

# IAEA TECDOC SERIES

IAEA-TECDOC-1737

## Modelling of Biota Dose Effects

*Report of Working Group 6  
Biota Dose Effects Modelling  
of EMRAS II Topical Heading  
Reference Approaches for Biota Dose  
Assessment*

*Environmental Modelling for  
RAdiation Safety (EMRAS II) Programme*



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# MODELLING OF BIOTA DOSE EFFECTS

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# MODELLING OF BIOTA DOSE EFFECTS

REPORT OF WORKING GROUP 6  
BIOTA DOSE EFFECTS MODELLING  
OF EMRAS II TOPICAL HEADING  
REFERENCE APPROACHES FOR BIOTA DOSE ASSESSMENT

ENVIRONMENTAL MODELLING FOR  
RADIATION SAFETY (EMRAS II) PROGRAMME

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Printed by the IAEA in Austria  
July 2014

### IAEA Library Cataloguing in Publication Data

Modelling of biota dose effects: report of Working Group 6 Biota Dose Effects Modelling of EMRAS II Topical Heading Reference Approaches for Biota Dose Assessment. — Vienna : International Atomic Energy Agency, 2014.  
p. ; 30 cm. — (IAEA-TECDOC series, ISSN 1011-4289; no. 1737)  
ISBN 978-92-0-101114-5  
Includes bibliographical references.

1. Ionizing radiation — Dose-response relationship. 2. Radioecology — Mathematical models. 3. Animals — Effect of radiation on. I. International Atomic Energy Agency. II. Series.

## FOREWORD

Environmental assessment models are used for evaluating the radiological impact of actual and potential releases of radionuclides to the environment. They are essential tools for use in the regulatory control of routine discharges to the environment and in planning the measures to be taken in the event of accidental releases. They are also used for predicting the impact of releases which may occur far into the future, for example, from underground radioactive waste repositories. It is important to verify, to the extent possible, the reliability of the predictions of such models by a comparison with measured values in the environment or with the predictions of other models.

The IAEA has been organizing programmes on international model testing since the 1980s. These programmes have contributed to a general improvement in models, in the transfer of data and in the capabilities of modellers in Member States. IAEA publications on this subject over the past three decades demonstrate the comprehensive nature of the programmes and record the associated advances which have been made.

From 2009 to 2011, the IAEA organized a project entitled Environmental Modelling for RAdiation Safety (EMRAS II), which concentrated on the improvement of environmental transfer models and the development of reference approaches to estimate the radiological impacts on humans, as well as on flora and fauna, arising from radionuclides in the environment.

Different aspects were addressed by nine working groups covering three themes: reference approaches for human dose assessment, reference approaches for biota dose assessment and approaches for addressing emergency situations. This publication describes the work of the Biota Effects Modelling Working Group.

The IAEA wishes to express its gratitude to all those who participated in the work of the EMRAS II programme and gratefully acknowledges the valuable contribution of T. Hinton (United States of America), A. Real (Spain), J. Garnier-Laplace (France), H. Vandenhove (Belgium), S. Mihok (Canada) and T. Sazykina (Russian Federation). The IAEA officer responsible for this publication was S. Fesenko of the IAEA Environment Laboratories.

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## SUMMARY

Radiological protection of the environment is advancing from the old paradigm which stated that if humans are protected then by default all other components of the environment are protected as well [1]. Indeed, humans are among the most sensitive organisms to radiation and their protection does ensure protection of much of the environment. However, a more satisfying approach for many stakeholders, and one that is in-line with protection strategies implemented for other types of environmental contaminants, is to explicitly show that the environment is protected. Therefore, the International Commission on Radiological Protection (ICRP) now recommends explicit consideration of the environment and new approaches are being developed to demonstrate such protection. Appropriately, the new developments strive to protect populations or higher organizational levels (e.g. communities, ecosystems [2]), rather than focusing on the protection of individuals. The protection of higher levels of biological organization was not originally addressed [3], but is now being considered further [4].

During the last 10 years, considerable progress has been made in deriving screening ecological benchmarks and in developing a tiered Ecological Risk Assessment (ERA) approach for radioecology [5–7]. While the ERA-type approach is a substantial advancement in radioecology, a lack of sufficient data prevents current ERA analyses from fully accounting for the realistic environmental conditions to which organisms are exposed. Data are insufficient to predict effects from chronic, low doses; variable dose rate regimes; multi-contaminant scenarios or multi-generational exposures; ecosystem level effects; or the influence of variable life-history traits [8]. Additionally, most measurements of effects are assayed on individuals within a population. Extrapolation is required to estimate population level effects from the individual-based measurements, or to account for the knowledge gaps mentioned above. The extrapolation uses assessment factors (or safety factors) that add conservatism and substantially increase uncertainties in risk assessments. Large uncertainties do little to promote confidence with the stakeholders of radioecology. Improvements are therefore needed.

The Biota Dose Effects Modelling Working Group (WG6) of the IAEA's EMRAS II Programme was formed due to a common interest in improving the science of estimating dose, effects and risks to wildlife exposed to radiation. The group made important contributions to this area of radioecology from five perspectives, namely:

- (1) A key component in evaluating the risks of ionizing radiation to wildlife is knowledge on the variety of biological effects that different types of radiation can produce in animals and plants, under different exposure situations. Thus, an initial task of WG6 participants was to update the existing FREDERICA Radiation Effects Database<sup>1</sup> (see Section 2). During the EMRAS II Programme, 222 references were added to the FREDERICA database (66 from the Russian literature), corresponding to more than 8300 new data entries. Key data in the area of field studies (as opposed to laboratory studies) and for specific wildlife groups for which information was scarce (i.e. insects and invertebrates) were added to the database. This is an important contribution of the IAEA's EMRAS II Programme because FREDERICA is freely available to the entire radioecology community. Periodic enhancements, as performed by members of WG6, are critical to the long term success of the database.

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<sup>1</sup> Available at: <http://www.frederica-online.org/mainpage.asp>

- (2) The data from the updated FREDERICA database was then used by participants to develop new dose-response relationships and taxonomically-specific screening values (see Section 3). All of the new data sets added to FREDERICA were evaluated for their adequacy in developing dose-response curves. The curves obtained from the data were then used to estimate key ecotoxicity values such as ED<sub>50</sub> (estimated dose causing a 50% effect) and EDR<sub>10</sub> (estimated dose rate causing a 10% effect). Data from the dose-response curves were used to develop Species Sensitivity Distributions (SSDs) and corresponding benchmark values (using methods from guidance documents common to other types of contaminants). Past work in ERICA<sup>2</sup> and PROTECT<sup>3</sup> developed SSDs for a generic community of organisms, based on data derived from laboratory experiments in which the organisms had been chronically exposed to external gamma radiation. Within the EMRAS II Programme, the PROTECT meta-analysis was updated and a new SSD for chronic external irradiation was derived. The resulting SSD\_HDR<sub>5</sub> was estimated at 10 µGy/h (95% confidence interval of 2–50 µGy/h). In addition, how this new set of data could be used to update group-specific screening benchmarks that were obtained within PROTECT was examined. For example, the new data allowed the SSD\_HDR<sub>5</sub> for invertebrates to be estimated with considerably more precision than was previously possible. In PROTECT, the SSD\_HDR<sub>5</sub> for invertebrates was estimated to be 505 µGy/h, with a 95% confidence limit of 55–4447. Due to additional data added during the work, the SSD\_HDR<sub>5</sub> for invertebrates was lowered to 43 µGy/h, with a narrower 95% confidence limit (5–744). Interestingly, the new data allowed comparison of the median value (HDR50) of the distribution established for organisms exposed in free-ranging field conditions at Chernobyl (about 100 µGy/h) to the HDR50 derived from organism exposed under controlled-laboratory experiments (about 850 µGy/h). The data suggest that organisms living in their natural environment were eight times more sensitive to radiation. This first comparison highlights the lack of mechanistic understanding and the potential confusion arising from sampling strategies in the field. To confirm the apparent higher sensitivity of wildlife in the Chernobyl Exclusion Zone, a robust field research strategy is needed with adequate design to deal with confounding factors and the spatial-temporal dynamics of a heterogeneously contaminated environment.
- (3) Advantage was also taken of unique environmental monitoring data from Canada that contains the activities of several <sup>238</sup>U-series radionuclides and other metals/metalloids in sediments of lakes that received uranium mining and milling effluents for several decades (see Section 4). The data consist of presence/absence information for 190 genera/species of benthic invertebrates at 132 sampling sites in Saskatchewan and Ontario, Canada. Four radioactive substances were measured (i.e. uranium, <sup>226</sup>Ra, <sup>210</sup>Pb and <sup>210</sup>Po) along with eight other contaminants (i.e. As, Cr, Cu, Mo, Ni, Pb, Se, V) for a total of 20 606 data points. These historical data were collated previously with benthic invertebrate community surveys to provide statistical insights into the potential effects of contaminants on the presence/absence of diverse organisms living in close contact with sediments. The Canadian Benthic Database was previously analyzed using a “univariate” approach. A Screening Level Concentration (SLC) method was used to derive Lowest Effect Level (LEL) and Severe Effect Level (SEL) concentrations for 9 metals and 3 radionuclides. Total dose and multivariate interactions among contaminants were not considered in the initial analysis, and hence these topics were

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<sup>2</sup> See <https://wiki.ceh.ac.uk/display/rpemain/ERICA>

<sup>3</sup> See <https://wiki.ceh.ac.uk/display/rpemain/PROTECT>

revisited during the EMRAS II Programme in order to obtain maximum value from this effort. For WG6, a “multivariate” analysis was performed to determine if changes in species diversity of the benthos community can be explained by changes in contaminant concentrations in sediment. The multivariate approach was conducted using constrained and unconstrained ordination methods (i.e. Redundancy Analysis (RDA) and Principal Component Analysis (PCA), respectively). These methods required building two matrices: (i) a species matrix containing the presence or absence of each of the 209 species along the 196 sites; and (ii) a contaminant matrix, containing the concentration of the 12 contaminants along the 196 sites. The Redundancy Analysis identified three significant contaminant variables: vanadium, copper and chrome; and results from the PCA method were very similar. However, the robustness of these results should be questioned because analyses were possible with only 31 sites. It was concluded that analyses could perhaps be improved by considering the total dose combined from all radionuclides as one of the components in the multivariate analysis, rather than considering individual radioisotopes (presence or absence, with no dose). These data from natural environments also provide an independent crosscheck on the laboratory-based thresholds. Two estimation methods were used to calculate total dose to a generic benthic invertebrate, the PSL approach and the ERICA approach. The LEL values derived using different statistical and dose calculation methods varied by about a factor of ten, i.e. 13–97  $\mu\text{Gy/h}$ , averaging 40  $\mu\text{Gy/h}$  for eight estimates. The mean value was very similar to the conceptually-equivalent threshold of effect (SSD\_HDR<sub>5</sub> of 43  $\mu\text{Gy/h}$ ) calculated from laboratory-based experiments with invertebrates. This finding is remarkable given the many contributing factors leading to the derivation of thresholds in each type of calculation and the different nature of the underlying data sets.

- (4) Substantial progress was also made in considering radiation within a mixture of contaminants (see Section 5). Most contaminant studies are conducted in isolation, as if that is how they occurred in nature; whereas, all organisms, including humans, are actually exposed to mixtures of contaminants and stressors. In addition to being unrealistic, studies that involve only one contaminant are unable to determine if interactions among contaminants might occur. Prior to the EMRAS II Programme there was no database and little knowledge on the extent to which radiation has been studied within a multi-contaminant, multi-stressor context. Therefore, within the framework of the IAEA’s EMRAS II Programme and the International Union of Radioecology (IUR) Mixture Toxicity Workgroup, a review was made specifically focussing on studies that included radiation or radionuclides in the mixture. The literature was reviewed and a database was compiled of studies that investigated combined effects of ionizing radiation and other stressors on non-human biota. It was found that the animals used in mixture studies included rat, mouse, frog, salmon, medaka, eel and brine shrimp. 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. All animal mixture studies that were found were based on laboratory experiments. About three-quarters of the papers reviewed suggested some form of interaction of effects existed among the stressors, however, no persistent pattern of interactions was apparent. That is, in some cases no interactive effects were observed, in others additive, synergistic or antagonistic effects were seen, and in some cases the effects were dose- or concentration-dependent. From the review it was concluded that although statements about additivity, synergism or antagonism were often made, these were mostly based on the incorrect principle of effect summation or on 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 were dose-response curves for the single stressors developed. The review concluded that the two most commonly used models in ecotoxicology, Addition (CA) and Independent Action (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 ecotoxicology. The database is an important step in establishing the state-of-knowledge in multiple stressor studies involving radiation, and will be useful for prioritizing and designing future experiments. The review indicates a lack of 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.

- (5) Advances were also made in modelling effects to populations of wildlife (see Section 6). Improving models that propagate effects from individuals to populations will decrease the uncertainties associated with current extrapolation methods. Participants recognized that the impact of a specific dose to a population of organisms is likely dependent on the life history characteristics of that species. Populations that produce an abundant number of offspring at frequent intervals are probably less sensitive to radiation than populations of species that reproduce much less frequently and with lower fecundity. Incorporating such life history characteristics into effects models is needed and will likely improve the predictions of effects. Advancements in this important area were made by reviewing existing population models, developing life history data sheets for key species, and incorporating population models into effects analyses. One of the objectives was to derive basic equations that govern population models while incorporating radiation effects, with an emphasis on finding an equation that is simple enough to be generally applicable across different species. Results were obtained from the analyses of several models, using a benchmark test scenario, that predicted effects from chronic exposure to several mammals with widely different life history characteristics (e.g. mice, rabbit, deer, wolf). General conclusions from the modelling exercise were:

- Length of life is important; population survival of short-lived species is better than that of long-lived animals;
- Dose rates of about 10 mGy/d for 5 years of chronic exposure produced significant reductions of wolf and deer populations;
- Dose rates of 20 mGy/d for 5 years of chronic exposure produced considerable reduction of all populations, except short-lived mice. The latter survived at levels of about 70% of the controls; and
- Larger animals = greater longevity = slower reproductive rate = populations with greater sensitivity to radiation.

Currently, it is not possible to validate these population models experimentally because of a lack of long-term experimental studies on population dynamics of free-ranging animals exposed to ionizing radiation. Nonetheless, WG6's work has helped to provide hypotheses to integrate population behaviour and radiological effects, and was successful in comparing the different approaches that are being developed. Furthermore, it is important to determine whether differences in predictions between models are due to design of the population algorithm, the radiation effect mechanism, or life history parameters.

## 1. INTRODUCTION

### 1.1. BACKGROUND OF THE EMRAS II PROGRAMME

The IAEA organized a programme from 2009 to 2011 entitled Environmental Modelling for RADIATION SAFETY (EMRAS II), which concentrated on the improvement of environmental transfer models and the development of reference approaches to estimate the radiological impacts on humans, as well as on flora and fauna, arising from radionuclides in the environment.

