the soils and eventually their uptake into crops. For this reason various countries have regulated this practice over the last few years or are in the process of doing so.

The content in heavy metals measured in water treatment sludges and in uncontaminated soils is given in Table 65. Most heavy metals in sludges are predominantly bound in forms not readily released into solution. From 76 to 87% of the Cr, Cu, Ni, Pb, and Zn were bound within an oxide or silicate matrix.

Table 65. Metals in water treatment sludges and uncontaminated soils (mg kg^{-1} d.w.) (Elliot et al., 1990).

	Sludges						
	Alum		$FeCl_3$		Soils		Maximum
Metal	Range	Mean†	Range	Mean†	Range‡	Typical§	Levels
Cd	1-2	1.6	<0.1-2	<1	0.01-0.7	0.4	25
Cu	135-230	171	135-485	272	2-100	12	1000
Cr	40-64	50	62-513	269	1-1000	50	1000
Ni	26-65	44	33-218	136	5-5000	25	200
Pb	47-439	204	18-840	245	2-200	15	1000
Zn	195-815	527	215-865	575	10-300	40	2500

† Means given are for three alum or $FeCl_3$, sludges.
‡ Data from Lindsay, 1979.
§ Data from Berrow and Reaves, 1984.
¶ Maximum contaminant levels allowed in Pennylvania for sludges used in agricultural production.

As with drinking water, (ground)waters used for irrigation may containNORM that may accumulate in certain soil fractions and eventually transferinto plant materials.For instance, in irrigated areas along the Arkansas river valley in southeasternColorado, uranium and salts are actively leached from marine shales. These contaminated saline irrigation waters eventually return to theriver, where U levels increase to concentrations as high as 200 ppb and,because of the accompanying high salinity, wetlands in this area do not trapU. In other much publicized wetland areas such as the KestersonWildlife Refuge in California, U and Se contamination isresponsible for wildlife death and deformities. Many other irrigationsystems in semi-arid areas that drain farmland on marine shales face similarstresses on water quality(Elliot et al. 1990).

7.9 Conclusions

As for the uranium industry, impact on the environment from the NORM industry is generally very much linked with heavy metals. As an example, soils and marshes in the vicinity of a phosphogypsum piles were much higher than Canadian guideline values for agricultural soil for Cd, Cu, Zn, As, Pb (Table 66). Concentrations in red gypsum from the tin industry, exceed the exemption limits for soil for V, Cr, Cd, Zn. Mean metal concentrations in drinking water treatment sludge exceed soil limits for Cd, Cu, Pb and Zn. Though no information was found on environmental concentrations in surroundings of oil and gas extraction plants, information from a Norwegian study, reports releases of BTX, phenols and heavy metals. Overall not so much information on environmental concentrations of co-contaminants could be extracted from the review on the NORM industry.

8. Overall conclusions

Though it is clear from the studies discussed that many co-contaminants should be considered when assessing the potential impact from nuclear fuel production, nuclear power plants, reprocessing plants, among chemical released substances, however, only Cu and to a minor extent hydrazine might exceed the (French) environmental guideline values and this only for the nuclear power plants

Also for low, medium and high level waste disposal, the expected environmental concentrations are much lower than the guideline values and as such all these co-contaminants released are not expected to affect the impact from radionuclides. Also for radionuclides the levels are extremely low and no impact is expected.

For the uranium mining and milling industry and NORM industries, the picture is different.

It is clear from this partial review of co-contaminants in the uranium mining and milling industry, that heavy metals (Cd, Cr, Cu, Pb, Zn, …) or metalloids like As may be present at concentrations higher that the guidelines but also other elements like Ba, Fe, Al, F, Cl may be of concern. Which other contaminants potentially matter is very much site specific.

Also for the NORM industry, concentrations exceeding the guideline values were mostly reported for heavy metals (Cd, Cu, Pb, Zn, …). For the oil and gas extraction industry important releases of organic components are also reported.

Though not the intention of this review, other site characteristics like pH, carbonate content, organic matter load, etc. may be much more important in determining the behaviour of the radionuclides of concern than are the concentrations of co-contaminants.
As reference for environmental guideline values, Table 66 gives the Canadian guideline values for soil, freshwater and sediments of the more important co-contaminants highlighted in this review.

Table 66. Canadian Environmental Quality Guidelines (CEQG, 2011) for sediment, freshwater and agricultural soil for a number of co-contaminants released by the industries/scenarios discussed

	Sediment		Freshwater$	Soil
Contaminant	ISQG*	PEL#		
	µg kg^{-1}	µg kg^{-1}	µg L^{-1}	µg kg^{-1}
As	5900	17000	5	12
Cd	600	3500	0.033§	1.4
Cr	37300	90000	No data	64
Cu	35700	197000	2.36§	63
Hg	170	486	0.026	6.6
Pb	3500	91300	3.18§	70
Zn	123000	315000	30	200
Benzo(a)pyrene	31.9	782	0.015	

*ISQG=InterimSediment Quality Guidelines; #PEL: Probable Effect Level; $For the protection of aquatic life; §for 100 mq l^{-1} CaCo$_3$.

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Annex 2: Evaluation of geochemical speciation model performance

1. Introduction

For each scenario, the following four runs were done:

- Including co-contaminants, not including dissolved organic matter (DOM)
- Not including co-contaminants, not including DOM
- Including co-contaminants, including DOM
- Not including co-contaminants, including DOM

For the purposes of the simulations, the co-contaminants were considered to be:

- Beaverlodge Lake scenario:As, Ba, Cu, Ni, Pb, Se, Zn
- Ritord scenario: Al, Ba, Fe, Mn

The carbonate system was simulated by inputting the carbonate concentrations given in the scenarios, i.e. equilibrium with atmospheric CO_2 was not assumed.

In the Canadian example, radionuclide activity concentrations in Bq l^{-1} were converted to mass concentrations (i.e. mg l^{-1}) using the specific activity of each radionuclide (Table 1).

Table 1. Specific activities of radionuclides, used to convert activities to mass concentrations for speciation modeling.

Radionuclide	Specific activity (Bqg^{-1})
Pb-210	2.84E+12
Po-210	1.66E+14
Ra-226	3.66E+10
Th-230	7.63E+08
U-238	1.24E+04

2. WHAM

Modelling was done with a version of WHAM 6.0, modified to allow the simulation of two processes not considered in the original version:

- Precipitation of hydroxides of Fe(III) and Al ($Fe(OH)_{3\,(s)}$ and $Al(OH)_{3\,(s)}$).
- Binding of ions to the surfaces of precipitated $Fe(OH)_{3\,(s)}$ and $Al(OH)_{3\,(s)}$.

Since concentrations of Al were present in only one scenario, for consistency the binding of ions to precipitated $Al(OH)_3$ was not simulated in either scenario. Binding of ions to precipitated $Fe(OH)_3$ was allowed in both scenarios.

Activity correction was done using the extended Debye-Hückel equation.

Simulations were performed with the default WHAM 6.0 database, default.dbm

In both scenarios, the temperature was fixed to 1°C.

Redox simulation is not simulated in WHAM. The following assumptions were thus made about the states of redox-active elements:

- Fe: all Fe was assumed to be Fe(III).
- U: all U was assumed to U(VI) (UO_2).
- N: in the Ritord scenario, where concentrations of NO_2^- and NO_3^- are provided, only NO_3^- was input. Since NO_2^- and NO_3^- are not a significant complexers of metals under environmental conditions, this simplification will have a negligible effect on the speciation predictions.

Precipitation was restricted to $Fe(OH)_{3\,(s)}$ and $Al(OH)_{3\,(s)}$. WHAM does not simulate the precipitation of other solids. Precipitated $Fe(OH)_3$ was assumed to have an 'active' surface with functional groups capable of binding metals. In the Beaverlodge Lake scenario, no measured Al concentration was available, so Al was computed assuming equilibrium with solid $Al(OH)_3$.

Dissolved organic matter was simulated by assuming it comprised a mixture of fulvic acid and an inert component, in line with previous application of WHAM to natural waters (e.g. Bryan et al., 2002). The percentage of DOM acting as fulvic acid was set to 65%, and the DOM was assumed to comprise 50% C.

In the Ritord scenario, a total organic carbon concentration was measured. From this, an estimated dissolved organic carbon concentration was obtained by assuming the suspended particulate matter in the water to be 5% organic carbon (10% organic matter). This produced an estimate of 9.21 mg l^{-1} DOC (the measured TOC was 9.57 mg l^{-1}), which corresponds to a fulvic acid concentration of 11.98 mg l^{-1}.

The input data were treated as follows:

- For the Beaverlodge Lake scenario, Po, Ra, As, Mo, Se, Ag, Ti and V were excluded from the inputs, since the WHAM default database does not have binding constants for these elements.
- For the Ritord scenario, NH_4, Si and NO_2 were excluded from the inputs, since the WHAM default database does not have binding constants for these elements.

3.1 Modelling results

3.1.1 Beaverlodge Lake scenario

In all the Beaverlodge Lake scenario runs the dominant predicted species in all four runs was $UO_2(CO_3)_2^{2-}$. (Figures 1 and 2). In the two runs not including DOM, this complex made up >96% of the predicted U. In the two runs including DOM, $UO_2(CO_3)_2^{2-}$was still predicted to be the most important species. There was a shift in predicted speciation in the absence of co-contaminants, where the predicted proportion of UO_2-DOM increased from 3.2 to 13.5% as a result of the removal of competition from Al and Fe(III) for DOM binding.

Thorium speciation

Thorium speciation was consistently predicted to be dominated by Th(OH)$_4$. In all cases except for the presence of DOM and the absence of co-contaminants, Th(OH)$_4$ was predicted to form over 99% of the Th. In the presence of DOM and the absence of co-contaminants, DOM-bound Th was predicted to comprise 32.2% of the total. The increased predicted binding of Th to DOM was due to removal of competition for DOM binding from Al and Fe(III).

Lead speciation

Lead speciation predictions are shown inFigure 3 and 4. In the absence of DOM, speciation is dominated by binding to colloidal iron oxide and the PbCO$_3$ complex. Removal of co-contaminants causes speciation to shift to inorganic complexes, since the removal of iron from the inputs removes the possibility of the formation of colloidal iron oxide and thus the binding of lead to this phase. In the presence of DOM, Pb-DOM complexes are predicted to dominate regardless of whether co-contaminants are considered or not. Removal of co-contaminants does cause an increase in the predicted Pb-DOM and concomitant decreases in the predicted PbCO$_3$ and Pb-FeOx. The Pb-FeOx species cannot form in the absence of Fe(III), while the reduced binding to PbCO$_3$ relative to Pb-DOM is caused by the decreased competition from Al and Fe(III) for binding to DOM.

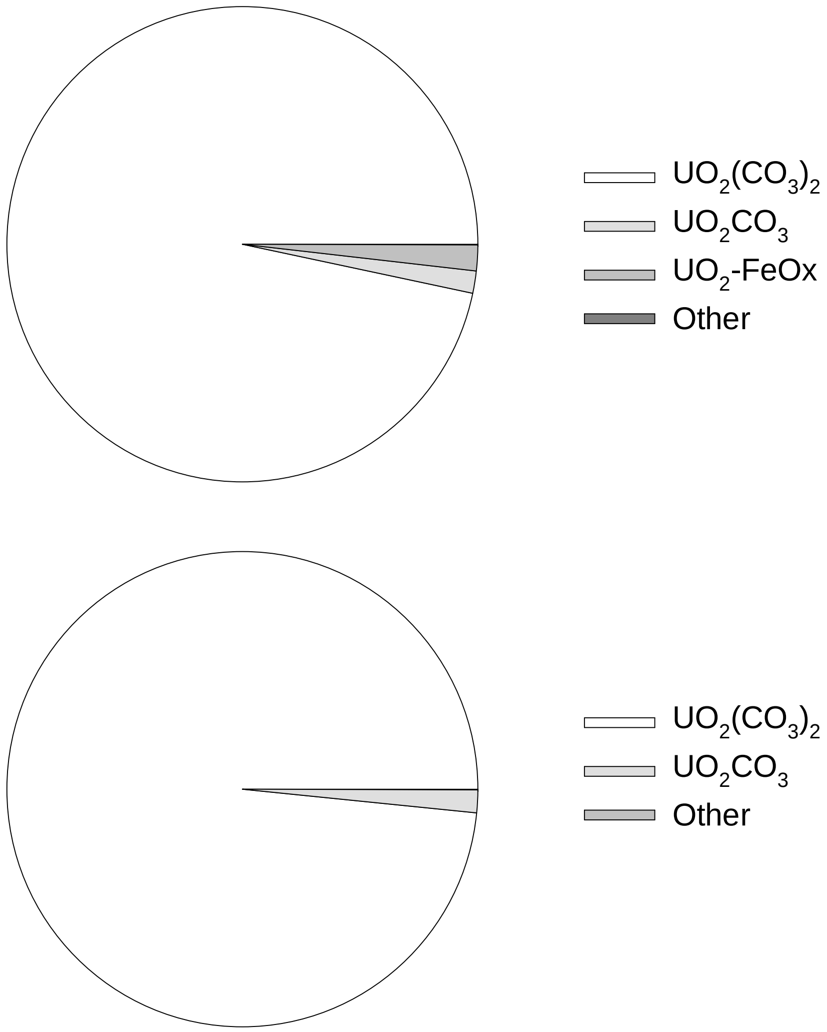


Figure 1. WHAM-predicted U speciation in the Beaverlodge Lake scenario, in the absence of DOM and in the presence (top) and absence (bottom) of the co-contaminants. Individual species are shown if their abundance is above 1% of the total U. The term UO_2-FeOx refers to UO_2 bound to colloidal iron oxide.

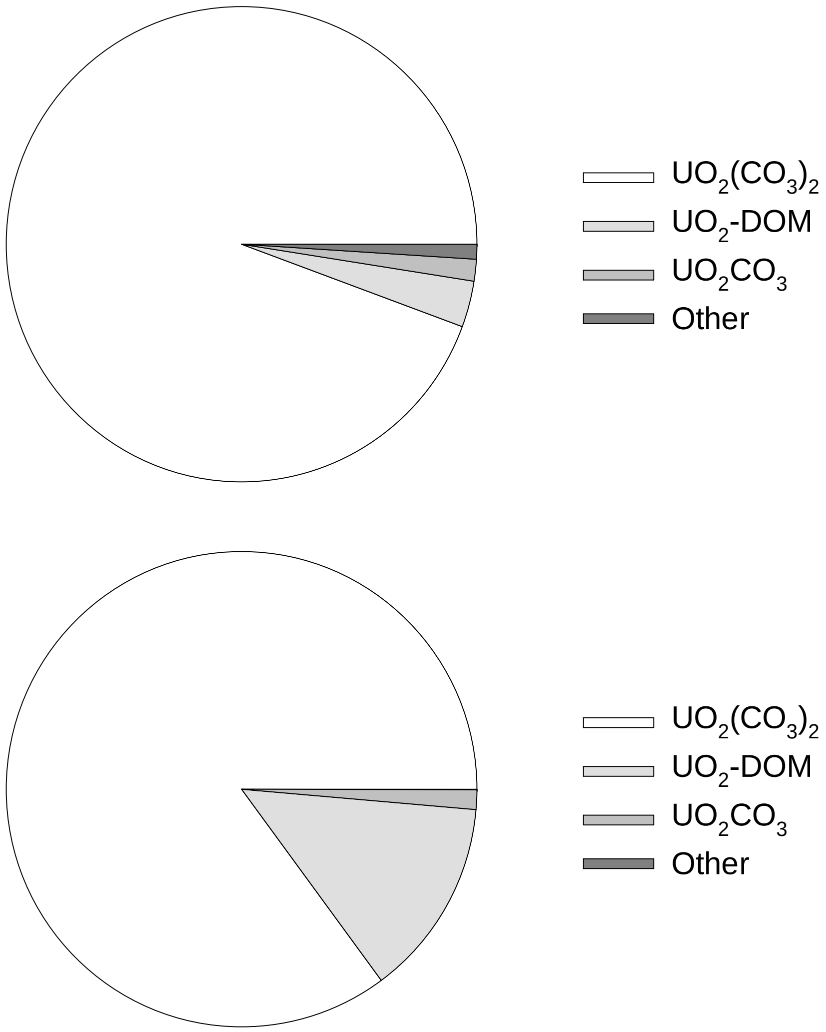


Figure 2. WHAM-predicted U speciation in the Beaverlodge Lake scenario, in the presence of DOM and in the presence (top) and absence (bottom) of the co-contaminants. Individual species are shown if their abundance is above 1% of the total U.