The following topics were addressed in nine working groups:

#### **Reference Approaches for Human Dose Assessment**

- Working Group 1: Reference Methodologies for Controlling Discharges of Routine Releases
- Working Group 2: Reference Approaches to Modelling for Management and Remediation at NORM and Legacy Sites
- Working Group 3: Reference Models for Waste Disposal

#### **Reference Approaches for Biota Dose Assessment**

- Working Group 4: Biota Modelling
- Working Group 5: Wildlife Transfer Coefficient Handbook
- Working Group 6: Biota Dose Effects Modelling

#### **Approaches for Assessing Emergency Situations**

- Working Group 7: Tritium Accidents
- Working Group 8: Environmental Sensitivity
- Working Group 9: Urban Areas

The activities and the results achieved by the Working Groups are described in individual IAEA Technical Documents (IAEA-TECDOCs). This report describes the work of the Biota Effects Modelling Working Group.

### 1.2. BACKGROUND FOR EMRAS II WORKING GROUP 6: BIOTA DOSE EFFECTS MODELLING

Radiological protection of the environment is advancing from the anthropocentric paradigm which stated that if humans are protected then by default all other components of the environment are protected as well [1]. Indeed, humans are among the most sensitive organisms to radiation and their protection does insure protection of much of the environment. However, a more satisfying approach for many stakeholders, and one that is in-line with protection strategies implemented for other types of environmental contaminants, is to explicitly show that the environment is protected.

Over the last 10 years, radiological protection for wildlife has advanced due to considerable international and national efforts (e.g. [3, 4]). Environmental protection is now referred to in the IAEA's Fundamental Safety Principles [9] and the revision of the International Basic Safety Standards [4]. Since 2005, the ICRP has had a fifth committee, which deals specifically with the protection of the environment from ionizing radiation. In 2007, the ICRP recommended explicit radiological protection of the environment, recognizing the need for advice and guidance,

including a clearer framework [10]. The new developments strive to protect populations or higher organizational levels (i.e. communities and ecosystems [2]), rather than focusing on the protection of individuals.

Regulators and the nuclear industry look to radioecology for help in determining environmental risks and in demonstrating legal compliance. Numerical benchmarks (e.g. dose rate limits) place the outputs of environmental assessments into context and help managers decide on the need for further assessment or regulatory/remedial action. Historically, the derivation of radiological benchmarks for environmental assessment has relied upon expert judgement. A benchmark relates to a protection goal, or can be a conservatively derived value which screens out sites where there is no cause for concern and identify those where further consideration is needed. Considerable progress has been made in deriving screening ecological benchmarks that are more transparent than expert judgment and in developing a tiered ERA approach for radioecology [5–7]. While the ERA-type approach is a substantial advancement in radioecology, a lack of sufficient data prevents current ERA analyses from fully accounting for the realistic environmental conditions to which organisms are exposed. Additionally, there is no agreement on what to do if advanced assessments estimate dose rates in excess of radiological screening benchmarks [11].

The endpoints currently considered to be most relevant in determining risks to populations of wildlife are increased mortality, increased morbidity and decreased reproductive output. These are endpoints that are assayed on individuals within populations. Thus, extrapolation is currently required to estimate population level effects from the individual-based measurements. The extrapolation uses assessment factors (or safety factors) that add conservatism and substantially increase uncertainties in risk assessments. Large uncertainties do little to promote confidence with the stakeholders of radioecology. Much more data are needed before population level impacts to wildlife can be confidently predicted as a function of radiological exposures [12]. Data are particularly scarce for the chronic, low-level exposures for which most assessments will be used, as well as insufficient to predict the effects from variable dose rate regimes; multi-contaminant scenarios; multi-generational exposures; variable life-history traits; or ecosystem level effects [8].

WG6 formed from a common interest in improving the science of estimating dose, effects and risks to wildlife exposed to radiation. Such work is needed because of the developing regulations; a disparity between what is measured (individual-based parameters) and what the regulations are trying to protect (i.e. populations, communities and ecosystems); frustration of regulators and industry in interpreting risk analyses; and the general lack of appropriate dose-effect data for wildlife.

## 2. FREDERICA DATABASE UPDATE

### 2.1. INTRODUCTION

A key component in evaluating the risks of ionizing radiation to wildlife is knowledge on the variety of biological effects that different types of radiation can produce in animals and plants, under different exposure situations. Thus, an initial task was to update the existing FREDERICA Radiation Effects Database.

The FREDERICA database is the foundation upon which new dose-response relationships and taxonomically-expanded screening level values are established. The FREDERICA database was last updated in 2006 and the need to update the database was recognized from three perspectives: (i) input of data published since 2006 in the open literature; (ii) the addition of some data within UNSCEAR thought to be missing within the database; and (iii) input from Japanese, Russian, and Ukrainian literature.

Some 650 papers were examined, from which 137 were deemed appropriate to add to the database. This represents about a 10% increase in the existing FREDERICA database. Some 250 of the 650 papers are in the Russian language and will need additional translation by native speaking scientists prior to their incorporation into the database. Several previously underrepresented classes of animals were enriched by the new papers (i.e. amphibians, insects and protozoa).

The FREDERICA database was originally developed during the European Project ERICA, and was the result of merging two existing effects databases: FRED and EPIC (developed during the FASSET and EPIC project, respectively), plus some extra additions (studies published up to 2006). At the end of the ERICA Project, FREDERICA contained over 1200 references (more than 29 000 data entries). Approximately 75% of these data concerned species inhabiting terrestrial ecosystems, twice as many data existed for acute than for chronic irradiations and most of the studies focused on external gamma irradiation [13, 14].

### 2.2. METHODS USED TO UPDATE THE FREDERICA DATABASE

For the task of updating the FREDERICA database, a literature survey was done, searching for new data published since 2006 and for Japanese and Russian literature. Over 600 papers were assembled, although not all of them were suitable for inclusion in the database (e.g. review articles without detailed experimental conditions).

As it can be seen in Table 1, many references (255 out of 670) on the radiation effects on wildlife are published in Russian. Thanks to the financial support of the IAEA, key information of many of these references has been translated into English, and included in the FREDERICA database.

TABLE 1. RESULTS OF THE LITERATURE SURVEY FOR NEW DATA ON EFFECTS OF IONIZING RADIATION ON ANIMALS AND PLANTS

Language	Number of references
Chinese	1
French	2
Japanese	7
Russian	255
English	405

Each reference newly included in FREDERICA was evaluated to determine whether or not contained enough information to be used in the process of defining biological effect levels. The criteria used for this quality control analysis were those established and used in the ERICA project [13, 14], and were related to dosimetry, experimental design and statistical methods used in the study. As summarized in Table 2, several specific aspects were evaluated for each of these three criteria. The final score of each paper, included in FREDERICA, reflects the quality and quantity of the available data.

It has to be noted that the aim of the QC evaluation was not to judge how the author(s) designed and performed the experiment(s), but to determine the amount of information provided within the reference, i.e. the information available to the reader in each reference included in the database.

The quality control analysis indicated that 75% of the references had a score > 35. A score of 35 was considered in the past as the minimal score that a reference has to have to use it in Species Sensitivity Distribution (SSD) analysis, and thus to further establish screening values [15].

### 2.3. RESULTS OF THE FREDERICA UPDATE

The data added to FREDERICA during the IAEA's EMRAS II Programme covers a wide variety of ecosystems, type of studies, radiation exposure, radiation type, wildlife groups and endpoints (Tables 3–10).

As Table 3 shows, nearly 50% of the data corresponded to species of the terrestrial ecosystem. Around 50% of the data added were field and controlled field studies, and 50% of the data included were obtained from chronic radiation exposures. The radiation type more commonly used in the studies was gamma and X rays (54.4% of the data). Plants and mammals were the wildlife groups more frequently used in the studies. However, around 5% of the data added to FREDERICA were from insect studies, a wildlife group poorly represented in the database. Regarding the biological endpoints, reproduction (35.0%) and morbidity (31.5%) were the more frequently assayed endpoints.

### 2.4. RELEVANCE AND IMPORTANCE OF THE FREDERICA DATABASE UPDATE

The data from the updated FREDERICA database has been used to develop new dose-response relationships and taxonomically-specific screening values. In summary, 222 references have been added to the FREDERICA database (66 from the Russian literature), corresponding to more than 8300 new data entries. Key data in the area of field studies (as opposed to laboratory studies) and for specific wildlife groups for which information was scarce (i.e. insects and invertebrates) have been added to the database.

The new information included in FREDERICA could also be used to update the “look-up” tables within the ERICA Tool (ERICA Tool uses the FREDERICA database to conduct ecological risk analyses based on a tiered approach). These tables could support any Environmental Risk Analysis, regardless of the tool used.

TABLE 2. ASPECTS TAKEN INTO ACCOUNT FOR EACH OF THE THREE CRITERIA USED IN THE EVALUATION OF THE PAPERS ON EFFECTS OF MULTIPLE STRESSORS

	<b>Maximum Score</b>
<b><i>Dosimetry</i></b>	<b>15</b>
<i>Determination of dose</i> (type, number and position of TLDs used; equations used for dose calculation)	
<i>Determination of dose-rate</i> (already calculated or able to be calculated)	
<i>Background levels</i> (dose or dose-rate values for background radiation reported)	
<b><i>Experimental design</i></b>	<b>40</b>
<i>Endpoint analyzed</i> : where (tissue, organ), when (in relation to treatment and lifespan of the organism), how (method used), ecological relevance	
<i>Control group</i> (held in appropriate conditions)	
<i>Exposure conditions</i> (range of doses, radiation source(s), single or multi contaminant)	
<i>Test organism</i> : Specie, sex, life cycle stage (embryonic/adult), whether bred in the lab or from the field, maintenance conditions (i.e. husbandry/maintenance of organisms)	
<b><i>Statistics</i></b>	<b>25</b>
<i>Number of replicates of the experiment</i>	
<i>Number of individuals per point</i>	
<i>Number of points per curve</i>	
<i>Number of combinations between the stressors assayed</i>	
<i>Method used for statistical analysis</i>	
<i>Confidence limits</i>	
<i>Statistical methods to determine the combined effect</i> (synergism, antagonism, etc.)	
<b>Maximum score</b>	<b>80</b>

TABLE 3. MAIN CHARACTERISTICS OF THE DATA INCLUDED IN FREDERICA DURING THE EMRAS II PROGRAMME

<b>Parameter</b>	<b>Sub-Category</b>	<b>N, data added</b>	<b>%</b>
Ecosystem	Agricultural	195	19.9
	Aquatic (general)	21	2.1
	Freshwater	98	10.0
	Marine	13	1.3
	Forest	104	10.6
	Semi-natural pasture	112	11.4
	Terrestrial (generic)	439	44.7
Type of study	Controlled field	169	17.2
	Field	332	33.8
	Laboratory	481	49.0
Radiation exposure	Acute	464	47.3
	Chronic	436	44.4
	Transitory	4	0.4
	Not stated	78	7.9
Radiation type	Alpha	17	1.7
	Beta	54	5.5
	Gamma & X rays	534	54.4
	Mixed	366	37.3
	Neutrons	7	0.7
	Protons	4	0.4
Wildlife group	Amphibians	7	0.7
	Aquatic invertebrates	23	2.3
	Aquatic plants	35	3.6
	Crustaceans	15	1.5
	Fish	42	4.3

TABLE 3. (Continued)

Parameter	Sub-Category	N, data added	%
	Fungi	1	0.1
	Insects	46	4.7
	Invertebrates	20	2.0
	Mammals	317	32.3
	Molluscs	4	0.4
	Moss/Lichen	1	0.1
	Plants	441	44.9
	Reptiles	3	0.3
	Soil fauna	13	1.3
	Zooplankton	12	1.2
	Review article	2	0.2
Endpoint	Adaptation	4	0.4
	Ecological	111	11.3
	Morbidity	309	31.5
	Mortality	109	11.1
	Mutation	102	10.4
	Reproduction	344	35.0
	Stimulation	1	0.1

TABLE 4. AGRICULTURAL ECOSYSTEM (195 DATA) DATA CHARACTERISTICS

Type of study (n data)	Radiation Exposure (n data)	Radiation Type (n data)	Wildlife Group (n data)	Umbrella Endpoint			
					n data		
Controlled field (111)	Acute (85)	Gamma (77)	Plants (77)	MB	28		
				MT	6		
				REPR	43		
		X rays (7)	Plants (7)	MB	6		
				MT	1		
				MB	1		
	Chronic (23)	Gamma (14)	Plants (14)	MB	10		
				REPR	4		
				MB	1		
		X rays (1)	Plants (1)	MB	1		
				MT	2		
				REPR	2		
Mixed (2)	Plants (2)	REPR	2				
		Gamma (3)	Plants (3)	MB	1		
		REPR	2				
Transitory (3)	Gamma (3)	Plants (3)	MB	1			
			REPR	2			
			REPR	2			
Field (28)	Acute (15)	Gamma (11)	Plants (11)	MB	3		
				REPR	8		
		X rays (4)	Plants (4)	MB	4		
				MUT	2		
	Chronic (4)	Mixed (3)	Plants (3)	MB	1		
				MB	3		
	Not stated (9)	Mixed (9)	Mammals (3)	MB	3		
				Plants (6)	MB	3	
			Plants (6)	MUT	3		
				MUT	3		
Laboratory (56)	Acute (50)	Gamma (38)	Plants (39)	MB	18		
				MT	15		
				MUT	3		
				REPR	2		
				Protons (4)	Plants (4)	MB	1
						MT	3
				Beta (2)	Amphibians (2)	MUT	2
		X Rays (6)	Amphibians (4)	MB	1		
				MT	1		
				REPR	2		
		Reptiles (2)	Reptiles (2)	REPR	2		
				Gamma (3)	Plants (3)	MB	2
				REPR	1		
		Chronic (4)	Gamma (3)	Plants (3)	MB	2	
REPR	1						
Beta (Sr-90) (1)	Reptile (1)	MT	1				
		Gamma (2)	Plants (2)	MUT	1		
Not stated (2)	Gamma (2)	Plants (2)	MUT	1			
			REPR	1			

TABLE 5. AQUATIC (GENERIC) ECOSYSTEM (21 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint	
				N° data	
Laboratory (21)	Acute (19)	Alpha (2)	Aquatic plants (2)	MB	2
		Gamma (1)	Amphibians (1)	MB	1
		X rays (16)	Fish (16)	MB	9
			MT	5	
			REPR	2	
	Chronic (2)	Gamma (2)	Crustaceans (2)	MB	1
			MT	1	