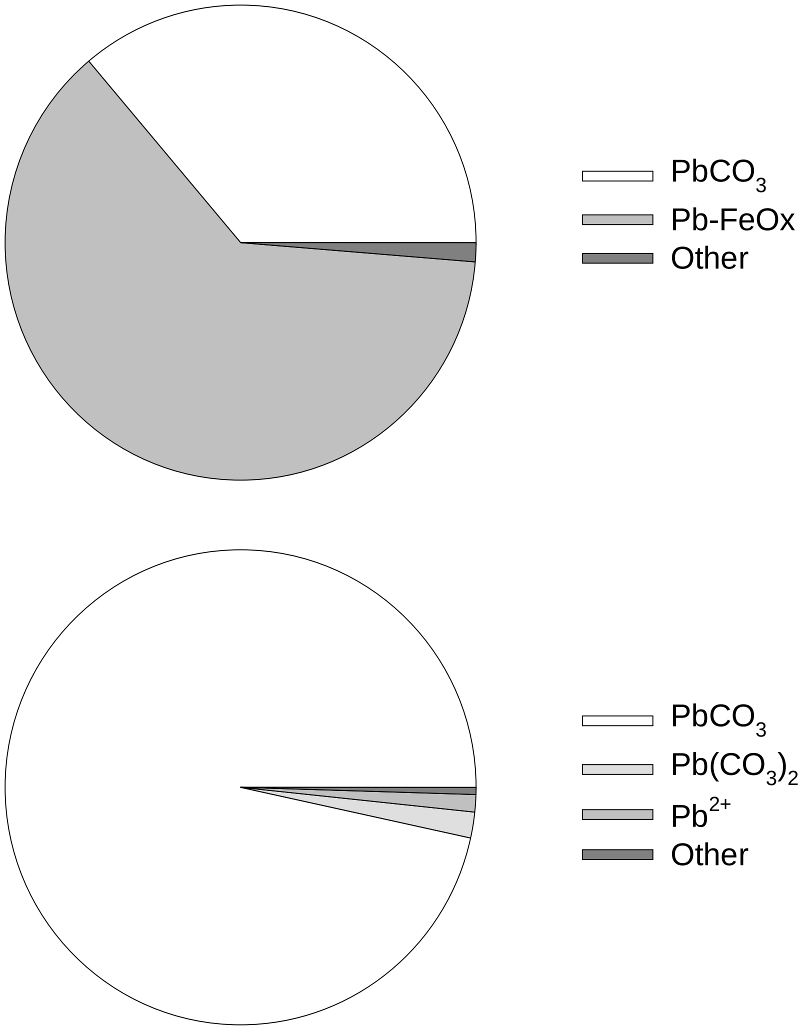


Figure 3. WHAM-predicted Pb speciation in the Beaverlodge Lake scenario, in the absence of DOM and in the presence (top) and absence (bottom) of co-contaminants. Individual species are shown if their abundance is above 1% of the total Pb. The term Pb-FeOx refers to Pb bound to colloidal iron oxide.

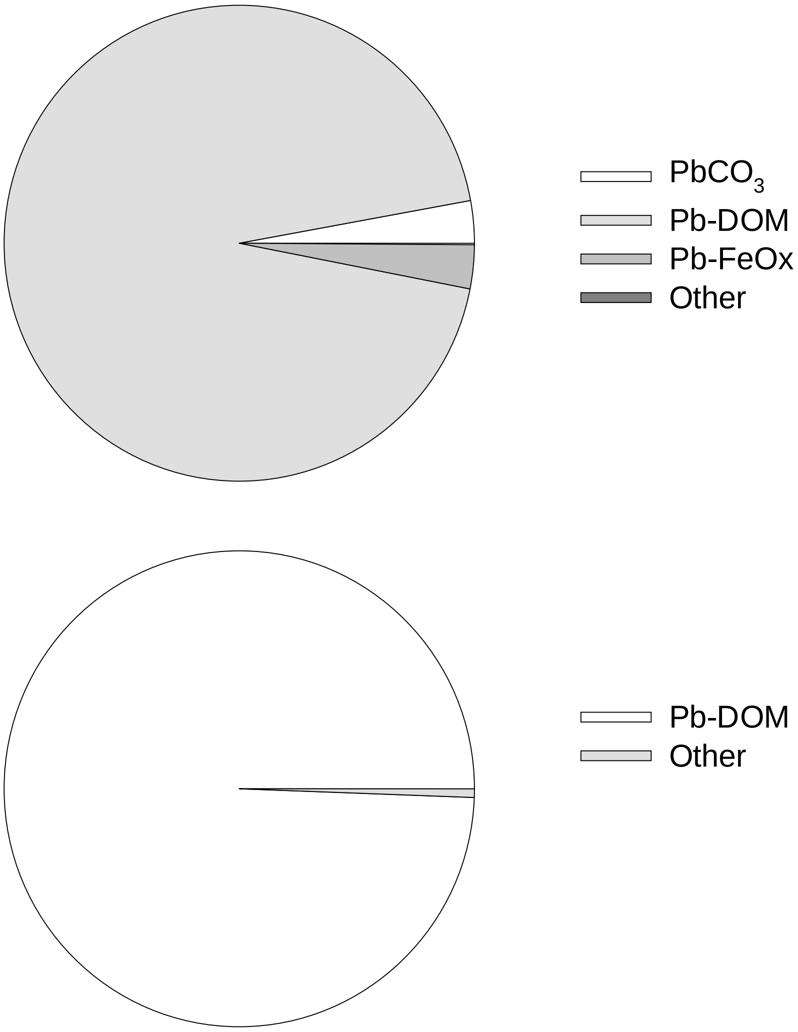


Figure 4. WHAM-predicted Pb speciation in the Beaverlodge Lake scenario, in the presence of DOM and in the presence (top) and absence (bottom) of co-contaminants. Individual species are shown if their abundance is above 1% of the total Pb.

3.2 Ritord scenario

Simulating the influence of co-contaminants

Colloidal iron oxide-bound UO_2 was predicted to be the dominant species, in both the presence and absence of co-contaminants. There was little effect of co-contaminants on the speciation (Figure 5).

Simulating the influence of DOM

Introducing DOM to the inputs produces a shift in the predicted speciation (Figure 6). Although UO_2 binding to colloidal iron oxide is predicted to remain dominant, it represents a smaller proportion of the total, and the majority of the remaining UO_2 is predicted to be bound to DOM. A small shift in the speciation is predicted in the absence of co-contaminants, with slightly less (~1.2% of the total) UO_2 predicted to be bound to colloidal iron oxide, and slightly more predicted to be bound to DOM.

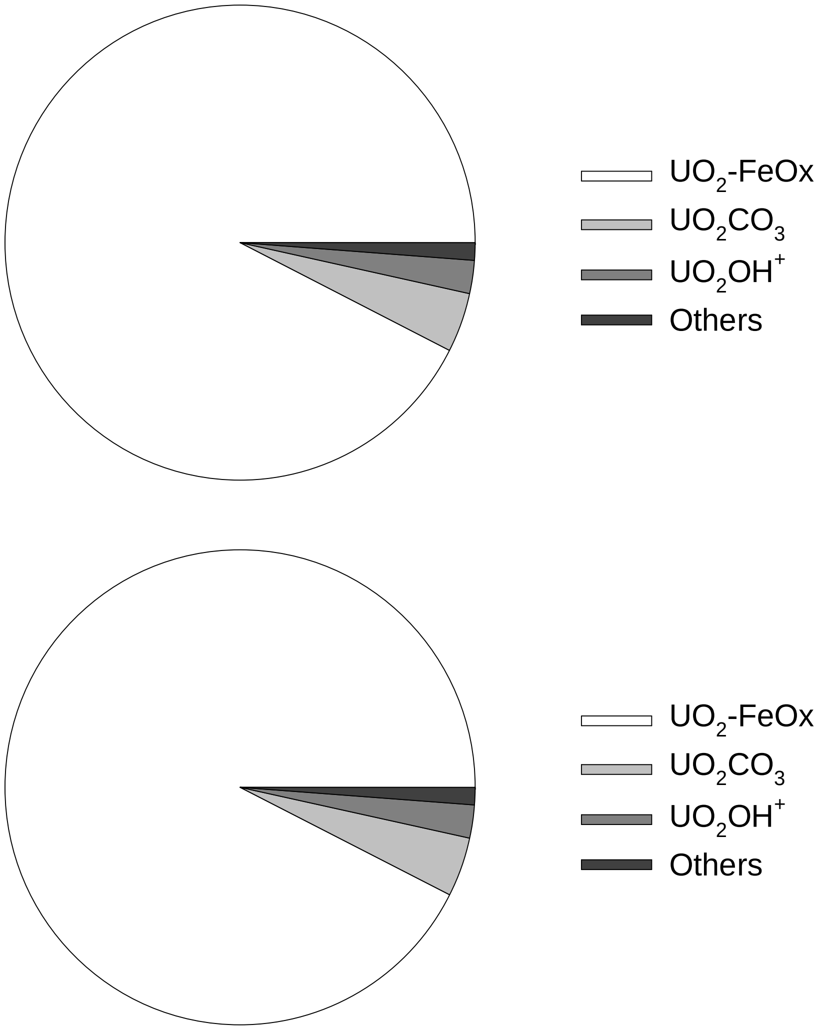


Figure 5. WHAM-predicted U speciation in the Ritord scenario, not including DOM, and including co-contaminants (top) and not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.

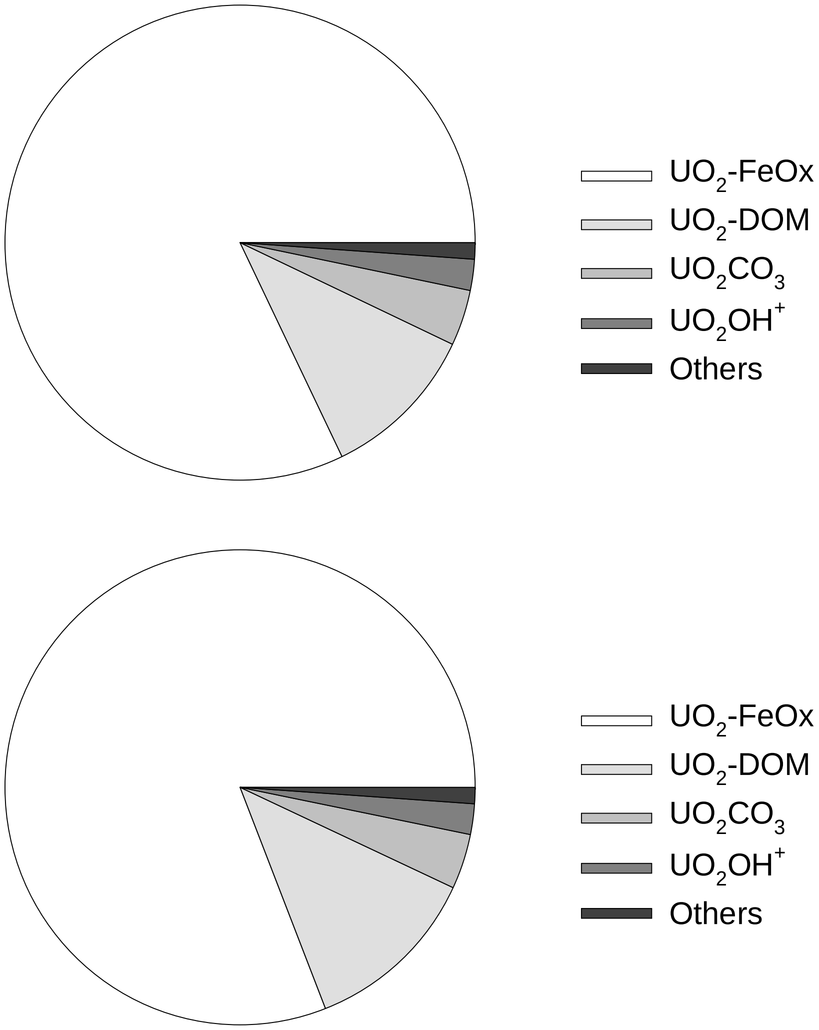


Figure 6. WHAM-predicted U speciation in the Ritord scenario, including DOM, and either including co-contaminants (top) or not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.

3. Visual MINTEQ

Modelling was done with Visual MINTEQ version 3.0.

Activity correction was done using the Davies equation.

Simulations were done using the standard MINTEQ thermodynamic database. This database is based on the MINTEQA2 model database, updated with new constants from the NIST thermodynamic database (NIST 46, Critical Stability Constants) versions 6.0 and 7.0.

Redox simulation was not enabled. Instead, it was assumed that those species having redox activity were present in the dominant form expected under oxic conditions. Thus, Fe was assumed to be present entirely as Fe(III) and U entirely as U(VI).

3.1 Modelling results

3.1.1 Beaverlodge Lake scenario

Uranium speciation

In the absence of DOM, the U speciation is predicted to be dominated by $Ca_2UO_2(CO_3)_3$ and $CaUO_2(CO_3)_3^{2-}$ (Figure 7). Removal of the co-contaminants from the inputs has a negligible influence on the predicted speciation. In the presence of DOM (Figure 8), UO_2-DOM is predicted to be non-negligible, but is a small (<3%) part of the total. Removal of co-contaminants has the effect of increasing UO_2-DOM somewhat, but it remains a small part of the total U.

Thorium speciation

In the absence of DOM, Th speciation was predicted to be dominated by the $Th(OH)_3CO_3^-$ species, with all other species negligible. In the presence of DOM, the predicted speciation was completely dominated by Th-DOM.

Lead speciation

In the absence of DOM, Pb speciation was predicted to be dominated by carbonate complexes, with small contributions from the free ion and hydrolysis complexes (Figure 9). The predicted speciation in the presence of DOM was dominated by Pb-DOM complexation (Figure 10). In both cases, removal of the co-contaminants had a minor influence on the speciation.

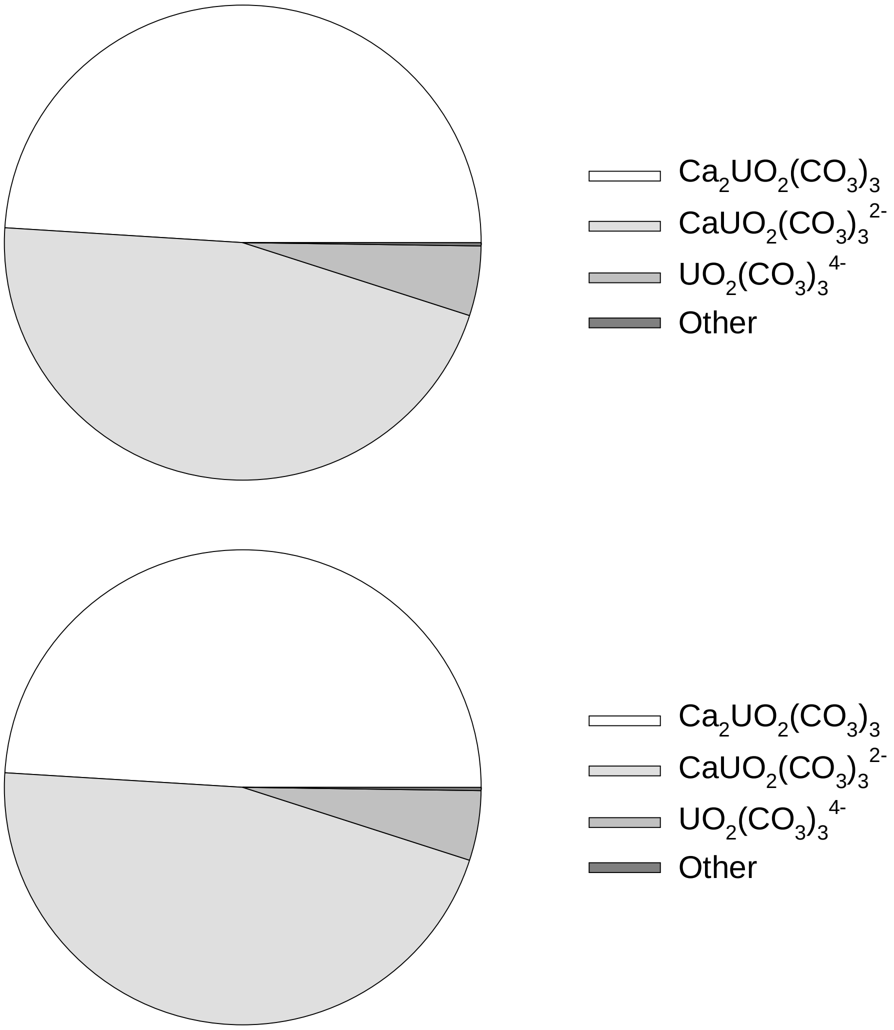


Figure 7. Visual MINTEQ-predicted U speciation in the Beaverlodge Lake scenario, not including DOM, and including co-contaminants (top) and not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.