TABLE 6. FRESHWATER ECOSYSTEM (98 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint	
				N° data	
Field (19)	Acute (1)	Gamma (1)	Zooplankton (1)	ECOL	1
	Chronic (18)	Mixed (18)	Fish (18)	MB	18
Laboratory (79)	Acute (71)	Alpha (3)	Aquatic plants (3)	MB	3
		Gamma (33)	Aquatic invertebrates (1)	MB	1
			Aquatic plants (27)	MB	15
				ECOL	8
				MT	4
			Crustaceans (2)	MB	2
		Zooplankton (3)	MB	3	
		Mixed (2)	Aquatic plants (2)	MB	2
	Chronic	X rays (33)	Aquatic invertebrates (18)	REPR	18
			Fish (8)	MB	4
				MT	4
			Insect (1)	MB	1
			Zooplankton (6)	MB	6
Alpha (Am-241)			Crustaceans (2)	REPR	2
Gamma			Aquatic invertebrates (4)	MB	4
	Zooplankton (1)	MB	1		
	X Rays	Zooplankton (1)	MB	1	

TABLE 7. MARINE ECOSYSTEM (13 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint	
				N° data	
Field (3)	Chronic (3)	Alpha (1)	Mollusc (1)	MB	1
		Mixed (2)	Mollusc (2)	MB	2
Laboratory (10)	Chronic (10)	Beta (P-32) (1)	Aquatic plants (1)	STIM	1
		Mixed (9)	Crustaceans (9)	MT	2
				REPR	7

TABLE 8. NATURAL FOREST ECOSYSTEM (104 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint	
				N° data	
Controlled Field (16)	Acute (3)	Gamma (3)	Plants (3)	REPR	3
	Chronic (13)	Gamma (13)	Plants (13)	ECOL	7
MB				6	
Field (88)	Chronic (86)	Mixed (79)	Plants (79)	ADAPT	1
				ECOL	3
				MB	47
				MT	1
				MUT	21
				REPR	6
					Gamma (7)
	Not stated (2)	Mixed (2)	Plants (2)	MB	2

TABLE 9. SEMI-NATURAL PASTURE ECOSYSTEM (112 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint	
					N° data
Controlled Field (2)	Chronic (2)	Mixed (2)	Plants (2)	ADAPT	2
Field (110)	Chronic (87)	Mixed (87)	Plants (87)	ECOL	57
				MB	5
				MT	2
				MUT	20
				REPR	3
	Not stated (23)	Beta (12)	Plants (12)	MB	9
				MUT	2
				REPR	1
				ECOL	10
				MUT	1
		Mixed (11)	Plants (11)	ECOL	10
				MUT	1

TABLE 10. TERRESTRIAL (GENERIC) ECOSYSTEM (439 DATA) DATA CHARACTERISTICS

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint				
					N° data			
Contr. Field (40)	Chronic (38)	Mixed (31)	Mammals (29)	MB	4			
				MUT	6			
				REPR	19			
			Plants (2)	MUT	1			
				REPR	1			
			Beta (H-3) (2)	Plants (2)	MB	2		
			Gamma (5)	Mammals (1)	REPR	1		
					Plants (4)	MB	4	
			Acute (2)	Gamma (1)	Plants (1)	MB	1	
					Mixed (1)	Mammals (1)	MT	1
			Field (84)	Acute (1)	Gamma(1)	Insects (1)	MT	1
						Plants (1)	ECOL	1
				Chronic (42)	Mixed (41)	Plants (9)	MB	4
							MUT	5
						Mammals (11)	MUT	10
REPR	1							
Soil fauna (4)	ECOL	4						
Insects (5)	MT	4						
	ECOL	1						
Invertebrates (11)	ECOL	3						
	MT	8						
Not stated (41)	Mixed (41)	Mammals (29)		MB	14			
			MUT	6				
			REPR	9				
		Insects (3)	ECOL	3				
		Invertebrates (9)	ECOL	9				
Laboratory (315)	Acute (217)	Alpha (9)	Insect (1)	MB	1			
			Soil fauna (1)	MB	1			
			Plants (7)	MB	7			
			Gamma (58)	Fungi (1)	MB	1		
				Insects (25)	MB	2		
		MT			14			
		REPR	9					
		Mammals (18)	MB	7				
			REPR	11				
		Mollusc (1)	MB	1				
		Plants (11)	ADPAT	1				
			MB	5				
			MUT	5				
			Soil fauna (2)	MB	2			

TABLE 10. (Continued)

Type of study (n° data)	Radiation Exposure (n° data)	Radiation Type (n° data)	Wildlife Group (n° data)	Umbrella Endpoint			
					N° data		
Laboratory (315) (Continued)	Acute (217) (Continued)	Mixed (12)	Insects (3)	MT	3		
			Mammals (9)	MB	5		
				MUT	2		
		REPR		2			
		Neutrons (6)		Mammals (6)	MB	2	
		X Rays (132)	Mammals (126)	REPR	4		
				Insects (6)	MT	6	
				REPR	114		
		Chronic (96)	Beta (29)	Mammals (24)	REPR	24	
					Moss/Lichen (1)	MB	1
					Plants (2)	ECOL	2
					Plants (2)	MUT	1
			Gamma (49)	Mammals (40)	REPR	1	
					MB	2	
					MT	11	
REPR	27						
Plants (3)	MUT				1		
REPR	2						
Mixed (12)	Mammals (6)		Soil fauna (6)	REPR	6		
			MB	4			
		MT	1				
		REPR	1				
		Plants (6)	ECOL	2			
		MB	1				
		MUT	2				
REPR	1						
X rays (6)	Mammals (6)	MB	6				
Transitory (1)	Gamma (1)	Insects (1)	MB	1			
Not stated (1)	Mixed (1)	Mammals (1)	MUT	1			

### 3. DOSE-RESPONSE RELATIONSHIPS AND DERIVATION OF SPECIES SENSITIVITY DISTRIBUTIONS

#### 3.1. INTRODUCTION

Benchmarks, or some form of criteria (usually numeric), allow the outputs of environmental assessments to be placed into context and aid decisions on the need for further assessment or regulatory/remedial action. For radiological protection of the environment, benchmarks are often referred to as the Predicted No Effect Dose Rate (PNEDR). A benchmark is often a value (e.g. contaminant concentration, dose, or dose rate) considered to be protective. If conservative risk calculations are well under the value then the situation being assessed is deemed to be compliant and does not require additional computations or analyses. Benchmark values are often used in Tier-1 risk calculations for screening many contaminants and as an aid to identify those that are thought not to contribute substantially to risks. Situations or contaminants that exceed the benchmark are flagged as potential concern and require more extensive data and calculations more accurately determine their risks. The next section describes methods to derive a PNEDR, the approaches used are often consistent with those used in the risk assessment of chemicals.

#### 3.2. ESTABLISH NEW DOSE-RESPONSE CURVES

In a first step, all the new data sets added to FREDERICA were evaluated for their adequacy to be used to develop dose-response curves. The curves obtained from the adequate data sets were then used to estimate key ecotoxicity values such as ED<sub>50</sub> and EDR<sub>10</sub> to be used for the implementation of Species Sensitivity Distributions (SSD).

The new references added to FREDERICA with QC score > 35 were further analysed for their adequacy to be used for building dose rate-effect relationships. IRSN in parallel merged this new information to the former data used within PROTECT. The full list summarizing the treated data are presented in Table 11 for chronic external gamma exposure conditions.

The details in the sets of estimated EDR<sub>10</sub> is given in Table 12.

TABLE 11. OVERVIEW OF THE REFERENCES DEDICATED TO CHRONIC GAMMA (OR X RAYS) EXTERNAL IRRADIATION IN LABORATORY OR CONTROLLED FIELD FOR NON-HUMAN SPECIES POPULATING THE UPDATED FREDERICA, INCLUDING THE WORK DONE UNDER IAEA PROGRAMMES (EMRAS II, RUSSIAN LITERATURE SURVEY)

Study types	Number of references	Number of species
Laboratory Gamma irradiation	89	51
Laboratory X ray irradiation	3	3
Controlled field Gamma irradiation	49	26
Total	141	70*
References and species useful for PROTECT meta-analysis	31	20
References and species useful for this meta-analysis	60	30

\*This total number of species is lower than the total number of species investigated per study type because some species were tested for several study types.

TABLE 12. LIST OF SPECIES INTEGRATED INTO THE CHRONIC GAMMA (OR X RAYS) EXTERNAL IRRADIATION EFFECT META-ANALYSIS

Species	Wildlife Group	Ecosystem	EDR <sub>10</sub> in Protect (n Ref)	EDR <sub>10</sub> in this meta-analysis (n Ref)	Lowest EDR <sub>10</sub> (μGy/h)	Maximum EDR <sub>10</sub> (μGy/h)
<b>Plants</b>						
<i>Abies balsamea</i>	Plant	Terrestrial	1 (1)	4 (2)	<u>1643</u>	2573
<i>Fagopyrum esculentum</i>	Plant	Terrestrial	1 (1)	1 (1)	<b>40153</b>	40153
<i>Pinus rigida</i>	Plant	Terrestrial	1 (1)	2 (2)	<b>710</b>	997
<i>Pinus banksiana</i>	Plant	Terrestrial	–	1 (1)	6802	6802
<i>Triticum monococcum</i>	Plant	Terrestrial	14 (1)	23 (3)	<u>6009</u>	39860
<i>Solanum tuberosum</i>	Plant	Terrestrial	–	4 (1)	<u>514</u>	3079
<i>Hordeum sp.</i>	Plant	Terrestrial	–	3 (1)	70321	652607
<i>Pisum sp.</i>	Plant	Terrestrial	–	1 (1)	2703	2702
<i>Vitis vinifera</i>	Plant	Terrestrial	–	7 (1)	<u>603</u>	14122
<b>Invertebrates</b>						
<i>Eisenia fetida</i>	Annelid	Terrestrial	6 (1)	9 (1)	<b>3369</b>	13012
<i>Ophryotrocha diadema</i>	Annelid	Marine	5 (1)	21 (1)	<b>36</b>	12390
<i>Neanthes arenaceodentata</i>	Annelid	Marine	–	9 (1)	134	24412
<i>Daphnia magna</i>	Crustacean	Freshwater	2 (1)	3 (1)	<b>16797</b>	18760
<i>Daphnia pulex</i>	Crustacean	Freshwater	5 (2)	7 (2)	<b>167045</b>	730319
<i>Porcellio scaber</i>	Crustacean	Terrestrial	3 (1)	3 (1)	<u>749</u>	6274
<i>Callinectes sapidus</i>	Crustacean	Marine	–	5 (1)	158747	251844
<i>Dahlbominus sp.</i>	Insect	Terrestrial	–	1 (1)	3031	3031
<i>Mercenaria mercenaria</i>	Mollusc	Marine	2 (1)	8 (1)	<b>14481</b>	315462
<i>Physa heterostropha</i>	Mollusc	Marine	4 (1)	6 (1)	<b>3851</b>	177796
<b>Vertebrates</b>						
<i>Gallus gallus</i>	Bird	Terrestrial	3 (2)	26 (2)	<b>13932</b>	31875
<i>Larus ridibundus</i>	Bird	Terrestrial	1 (1)	1 (1)	<b>3696</b>	3695
<i>Oncorhynchus tshawytsch</i>	Fish	Freshwater	1 (1)	2 (1)	<b>2046</b>	3518
<i>Oryzias latipe</i>	Fish	Freshwater	6 (3)	9 (5)	<b>2012</b>	88732
<i>Pleuronectes platessa</i>	Fish	Marine	5 (1)	7 (3)	<b>47</b>	10982
<i>Poecilia reticulata</i>	Fish	Freshwater	2 (2)	3 (2)	<b>105</b>	2423
<i>Mus musculus</i>	Mammal	Terrestrial	25 (6)	59 (12)	<b>25</b>	2.9×10 <sup>6</sup>
<i>Rattus norvegicus</i>	Mammal	Terrestrial	6 (2)	6 (2)	<b>24</b>	631
<i>Sus crofa</i>	Mammal	Terrestrial	7 (1)	9 (2)	<b>3.6</b>	2723
<i>Canis familiaris</i>	Mammal	Terrestrial	–	3 (2)	155	22911
<i>Capra hircus</i>	Mammal	Terrestrial	–	3 (2)	12	1968
<b>Total</b>			100 (31)	246 (60)	30	–
<b>Including data from controlled field</b>			0	44	4	–
<b>Hormesis models</b>			6	32	1	–
<b>Sigmoidal models</b>			94	214	29	–

NOTES: The number of estimated EDR<sub>10</sub> is reported also with the corresponding number of references (within brackets) used under the PROTECT project and in this meta-analysis. The resulting minimum and maximum EDR<sub>10</sub> values are expressed in μGy/h. **Bold characters** refer to similar EDR<sub>10</sub> than the one used in PROTECT, **bold italic characters** mean that the value corresponds to a similar species but a lower EDR<sub>10</sub> value than the one in PROTECT and normal characters mean that the EDR<sub>10</sub> is estimated for a species absent from PROTECT. Lowest EDR<sub>10</sub> estimated on the basis of data sets from controlled field are underlined. All are derived from logistic model fit except the one with dotted underline (hormetic model).

### 3.3. DEVELOP CHRONIC SSDS AT TAXONOMIC LEVEL

Data from the dose-response curves were used for the implementation of species sensitivity distributions (SSDs) and the corresponding derivation of threshold protection values. Past work in ERICA and PROTECT developed ecosystem-level SSDs based on data derived from laboratory experiments in which the organisms had been chronically exposed to external gamma radiation. Within the EMRAS II Programme, this meta-analysis was updated and participants derived a new SSD for chronic external irradiation (Figure 1). The resulting SSD\_HDR<sub>5</sub> was estimated at 10 µGy/h (IC95% 2–50 µGy/h).

In addition, participants examined how this new set of data could be used to update the transitional group-specific screening benchmarks that were obtained within PROTECT (see Table 13).

### 3.4. PUBLICATIONS

A paper was published [16] which uses data treated by some participants of this task group. The best estimate of the median value (HDR<sub>50</sub>) of the distribution established for field conditions at Chernobyl (about 100 µGy/h) was found to be eight times lower than the one from controlled experiments (about 850 µGy/h), suggesting that organisms in their natural environment were more sensitive to radiation. This first comparison highlights the lack of mechanistic understanding and the potential confusion coming from sampling strategies in the field. To confirm the apparent higher sensitive of wildlife in the Chernobyl Exclusion Zone, a more robust field strategy, with adequate design to deal with confounding factors, is needed.

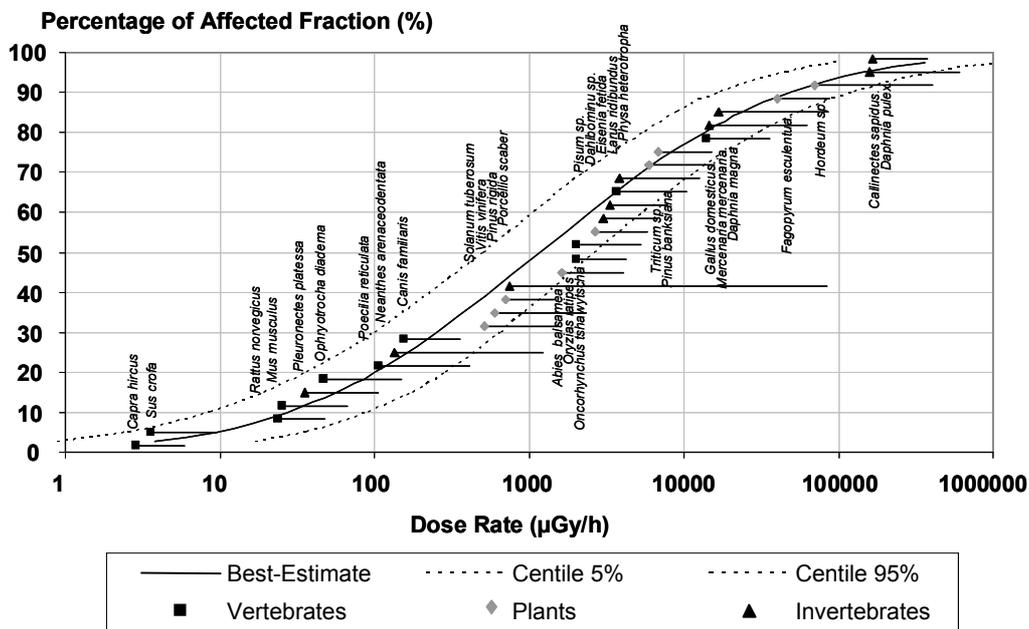


FIG. 1. Species Sensitivity Distribution (SSD) for generic ecosystems (freshwater, marine or terrestrial) and chronic external gamma exposure conditions. The log normal distribution is fitted to the set of lowest EDR<sub>10</sub> values (30 data which are the lowest per species). The estimation standard error is represented by horizontal bar for each EDR<sub>10</sub>.

TABLE 13. PROPOSED ORGANISM GROUP AND GENERIC ECOSYSTEMS HDR<sub>5</sub> VALUES (μGy h<sup>-1</sup>) ESTIMATED USING SSD

Group	Number of species	Lowest EDR <sub>10</sub>	Most sensitive wildlife Group (and species)	SSD_HDR <sub>5</sub> * from EMRAS II (μGy/h)	r <sup>2</sup>	SSD_HDR <sub>5</sub> ** from PROTECT (μGy/h)
Plants	9	514	Plant ( <i>Solanum tuberosum</i> )	192 (79–721)	0.92	n/a
Invertebrates	10	36	Annelid ( <i>Ophryotrocha diadema</i> )	43 (5.5–744)	0.96	505 (55–4447)
Vertebrates	11	2.9	Mammal ( <i>Capra hircus</i> )	1.4 (0.2–13)	0.95	2.1 (0.3–62)
Generic ecosystem	30	2.9	Mammal ( <i>Capra hircus</i> )	9.5 (2.0–47.2)	0.98	17 (2–211)

\* HDR<sub>5</sub> estimated within EMRAS II using SSDs: best estimate and associated 95% confidence limits (in parenthesis).

\*\* See Reference [17] for details.

## 4. BENTHIC DATABASE FROM URANIUM MINING AND MILLING IN CANADA

Routine environmental monitoring in Canada has generated a unique database on the activities of several  $^{238}\text{U}$ -series radionuclides and other metals/metalloids in sediments in water bodies that have received uranium mining and milling effluents for up to several decades [18]. These historical data were collated previously with benthic invertebrate community surveys to provide statistical insights into the potential effects of contaminants on the presence/absence of diverse organisms living in close contact with sediments (Figure 2). Total dose and multivariate interactions among contaminants were not considered in that initial analysis, and hence these topics were revisited in EMRAS II to obtain maximum value from this effort. These data from natural environments provide an independent crosscheck on the laboratory-based thresholds. Results from experimental studies in Table 13 suggest a low radiation effects threshold for invertebrates (SSD\_HDR<sub>5</sub> of 43  $\mu\text{Gy}/\text{h}$ ). Invertebrates as a group are considered to be relatively insensitive to radiation effects, although experimental data are few [2].

### 4.1. DOSE THRESHOLDS OF EFFECT FOR BENTHIC INVERTEBRATES ASSOCIATED WITH URANIUM MINING AND MILLING IN CANADA

#### 4.1.1. Introduction

The Species Sensitivity Distribution (SSD) approach is often used for the derivation of effects benchmarks for hazardous substances, as in the derivation of water quality guidelines for the protection of aquatic life by the Canadian Council for Ministers of the Environment [19]. In radioecology, the SSD approach was used to derive a generic screening value for non-human biota of  $10 \mu\text{Gy h}^{-1}$  (Predicted No-Effect Dose Rate, PNEDR) in an ecosystem protection context by the European PROTECT project [20]. It represents a statistical model of the 5<sup>th</sup> percentile of chronic radiation effects studies in the laboratory for conservatively-chosen, species-specific, 10% effects levels. An analogous non-parametric approach has also been used for the derivation of sediment quality guidelines, the Screening Level Concentration (SLC) approach. It was developed to derive thresholds of effect based on the presence of various taxa of benthic invertebrates relative to environmental gradients of single contaminants [21].

For a given contaminant and a given wildlife species, a frequency distribution of the concentrations is done for all sites where the species is present. The corresponding contaminant concentrations are ordered and the frequency distribution is then assembled. From this distribution the concentration corresponding to the 90<sup>th</sup> percentile (the Species Sensitivity Limiting Concentration, SSLC) is determined. In a second step, all the SSLCs derived previously for a given contaminant (one for each species), are ordered and the frequency distribution of the SSLCs is assembled. Note that the SSLC is conceptually similar to the 10<sup>th</sup> percentile for effects in SSD analysis. The Lowest Effects Level (LEL) and the Severe Effects Level (SEL) are next estimated as the concentrations corresponding respectively to the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the SSLCs. If the sediment contamination of a site is found to be below the LEL, environmental risk is likely to be low. If the sediment contamination is above the SEL, there is likely to be an environmental risk.



FIG. 2. Examples of benthic invertebrates in the Canadian data set (left to right; fingernail clam (*Pisidium* spp.), Chironomids, and caddisflies (*Nemotaulius* spp.).

The SLC approach was chosen by Thompson et al. [18] to make best use of co-located sediment and benthic invertebrate community monitoring data collected for diverse purposes in the uranium mining and milling regions of Canada up to about 2001. Thompson et al. [18] derived LELs and SELs for several metals/metalloids and a few radionuclides. However, they did not include an estimate of total dose. To calculate total dose requires consideration of the presence of many uranium decay chain daughters that are not typically measured in environmental monitoring programmes.

Methods for integrated dose calculations have matured considerably in recent years [18, 22, 23]. Hence, this unique historical data set was revisited to estimate total dose for a similar LEL/SEL analysis to put previous results into a modern environmental radiation protection context. In parallel, multivariate techniques were also used to obtain insights into the potential interactions between dose effects and the effects of other hazardous substances found in uranium mining and milling effluents. To put results into a modern context of current practices in uranium mining and milling in Canada, potential doses to benthic invertebrates based on recent sediment monitoring data are also briefly reported here.

#### 4.1.2. Methods

##### 4.1.2.1. Historical sediment data

The LEL/SEL data archive was verified for consistency relative to the original statistical analysis in [18]. The data consist of presence/absence information for 190 genera/species of benthic invertebrates at 132 sampling sites in Saskatchewan and Ontario, Canada. Four radioactive substances were measured (uranium,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ ) along with eight other contaminants (As, Cr, Cu, Mo, Ni, Pb, Se, V) for a total of 20 606 data points. Values for natural uranium ( $\mu\text{g/g}$ ) and  $^{226}\text{Ra}$  (Bq/g) in sediments were available for nearly every site;  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  were measured at about three quarters of the sites.

Two estimation methods were used to calculate total dose to a generic benthic invertebrate, the PSL approach [24] and the ERICA approach [22]. The PSL approach refers to the methodology developed during the “Priority Substances List” assessment of releases of radionuclides from nuclear facilities (impact on non-human biota) by Environment Canada and Health Canada. The PSL approach is routinely used for environmental risk assessments for regulatory purposes in Canada. It includes many simplifying assumptions, but remains fit for purpose [25]. Two key features are the use of conservative dose conversion coefficients [26], and the use of a high weighting factor of 40 for alpha particles [27–29]. The ERICA approach was developed in Europe and is used for risk assessments worldwide. It is kept up to date for many parameters in radioecology through an accompanying software tool. In contrast to the PSL approach, ERICA uses more realistic dose conversion coefficients, as adopted for reference animals and plants [23], and includes a default alpha weighting factor of 10 rather than 40. All doses reported here include weighting factors and are thus “biota effective doses” in  $\mu\text{Gy/h}$ .

For simplicity, the conceptual model for a benthic invertebrate (default insect larvae in ERICA) was taken from the PSL approach. Results are hardly affected by size considerations as most of the dose is internal and comes from alpha emitters. A benthic invertebrate was assumed to contain radionuclides at an activity equal to its surrounding sediments on an equivalent dry weight basis (dry weights measured for sediments; invertebrates assumed to be 10% dry weight). Equivalent activity is based on relevant measurements of maximally-exposed benthic invertebrates such as chironomids [24]. In the absence of robust transfer factors for diverse species and environments; this simple approach appeared to be most practical for interpretation of generic results from different screening tools [30]. Total doses calculated here are likely conservative, as uptake of radionuclides by benthic organisms varies considerably [4]. Underestimation of dose is unlikely, except for  $^{226}\text{Ra}$ , which can accumulate in the shells of gastropods and mollusks (as an analog of calcium). Transfer factor compilations (which exclude shells) and dosimetry methods (whole body only) have not yet considered the implications of this phenomenon.

Calculations for an integrated dose estimate in a uranium mining and milling context require activity values for many unmeasured radionuclides. For accuracy, the assumed activities of the three key uranium isotopes should also be used for explicit estimates (the PSL approach ignored this and lumped all uranium isotopes with a single calculation using the dose conversion coefficient for  $^{238}\text{U}$ ). From uranium abundance and specific activity data [31]<sup>4</sup>, generic multipliers for conversion of mg natural uranium to Bq of each isotope are 12.35 for  $^{238}\text{U}$ , 12.29 for  $^{234}\text{U}$ , and 0.5689 for  $^{235}\text{U}$ . Recent peat, soil and rock samples in Canada indicate that the generic conversion parameter for  $^{235}\text{U}$  is reasonable (expected 0.71%  $^{238}\text{U}$ , observed 0.72–0.74% for eight samples) [32]. Dose from  $^{235}\text{U}$  decay chain daughters is potentially large and hence inclusion of realistic estimates of daughters in dose calculations is important, even though very few measurements are available.

For historical continuity, PSL calculations were performed exactly as per EC & HC [24]. In addition to uranium isotopes being lumped in one calculation, external doses were not tallied, and  $^{234}\text{Th}$  and the daughters of  $^{235}\text{U}$  were not included in calculations. To estimate contributions from other daughters,  $^{230}\text{Th}$  was set equal to the activity of  $^{226}\text{Ra}$  rather than

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<sup>4</sup> Natural uranium contains equal quantities, by activity, of  $^{234}\text{U}$  ( $T_{1/2} = 7.69 \times 10^{12}$  sec) and  $^{238}\text{U}$  ( $T_{1/2} = 1.41 \times 10^{17}$  sec), and 569 Bq  $\text{g}^{-1}$  of  $^{235}\text{U}$  ( $T_{1/2} = 2.22 \times 10^{16}$  sec). The equality of activities,  $1.26 \text{ H } 10^4 \text{ Bq g}^{-1}$ , of  $^{234}\text{U}$  and  $^{238}\text{U}$  is due to the fact that  $^{234}\text{U}$  is in secular equilibrium with its parent  $^{238}\text{U}$ . The mass percentages are 99.2837%, 0.7110% and 0.0053% for  $^{238}\text{U}$ ,  $^{235}\text{U}$  and  $^{234}\text{U}$  respectively and the specific activity is  $2.52 \times 10^4 \text{ Bq g}^{-1}$  [31].

$^{234}\text{U}$  (based on available data at that time for sediments),  $^{222}\text{Rn}$  and  $^{210}\text{Bi}$  were set at 30%  $^{226}\text{Ra}$  (based on long-term retention of  $^{222}\text{Rn}$  in vertebrate bone as in human dosimetry). This assumption is also reasonable in terms of measured emanation coefficients for the partitioning of radon in air/water in solids [33]. When values for  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  were not available (about 25% of the time), they were set at 30%  $^{226}\text{Ra}$  (assuming secular equilibrium with  $^{222}\text{Rn}$  retained in tissue). These assumptions are briefly outlined in [24].

For the ERICA approach, fully comprehensive calculations were performed with internal and external doses summed for all daughters in the  $^{238}\text{U}$  and  $^{235}\text{U}$  decay chains. The ERICA dose conversion coefficients are parameterized in terms of daughters such that  $^{222}\text{Rn}$  dose is included in  $^{226}\text{Ra}$ . Hence, it is inherently assumed to be at equal activity to  $^{226}\text{Ra}$ . To provide conservative estimates, doses from other missing daughters were calculated in a similar fashion by assuming secular equilibrium (i.e. daughter = parent). As a result, the ERICA approach accommodates the many uncertainties in using sediment concentrations as surrogates for actual tissue concentrations, while using a more realistic dosimetry model than the PSL approach. For clarity, key features of the PSL and ERICA calculation methods are compared in Table 14.

TABLE 14. COMPARISON OF PSL AND ERICA APPROACHES FOR ESTIMATING TOTAL DOSE TO A BENTHIC INVERTEBRATE FROM MEASURED SEDIMENT DATA; CALCULATIONS ASSUME THAT ACTIVITY IN A BENTHIC INVERTEBRATE EQUALS THE ACTIVITY IN SEDIMENTS ON A DRY WEIGHT BASIS

Parameter	Data	PSL	ERICA
<b><math>^{238}\text{U}</math> decay chain</b>			
Natural uranium	Yes, 96%	25.21 Bq mg <sup>-1</sup> , using $^{238}\text{U}$ DCC	$^{238}\text{U}$ at 12.35 Bq mg <sup>-1</sup> using isotope-specific DCC = parent $^{238}\text{U}$
$^{234}\text{Th}$		Ignored	$^{234}\text{U}$ at 12.29 Bq mg <sup>-1</sup> using isotope-specific DCC = parent $^{234}\text{U}$
$^{234}\text{U}$		Included in natural uranium	
$^{230}\text{Th}$		= daughter $^{226}\text{Ra}$	
$^{226}\text{Ra}$	Yes, 99%	If missing = $^{230}\text{Th}$	If missing = $^{230}\text{Th}$
$^{222}\text{Rn}$		= 30% $^{226}\text{Ra}$	= $^{226}\text{Ra}$
$^{210}\text{Pb}$	Yes, 74%	If missing = 30% $^{226}\text{Ra}$	If missing = $^{226}\text{Ra}$
$^{210}\text{Bi}$		= $^{210}\text{Pb}$ , if also missing = 30% $^{226}\text{Ra}$	= $^{210}\text{Pb}$ , if also missing = $^{226}\text{Ra}$
$^{210}\text{Po}$	Yes, 70%	If missing = $^{210}\text{Pb}$ , if also missing = 30% $^{226}\text{Ra}$	If missing = $^{210}\text{Pb}$ , if also missing = $^{226}\text{Ra}$
<b><math>^{235}\text{U}</math> decay chain</b>			
$^{235}\text{U}$		Included in natural uranium	$^{235}\text{U}$ at 0.5689 Bq mg <sup>-1</sup> using isotope-specific DCC = $^{235}\text{U}$
$^{231}\text{Pa}$		Ignored	= $^{235}\text{U}$
$^{227}\text{Ac}$		Ignored	= $^{235}\text{U}$
$^{227}\text{Th}$		Ignored	= $^{235}\text{U}$
$^{223}\text{Ra}$		Ignored	= $^{235}\text{U}$
<b>Other parameters</b>			
Dose conversion coefficients		Amiro [26]	Brown et al. [22]
Exposure pathways		Internal only	Internal and external
Alpha weighting factor		40	10
Benthic invertebrate		Conceptual chironomid	Insect larvae

Notes: DCC = dose conversion coefficient; a very small number of missing values that could not be readily assigned to a parent were estimated from the nearest logical parent or daughter; the two calculation methods incorporate other daughters in a similar way in DCCs with the exception of  $^{222}\text{Rn}$ ; a benthic invertebrate is assumed to be 10% dry weight.