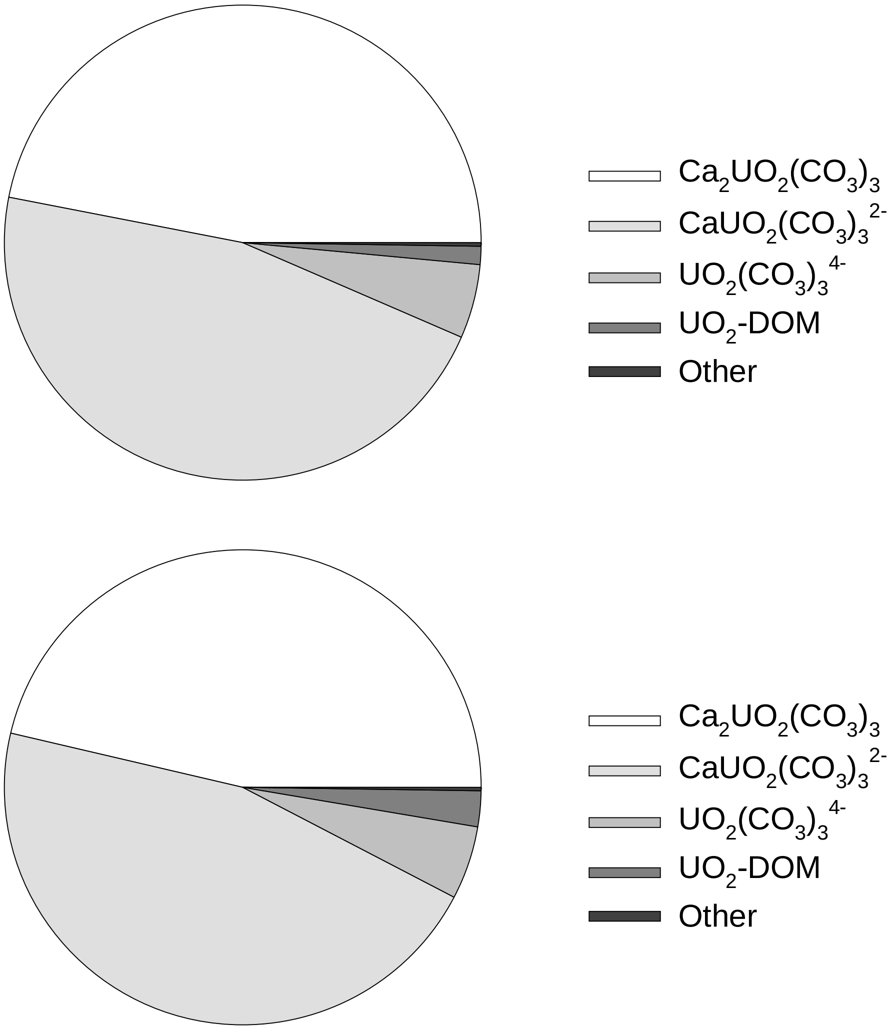
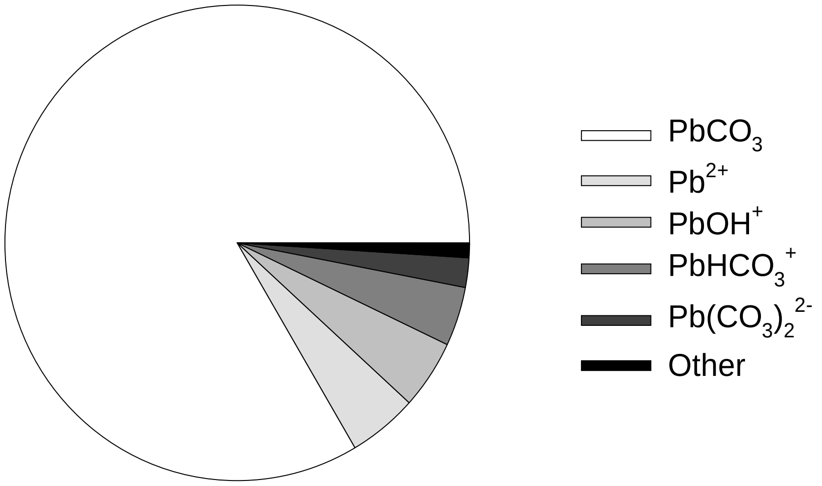


Figure 8. Visual MINTEQ-predicted U speciation in the Beaverlodge Lake scenario, including DOM, and either including co-contaminants (top) or not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.



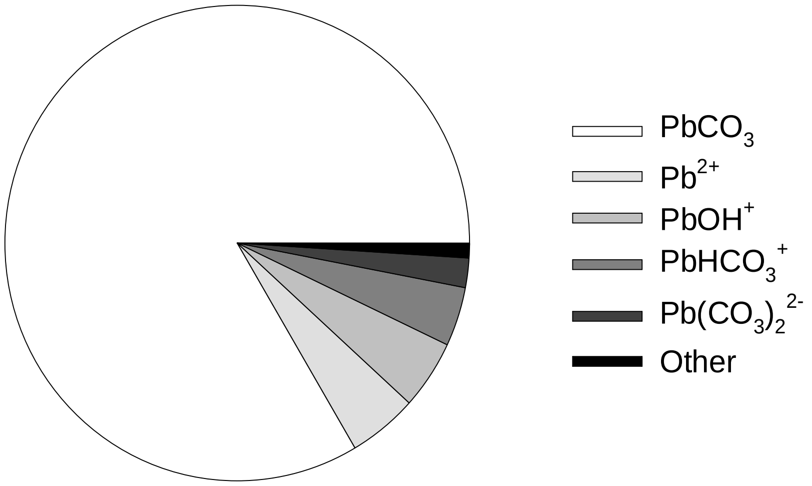


Figure 9. Visual MINTEQ-predicted Pb speciation in the Beaverlodge Lake scenario, not including DOM, and including co-contaminants (top) and not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total Pb.

Figure 10. Visual MINTEQ-predicted Pb speciation in the Beaverlodge Lake scenario, including DOM, and including co-contaminants (top) and not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total Pb.

3.1.2 Ritord scenario

Uranium speciation

Simulating the influence of co-contaminants

Carbonate complexes dominate, although ternary UO_2-Ca-CO_3 complexes, are less important than in the Beaverlodge scenario, presumably due to the lower pH in the Ritord scenario. Hydrolysis complexes of UO_2 are also predicted to be important, along with a UO_2-Si complex. Removing co-contaminants from the inputs produces a negligible change in the predicted speciation.

Simulating the influence of organic matter

Adding DOM to the model inputs produces a significant shift in speciation (compare the top panes of Figures 11 and 12) with UO_2-DOM predicted to dominate. Removing co-contaminants has a minor effect, increasing the predicted proportion of UO_2-DOM somewhat.

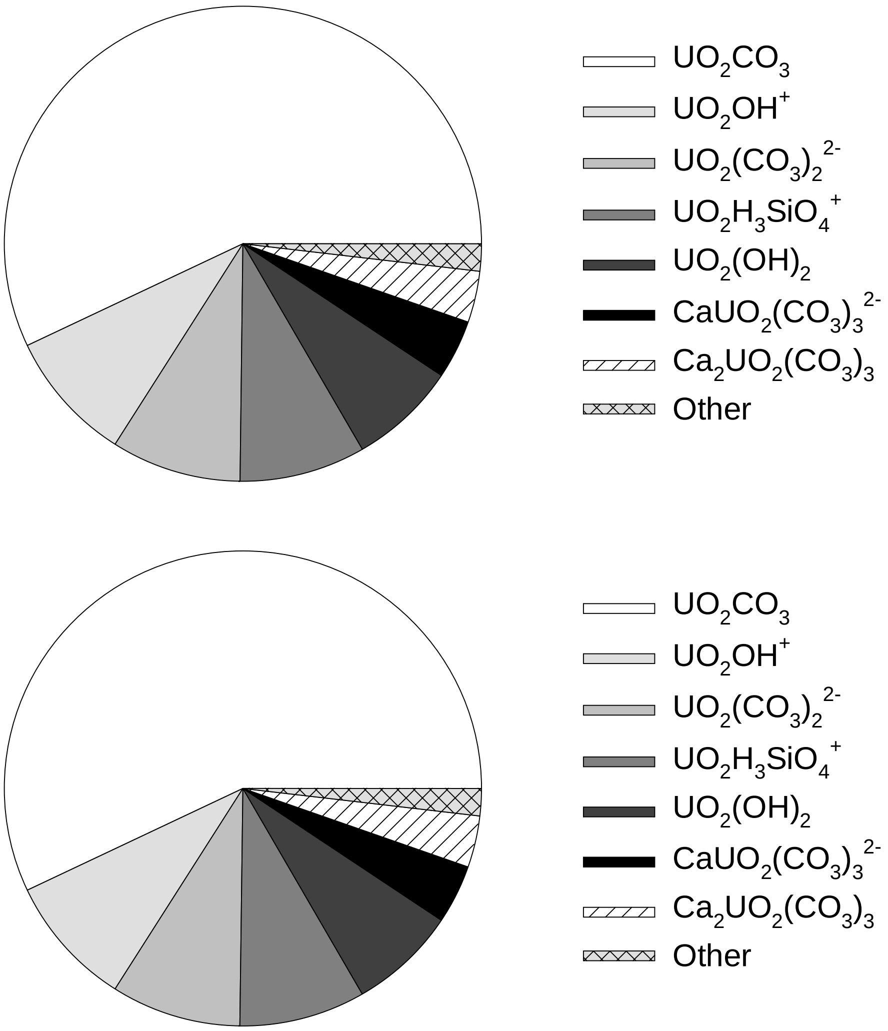


Figure 12. Visual MINTEQ-predicted U speciation in the Ritord scenario, not including DOM, and including co-contaminants (top) and not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.

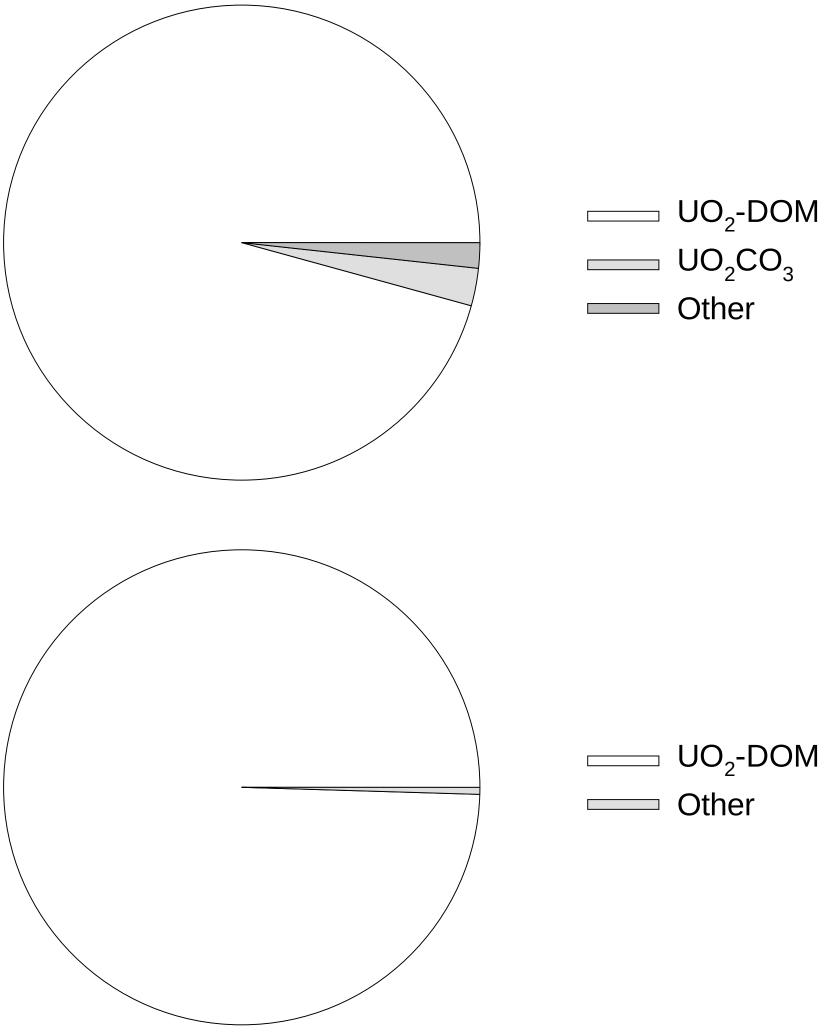


Figure 12. Visual MINTEQ-predicted U speciation in the Ritord scenario, including DOM, and either including co-contaminants (top) or not including co-contaminants (bottom). Individual species are shown if their abundance is above 1% of the total U.

4. Geochemists Workbench

Modelling was done with the Geochemist's Workbench version 8.0.12.

Simulations were performed using three binding constant databases:

- Thermo.com.v8.r6+.dat: a thermodynamic database based on the Lawrence Livermore National Laboratory dataset. The database is an update of the thermo database, containing more radionuclides than the latter.
- NEA(03 update).dat
- Thermo_minteq.dat: The thermodynamic database from the current release of Visual MINTEQ, for use with GWB releases 5.0 and later. This dataset invokes activity coefficient calculations consistent with the implementation in MINTEQ. This database contains binding constants for citrate and EDTA, which were used to model the effect of DOM by assuming either citrate or EDTA to be an analogue for DOM.

In both scenarios, the temperature was fixed to 25°C.

Redox simulation was enabled for both simulations. In the Ritord scenario, the nitrite and nitrate concentrations provided were summed and input as nitrate. In both scenarios iron was added as iron(II).

Precipitation of solids was disabled since simulations were done only on the filterable phase of the water.

Organic matter was simulated assuming organic matter to comprise either citrate or EDTA. This was done only for the simulations using the THERMO_MINTEQ.DAT database, since only this database has the required binding constants.

The input data were treated as follows:

- Po was excluded from the calculations, as none of the databases used contain binding constants for this element.

Results of the U speciation in the two test scenarios are given in the following figures (only species >1% are given) for different situations. Apart from U, concentrations of ^{230}Th, ^{210}Pb, ^{210}Po and ^{226}Ra were available in the scenario, but the databases used contained complexation constants for Th and Pb only. Therefore,only Th and Pb speciation were assessed in the Beaverlodge Lake scenario.

4.1 Modelling results

4.1.1 Beaverlodge Lake scenario

Uranium speciation

Simulating the influence of co-contaminants

From these data it seems that addition of the co-contaminants to the Canadian scenario did not affect the predicted U speciation. U is predicted to be present almost entirely in the uranyl form, as carbonate complexes. Simulations using the Thermo.Minteq or NEA databases (the latter with updated information for U-constants) gave similar results.

Simulating with different databases

Uranium speciation

A clear difference in the speciation of U was found depending on the database used. For Thermo.com.v8.r6+.dat (Figure 13) the main U-species was $UO_2(CO_3)_3^{4-}$ (almost 75%) whereas for Thermo-MINTEQ (Figure 14) $Ca_2UO_2(CO_3)_3$ was the dominant species. When the NEA database was used the predominant predicted species was $UO_2(CO_3)_3^{4-}$ (Figure 15). Figure 16 clearly shows how different the predicted speciation are for the different databases. The NEA database contains the most updated U-parameters of the three databases used. When comparing the speciation in presence or absence of co-contaminants, it is clear from Figures 13-15 that no effect of co-contaminants on speciation is observed.

Lead and thorium speciation

For Pb although differences were found in speciation depending on the database used, both databases predict $PbCO_3$ as the most predominant species. Pb speciation was not performed with the additional co-contaminants as stable lead was among the co-contaminants in a high concentration.

The speciation is not shown for Th as only one species was dominant. in both speciation databases However, for Thermo.Minteq this was $Th(OH)_3CO_3^-$ (99.999%), whereas for Thermo.com.v8.r6+.dat it was $Th(OH)_4$ (99.99%). Co-contaminants did not influence U-speciation.

Simulating the influence of organic matter

No significant differences in the speciation were found in the Beaverlodge Lake scenario when either citrate or EDTA were added to the inputs as proxies for natural organic matter (Figure 17). Also in the presence of DOM, no influence of co-contaminants on U-speciation was observed.