#### 4.1.2.2. Calculation of LEL/SEL values for total dose

SSLCs and LEL/SEL values were calculated as in [18]. The “weighted method” is the original method of [21]; the “closest observation method” is one of many other possible options for estimating percentiles and is often more conservative (i.e. producing lower values).

For comparison with a typical SSD analysis, SSLCs calculated by the above non-parametric methods (some SSLCs are based on small sample sizes, hence parametric fitting is not appropriate) were fit to a lognormal distribution ( $n = 59$ ). The 5<sup>th</sup> and 95<sup>th</sup> percentiles of this parametric fit represent the LEL and SEL, respectively.

#### 4.1.2.3. Modern sediment data

Radionuclide and other contaminant concentrations in sediments at uranium mines and mills are now routinely measured as part of Canadian licensing requirements. Current surveys include a complete and consistent set of measurements, i.e. natural uranium, <sup>230</sup>Th, <sup>226</sup>Ra, <sup>210</sup>Pb and <sup>210</sup>Po. Most samples consist of surficial sediments collected with Tech/Ops corers at similar times of the year from depositional zones in lakes, with a few creeks and rivers also represented. From a recent compilation [34], data were selected from six areas sampled between 2002 and 2009 to place historical data into a current context, calculating total dose using only the ERICA approach.

### 4.1.3. Results

The lowest effects level (LEL) values derived using different statistical and dose calculation methods varied by about a factor of ten from 13–97  $\mu\text{Gy/h}$ , averaging 40  $\mu\text{Gy/h}$  for eight estimates (Table 15). The LEL values with the closest observation method were lower than the weighted method. ERICA values were also lower than PSL values, as expected from the use of conservative DCCs and a high alpha weighting factor in the PSL approach. SEL values followed similar patterns among methods; estimates varied by a factor of ten from 175–2113  $\mu\text{Gy/h}$ .

Although the SSLCs did not fit a lognormal distribution (all  $P < 0.002$ ), fitting SSLC non-parametric results to this distribution did not produce substantially different LEL/SEL values relative to a fully non-parametric approach (Table 15).

Altogether, the average LEL calculated here (40  $\mu\text{Gy/h}$ ) was very similar to the conceptually-equivalent threshold of effect (SSD\_HDR<sub>5</sub> of 43  $\mu\text{Gy/h}$  in Table 13) calculated from laboratory-based experiments with invertebrates. This finding is remarkable given the many contributing factors leading to the derivation of thresholds in each type of calculation and the different nature of the underlying data sets.

Doses based on the ERICA calculation approach for modern sediment data were low relative to the historical data set used to develop LEL/SEL values (Table 16), reflecting modern improvements in pollution prevention at uranium mines and mills. Average values at five exposure sites ranged from 2.5–47.6  $\mu\text{Gy/h}$ ; values lower than or similar to the ERICA LEL estimates (13–43  $\mu\text{Gy/h}$ ). The average dose at the newest mine and mill (2.5  $\mu\text{Gy/h}$ ) was nearly identical to the average dose at reference sites (2.6  $\mu\text{Gy/h}$ , excluding one reference outlier in the reference average, Zimmer Lake).

TABLE 15. LEL/SEL VALUES FOR 59 SSLCS FOR TOTAL DOSE TO A BENTHIC INVERTEBRATE ( $\mu\text{Gy/h}$ ) USING VARIOUS STATISTICAL APPROACHES TO ESTIMATING PERCENTILES

Approach	Percentile	ERICA	PSL
<b>Non-parametric</b>			
LEL	Closest observation	13	36
	Weighted	43	97
SEL	Closest observation	175	1544
	Weighted	499	2113
<b>Lognormal fit for non-parametric SSLCs</b>			
LEL	Closest observation	12	31
	Weighted	24	60
SEL	Closest observation	552	1337
	Weighted	685	1701

NOTES: Dose is based on extrapolations from natural uranium,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  measured in sediments and consideration of unmeasured daughters in the  $^{238}\text{U}$  (ERICA & PSL) and  $^{235}\text{U}$  decay chains (ERICA only).

TABLE 16. ERICA ESTIMATES OF TOTAL DOSE TO A BENTHIC INVERTEBRATE ( $\mu\text{Gy/h}$ ) BASED ON MEASURED NATURAL URANIUM,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$  AND  $^{210}\text{Po}$  ACTIVITY IN THE 0–2 cm SEDIMENT LAYER AT SITES RECEIVING URANIUM MINE AND MILL EFFLUENTS IN NORTHERN SASKATCHEWAN, CANADA COMPARED WITH REFERENCE SITES FROM 2002 TO 2009

Context	Area	Mean	Std Dev	N
Reference	All data	17.0	61.3	188
	Zimmer Lake	273.1	34.7	10
	All data excluding outlier	2.6	1.3	178
Exposure	Modern mine and mill	2.5	1.4	58
	Mine and mill	29.8	34.6	50
	Mine and mill	47.6	77	130
	Mine only	14.6	11.5	27
	Mine under development	3.2	2.8	8

#### 4.2. MULTIVARIATE APPROACH TO BENTHIC INVERTEBRATES ASSOCIATED WITH URANIUM MINING AND MILLING IN CANADA

##### 4.2.1. Introduction

The data are from Uranium mining areas located in Saskatchewan and Ontario, Canada. Benthos was identified at least to the family level, but generally to the genus/species level and recorded as presence/absence – number of individuals. The biological data were coupled with measurements of radiological and non-radiological contaminants in water and sediment.

The Canadian Benthic Database was previously analyzed through a “univariate” approach (see Section 4.1). A Screening Level Concentration (SLC) method was used to derive Lowest Effect Level (LEL) and Severe Effect Level (SEL) concentration for 9 metals and 3 radionuclides. For the Biota Effects Working Group, a “multivariate” analysis was performed to determine if changes in species diversity of the benthos community can be explained by changes in contaminant concentrations in sediment.

#### 4.2.2. Methods

The multivariate approach was conducted using constrained and unconstrained ordination methods (i.e. Redundancy Analysis (RDA) and Principal Component Analysis (PCA), respectively). Redundancy Analyses can be seen as an extension of Principal Components Analysis because the canonical ordination vectors are linear combinations of the response variable Y. These methods required building two matrices: (1) a species matrix containing the presence/or absence of each of the 209 species along the 196 sites; and (2) a contaminant matrix, containing the concentration of the 12 contaminants along the 196 sites.

Because the contaminant matrix contained many N/A (not available or missing) values, only 31 sites had complete concentration data and were able to be analyzed with classical ordination methods RDA and PCA analyses. This dataset was called “complete dataset”. Ordination methods, realized after Hellinger transformation of the species matrix, gave similar results and permit to bring to light some “contaminant—species” combinations (e.g. Vanadium contaminant and *Pisidium* species).

Another method of analyze was tried in order to take in account all the sites (even those having missing data). This dataset was called “all data set”. This approach consisted of doing a PCA analysis on the presence/absence species matrix (after Hellinger transformation) making groups of sites using ‘kmeans’ method with the PCA coordinates characterizing groups in terms of contaminants concentration characterizing groups in terms of species too, in order to find species/contaminant combinations. The method developed has the advantage that it takes into account all the available information (i.e all the sites, not just the 31 out of 196). However, it appears that it also suffers several drawbacks (Table 17): (1) it does not consider sites, species and contaminants individually but into groups; thus it is not directly comparable with classical ordinations approach; (2) it does not bring to light the association of several species with a specific contaminant but instead with a mixture of contaminants; (3) it does not order the contaminants according to their “power” on the species distribution; (4) it only considers the presence of a species (not the absence).

TABLE 17. COMPARISON OF STRENGTHS AND WEAKNESSES OF THE MULTIVARIATE METHODS

	<b>Developed method</b>	<b>“PCA + vector fitting” or RDA</b>
Strength	Take all the available information (less influence of possible confusing variables)	Consider sites, species and contaminants individually  Bring to light association of several species with a specific contaminant  Consider the presence and the absence of species
Weakness	Does not consider sites, species and contaminants individually but into groups  Does not bring to light association of several species with a specific contaminant but with a mixture of contaminants  Does not order the contaminants according to their “power” on the species distribution  For the moment only permits to consider the presence of a species (not the absence)	Can only be used with no N/A values, so loss of information and perhaps influence of possible confusing variables on the results

### 4.2.3. Results

The Redundancy Analysis (RDA) identified three significant contaminant variables: vanadium, copper and chrome (Figure 3).

Observed ‘contaminant-species’ associations based on the RDA were:

- **Vanadium:** Negative correlation with three benthic species (*Parakiefferiella*, *Ablamesmyia*, *Stichtochironomus*) and positive correlation with another three benthic species (*Pisidium*, *Microspectra*, *Eukiefferellia*).
- **Chrome:** Negative correlation with two benthic species (*Microspectra*, *Proladius*) and positive correlation with three benthic species (*Caenis*, *Hexagenia*, *Clinotanypus*).
- **Copper:** Negative correlation with two benthic species (*Chaoborus*, *Cryptochironomus*) and positive correlation with four benthic species (*Paracladepelma*, *Probezzia*, *Ilyodrilus*, *Templetoni*).

Principal Components Analysis, with vector fitting, was then used on the same data set. PCA is an unconstrained ordination method that displays all the compositional variation, rather than just the variation explained by the environmental variables, as in RDA. The results (Figure 4) were very similar to those obtained from the RDA.

RDA seems to be an appropriate method because it permits to highlight the relationship between some species (presence and absence) and some contaminants. However, the robustness of these results should be questioned because only analyses were possible with only 31 sites. The RDA permitted a causality link between the contaminants and the distribution of the benthic species. The PCA method is strictly correlation, no causality link; however, it does take in account all the variation. Use of both methods were restricted to the 31 sites.

The All-data method, developed within WG6, permitted analyses of all 196 sites (Figure 5). In both the analyses using 31 sites and the “All-data” analyses using 196 sites, *Procladius* was the most present species in sites characterized by high concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  (25.4% of sites in “all data” set and 30.25% of sites in “complete data” set). For both datasets, *Chironomus*, *Chaoborus* and *Pisidium* were members of the five most frequent species in groups of sites characterized by a low concentration level in  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ . However, in these last groups of sites (characterized by low concentrations in  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ ) the *Procladius* species is also relatively frequent (in 21.5% of the sites from the “all data” set and in 20.12% of the sites from the “complete data” set). Because “all-data” and “complete data” some same “contaminants-species” combinations appear, it seems that the method developed is able to bring to light real “contaminant-species” combinations. Importantly, the method developed is able to support the hypothesis of contaminant control of the benthos distribution. However, because some species (e.g. *Procladius*) were present in both groups of sites characterized by high and low concentrations of  $^{210}\text{Pb}$  and  $^{210}\text{Po}$ , it means that the method lacked precision.

The group concluded that analyses could perhaps be improved by considering the total dose combined from all radionuclides as one of the components in the multivariate analysis, rather than considering individual radioisotopes (presence or absence, with no dose). Finally, the absence of species, rather than their presence needs to be explored. A step-wise regression approach might increase data availability.

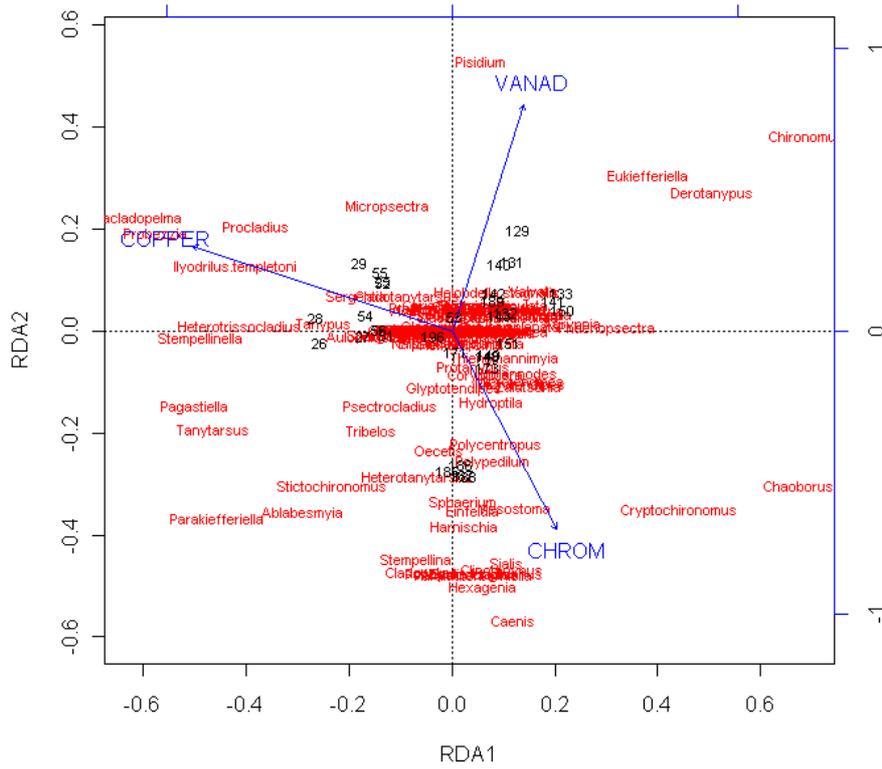


FIG. 3. Results of a Redundancy Analysis (RDA) of the “complete data set” (31 sties) within the Canadian benthic data. Sites are represented by black numbers, species by red names, and contaminants by blue arrows.

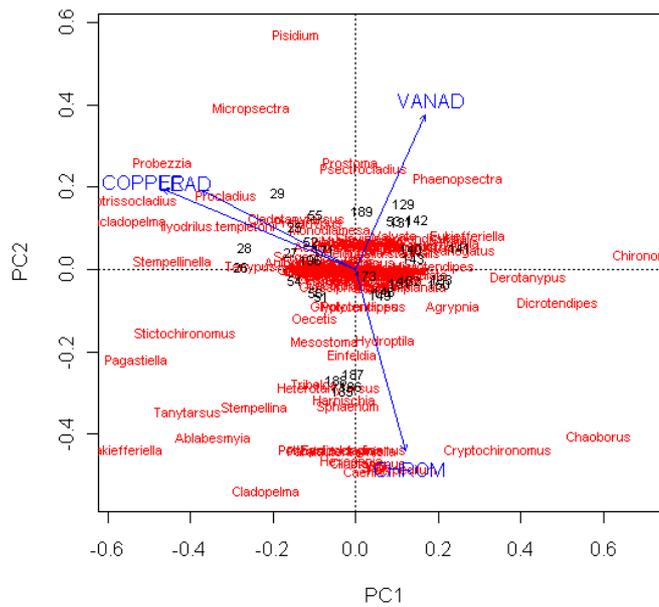


FIG. 4. A plot of the Principal Components Analyses (PCA) with vector fitting. Sites are represented by black numbers, species by red names, and contaminants by blue arrows.

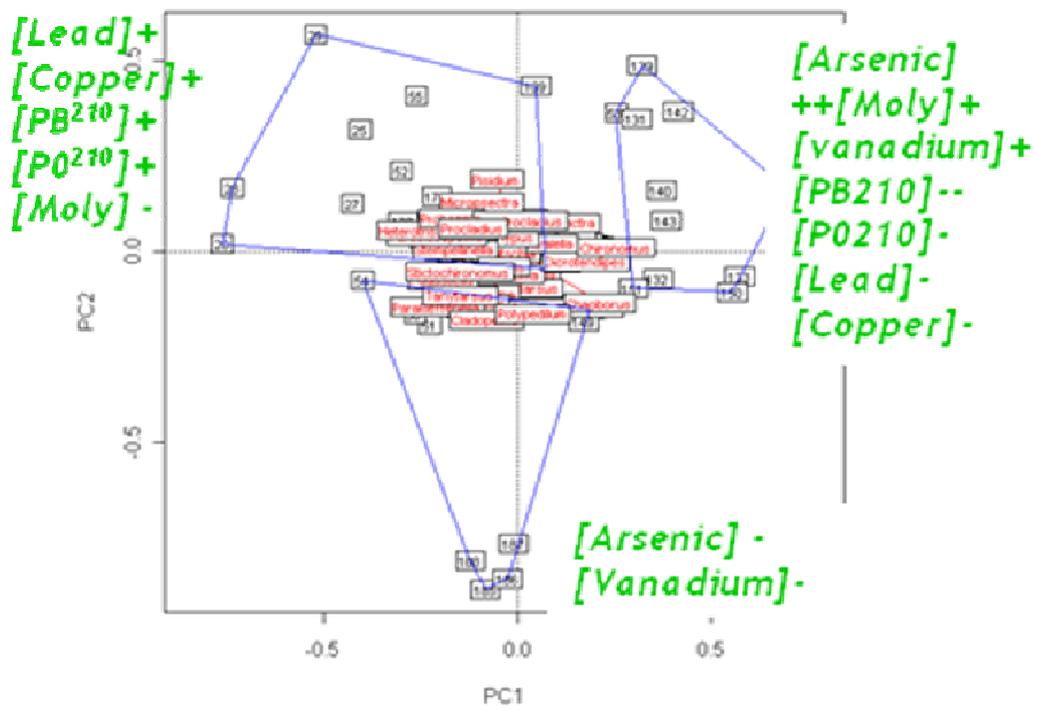


FIG. 5. Distribution of contaminants, species and all 196 sites using a method developed with the working group.

## 5. MIXED CONTAMINANT WORKING GROUP

### 5.1. GENERAL AIMS

All organisms are exposed to a complex mixture of stressors; and yet, contaminants are managed as if they occurred in isolation within the environment. The chemistry industry is spending much time and money examining multiple stressors. The Biota Dose Effects Group (WG6) reviewed the literature for multiple stressor data in which radiation was among the mix, and reviewed approaches used in ecotoxicology to determine their applicability to multiple stressor effects assessments for situations including radiation.

The work was done in collaboration with the International Union of Radioecology (IUR).

### 5.2. LITERATURE REVIEW ON MULTIPLE STRESSOR STUDIES INCLUDING RADIATION OR RADIONUCLIDE EXPOSURE AS ONE OF THE STRESSORS

Ecosystems are exposed to combinations of anthropogenic and natural stressors. Studies were reviewed that investigated combined effects of ionizing radiation and other stressors on non-human biota, with the aim of clarifying if the observed combined effects are generally additive, synergistic or antagonistic [35]. Therefore, a multiple stressor database was established and data were collected for terrestrial, freshwater and marine ecosystem animals and plants. Information was collected on ecosystem type, species considered, stressors applied and effects evaluated. In total, approximately 50 papers were included, covering terrestrial plants, aquatic plants, terrestrial animals, freshwater animals, aquatic microcosms, and marine and estuarine animals. Experiments on tumour induction were not included in the final analysis. As a result of their evaluation and quality control analysis, 35 papers were included in the final review: 20 on aquatic (freshwater, estuarine and marine) animals, 10 on terrestrial animals, 4 on terrestrial plants and 1 on aquatic plants.

For terrestrial and aquatic plants, studies investigated two-component mixtures with one radionuclide or one radiation type in combination with metals, other radionuclides or radiation types, pro-mutagens and herbicides. All plant studies but one were based on laboratory experiments. The animals studied were rat, mouse, frog, salmon, medaka, eel and brine shrimp. 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. All animal studies reviewed were based on laboratory experiments.

There was no overall pattern of interactive effects in the papers reviewed. In some cases no interactive effects were observed, in others additive, synergistic or antagonistic effects were seen, and in some cases the effects were dose- or concentration-dependent. The database is an important step in establishing the state-of-knowledge in multiple stressor studies involving radiation, and will be useful for prioritizing and designing future experiments. Further research in the interdisciplinary field of multiple stressors (including radiation) is needed to allow prediction of potential combined effects of low exposure levels on biota. The lack of systematic mechanistic understanding and quantitative assessments of combined exposures and the resulting possible interactions urgently needs to be resolved.

### 5.3. STATE OF THE ART ON APPROACHES AND TOOLS FOR EFFECTS ASSESSMENT UNDER MIXED CONTAMINANT CONDITIONS AND APPLICABILITY TO MIXTURES INCLUDING RADIATION

#### 5.3.1. Introduction

Increasing industrialization and population density have led to situations where mankind and its environment are exposed to a multitude of stressors and contaminants of which very often little is known about their mid- and long-term health and ecological consequences. Legislation is still mainly based on effects of single compounds, but in real life there is no such thing as ‘a single chemical exposure’. Although the number of studies addressing possible mixture interactions is steadily growing, there is still a pressing need to obtain sufficient experimental data to support ecological risk assessment (ERA) of mixtures and to identify mixture exposure situations that may cause unexpectedly high risks compared to the standard null hypothesis of concentration addition. The aim of this report was to give an overview on how effect assessment in multiple stress situations is currently performed and how to apply this within a radiological context.

Within the STAR Network of Excellence an extensive effort was made to critically review approaches for multiple stressor research from exposure, effect and risk assessment point of views [36]. As a number of people involved in the IAEA workgroup on multiple stressors were also involved in writing the STAR deliverable, the report given here benefits from the work that went into making this review and is hence, mainly based on Chapter 4 of that deliverable, found at: [www.star-radioecology.org](http://www.star-radioecology.org).

This chapter is introduced by giving a general description of modes of actions for effects from ionizing radiation and a comparison with the general modes of actions seen by various toxicants. This is followed by a section dealing with the two most commonly used component based reference models, concentration addition (CA) and independent action (IA). Their weaknesses and strengths and possible deviations from the models are described. An overview of different experimental designs used to test CA/IA is presented. The next section focuses on whole mixture approaches, including Whole Effluent Toxicity testing (WET), Effect-Directed Analysis (EDA) and Toxicity Identification Evaluation (TIE). These are diagnostic approaches that include step-by-step assessments of toxicity from an environmental sample and have close connection to approaches used in risk assessments. This is followed by a section which describes an overview of bioassays and biomarkers in radioecology. Then toxicokinetic (TK) applications are described, and include the fate of toxic components in the organism from the point of absorption, internal distribution, metabolism and excretion. Toxicodynamic (TD) approaches of a toxic mixture are then described. TD approaches incorporate how components affect the organism over time. Dynamic Energy Budget (DEB) theory is also discussed. DEB provides a conceptual framework which specifies how energy is taken from food and allocated to growth and reproduction, including reallocation of energy required following exposure to contaminants. Finally, issues are considered such as quality criteria and data demands needed for the different approaches.

##### 5.3.1.1. *Short description of mode of action of ionizing radiation*

Radiation is the physical process in which energetic particles or energetic waves move through a medium. Ionizing radiation is any kind of radiation that when it interacts with material can induce (directly or indirectly) ionization. Ionisation occurs if the radiation has

sufficient energy to eject one or more orbital electrons from the atom in which it interacts. There are different types of ionizing 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 aluminum and for gamma rays a denser material, such as lead, is needed. Alpha particles are not harmful to organisms as long as they are outside of the body because they cannot penetrate through the outer (dead) cell layer of skin. However, once taken up, alpha particles form a greater risk to sensitive linings of lung or gut. The term relative biological effectiveness (RBE) is used to indicate 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 (i.e. alpha particles are 20 times more effective at causing ionization).

Biological effects induced by ionizing radiation in organisms originate from the deposition of energy from the radioactive material to biomolecules (e.g. DNA, proteins). Ionizing 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 ionizations. As for a great number of other biotic stressors, ROS can be formed, for instance, through the radiolysis of water. Hence, ionizing radiation can lead to DNA lesions, including oxidized and methylated bases, DNA adducts, and single- and double stranded breaks [37]. 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 mis-repaired then cell death can occur, or DNA mutations can form 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 are necessary for an appropriate description of the overall toxicity.

Currently, for protection of wildlife and ecosystems, population-linked individual endpoints (morbidity, reproductive capacity, mortality) are required and not DNA damage, which can be considered as an early marker of a potential effect. A challenge, that has yet to be met in radioecology, is to quantitatively link molecular and individual endpoints to effects such that predictions of effects to populations can be reliably made [38].

#### *5.3.1.2. 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 [39]. 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 utilizes four categories [40]:

- **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 [41] than non-polar narcotics.
- **Non-polar reactive compounds** react non-selectively with chemical structures within cells [42];
- **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 [43].

### 5.3.2. Component-based modelling concepts and deviations of these reference models

#### 5.3.2.1. 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 (CA) 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 [44–46]. It is written mathematically as follows:

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

with  $c_i$  the exposure concentration (EC) of chemical  $i$  in the mixture which elicits  $x\%$  effect,  $ECx_i$  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 [47], 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:

$$\sum_{i=1}^n TU_i = TU_{mix} \quad (2)$$

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  $\Sigma TU$  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:

$$ECx_{mix} = \left( \sum_{i=1}^n \frac{p_i}{ECx_i} \right)^{-1} \quad (3)$$

The concept of Independent Action (IA), 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:

$$E_{A,B} = E_A + E_B - (E_A * E_B) \quad (4)$$

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:

$$E_{mix} = 1 - (1 - E(c_1)) (1 - E(c_2)) \dots (1 - E(c_n)) \quad (5)$$

$$E_{mix} = 1 - \prod_{i=1}^n [1 - E(c_i)] \quad (6)$$

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 [46, 48].

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 [46].

#### 5.3.2.2. *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 [45]. 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 [49]. Hence, for the use of this kind of model it is highly recommended to obtain the best set of parameters for the sigmoidal regression curve, see [50] for a description of 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 [51, 52]. 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 [53, 54]. 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 [55, 56].

A so-called Two-Step Prediction (TSP) model can be applied to deal with mixtures containing components that have similar and dissimilar modes of action [57]. 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. Since results from CA/IA do not differ too much, often the more conservative CA is used in risk assessment purposes.

#### 5.3.2.3. *Use of Concentration Addition (CA) and Independent Action (IA) 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 [45]. 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 [44, 55]. 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 [48].

The basic principles of CA are used for risk assessment purposes in methods like hazard index (HI), relative potency factor (RPF) and toxicity equivalency factor (TEF) [51].

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, it was found that IA was better than CA for dissimilarly acting pharmaceuticals and personal care products [58]. 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 [45].

The principle of strict independent effects may only rarely hold true due to converging stress signaling 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 (ERA), CA has generally been indicated as the more broadly applicable option. Theoretically (assuming infinitely steep dose-response curves for the single components) the EC<sub>50</sub> 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 [58]. However, in reality dose-response curves are not infinitely steep and CA and IA predictions are often close together. In a study based 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 [58]. For chemicals inducing dose-response curves with a log-logistic slope around one, the predictions of IA and CA were the same [59, 60].

#### 5.3.2.4. *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 [61] and endpoint considered [62] 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 [63]. 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 not equalling one. 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 the sum of TU observed deviates from the theoretical one it is said that there is an interactive mixture effect. 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 [64]. A MIXTOX model based on CA and IA has been developed to characterize mixture interaction effects by quantifying the degree of deviations of the data from either reference model [65]. 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 and in practice it is hard to reproduce dose-ratio or dose-level dependent interactions [62]. 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 [66, 67].

#### 5.3.2.5. *Limitations of Concentration Addition (CA) and Independent Action (IA)*

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 characterized 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 [45]. 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 [45].

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 concentrations or 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 [48]. Whereas, the dynamic DEBtox model 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 precautionous assessments, it was suggested that the relationships between CA and IA should be considered [68]. 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 [69].

#### 5.3.2.6. *Effects of low doses in mixtures*

From an environmental toxicological as well as risk assessment perspective, it is necessary to know whether or not several 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 [58] “*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 consequently reviewed [70, 71] and has been referred to as “Something from Nothing” [72].

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 [58]. A NOEC 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 [58, 71]. A number of studies on mixtures of dissimilarly acting compounds present at threshold values were summarized and showed that clear joint mixture effects sometimes above 50%, were detected in different studies [70, 71].

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” [58], these concentrations might however only exist in theory.

### 5.3.2.7. *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.

#### *5.3.2.8. Use of CA and IA approaches in mixtures including radiation or radionuclides as one of the components*

Within the framework of the IAEA’s 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 [35]. 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.

### **5.3.3. 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 5.3.4). 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.

#### 5.3.3.1. *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) [73]. 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 results obtained 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 [74, 75].

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 [76]. 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 [71]. 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 organizations 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 bioassay). 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 [76]. EDA is less widely used in risk assessment and the method is less standardized 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.