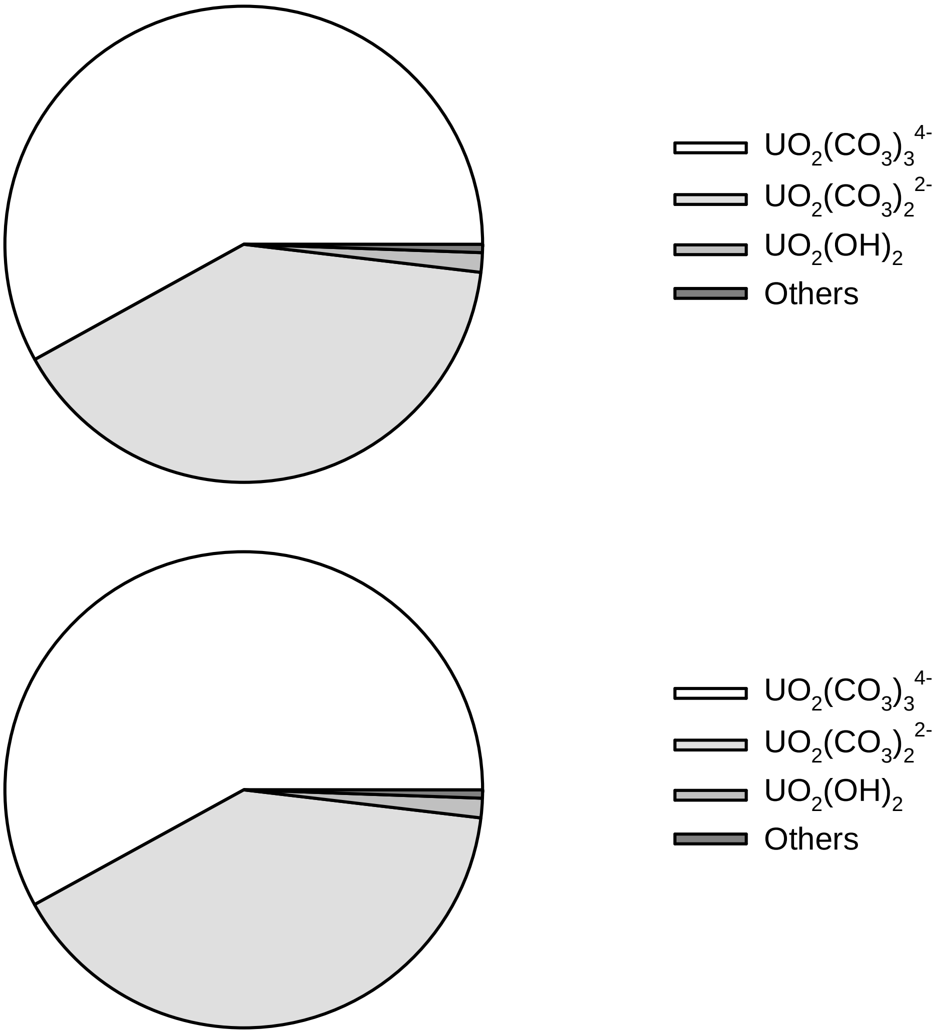


Figure 13. U speciation in the Beaverlodge Lake scenario run with Thermo.com.v8.r6+.dat without (top) or with (bottom) co-contaminants simulated.

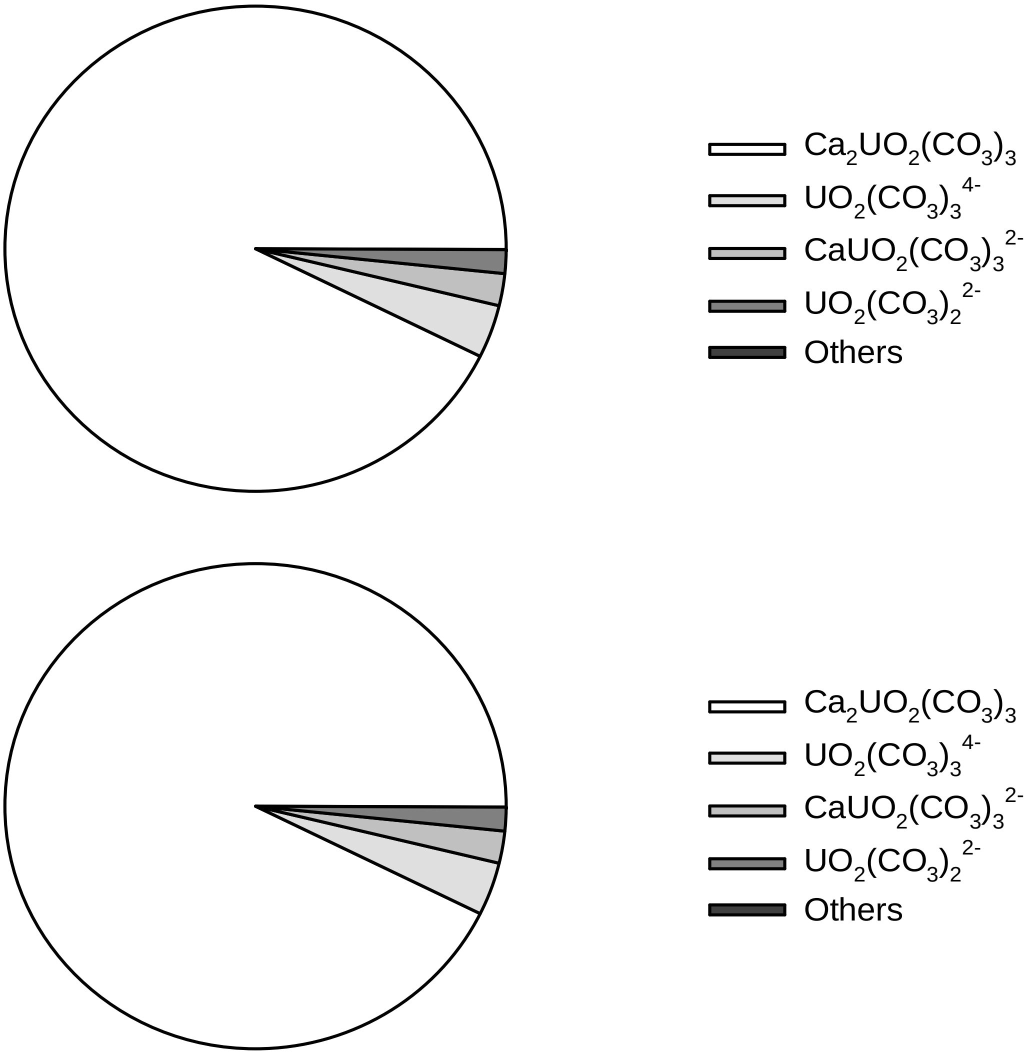


Figure 14. U speciation in the Beaverlodge Lake scenario run with the Thermo.Minteq database with (top) or without (bottom) co-contaminants simulated.

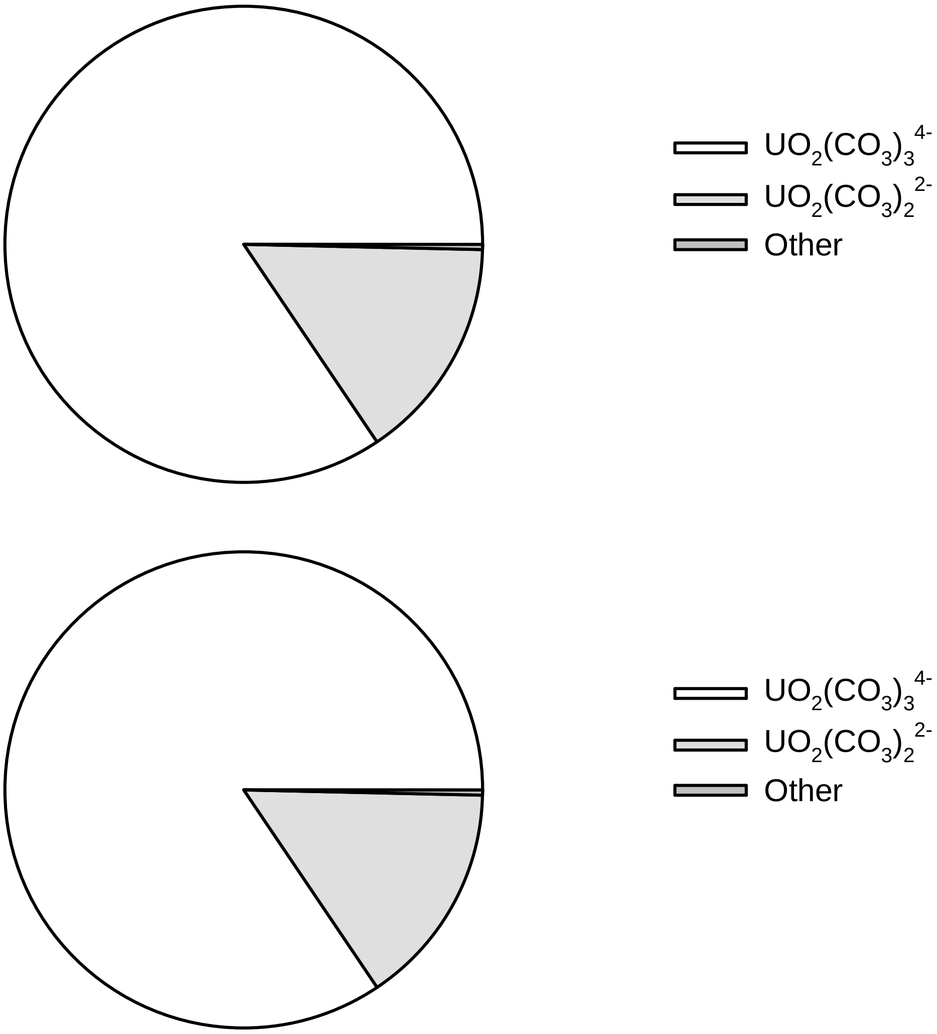


Figure 15. U speciation in the Beaverlodge Lake scenario run with NEA/NO3 without (top) or with (bottom) co-contaminants simulated.

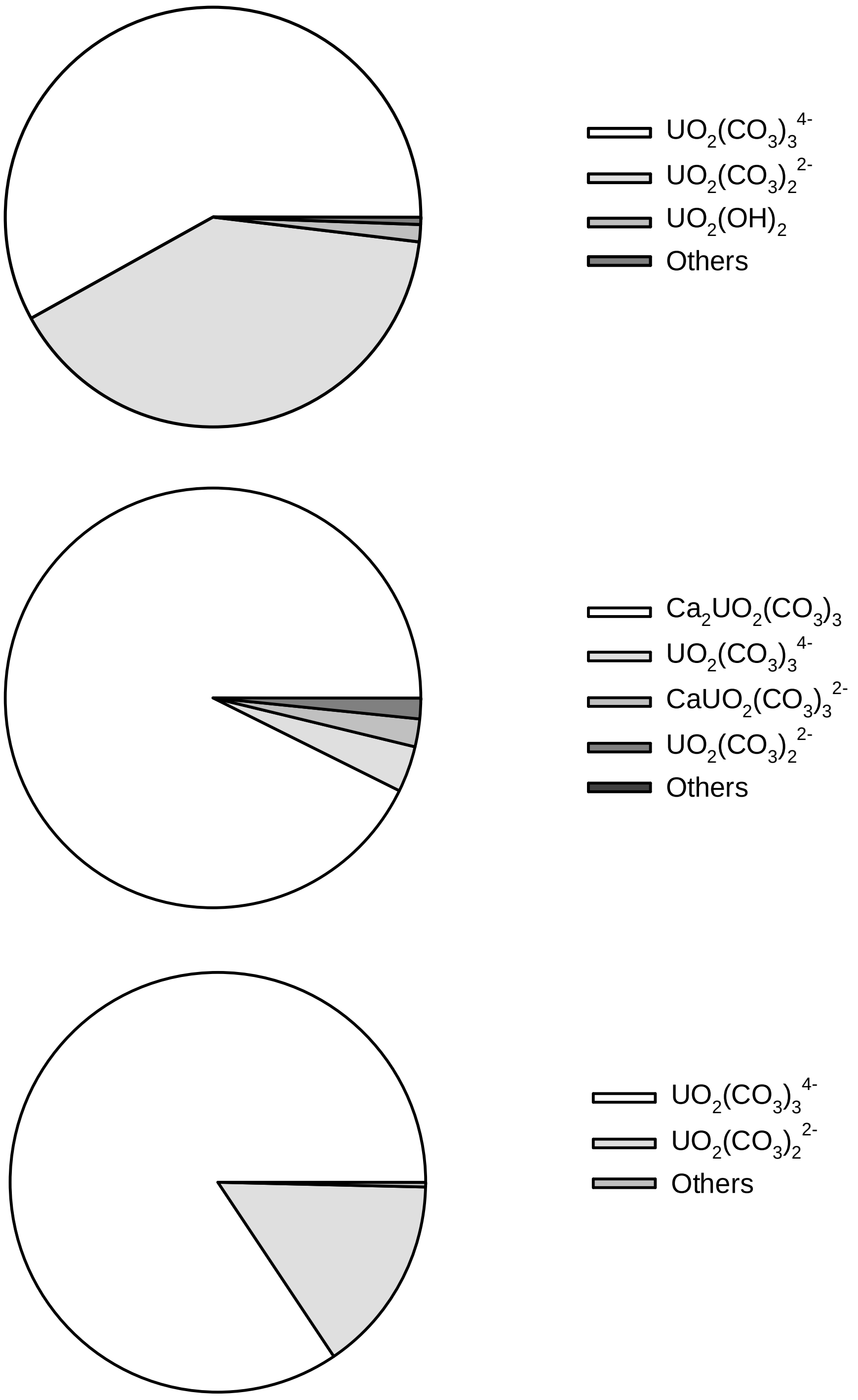


Figure 16. U speciation in the Beaverlodge Lake scenario run with Thermo.com.v8.r6+.dat (top), with Thermo-Minteq (middle), or NEA/NO3 (bottom) as the speciation database. The co-contaminants were included in the input.

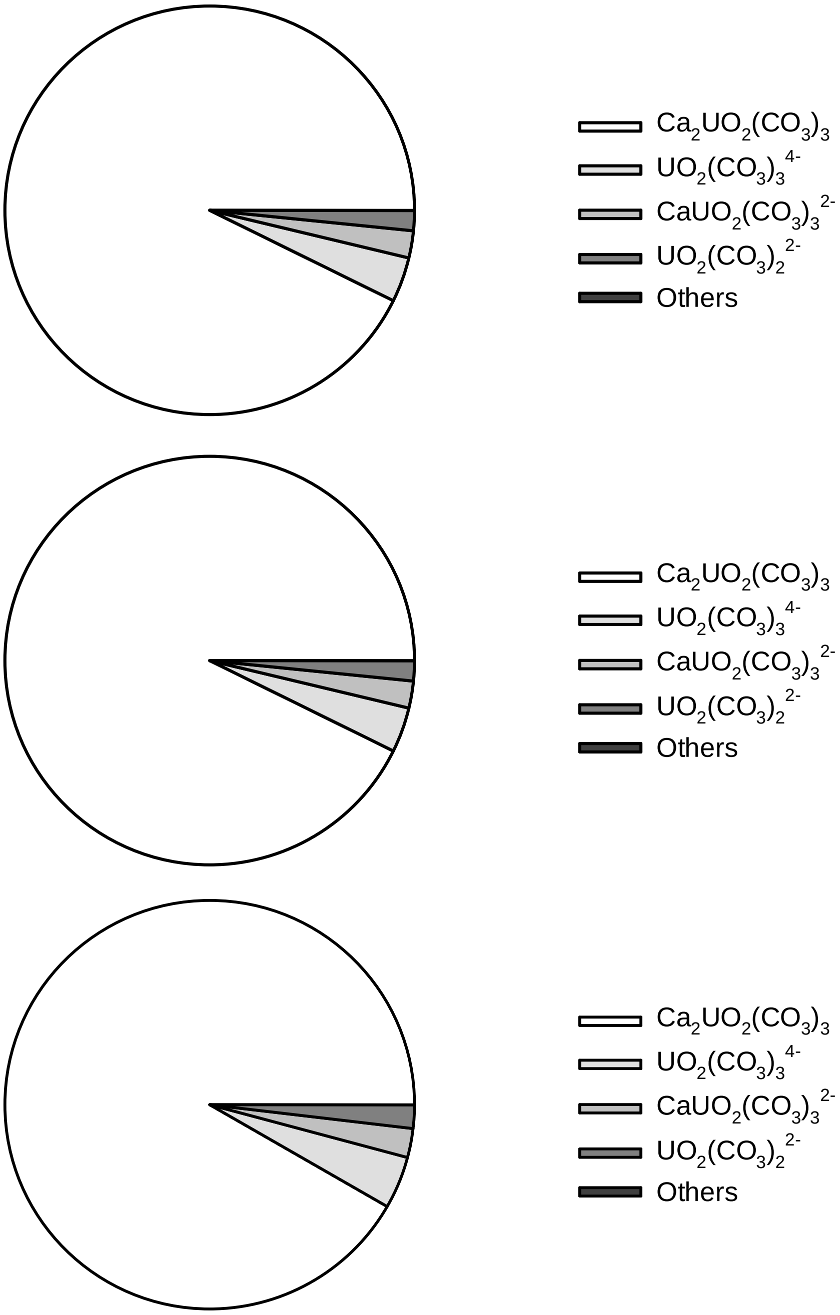


Figure 17. Effects of organic matter on U speciation in the Beaverlodge Lake scenario calculated using GWB, simulated with the Thermo-MINTEQ database. Top: no organic matter simulation; middle: organic matter simulated using EDTA as a proxy; bottom: organic matter simulated using citrate as a proxy.

4.1.2 Ritord scenario

Simulating the influence of co-contaminants

Addition of the co-contaminants to the Ritord scenario did not affect the predicted U speciation (data not shown). U is predicted to be present mostly as $UO_2(OH)_2$when speciation calculations are performed with Thermo.comv8.r6+ database. The dominant U species is $UO_2CO_3$when using the Thermo-MINTEQ database.

Simulating the influence of the database:

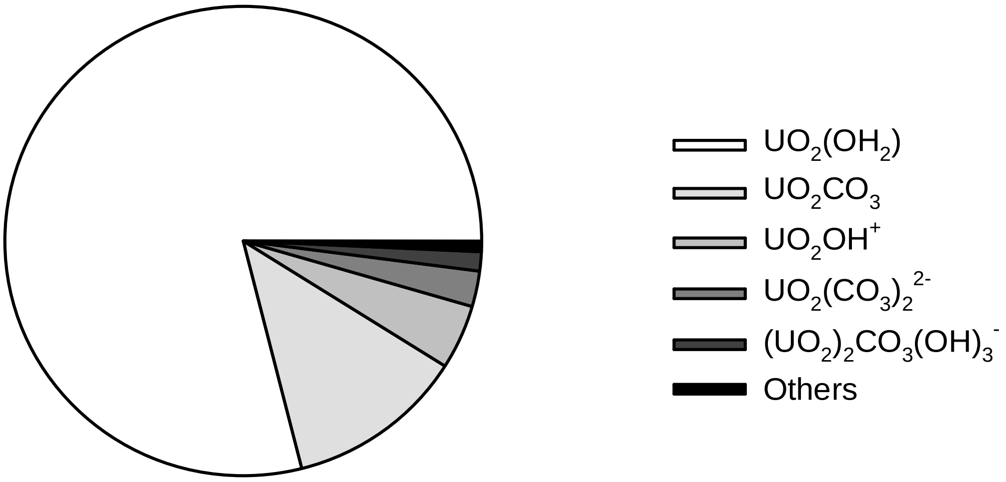
The speciation of U is clearly different depending on the database used (Figure 18). When using the Thermo.comv8.r6+ database, the dominant U species is the second hydrolysis product of uranyl, $UO_2(OH)_2$. When using the Thermo-MINTEQ database, the uranyl carbonate complex UO_2CO_3 dominates.

Simulating with organic matter

Addition of organic matter as EDTA did not significantly change the U speciation in the Ritord example, whereas addition as citrate shifted the speciation towards UO_2-citrate as the most abundant species (+80%) (Figure 19).

Simulating the effect of temperature

Shifting the temperature from 25 °C to 17 °C did not change the predicted order of abundance of the different U species and had only minor effects on the abundances (Figure 20).



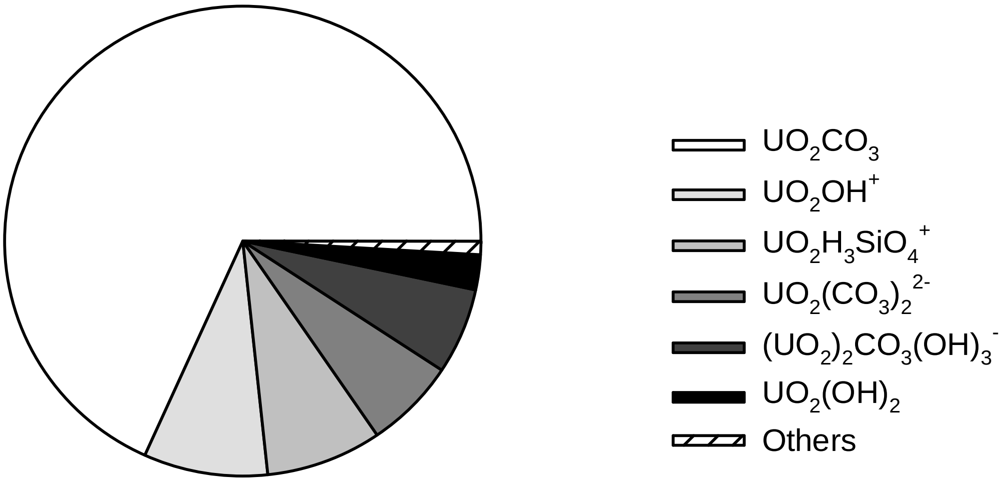


Figure 18. U speciation in the Ritord scenario, using with Thermo.com.v8.r6+.dat (top) or Thermo-MINTEQ (bottom) as the speciation database.

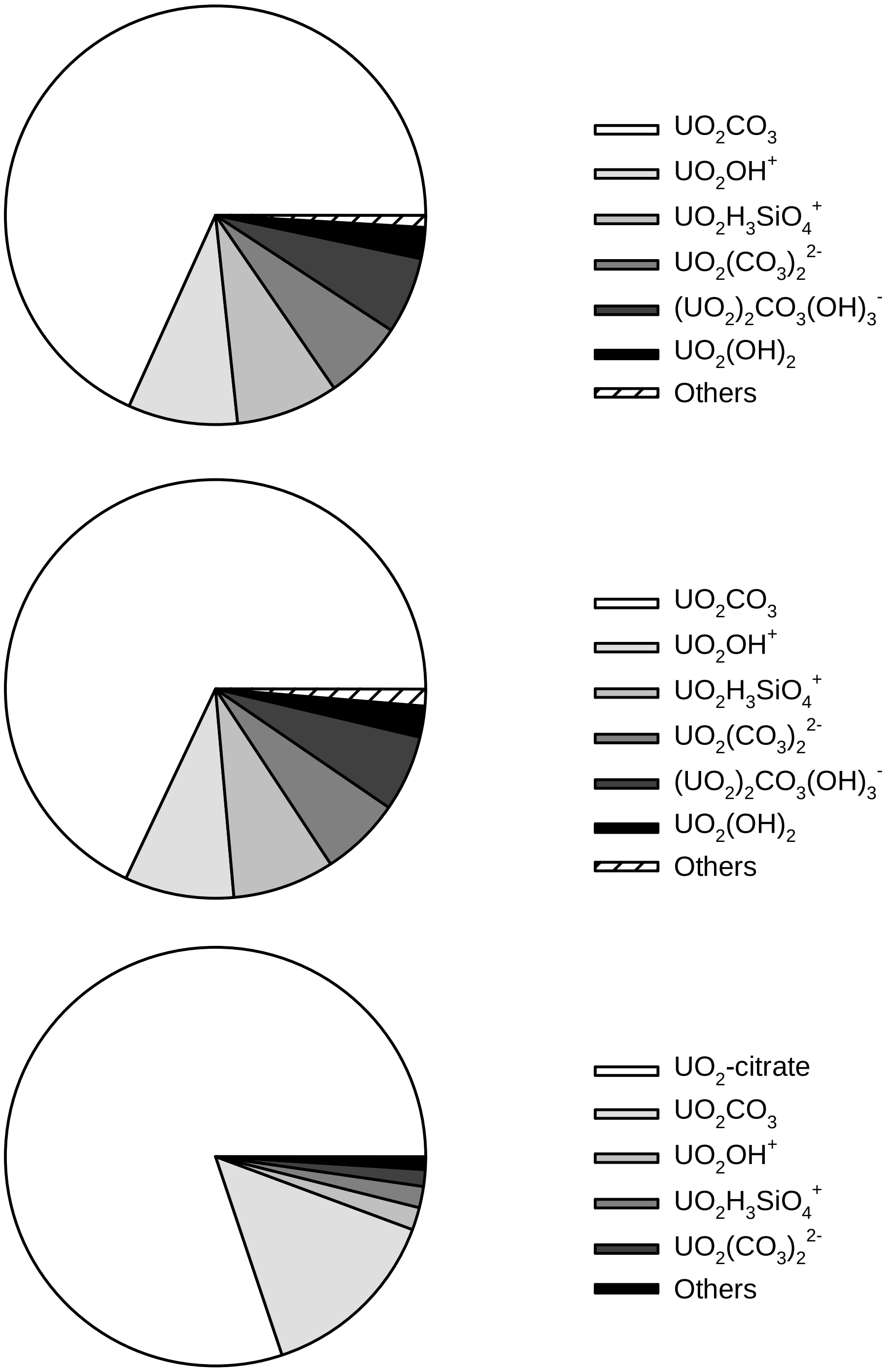


Figure 19. U speciation in the Ritord scenario run with the Thermo-MINTEQ database, without (top) or with EDTA (middle) or citrate (bottom) added as proxies for the measured total organic carbon of 9.57 mg L-1.

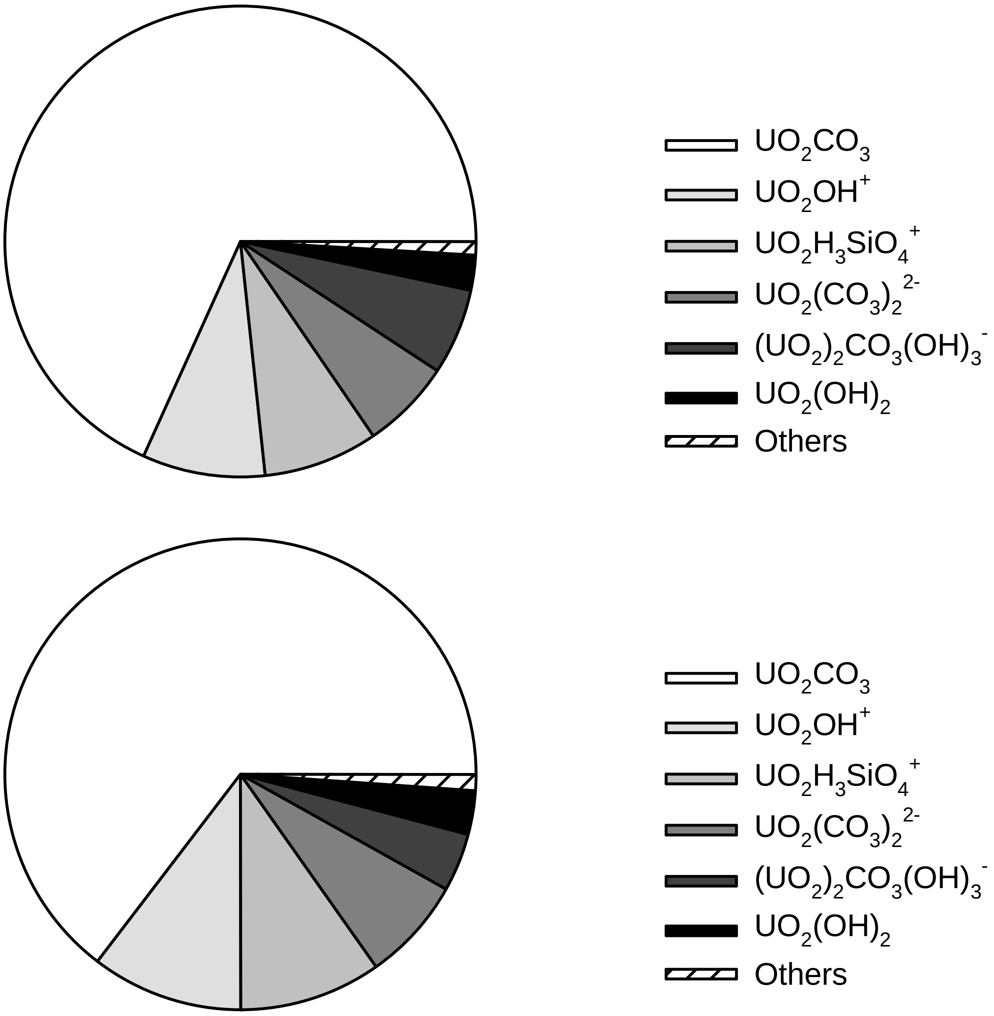


Figure 20. U speciation in the Ritord scenario, run with the Thermo-MINTEQ database at 25°C (A) or at 17°C (B).

5. CHESS

Modelling was done with CHESS version JCHESS 2.0 (based on CHESS 3.0).

Activity correction was done with the truncated-Davies equation.

Two databases were used in the evaluation:

- Chess.tdb: based on the Lawrence Livermore National Laboratory database EQ3/6.
- Ctdp_v3_Dong: updated database based on the merger of the irsn_lre database (Denison, 2002) and the LLNL database. Data for U were based on the latest publicly available OECD/NEA, with a few modifications concerning the hydrolysis constants of uranyl ions, compared to the NEA database, in particular on the formation of the polynuclear uranyl hydroxo complexes. Updated data of ternary complexation of uranyl, carbonate and alkaline earth metals (Dong and Brooks, 2006, 2008; Geipel et al., 2008) have been also included.

Simulation temperatures were set to 25^{o}C for the BeaverlodgeLakescenario and 17^{o}C for the Ritord scenario.

Redox reactions:

- In the Ritord scenario, redox was enabled for all compounds except for NO_2^- and NO_3^- where thermodynamic equilibrium was obviously not reached for these species. Therefore, these two species have been considered as two independent species.
- In the Beaverlodge Lake scenario, as data on O_2(aq) or E_H were absent, redox was disabled for all species.
- As it was not clear whether Fe should be considered as Fe^{2+} or Fe^{3+} different simulations were done to test the sensitivity of this input on the U speciation.

Precipitation: As data were given on filtered waters, all reactions leading to a precipitation and to a decrease of soluble concentrations have been excluded. Both systems were thus over-saturated for a lot of minerals. This was especially the case for minerals containing of Al, K and/or Si in the Ritord example and for precipitation of calcium and magnesium carbonates in the Beaverlodge Lake example.

Organic matter: In the CHESS database, the organic matter data relates to Aldrich Humic Acid (AHA). Therefore, DOM was considered to be 100% AHA and the concentration of AHA was considered as twice that of DOM (considering that AHA contains around 50 % C). Simulations without any organic matter were also done.

Input data:

- Note that Ctdp_v3_Dong does not contain any binding constants for organic matter. Therefore DOM was not considered during simulation with these databases.
- None of the databases contain Po. Thus this element was excluded from the calculations.

The carbonate system was simulated by inputting the carbonate concentrations givenin the scenarios, i.e. equilibrium with atmospheric CO_2 was not assumed.

5.1 Modelling results

5.1.1 Beaverlodge Lake scenario

Uranium speciation

The water has an alkaline pH, which leads to a predicted precipitation of calcite and dolomite in the media, or of calcite, dolomite and hematite if Fe is considered to be under its +II or +III oxidation state respectively.

In this condition, uranium speciation in the absence of co-contaminants and DOM is predicted to be dominated by the $UO_2(CO_3)_3^{4-}$ and $UO_2(CO_3)_2^{2-}$ complexes with the Chess database or by the ternary uranyl-calcium-carbonates complexes - $CaUO_2(CO_3)_3^{2-}$ and $Ca_2UO_2(CO_3)_3^{0}$ when using the ctvdpv3Dong database (Figure 21).

With the ctdpv3Dong database, uranium is predicted to be only in the form of ternary complexes of uranyl and carbonate with alkaline earth metals, either Ca or Mg (Figure 22 bottom) when precipitation reactions are disabled (since more carbonate is available for complexation with U).
The choice of oxidation state for Fe does not affect the predicted speciation, when either database is used.

Lead speciation

Lead speciation in absence of co-contaminants and DOM is dominated by the $PbCO_3^0$ complexes with the Chess database or by a mix of $PbOH^+$ and $PbCO_3^0$ complexes with the ctdpv3Dong database (Figure 23). Considering precipitation in the media (Figure 24) leads only to minor changes in the relative percentage of these two species. The choice of oxidation state for Fe does not affect the predicted speciation, when either database is used, regardless of whether precipitation is enabled or disabled.