#### 5.3.3.2. *Drawbacks of TIE and EDA approaches and their relevance for studies of mixtures including radiation*

Extraction can chemically alter the speciation 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 [77]. It also requires sophisticated preparative and analytical tools to identify the pertinent compounds [76].

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, bioassays specific to radioactivity do not exist and thus can at this point not be used to narrow down the toxicant/biotest combinations.

### 5.3.4. Biological testing of mixtures

#### 5.3.4.1. Different biological testing approaches

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 standardized 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 that biological 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 [50].

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 18. In contrast to *in vivo* bioassays, the methodology for *in vitro* tests is less well standardized.

TABLE 18. EXAMPLES OF *IN VIVO* AND *IN VITRO* BIOASSAYS

<i>in vivo</i>	<i>in vitro</i>
Invertebrates e.g. <i>Daphnia</i> , <i>Hyalella</i> , <i>Artemia</i> , <i>Mysidopsis</i> , <i>nematode</i>	inhibition of bacteria <i>Vibrio fischeri</i> (Microtox)
Fish e.g. trout, minnow, zebrafish, medaka	enzyme induction e.g. EROD, cytochrome P450, CYP1A
Single-celled algae e.g. <i>Scenedesmus</i> , <i>Selanestrum</i>	aryl hydrocarbon receptor (AhR) agonists using the DRCALUX assay
Algae and plants e.g. <i>Ceramium</i> , <i>Champia parvula</i> , <i>Lemna</i>	mutagenic activity using the Mutatox assay
Embryo tests e.g. sea urchin, <i>Crassostrea</i> (oyster), FETAX ( <i>Xenopus</i> 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 [78]. 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) [79] and are more indicators of general stress in an organism/biological system. Brooks [80] 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.

#### 5.3.4.2. *Applicability to radioactive mixtures*

The mode of action of radiation is described in the introduction. 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. An overview of different studies that aimed at identifying radiation specific biomarkers or markers that distinguish between radiosensitive and radioresistant species was recently published [50]. These include examples of biomarkers of both exposure and sensitivity that can be utilized within both human and ecological toxicology to identify the response to ionizing radiation on different levels complexity (from molecular, cellular to organism levels). The possible biomarkers can also be classified according to whether they test for DNA damage and repair mechanisms (e.g. antibodies against gamma-H2AX, ploidy determination, quantification of chromosomal aberration, comet assay), oxidative stress (e.g. ROS determination, antioxidative enzyme assays, determination of oxidative induced lipid peroxidation) or general stress responses (determination of cytp450 activity or quantification of HSP70).

### 5.3.5. 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 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. It has been demonstrated 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 [81].

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, metabolism 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 [82–84], starry flounder [85], salmon [86], channel catfish [87] and beluga [88]. 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 [89]. Asiatic clams and zebra mussels were exposed to  $^{57}\text{Co}$ ,  $^{110\text{m}}\text{Ag}$  and  $^{134}\text{Cs}$ , in mixtures with Zn, Cd or Cd+Zn. Zn and the Cd+Zn treatment increased the  $^{110\text{m}}\text{Ag}$  uptake in mussels and clams and also increased the  $^{110\text{m}}\text{Ag}$  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 [90]. A one-compartment model was also used for the analysis of time-series survival data for the springtail *Folsomia candida*, but without taking into account the toxicokinetic interactions [61].

TK interactions between metals and organic compounds have also been shown. For example, in the amphipod *Hyaella azteca*, chlorpyrifos enhances the accumulation of methylmercury, but as methylmercury presumably forms a chlorpyrifos-MeHg complex, the toxic effect (acetylcholinesterase inhibition) is reduced [91].

### **5.3.6. Toxicodynamics including Dynamic Energy Budget**

#### *5.3.6.1. 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 [92].

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  $\text{LC}_{50}$  or  $\text{EC}_{50}$  can be obtained.

The CBR approach has been applied to mixture toxicity of narcotic chemicals at a single time-point [93, 94]. 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  $\text{LC}_{50}$  [95].

The stochastic approach of Bedaux and Kooijman [96] has been extended to mixtures by Baas et al. [61]. 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, studies showed that the apparent mixture interactions change with time [97, 98]. Different interactions were also observed for the toxicity of a mixture of U and Se on the daphnids, depending on the endpoint studied [90]. The statistical analysis method of Jonker [98] 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 [99]. 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.

### 5.3.6.2. *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 [100, 101]. 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. 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)<sup>5</sup>.

$$\begin{cases} s = 0 & \text{if } c \leq NEC \\ s = \frac{I}{c_T}(c - NEC) & \text{if } c > NEC \end{cases} \quad (7)$$

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 [102].

The approach has already been successfully applied to a range of chemicals and biological species to understand effects of mixtures on growth, reproduction and survival [61, 66, 67, 101, 103, 104]. In a mixture context effects on organisms result from the combined individual actions of each single compound composing the mixture. Figure 6 shows the structure of the DEB approach for mixture toxicity.

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 [67], 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);

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<sup>5</sup> 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.

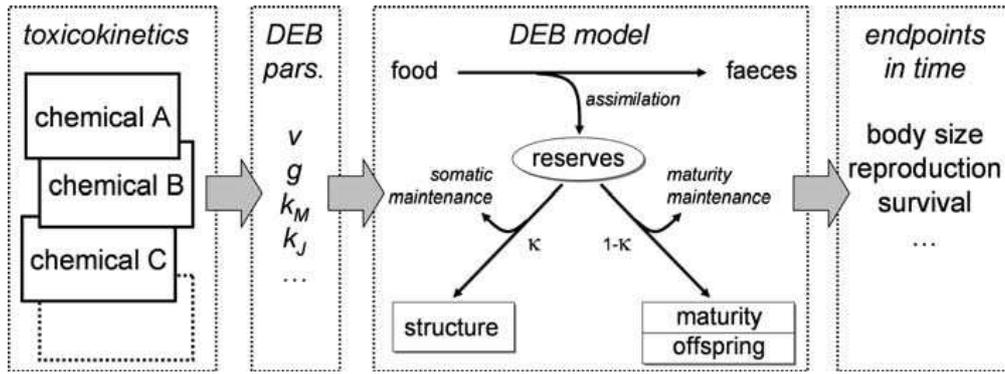


FIG. 6. Modelling approach, including a toxicokinetic module as a first step, followed by a description of how processes are affected by each toxicant and a feedback on the kinetics as a result of observed effect on growth [67].

- 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:

$$(1 - s_A) \times (1 - s_B)_{\text{or}} (1 + s_A) \times (1 + s_B) \quad (8)$$

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:

$$c_+ = c_A + W_B \cdot c_B \quad (9)$$

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 [67]. This in its turn is similar to the additivity principle that is also behind the concept of CA for single dose-response curves.

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 [66, 100]. 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 (*Pimephales promelas*), the flour beetle (*Tribolium castaneum*), the nematode (*Caenorhabditis elegans*) and the microcrustacean *Daphnia magna* (Table 19). The recent study from [67] was the first to apply a biology-based approach for mixture toxicity of multiple endpoints over the life cycle on daphnids for two PAHs.

TABLE 19. LIST OF STUDIES USING DEB-TOX FOR THE DESCRIPTION OF COMBINED EXPOSURE OF TOXICANTS

Tested species	Type of mixture	Endpoints	Conclusion	Reference
Flour beetles ( <i>Tribolium castaneum</i> )	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	[101]
Fathead minnows ( <i>Pimephales promelas</i> )	14 PAH mixture with known Kow values	Survival	Same conclusions as above	[66] and references therein
Fathead minnows ( <i>Pimephales promelas</i> )		Survival	Application of the hazard model from DEBtox to survival data. Different modes of action resulted in different patterns in the parameter estimates.	[104] and references therein
Collembolan ( <i>Folsomia candida</i> )	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.	[61]
Waterflea ( <i>Daphnia magna</i> ) 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	[103]
Waterflea ( <i>Daphnia magna</i> )	Pyrene and fluoranthene = narcotic mode of action, with negligible metabolism	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.	[67]

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 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 organization (coupling of DEBtox outcomes with population dynamics using Leslie matrices).

The key strengths of DEBtox approaches can be summarized 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 parameterize the model both for the organism as for the toxicant.

### 5.3.7. General discussion

#### 5.3.7.1. *Comparing different approaches: Challenges and knowledge gaps*

For the different approaches described above, Table 20 gives a comparative overview of the different data requirements, applicability and capacity to predict mixture effects.

A number of empirical and conceptual knowledge gaps of mixture toxicity approaches can be defined [58, 71]. 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 [58]. 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 action 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. 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 [50].

TABLE 20. 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	<ul style="list-style-type: none"> <li>– Dose-response curve of individual toxicants (if not CA/IA lose their capacity to predict)</li> <li>– Concentrations of all toxicants in the mixture</li> <li>– Monotonic dose-response curves</li> </ul>	<ul style="list-style-type: none"> <li>– Parameters describing growth, survival, reproduction of individual species</li> <li>– Parameters describing toxic effects of individual toxicant</li> <li>– Concentration of all toxicants in the mixture</li> </ul>	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 parameterized and toxicants for which a toxicokinetic module has been developed	<ul style="list-style-type: none"> <li>– Whole mixture is tested; as such results only applicable to that specific mixture</li> <li>– Identification of different toxicants and of effect contributing toxicants by TIE or EDA depends on available fractionation techniques</li> </ul>
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

Whole mixture approaches are normally not conducted with the aim of elucidating interacting effects or be able to predict mixture effects (Table 20). 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 [74]). 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 [62]. 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 [62]. 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 [105]. 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.

#### 5.3.7.2. *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 on 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 [35]. 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 statements about additivity, synergism or antagonism were often 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 dose-response curves for the single stressors were rarely 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 [35] 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 report 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 summarized in Table 20 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 are containing radionuclides (for an overview see Annex 1 of the STAR Deliverable 4.1 [106]), 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 [15, 107]. 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) [108–110] and as such do not deliver monotonic dose-response curves. The effect of hormesis in binary mixtures was studied [111] 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 [111]. 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 [112]. Recent studies have shown the possibility of applying DEBtox for combined exposures (see Table 19). 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 [113] and fish [114]. As such the success to apply DEBtox to mixtures containing radiation or radionuclides depends largely on obtaining the necessary data for parameterizing 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.

## 6. POPULATION MODELLING

### 6.1. INTRODUCTION

Radiological protection of the environment has advanced from the old paradigm that stated that if humans are protected then all other components of the environment are protected as well. Now, the ICRP recommends explicit consideration of the environment and new approaches are being developed. These new developments do not focus on the protection of individuals within the environment, but strive to protect populations of individuals or higher organizational levels (communities, ecosystems; [2]).

Most effects measurements, however, are taken on individuals within a population. Extrapolation is generally required to estimate population level effects from the individual-based measurements. Improved models that propagate effects from individuals to populations will decrease the uncertainties associated with current extrapolation methods. The impact of a specific dose to a population of organisms is likely to be dependent on the life history characteristics of that particular species. Populations that produce an abundant number of offspring at frequent intervals are probably less sensitive to radiation than populations of species that reproduce much less frequently and with lower fecundity. Incorporating such life history characteristics into effects models is needed and will likely improve predictions of effects.

The Population Modelling Group<sup>6</sup> reviewed existing population models, developed life history data sheets for key species, and incorporated population models into effects analyses. One of their objectives was to derive basic equations that govern population models while incorporating radiation effects, with an emphasis on finding an equation that is simple enough to be generally applicable across different species.

### 6.2. REVIEW OF EXISTING POPULATION MODELS APPROPRIATE FOR ADAPTATION IN RADIATION EFFECT ASSESSMENT (NON-HUMAN BIOTA)

A questionnaire was distributed among participants in 2009, which was intended to help the participants in reviewing the existing population models and analyzing the possibility of adapting the models for predicting effects from radiation. Existing population models [115–124] were reviewed, and a set of eight population models was selected, most of which were specially designed to describe radiation effects in populations (Table 21). These models formed a good basis for developing a generic population approach, to simulate the main features of radiation effects in a population, and to show the key parameters responsible for the response of populations to radiation damage. It should be noted that most of the models existing in 2009, and had not been validated with real data. Thus, a strong need existed for a scenario of model inter-comparison. It should also be noted that the models reviewed were designed earlier for different purposes, and they were not exactly generic. A detailed description of the models used is available [125].

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<sup>6</sup> A subgroup within EMRAS II Working Group 6.

TABLE 21. PARTICIPANTS IN THE POPULATION MODELLING SUB-GROUP WITH SOME GENERIC INFORMATION ABOUT THEIR MODELS

Authors	Type of model	Generic or specific	Environmental stresses considered	Validation or parametrization of parameters
Vives i Batlle, J., et al.[125]	Logistic growth model, age classes	Specific for European lobster	Radiation, fishing	Parametrization
Doi, M., Kawaguchi, I., [115]	Aquatic microcosm model, 3 species	Specific for experimental microcosm	Radiation, ecological interactions	Validation on experimental data
Kryshev, A., et al. [120]	Dynamic population model, repair	Generic fish	Radiation, parasites	Parametrization comparison with data
Alonzo, F. et al. [122]	Model of age-structured population	applied to earthworm and Daphnia	Radiation	Parametrization
Monte, L., [124]	Model based on Lotka-Volterra equations (resources and consumers)	Generic terrestrial	Radiation, migration	Parametrization
Sazykina T. et al. [126]	Ecosystem model with limited resources	Generic aquatic	Can be applied to radiation	

### 6.3. DEVELOP GENERIC POPULATION MODEL FOR RADIOLOGICAL ASSESSMENT

The objective of this exercise was to develop a mathematical model that adequately simulates radiation effects in an isolated, chronically exposed population at different dose rates, and that shows the key parameters responsible for the response of the population to radiation exposure.

The following three umbrella endpoints were considered: morbidity, reproduction, and decrease of the population size. Several generic models were suggested:

- Logistic growth model (all; Figure 7);
- Population model with 2 stages – young and mature organisms [122, 124, 125];
- Population model with stochastic parameters (risk of extinction) [115];
- Population in a limited environment with “dose rate-effect” formulas for model parameters [126] (Figure 8)

### 6.4. DEVELOP SCENARIO FOR MODEL APPLICATION

The Benchmark scenario “Population response to chronic irradiation” was formulated distributed among participants. The benchmark scenario included chronic, low-LET radiation exposure with dose rates between 1–50 mGy/d to 4 generic mammal populations (e.g. mice, hare/rabbit, wolf/wild dog and deer) with different life characteristics. Before irradiation, each population was in a stable state and consisted of 1000 animals, which corresponded to the carry capacity of the environment. The benchmark endpoints included predictions of the decrease in population size at the end of each year of exposure (total duration of exposure – 5 years), as well as time of population recovery to initial size after termination of irradiation.