Thorium speciation

Considering precipitation reactions with the Chess database has a major effect on the predicted speciation of thorium. In condition where precipitation is enable, thorium is predicted to be precipitated at 98 % under the form of thorianite (ThO_2) with the Chess database whereas it remains at 99.9 % under the form of a neutral hydroxo complex - $Th(OH)_4^0$ when precipitation is disabled. These results are the same regardless of the choice of oxidation state for iron.

The speciation of thorium is very sensitive to the choice of the database. With the ctdpv3Dong database, which includes thorium-carbonates complexes, thorium is predicted to be 99.7 % the $Th(OH)_3CO_3^-$ form whatever the iron oxidation state and this result is not affected by the consideration of precipitation reactions. Thorianite is included in the ctdpv3Dong database, but when using this database a greater degree of carbonate complex formation is predicted, increasing the solubility of thorium sufficiently that precipitation of thorianite is not predicted to occur.

Simulating the influence of co-contaminants

Considering co-contaminants in the media has no influence on either uranium, thorium or lead speciation, whatever the form of Fe considered (either Fe(+II) or Fe(+III)).

Simulating the influence of organic matter

Influence of organic matter was appreciated only with the chess database. But as this database contains no data with U and DOM, introducing organic matter does not change the predicted speciation of uranium. The speciation of lead and thorium remains also unchanged.

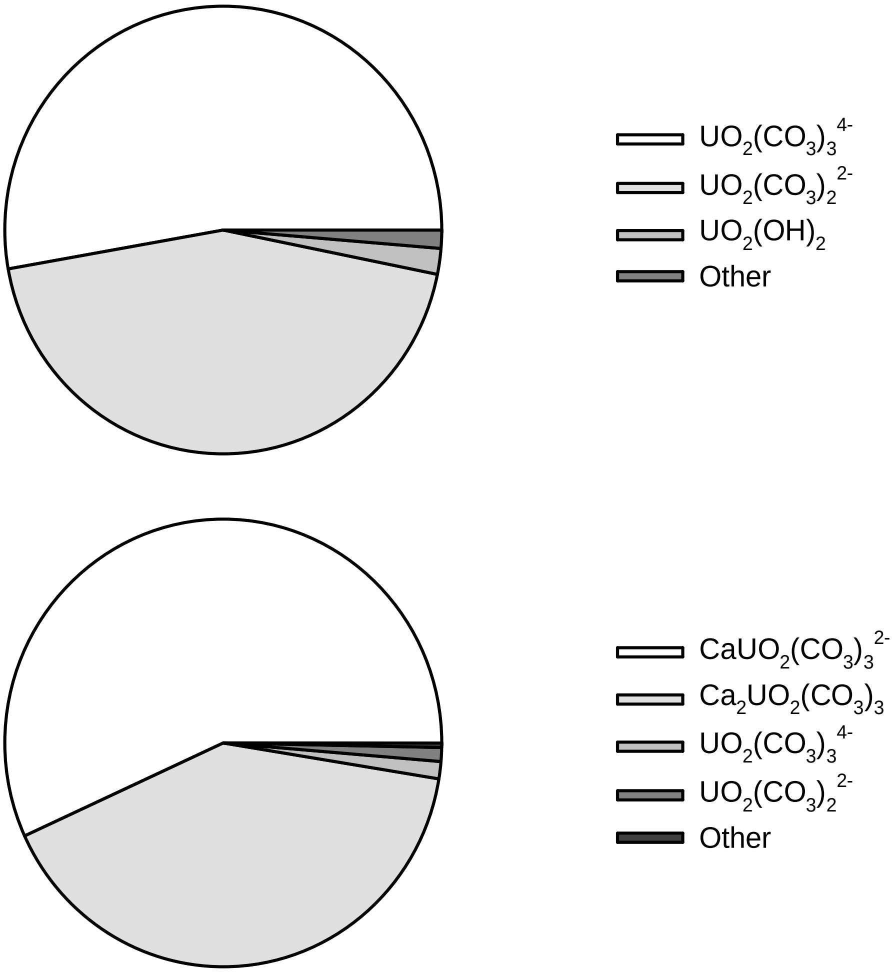


Figure 21. U speciation modeling in the Beaverlodge Lake scenario, using the Chess.tdb database (top) and the ctdp_v3Dong.tdb database (bottom). Co-contaminants and DOM are not considered, iron is simulated as iron(III) and precipitation/dissolution equilibria are enabled.

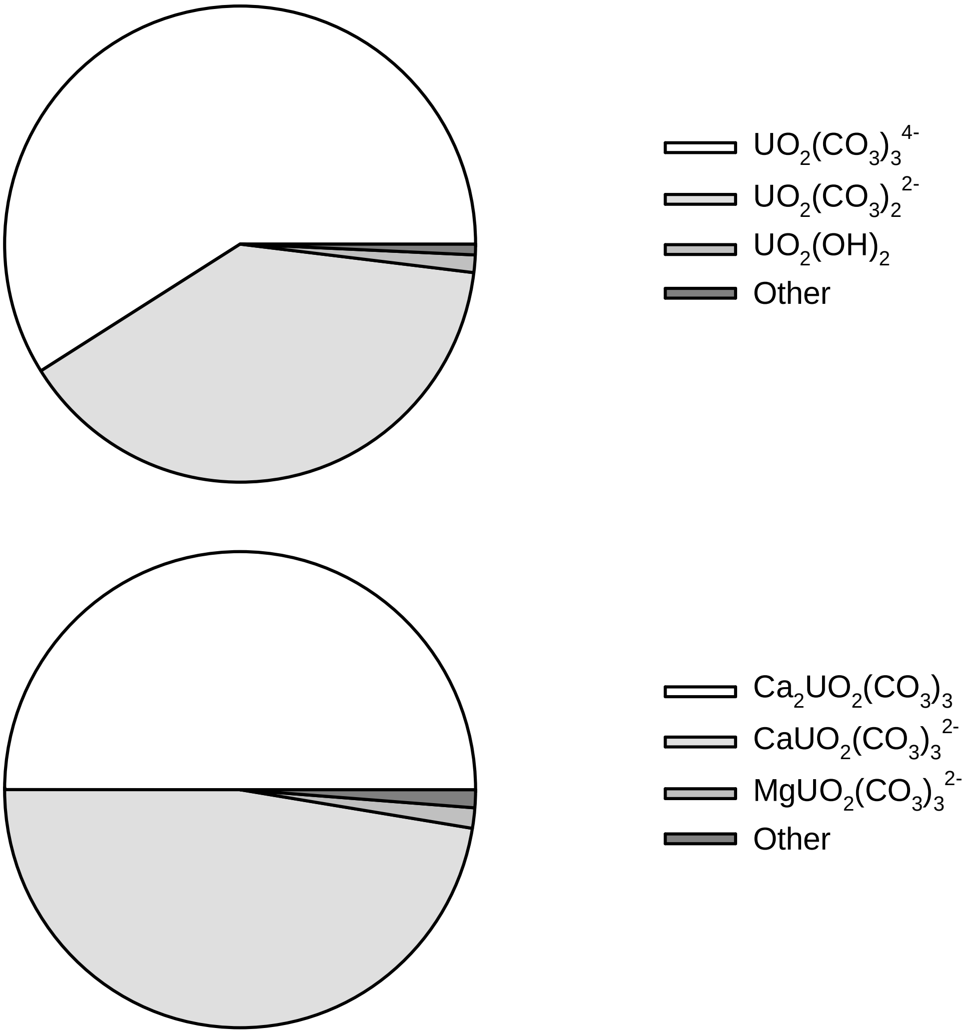


Figure 22. U speciation modeling in the Beaverlodge Lake scenario, using the Chess.tdb database (top) and the ctdp_v3Dong.tdb database (bottom). Co-contaminants and DOM are not considered, iron is simulated as iron(III) and precipitation/dissolution equilibria are disabled.

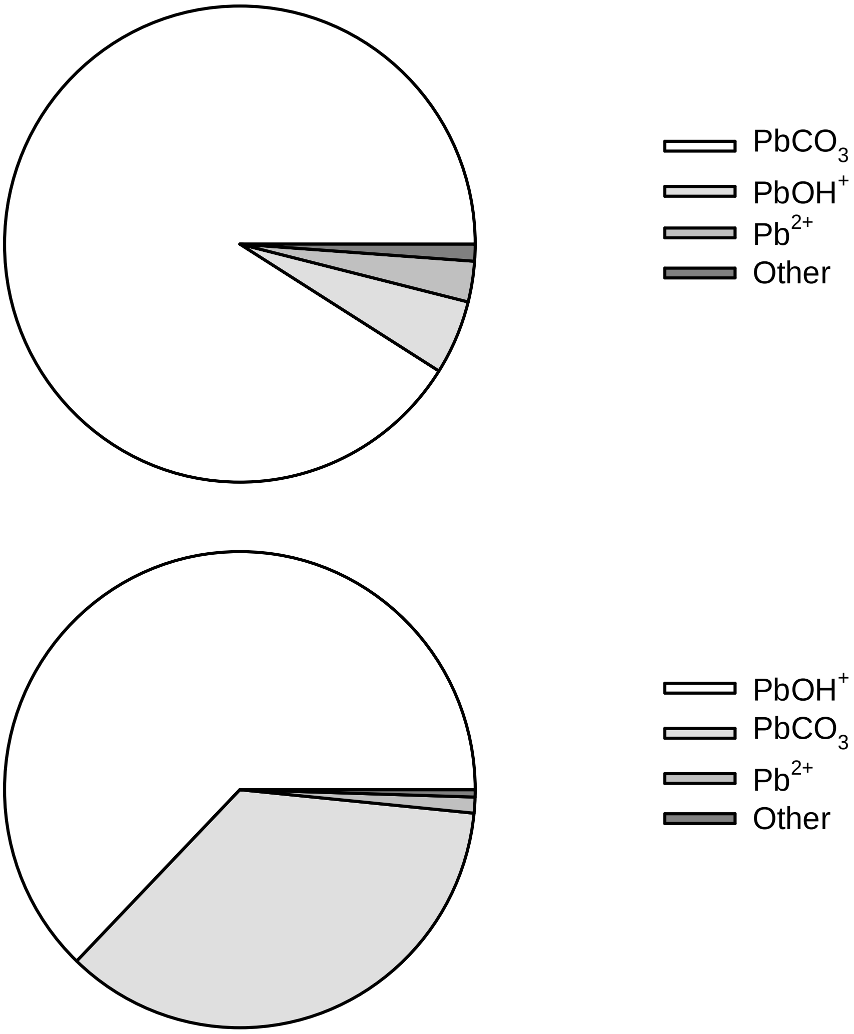


Figure 23. Pb speciation modeling in the Beaverlodge Lake scenario, using the Chess.tdb database (top) and the ctdp_v3Dong.tdb database (bottom). Co-contaminants and DOM are not considered, iron is simulated as iron(III) and precipitation/dissolution equilibria are enabled.

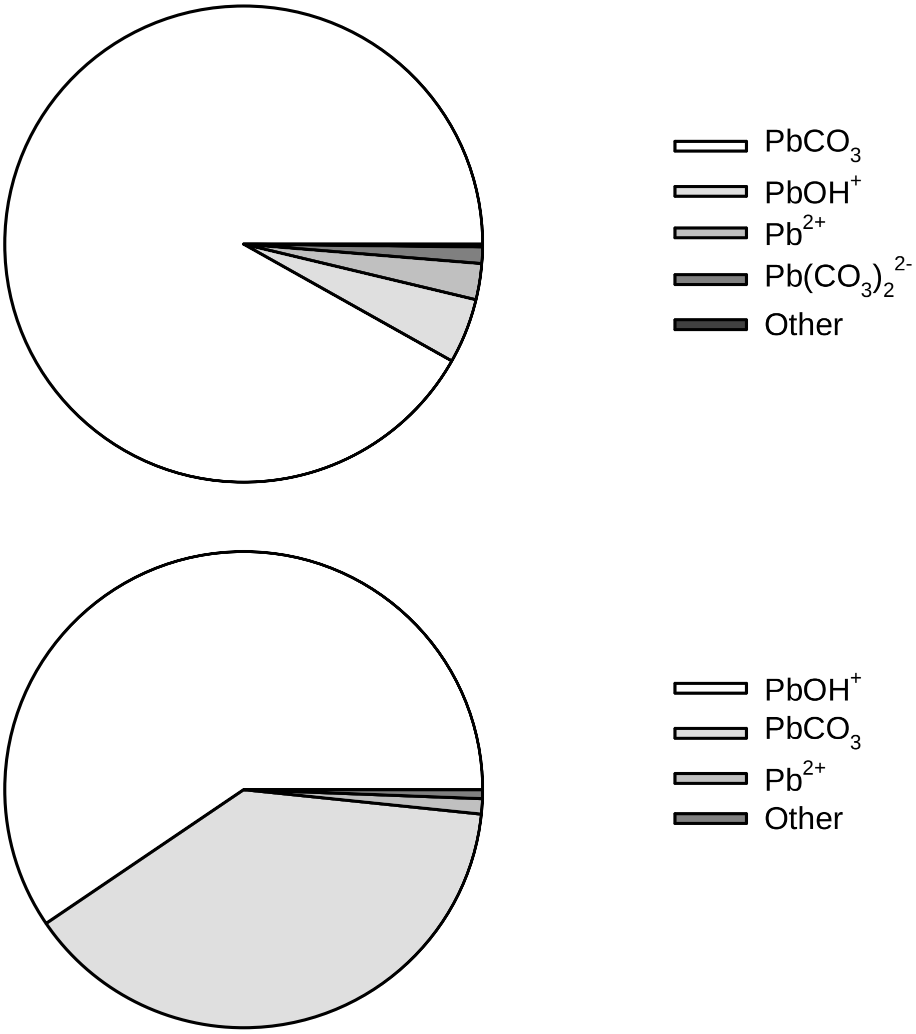


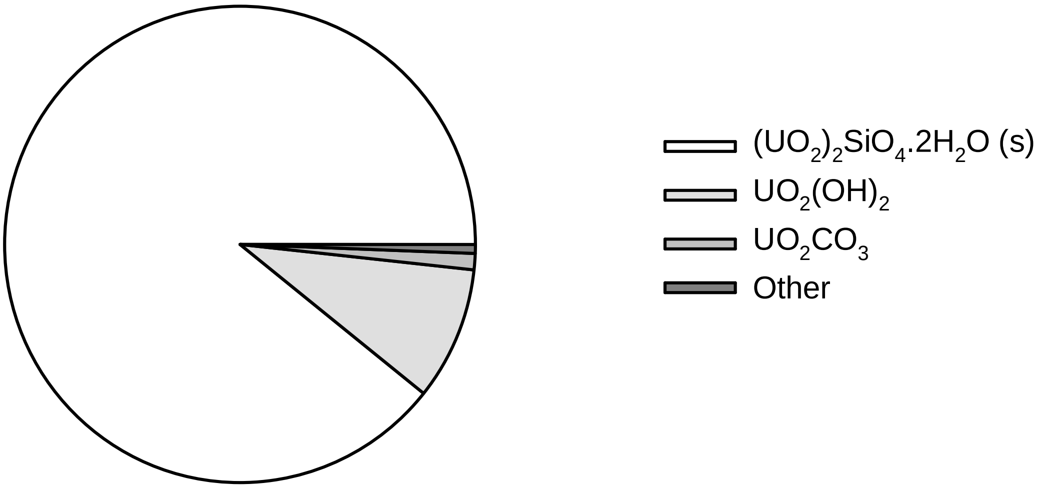
Figure 24. Pb speciation modeling in the Beaverlodge Lake scenario, using the Chess.tdb database (top) and the ctdp_v3Dong.tdb database (bottom). Co-contaminants and DOM are not considered, iron is simulated as iron(III) and precipitation/dissolution equilibria are disabled.

5.1.2 Ritord scenario

Uranium speciation

In absence of co-contaminants and DOM, the speciation of uranium predicted with the Chess database is strongly influenced by the integration of precipitation reactions. When precipitation is enabled, 89 % of the uranium is predicted to be in a precipitated form, as Soddyite,$(UO_2)_2SiO_4.2H_2O$ (Figure 25 top). When precipitation is disabled, U speciation is predicted to be dominated by the $UO_2(OH)_2^0$ complex (Figure 26 top).

The effect of the database is also highlighted in this scenario, since the uranium speciation predicted with the ctdpv3Dong database differs greatly from that predicted with the Chess database. With the ctdpv3Dong database, uranium remains in soluble form even where precipitation is possible (Figure 25 and Figure 26 bottom). The major complexes are the same as those of Figure 26 (top), but their relative contribution is notably different. The speciation is dominated by a mix of carbonates, hydroxo-carbonates and hydroxo complexes – mainly $UO_2CO_3^0$ and $(UO_2)_2CO_3(OH)_3^-$,and to a minor extent $UO_2(OH)_2^0$.



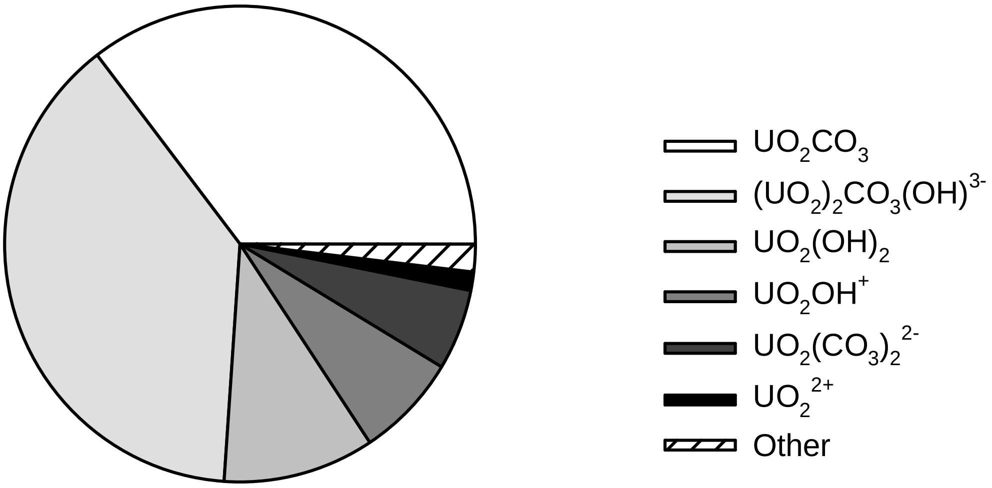


Figure 25. U speciation modeling in the Ritord scenario, using the Chess.tdb database (top) and the ctdp_v3.tdb database (bottom). Co-contaminants and DOM are not considered and precipitation/dissolution equilibria are enabled.

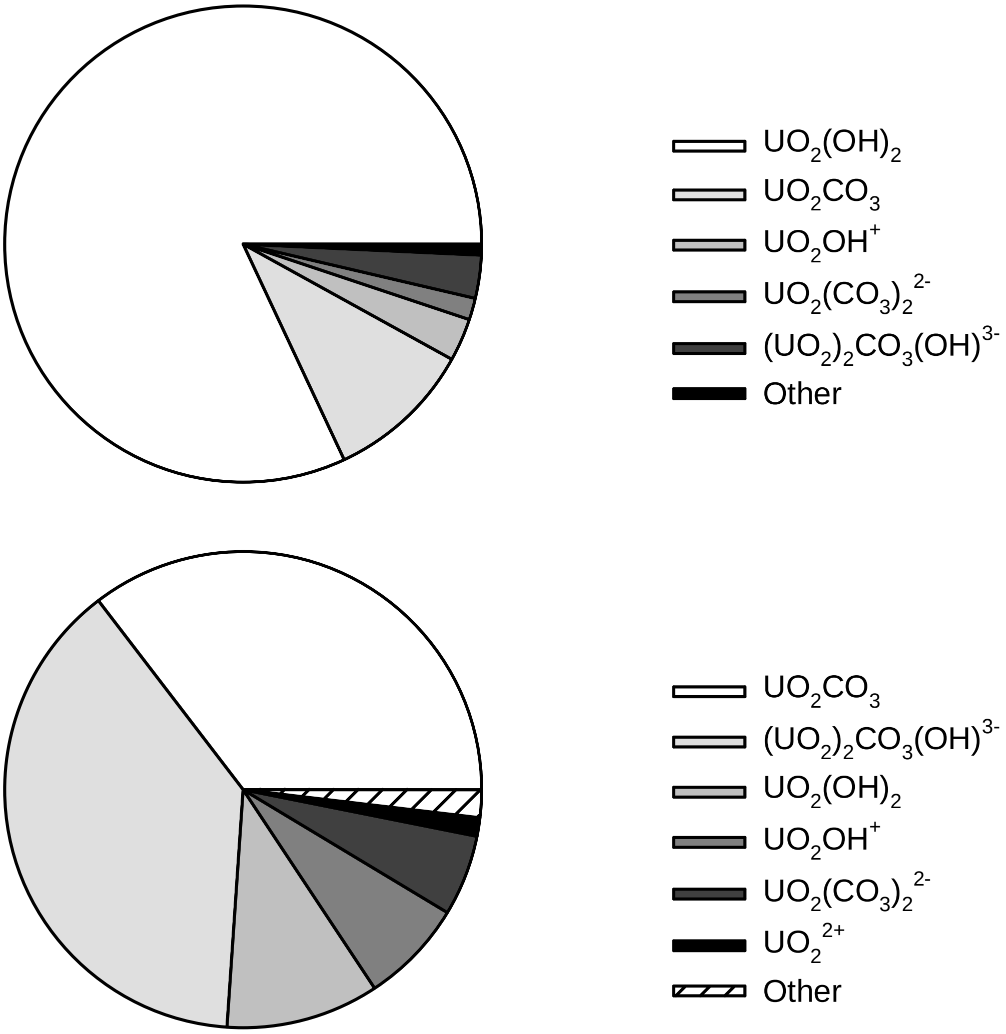


Figure 26. U speciation modeling in the Ritord scenario, using the Chess.tdb database (top) and the ctdp_v3.tdb database (bottom). Co-contaminants and DOM are not considered and precipitation/dissolution equilibria are disabled.

Simulating the influence of co-contaminants

Introducing co-contaminant in the scenarios does not change the predicted speciation of uranium compared to Figure 25 and Figure 26 with the Chess or ctdpv3Dong databases respectively (results not shown).The choice of oxidation state for Fe (Fe(II) or Fe(III)) does not affect the simulation significantly.

Simulating the influence of organic matter

As for co-contaminants, introducing organic matter in the simulation with the chess database does not change the predicted speciation of uranium compared to Figure 25 and Figure 26 (top).

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Annex 3: How is mixture risk assessment handled in a regulatory context

This chapter will focus on legislation, guidance, responsibilities and – to a certain extent – application of different multi-contaminant assessment methods in various countries/regions of the world, concentrating on the USA and the EU. Guidance from various international bodies such as the WHO and OECD will also be considered briefly. The main part of the chapter (i.e. sections 1-3) will deal with mixtures of non-radioactive substances (i.e. pesticides, metals, PCBs, PAHs etc). These sections are largely based on the "State of the art report on mixture toxicity" by Kortenkamp et al. (2009) and the book "Mixture toxicity" edited by van Gestel et al. (2011) – particularly the part by Ragas et al. (i.e. Chapter 5). In light of the perspective that two or more radioactive substances, together, may also be viewed as a "mixture of contaminants", guidance from the ICRP and IAEA is also provided (section 4.4). Due to its topical relevance, key findings from the EURATOM-EU financed project PROTECT (i.e. 'Protection of the environment from ionising radiation in a regulatory context') is also included here.

1. International bodies

1.1 World Health Organisation (WHO)

Through the International Programme on Chemical Safety (IPCS), WHO works to establish the scientific basis for the sound management of chemicals, and to strengthen national capabilities and capacities for chemical safety. IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the WHO. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

The IPCS has, during the last two decades, established toxic equivalency factors (TEFs) for dioxins, furans, and dioxin-like PCBs. WHO-TEF values have been established for humans and mammals, birds and fish. These Internationally accepted TEFs have been used for the risk management in various UNMember States and have been adopted formally by a number of countries and supranational bodies, including, amongst others, Canada, Japan, the United States and the European Union. TEF values are re-evaluated on a regular basis, preferably at five-year intervals (Kortenkamp et al., 2009).

In 2007, IPCS convened an international workshop to discuss methods for assessing the combined risk from exposure to one or more agents via all relevant routes and pathways (IPCS, 2009). In connection with this meeting the development of a framework for such assessments was initiated, and recently the result of this work has been published (Meek et al., 2011). The WHO/IPCS framework for risk assessment of combined exposure to multiple chemicals is "designed to aid risk assessors in identifying priorities for risk management for a wide range of applications where co-exposures to multiple chemicals are expected. It is based on a hierarchical (phased) approach that involves integrated and iterative consideration of exposure and hazard at all phases, with each tier being more refined (i.e. less cautious and more certain) than the previous one, but more labour and data intensive. It includes reference to predictive and probabilistic methodology in various tiers in addition to tiered consideration of uncertainty. The paper also annexes two case studies that have been developed to test and refine the framework". One annex is based on an existing screening assessment for polybrominated diphenyl ethers (PBDEs); the second is a fictionalized example, for a range of substances that co-occur in drinking water (Meek et al., 2011).

1.2 Organisation for Economic Cooperation and Development (OECD)

OECD Member countries and the OECD Secretariat cooperate to develop and co-ordinate chemical and pesticide related activities on an international basis. The main objectives of the OECD Chemicals Programme are to assist Member countries' efforts to protect human health and the environment through improving chemical safety, to make chemical control policies more transparent and efficient and save resources for government and industry, and to prevent unnecessary distortions in the trade of chemicals and chemical products (Kortenkamp et al., 2009).

In 2004, the UN sub-committee of experts on the Globally Harmonised Systems (GHS) mandated OECD to work on classification criteria for toxic gas mixtures. For this purpose, the OECD proposed a formula equivalent to concentration addition (CA) to determine the concentration at which a mixture of hazardous gases would be classified in a GHS category (OECD, 2005).

OECD (2007) produced guidance on limiting the number of toxicological tests to be carried out by grouping chemicals into closely related categories. In this so-called category approach, not every chemical has to be tested. In principle, the category approach can also be used to define groups of chemicals to be subjected to mixtures risk assessment. An example is the Toxicity Equivalent approach (TEQ) that is used for polychlorinated dioxins and furans (PCDD/F). Originally designed to estimate the toxicity of untested congeners, TEQ has matured into a framework for assessing mixtures of PCDD/F.

2. United States of America (USA)

The USA has several laws that authorise regulatory agencies to address risks of chemical mixtures. Important examples being the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA); the Food Quality Protection Act (FQPA); the Safe Drinking Water Act Amendments; and the Occupational Safety and Health Act (OSHA) (Ragas et al., 2011). The practice of Cumulative Risk Assessment[6] (CRA) is also furthest developed in the USA compared to other countries (Kortenkamp and Hass, 2009), and guidance is provided by different agencies and for different risk assessment areas. The most extensive guidance for human health assessments has been developed by the US Environmental Protection Agency (USEPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) (Ragas et al., 2011). More limited regulatory rules of mixtures exist for ecological risk assessment. In general, legislation provides the opportunity to account for mixture effects, but explicit guidance is often lacking. However, the USA has extensive guidelines on ecological risk assessments of the overall toxicity of effluents, receiving waters, sediments, and terrestrial sites (Kortenkamp et al., 2009).

2.1 The US Environmental Protection Agency (USEPA)

The USEPA has developed a very elaborate framework of documents for dealing with cumulative risk assessment (CRA) for chemical mixtures. Various areas are summed up in the data sheets shown in the following tables (all mainly based on information from Kortenkamp et al. (2009); Ragas et al. (2011)):

Table1: Risk Assessment Guidance for Superfund Waste Sites (US EPA, 1989)

Protection aim	Humans
Short description	Superfund sites are polluted locations requiring a long-term response to clean-up hazardous material contaminations. These sites are designated under CERCLA, which authorises USEPA to identify parties responsible for contamination of sites and compel

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[6] Risk assessment approaches that consider the impact of multiple chemical exposures, from multiple sources, routes and pathways, over multiple time frames (Kortenkamp and Haas, 2009)

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	the parties to clean up the sites.
Chemicals	Specified on the USEPA target component and target analyte list (TCP/TALs): Volatile chemicals (52), pesticides and aroclors (30), metals (23), cyanide, and semi-volatile chemicals (67) (numbers of chemicals per May 2008).
Exposure pathways and routes	All relevant
Approach	Component based
Basic concepts	Independent action for carcinogenic substances independent Action for non-cancer end-points
Specific methodologies	For each non-carcinogenic chemical a hazard quotient (HQ) is calculated – summation is over all exposure pathways and time frames. An overall summary hazard index (HI) is calculated for the mixture based on the HQs.
End-points considered	Cancer and non-cancer
Interactions (Synergy/antagonism)	Not considered

Table 2. Pesticides Cumulative Risk Assessment(US EPA, 1999; 2002a; 2002b; 2006a; 2006b; 2007a)

Protection aim	Humans
Short description	Health risk from a combination of pesticides with a common mode of action (MoA) as described in the Food Quality Protection Act (FQPA).
Chemicals	• Organophosphates • Carbamates • Triazines • Choloroacetanilides
Exposure pathways and routes	All (including simultaneous exposures from food, drinking water, residential use). Oral, dermal, inhalation
Approach	Component based. Identifying common mechanism groups (CMGs) and from that a relevant sub-set termed cumulative assessment group (CAG) subject to quantitative analyses.
Basic concepts	Concentration addition (CA) is normally used for estimation of combined risks for the CAG
Specific methodologies	Index chemical representing the CAG is selected. Used as a reference point for other CAG membersin terms of relative potency factors (RPF) (i.e. similar to dioxin-like chemicals)
End-points considered	
Interactions (Synergy/antagonism)	Not considered
Comments	Only relevant for the common MoA pesticides

Table 3. Safe Drinking Water Act amendments (Teuschler *et al.*, 2004)

Protection aim	Humans
Short description	1996 amendments to Safe Drinking water Act required consideration of chemical mixtures in drinking water.
Chemicals	Disinfection by-products in particular
Exposure pathways and routes	Intake?
Approach	Component based
Basic concepts	Concentration addition (CA)
Specific methodologies	Relative potency factors (RPF)
End-points considered	
Interactions (Synergy/antagonism)	Not considered

Table 4. National Air Toxics Assessment (NATA) (US EPA, 2006c; 2007b)

Protection aim	Humans
Short description	Estimate health effects of air pollutants across the entire USA. Non-chemical stressors were not taken into account
Chemicals	177 (in 2006)
Exposure pathways and routes	Inhalation
Approach	Component based
Basic concepts	Independent action (IA) for carcinogenic substances (inhalation exposure) Concentration addition (CA) for respiratory irritants
Specific methodologies	For each non-carcinogenic chemical a hazard quotient (HQ) is calculated. An overall "respiratory hazard index "(HI) is calculated for the mixture based on the HQs.
End-points considered	Cancer (inhalation exposures) and non-cancer (respiratory irritation)
Interactions (Synergy/antagonism)	Not considered
Comments	Dispersion models were used to estimate concentrations in ambient air (input values). Follows the precedents in the Superfund site assessments

2.2 The Agency for Toxic Substances and Disease Registry (ATSDR)

According to Kortenkamp *et al.*(2009), the ATSDR is – like the USEPA – engaged in Superfund sites, but the agency does not perform site specific assessments. The ATSDR instead assesses whether adequate information on health effects is available for the priority hazardous substances. To fulfil legislative mandates under CERCLA and FQPA, ATSDR's Division of Toxicology has developed a chemical mixtures program. As part of this program, a mixture guidance manual and a series of interaction profiles have been devised by the agency:

Table 5. Mixture guidance manual (ATSDR, 2004)

Protection aim	Humans
Short description	Determine whether exposure to chemical mixtures at hazardous waste sites may impact public health. Basis of development for interaction profiles (see next template)
Chemicals	E.g. coke oven emissions, groundwater contaminant cocktail
Exposure pathways and routes	
Approach	Preferably whole mixture approach, otherwise "similar" mixture. If these are not available component based approaches are adopted
Basic concepts	
Specific methodologies	Component based approach – HI fully compatible with US EPA approaches Two departures: Target organ toxicity dose (TTD) and Weight of evidence (WOE) modifications of HI
End-points considered	"Health effects"
Interactions (Synergy/antagonism)	Addressed: WOE modification to HI. Using additional uncertainty factors to accommodate the possibility of deviation from expected additivity.
Comments	

Table 6. Interaction profiles

Protection aim	Humans
Short description	Evaluate data on the toxicology of "whole" priority mixtures
Profiles (final)	• Arsenic, Cadmium, Chromium, Lead • Benzene, Toluene, Ethylbenzene, Xylenes • Lead, Manganese, Zinc, Copper • Persistent chemicals found in breast milk • Persistent chemicals found in fish • 1,1,1-TCE, 1,1-DCE, TCE, PERC • Cesium, Cobalt, Polychlorinated Biphenyls, Strontium, and Trichloroethylene • Arsenic, Hydrazines, Jet Fuels, Strontium-90, and Trichloroethylene • Cyanide, Fluoride, Nitrate, and Uranium • Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine • Chlorpyrifos, Lead, Mercury, and Methylmercury
Exposure pathways and routes	
Approach	
Basic concepts	
Specific methodologies	
End-points considered	"Health effects"
Interactions (Synergy/antagonism)	
Reference	http://www.atsdr.cdc.gov/interactionprofiles/index.asp
Comments	Note that some of the profiles include radioactive substances

3. European Union (EU)

Like the USA, the EU has legislation that enables regulatory agencies to address mixture risk. Examples being – according to Ragas et al. (2011):

- Regulation (EC) 1488/94 on risk assessment of existing substances;
- Directive 98/8/EC on the placing of biocidal products on the market;
- Directive 89/39/EEC on safety and health of workers at work and
- Regulation 315/93/EEC on contaminants in food.