## Basis of the population model

- Logistic function with a built-in self-recovery capacity:

$$\frac{dN_0}{dt} = rF \left( 1 - \frac{N_0 + N_1}{K} \right) \left( 1 - \frac{W}{N_1} \right) - (s + d_0)N_0$$

$$\frac{dN_1}{dt} = sN_0 - d_1N_1$$

$$\frac{dF}{dt} = -rF \left( 1 - \frac{N_0 + N_1}{K} \right) \left( 1 - \frac{W}{N_1} \right) - fF \left( 1 - \frac{F}{L} \right)$$

- Where:
  - $N_0, N_1$ : Population numbers for young and adult
  - $F$ : Fecundity
  - $K = L$ : Carrying capacity and fecundity recovery constant
  - $r = f$ : Reproduction and fecundity rates
  - $s, d_0, d_1$ : growth and death rates

FIG. 7. Basis of a population model built on a logistic function with recovery capacity [125].

## Effects and repair model

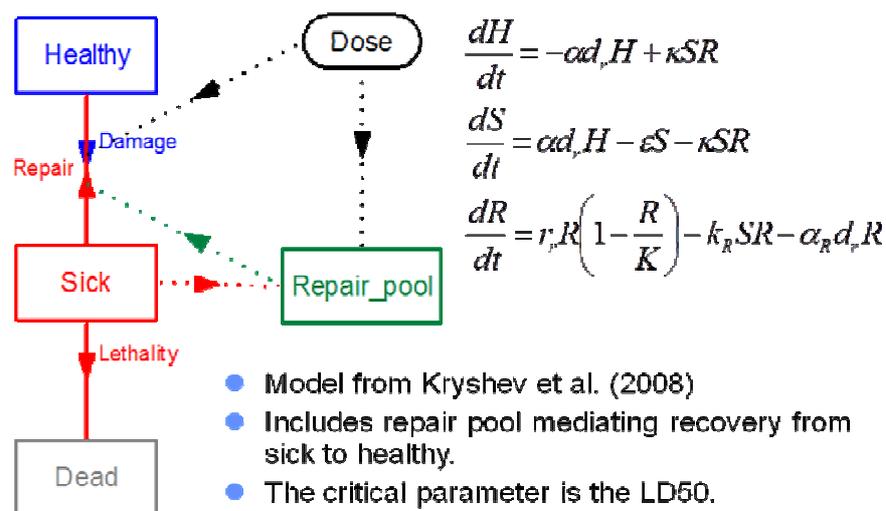


FIG. 8. An example of a model with radiation effects, as well as repair simulated [125].

## 6.5. DEVELOP LIFE HISTORY DATA SHEETS

Life history characteristics are needed as input data for population models (Table 22). Data sheets on life characteristics of reference animals were collected, including the following: longevity; longevity of immature and mature states; growth rate (estimated from logistic or Gompertz' models); basic metabolic rate; mortality rate (IMR and MRDT); adult weight, weight at birth; and reproduction (number of newborns per year) [127]. A large database on mammalian basal metabolic rates was also used [128].

## 6.6. RUN MODELS, COMPARE RESULTS

Several generic models were run for calculations on the benchmark scenario (e.g. Figure 9). The following results were obtained from the analyses of models:

- Length of life is important; population survival of short-lived species is better than that of long-lived animals;
- Dose rates of about 10 mGy/d for five years of chronic exposure produced significant reductions of wolf and deer populations of controls (Figure 10);
- Dose rates of 20 mGy/d for five years of chronic exposure produced considerable reduction of all populations, except short-lived mice. The latter survived at levels of about 70% of the controls (Figure 11);
- Larger animals = greater longevity = slower reproductive rate = populations with greater sensitivity to radiation.

Currently, it is not possible to validate these population models experimentally because of a lack of long-term experimental studies on population dynamics of free-ranging animals exposed to ionizing radiation. Nonetheless, the work carried out by the participants of EMRAS II Working Group 6 has helped to provide hypotheses to integrate population behavior and radiological effects, and was successful in comparing the different approaches that are being developed. Furthermore, it is important to determine whether differences in predictions between models are due to design of the population algorithm, the radiation effect mechanism, or life history parameters.

TABLE 22. AN EXAMPLE OF THE MODEL PARAMETERS NEEDED TO SIMULATE POPULATION DECLINE FOLLOWING EXPOSURE TO CHRONIC IRRADIATION [125]

Sub-model	Parameter	Description	Mouse	Hare/rabbit	Wolf/wild dog	Deer
Young	$d_0$	Death rate for young ( $d^{-1}$ )	2.74E-05	1.34E-05	9.68E-06	5.80E-06
	$m_0$	Mass for young (kg)	1.90E-03	8.25E-02	4.50E-01	6.71E+00
Adult	$d_1$	Death rate for adult ( $d^{-1}$ )	1.42E-03	6.40E-04	3.15E-04	2.93E-04
	$m_1$	Mass for adult (kg)	2.32E-02	3.00E+00	3.33E+01	1.49E+02
General	Allom_int_LD50	Intercept for LD <sub>50</sub> ( $Gy\ kg^{0.1297}$ )	7.21E+00	7.21E+00	7.21E+00	7.21E+00
	Allom_slo_LD50	Slope for LD <sub>50</sub> (dimensionless)	-1.30E-01	-1.30E-01	-1.30E-01	-1.30E-01
	$s$	Growth rate ( $d^{-1}$ )	4.12E-02	2.10E-02	2.11E-02	4.87E-03
	$f$	Recovery rate for fecundity ( $d^{-1}$ )	7.88E-02	3.98E-02	1.48E-02	2.69E+02
	$r$	Reproduction rate ( $d^{-1}$ )	7.88E-02	3.98E-02	1.48E-02	3.47E-03
	$K_f$	Carrying capacity of fecundity (individuals)	1.00E+03	1.00E+03	1.00E+03	1.00E+03
	$K_c$	Carrying capacity of ecosystem (individuals)	1.00E+03	1.00E+03	1.00E+03	1.00E+03
	$w_1$	Allee parameter (individuals)	2.00E+00	2.00E+00	2.00E+00	2.00E+00
	$e$	Lethality rate ( $d^{-1}$ )	2.30E-02	2.30E-02	2.30E-02	2.30E-02
	$d_r$	Dose rate (Gy)	0.01 - 0.05	0.01 - 0.05	0.01 - 0.05	0.01 - 0.05
	$T_c$	Cut-off time for exposure (d)	2.00E+03	2.00E+03	2.00E+03	2.00E+03

# Results at lower doses

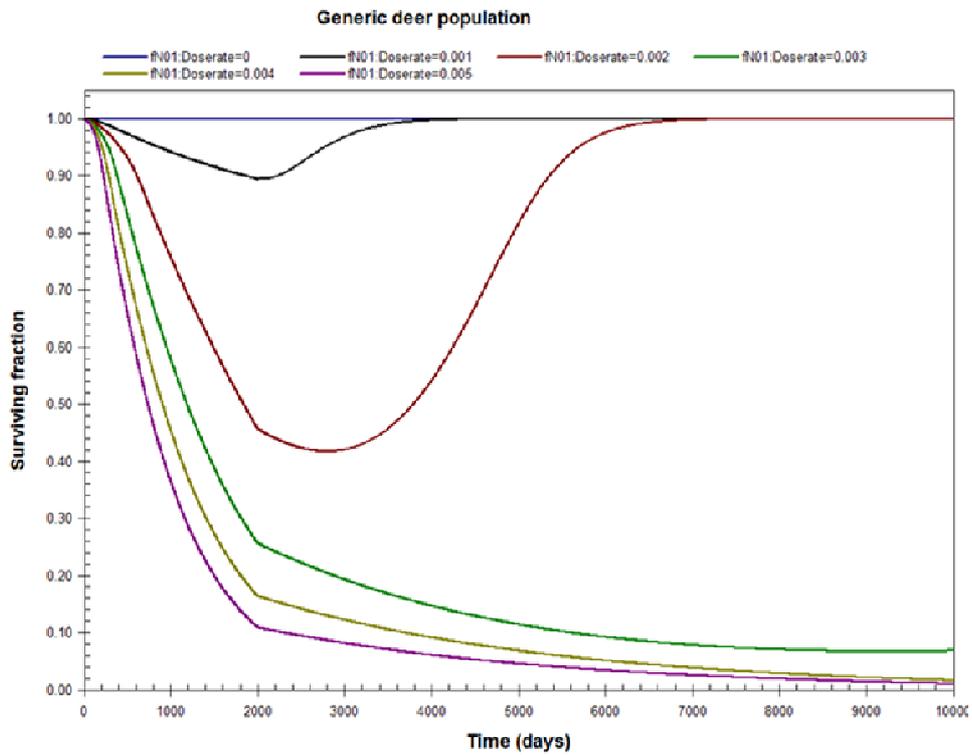


FIG. 9. Example of the dynamics of a deer population over 10000 days with chronic exposure to irradiation at dose various dose rates [125].

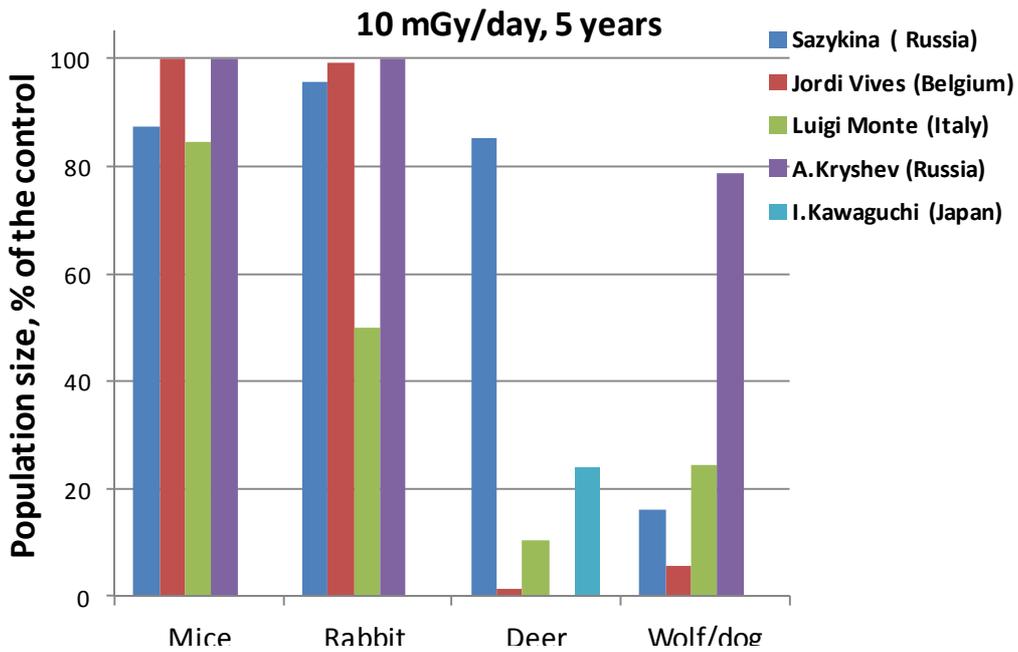


FIG. 10. Results of an inter-model comparison of population size for mice, rabbit, deer, and wolf following five years of chronic exposure to 10 mGy/d.

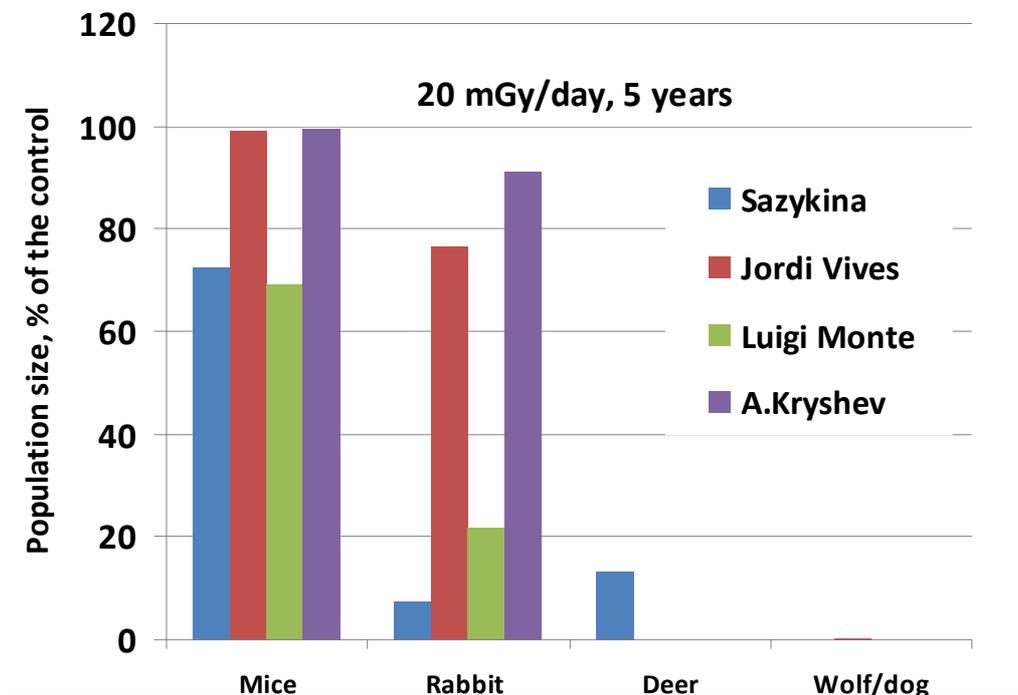


FIG. 11. Results of an inter-model comparison of population size for mice, rabbit, deer, and wolf following five years of chronic exposure to 20 mGy/d.

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## LIST OF ABBREVIATIONS

CBR	critical body residue
CA	concentration addition
DBTK	data-based toxicokinetic
DCC	dose conversion coefficient
DEB	dynamic energy budget
EC	exposure concentration
EDA	effect-directed analysis
ERA	ecological risk assessment
HI	hazard index
IA	independent action
ICRP	International Commission on Radiological Protection
ILSI	International Life Sciences Institute
IUR	International Union of Radioecology
LEL	lowest effect level
NEC	no-effect concentration
NOEC	no observed effect concentration
PBTK	physiologically based toxicokinetic
PCA	principal component analysis
PNEDR	predicted no effect dose rate
PSL	priority substance list
QSAR	quantitative structure–activity relationships
RBE	relative biological effectiveness
RDA	redundancy analysis
ROS	reactive oxygen species
RPF	relative potency factor
SEL	severe effect level
SLC	screening level concentration
SSD	species sensitivity distribution
SSLC	species sensitivity limiting concentration
TD	toxicodynamic
TEF	toxicity equivalency factor
TIE	toxicity identification evaluation
TK	toxicokinetic
TSP	two-step prediction
TU	toxic unit
WET	whole effluent toxicity
WG6	Working Group 6 “Biota Dose Effects” of the IAEA’s EMRAS II Programme



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**EMRAS II Technical Meetings, IAEA Headquarters, Vienna**

Vienna, Austria: 19–23 January 2009, 25–29 January 2010, 24–28 January 2011

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ISBN 978-92-0-101114-5  
ISSN 1011-4289