Unlike the USA, explicit guidance on the assessment and regulation of chemical mixtures in human risk assessment is often lacking. The regulatory rules of dealing with mixture in ecological risk assessment are even more limited in number.

3.1 Current provisions for taking into account hazards and risks arising from mixture toxicity in EU legislation

Kortenkamp et al. (2009) analysed 21 pieces of EU legislation with respect to their scope in dealing with multiple chemicals, and found that four out of these appeared to be particularly noteworthy from a mixture toxicity perspective. Condensed information from that review is provided in the following:

- **Regulation (EC) No 1907/2006** concerning registration, evaluation, authorisation and restriction of chemicals (REACH). Covers the obligations of a manufacturer/importer of a substance (on its own and in a mixture) with respect to a chemical safety assessment (CSA) before it is placed on the market. The main purpose of the CSA is to determine the intrinsic hazard of a compound or mixture by estimating Derived No-Effect Levels (DNEL) for human health and Predicted No-Effect Concentrations (PNEC) for environmental assessments and to assess substance properties relating to persistence, bioaccumulation and toxicity (PBT/vPvB) properties. This information is then used to derive hazard threshold levels for human health and the environment. Three categories of chemicals are considered: (1) Preparations/isomeric mixtures (e.g. paints); (2) multi-constituent substances (MCS); and (3) substances of unknown or variable composition (UVCB), such as petroleum products. For preparations registration applies to individual chemicals in the mixture, whereas MCS/UVBC are generally treated as single substances. Protection aim: Humans and the environment.
- **Regulation (EC) No 1272/2008** on classification, labelling and packaging of substances and mixtures (CLP). Makes detailed prescriptions for the toxicity assessment of intentionally prepared commercial mixtures. The approaches prescribed are (1) Whole mixture testing, (2) concentration addition, or (3) the summation method, which is the toxicity weighted summation of the relevant mixture components and the subsequent analysis whether or not the relative amount of relevant components is above or below a pre-defined threshold. CLP largely follows the Globally Harmonised System (GHS) for the classification and labelling of Chemicals as developed by the UN. The regulation applies to all chemical and mixtures that are put on the European market – with certain exceptions, including radioactive substances. The aim of protection is human health and the aquatic environment.
- **Regulation (EC) No 396/2005** on maximum residue levels (MRLs) of pesticides in or on food and feed of plant or animal origin. Provides incentives for the development of methodologies for mixture risk assessments. The task of developing viable assessment methods has been assigned to EFSA (European food safety authority). EFSA has, since 2006, been working on a methodology for assessing cumulative risks that may result from human exposure to combinations of pesticide residues, based upon work previously carried out in the USA. Aim of protection is human health?
- **Directive 2008/1/EC** (EC, 2008) concerning integrated pollution prevention and control (IPPC). The purpose is to prevent or reduce emissions to the air, water and land to achieve a high level of protection of the environment taken as a whole. Application of the best available

technology/techniques (BAT) is a governing principle for all measures taken against pollution under the IPPC Directive. It refers to the directive on waste incineration as a complementary piece of legislation and this in turn includes emission limit values for mixtures of dioxins and furans that are based on the toxicological concept of Toxic Equivalence Factors (TEF).

For more detailed information about the four individual Regulations/Directives described above (and the 17 other) Kortenkamp et al. (2009) should be consulted.

3.2 Cumulative risk assessment (CRA) in EU – present and future

As mentioned above, EU legislation generally provides the opportunity to account for mixture effects, but explicit guidance is often lacking. Most of the Directives and Regulations examined by Kortenkamp *et al.* (2009) are substance or product-oriented. Typically, hazards and risks of these substances and products are treated as if they were present in isolation.

With the exception of the recent changes in European pesticides regulations, where mixture risk assessment is mandated, comparative legal frameworks that clearly address cumulative risk assessment (CRA) do currently not exist in Europe. In REACH, for example, CRA for multiple chemicals from multiple sources, routes and pathways is only addressed to a very limited extent in the current guidance. Other relevant European legislation does not contain a mandate for CRA for multiple chemicals from multiple sources, routes and pathways (Kortenkamp and Hass, 2009).

Process-oriented pieces of environmental legislation that control emissions from production, transportation, and recycle processes, such as the IPPC (Directive 2008/1/EC, EC, 2008), provide a basis for assessing mixtures of chemicals released from a definite source. However, media-, site-, or population-oriented elements of legislation, such as the Water Framework Directive (2000/60/EC, EC, 2000), the Marine Strategy Directive (2008/56/EC), or the proposed Soil Directive, should form a better starting-point for assessing mixtures occurring in soil, water, biota, and humans in the future (Kortenkamp et al., 2009).

Transfer of scientific knowledge into appropriate regulatory approaches is not a trivial task. For instance, the USEPA spent many years on the development of its guidelines for the health risk assessment of chemical mixtures. Without the legal mandates laid down in the US American CERCLA and FQPA, cumulative risk assessment would not have been implemented in the USA (Kortenkamp and Hass, 2009). Thus consistent and clear mandates are needed in Europe for taking mixture toxicity into account in the numerous pieces of legislation that contribute to the protection of human health and the environment from chemical risk. This seems to be an essential prerequisite for better dealing with the challenging issue of potential mixture toxicity effects (Kortenkamp et al., 2009).

3.3 Approaches and practical experiences in assessing the mixture toxicity of complex environmental samples and waste samples in EU member states

The limited amount of EU guidance presently available regarding mixture risk assessment does not imply that the issue of mixture toxicity is not addressed by individual member states/agencies. Many environmental authorities and collaborating research institution in EU member states have extensive experience with whole-mixture testing approaches (WMA), and various types of component based approaches (CBA) are also generally applied (as shown in Table 7).

Table 7. Approaches and methods for mixture toxicity used in various EU member states. Selected results from a questionnaire study with participants from 25 authorities / collaborating research institutions in 14 countries (see Kortenkamp et al., 2009). WMA = whole-mixture approaches; CA = Concentration Addition (TUS = Toxic unit summation; TEF = Toxic equivalence factor; RPF = Relative Potency Factor; PODI = Point of Departure Index; HI = Hazard Index); IA = Independent Action; CBA = Component based approaches

Country **Method**	**BE**	**DK**	**EE**	**ES**	**FI**	**FR**	**HU**	**IE**	**IT**	**NL**	**SE**	**SI**	**SK**	**UK**
Various WMA[1]	x	x	x	x	x	x	x	x	x	x		x	x	x
CA Dir[2]		x					x			x				
CA TUS		x	x	x			x			x				
CA TEF		x		x		x				x				
CA RPF		x					x			x				
CA PODI							x							
CA HI				x			x							
IA Dir[2]		x		x						x				
IA Other[3]										x				
CBA Other[4]				x						x				

[1] Biotests: sub-cellular assays (enzyme, immuno, receptor etc.), cell culture, bacteria, algae, other plants, protozoa, daphnids, other invertebrates, fish, other vertebrates, multi-species assays, others

[2] Direct application of the CA or IA formula

[3] Other IA based approaches

[4] Any other CBA based approaches

WMA are mainly used for toxicity assessments of waste water, and waste water treatment plant effluents for the control of emission permits under IPPC (Directive 2008/1/EC, EC, 2008). Such approaches are also applied to practically all other types of environmental samples for the purpose of general environmental monitoring, risk assessment of contaminated sites, priority setting for risk reduction measures, and the control of remedial work (Kortenkamp et al., 2009). Whole-mixture approaches will not be considered further here.

Regarding CBA, most member states included in Table 7use one or more approaches of concentration addition (CA), whereas only a few apply independent action (IA) (i.e. Denmark, Spain and the Netherlands) or mixed models – combining CA and IA (i.e. Spain and the Netherlands). CA is used for substances with an assumed similar mechanism/mode of action (MOA), such as dioxins, furanes, dioxine like PCBs, substances with estrogenic activity, PAHs, phenols, some metals, pharmaceuticals, and pesticides (Kortenkamp et al., 2009).

More details regarding use of CBA will be provided in the next section, using the Netherlands as an example. The reason for this selection is that the Dutch information shown in Table 7 was – in contrast to the other member states – based on an integrated reply, involving various research institutes and policy makers[7].

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[7] Directorate-General of Public Work and Water management / Rijkswaterstaat, Centre of Water Management; Ministry of Housing, Spatial planning and the Environment / National Institute of Public health and the Environment.

3.4 Example – How are multi-contaminants treated in the Netherlands?

There are no legal requirements in the Netherlands to perform complete mixture toxicity tests and no plans to introduce such legal requirements. Nevertheless, there is extensive experience in dealing with mixtures. Generally this work has been people-driven – focused on gaining scientific insights; or considered from a precautionary principle perspective (Kortenkamp et al., 2009). The use of component based approaches in the Netherlands is summed up in Table 8.

Table 8: Application of various component based approaches (CBA) used in the Netherlands (Kortenkamp et al., 2009). CA = Concentration Addition (TUS = Toxic unit summation; TEF = Toxic equivalence factor; RPF = Relative Potency Factor; PODI = Point of Departure Index; HI = Hazard Index); IA = Independent Action; msPAF = multi-substance probably affected fraction.

Method	**Details**	**Chemicals**	**Samples**	**Toxicological End points assessed**
CA Dir[1]		Metals	Surface waters	Acute or chronic toxicity
CA TUS	Based on NOEC or EC_{50}	Pesticides, metals, PCBs, PAHs	Surface water, sediment, waste waters	Acute or chronic toxicity: algae, daphnids, bacteria
CA TEF	WHO values	Dioxins, PCBs	Biota, sediment	Dioxin-like activity
CA RPF	USEPA RPFs derived by RIVM	Dioxins, estrogens, pesticides	Surface water, waste water, sediment	Dioxin-like activity, estrogenic activity etc
IA Dir[1]	Model OMEGA; msPAF[4]	Metals, PCBs, PAHs, OCBs, pesticides	Surface water, biota, sediment	Effect on ecosystem level or on species level
IA Other[2]	msPAF[4]	Metals, PAHs	Soil	Effects on species level
CBA Other[3]	msPAF[4]	Metals, PCBs, PAHs, OBCs etc.	Surface water, soil, sediment	Effect on ecosystem or species level

[1] Direct application of the CA or IA formula
[2] Other IA based approaches
[3] Any other CBA based approaches
[4] Mixed model approach

The multi-substance probably affected fraction (msPAF) approach has recently been adopted in the Netherlands for retrospective assessments of the possible impact of mixtures in sediments (Ragas et al., 2011).

4. Regulations for radioactive substances

As noted in the introductory part of the chapter, radioactive substances may be considered as mixtures of contaminants. In this respect it is of some relevance to touch briefly upon the way in which mixtures of radionuclides are regulated.

Most countries use the recommendations of the International Commission on Radiological Protection (ICRP, 2007) and the various safety standards, safety and technical reports of the International Atomic Energy Agency (e.g. IAEA, 1996) to guide the development and formulation of national regulations. In Europe, this is elaborated at a regional level by a European Basic Safety Standard (EC, 1996). Traditionally the focus in most countries has been on the protection of humans. The ICRP (2007) expressed this as to manage and control exposures (of people) to ionising radiation so that deterministic effects are prevented, and the risks of stochastic effects are reduced to the extent reasonably achievable. This is achieved through three fundamental principles of radiological protection as applied to planned, emergency, and existing exposure situations: Justification, Optimisation of protection, and application of dose limits (ICRP, 2007).

It was considered too onerous a task to consider and provide details on how various countries translate the various high level guidance considered above into the regulation of radioactive substances at a national level. Suffice to say that national regulations are highly developed in most countries and relate to all foreseeable human exposure situations including restrictions and remediation in the aftermath of nuclear accidents, the transport and operation of radioactive sources, the exploitation and use of naturally occurring radioactive material (NORM) and exposures to radon gas – the list is extensive. In many cases the situation becomes convoluted by the observation that numerous regulatory instruments, pertaining to different aspects of radiation exposure and risk, may come into play for any given situation. A case in point can be provided for a case in Norway involving the assessment of risk and consideration of remediation options at an abandoned mining site. Even for this isolated and highly specific case, NGI (2009) identified a long list of pertinent acts and regulations (seven in total) that were considered to warrant attention at various stages in the risk assessment.

The science base upon which radioactive regulations and concomitant (normally human) risk assessment requirements are constructed are also well elaborated. Many years of development and effort have been expended upon scientific disciplines that underpin the various reference levels, limits and constraints used in radiological protection regulation. This includes investigating the behaviour and fate of radioisotopes within the human body (biokinetics), the physical interaction of radiation with matter resulting in an absorbed dose (dosimetry), the relative sensitivity of various organs in the human body to irradiation and the long term study of stochastic effects in large populations with time using the tools of epidemiology.

In the sense of looking at the combined action of radioactivity with other contaminants/stressors the EU-EURATOM funded project PROTECT (Howard et al., 2010) did not consider mixture toxicity explicitly. The project, however, provided some useful observations on approaches to consider environmental impacts of both chemical and radionuclides within a regulatory context.

Part of the work in PROTECT involved the collation of information, through circulation of a bespoke questionnaire and interviews within a community of national authorities, industry and Non-Governmental-Organisations, in relation to application of approaches to protect the environment from chemical and radioactive stressors (Hingston et al., 2007). Although most of the respondents working in the field of radiological protection replied in the affirmative when asked whether they regulated to protect the environment from radioactive substances, it became evident that this widely involved the application of the old axiom of the ICRP whereby ensuring human protection also safeguards the environment (ICRP, 1991). At the time of writing of the report (i.e. 2007) only the United Kingdom, Finland and Sweden indicated that they used additional approaches, which explicitly evaluated harm to wild-life although more recently additional European countries like Norway are now also in the process of implementing explicit environmental protection legislation for radioactivity (Hosseini et al., 2011). Outside of Europe Hingston et al. (2007) reported that Canada and the USA also had elaborated regulations with regards environmental protection from radioactivity. The USA, for example, has technical standards pertaining to thresholds of protection for plants and animals and in the case of aquatic animals this is enshrined in regulation as a dose limit through a DOE Order (US DOE, 2002).

With regards to the drivers for regulating to protect the environment from radioactive impacts, most European countries cited their own national regulation, most of which was firmly focussed on issues related to human health, and guidance from international bodies such as the ICRP and WHO (Hingston et al., 2007). Interestingly, only England & Wales and Scotland quoted EC directives in the form of the Habitats (Council Directive 92/43/EEC) and Birds (Council Directive 79/409/EEC) Directives, as having any relevance in terms of precipitating the requirement to address environmental impacts from radioactive substances. This was considered to be in some contrast to regulations for chemical wherein European directives have been widely adopted and implemented. The PROTECT project noted that for chemicals, key European legislation is covered in REACH and the WATER

Framework Directive, WFD (2000/60/EC, EC, 2000). Whilst the WFD makes passing reference to radionuclides as a possible pressure on water quality there is limited work being done in this area. Radionuclides are not covered by REACH.

Of further interest from PROTECT was the observation that most respondents considered optimisation to be important when regulating discharging industries and that cost-benefit criteria were integral to this (Hingston et al., 2007). Therefore, the optimisation principle As Low As Reasonable Achievable (ALARA) is often implemented in this process through studies of the Best Available Technology/Techniques (BAT). The important point is that this view is held for both radioactive and non-radioactive substances in the licencing of industrial discharges in some countries. A good example is provided by the ECs Integrated Pollution Prevention and Control (IPPC) Directive (2008/1/EC), as discussed above, where the BAT approach is strongly promoted and that is the process of being implemented in countries like the UK.

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