IAEA EMRAS II
Biota Effects Group

Advances of the Multiple Stressor group

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SCK•CEN
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

Objectives

- Review literature for multiple stressor data in which radiation was among the mix
- Query ecotoxicologists from the chemical industry to see what their most recent conclusions are relative to the need for multiple stressor analyses
- Report to the IAEA on whether this should be a topic requiring further exploration in the future
- If sufficient interest and resources, collaborate on a common, multi-stressor, radiological experiment
- This work is performed in conjunction with IUR.

Multiple Stressor database

- Scope: multiple stressor exposure with one of stressors external radiation or uptake of radionuclides
  - Including natural stressors (t°, pH, ...)
- Aim: Get an overview of what has been done so far, how it has been done, generalities on outcome
  - Status of the research in this area
- Approach
  - Literature review
  - Data compiling
    - Description of exp set-up, summary of results, limited QA/QC
    - In later stage, data compilation can be more detailed, if this has additional value

Multiple Stressor database

Study type
Ecosystem
Multispecies studies
Species studied
Species 1 Common name
Species 1 Latin name
Species 2 Common name
Species 2 Latin name
Species 3 Common name
Species 3 Latin name
Species 4 Common name
Species 4 Latin name
List of stressors
Stressor 1
Exposure levels stressor 1 single
Stressor 2
Exposure levels stressor 2 single
Stressor 3
Exposure levels stressor 3 single
Stressor 4
Exposure levels stressor 4 single
Stressor 5
Exposure levels stressor 5 single
Exposure levels combination
Short description of the experimental set up and conditions

Effects endpoints and results	
Umbrella effect	
Reproduction	**Which effect ?**
Morbidity	**Which effect ?**
Mortality	**Which effect ?**
Genetic	**Which effect**
Physiological	**Which effect ?**
Population relevant endpoints for multipspecies studies (e.g. Numbers of species)	**Which effect ?**
Other	**Which effect ?**
Other 2	**Which effect ?**
Short description of Results	
Please express results as far as possible in terms of : no deviation from addition, potentiation, synergy, antagonism	
Major conclusions from the study	
QA/QC	
Are we confident about the data?	
Can we use the data for dose response curve development?	**Are we confident about the statistics and associated experimental design used to identify the interaction?**
Reference	
ID of person who put in data	

Data collection finalised

- Terrestrial plants: 5
- Aquatic plants: 1
- Terrestrial animals: 14 (21 if including tumors)
- Aquatic animals: 5
- Aquatic microcosm: 1
- Marine estuaries: 19
- Yeast: 2
- (Cell culture: TA(2), AA (9)

- Big thanks to CLARE, ALMUDENA, NATHALIE

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Some generalities

- Binary mixtures/exposures (except for 2 cases)
- Toxicants or environmental parameters (acidity, T°, starvation)
- Only one case where dose response curve was established for single stressors (prerequisite to say anything about synergism/antagonism!!!

Some specific info (1)

- *1. Terrestrial plants*
  - 5 papers
  - Mostly laboratory studies, 1 field study
  - All different test organisms (Arabidopsis, barley, birch …)
  - Mostly gamma/X-irradiation combined with alpha/uranium, promutagens or heavy metals
  - Endpoints: mostly genetic effects, growth and oxidative stress
  - Antagonism/additive/synergism depends on exposure pattern, no clear trend
- *2. Aquatic plants*
  - 1 paper
  - Laboratory study
  - Lemna
  - Combination of uranium and copper
  - Endpoint: frond growth rate
  - Antagonism

Some specific info (2)

- *3. Terrestrial animals*
  - 14 papers (21 papers including tumors)
  - All laboratory studies
  - Rats and mice were used as test species
  - Mostly gamma/X-irradiation combined with heat shock, caffeine, heavy metals, N-nitroso-N-ethylurea (ENU) (for tumor research) …
  - Endpoints: mostly fetal death, malformations, growth retardation, tumor formation …
  - No clear trend for antagonism/additive/synergism
- *4. Aquatic animals*
  - 4 papers
  - All laboratory studies
  - Salmons and frogs were used as test organisms
  - Gamma/X-irradiation combined with metals, temperature or starvation
  - Endpoints: oxygen consumption, survival, bystander effects …
  - Little information available for antagonism/additive/synergism

Some specific info (3)

- *5. Freshwater microcosm*
  - 1 paper
  - Laboratory study
  - Euglena gracilis + Tetrahymena thermophila + Escherichia coli (= 1 system)
  - Gamma irradiation combined with acids
  - Endpoints: cell densities, chlorophyll *a* and ATP concentrations
  - Additive

- *6. Marine estuarine*
  - 19 papers
  - All laboratory studies
  - Medaka or Japanese killifish, mummichog, eel, brine shrimp, salmon …
  - Mostly gamma/X-irradiation combined with temperature, salinity …
  - Endpoints: Egg hatchability, mortality, growth, development primordial germ cells …
  - Often addition/synergism but most of the time no information available

Draft paper (JER) outline and request/proposal for contributors (1)

- Multiple stressor environmnent
  - #combinations is innoumerous
    - Environmental stressors (pH, T°, predators, ..)
    - Chemical mixtures
- Environmental standards and their requirements
  - Generally developed for single contaminants
  - Ecotox tests: contaminants in isolation
  - Environ characteristics (including other stressors) will influence effect tresholds
- Approaches to dose response curves (NV)
  - Independent action/concentration addition and deviations thereoff
  - Including other MS effects models
  - How far dose additivity correct assumption: alpha, beta, gamma
- Combined effect of substances
  - Different exposure modes/diff modes of action/diff target organs
  - Interaction can occur at all levels – adsorption, metabolisation, decontamination mechanisms, damage repair mechanisms

Draft paper (JER) outline and proposal for contributors (2)

- Presentation of data from the different ecosystem - organisms combination. Discussion in light of
  - Type of stressors studied
  - Methodology/approach used
  - Lab/fields
  - Endpoints considered
  - Effects observed
  - Validity of approach and (hence) data
  - Terrestrial and aquatic plants + microcosm (NV, HV)
  - Terrestrial animals (Almudena)
  - Aquatic animals (Karolina/Carmel and C°?)
  - Marine animals (Clare)
- Conclusions and recommendations for future research (all)
- Draft May 2010

Look what has been done for chemicals

- Contributors: Tom, Tamara, Nele, Carmel, a colleague of David, Hildegarde
- Learn from chemical ecotoxicology concentrating on NoMiracle
- Report: approach with chemicals and how it can be transferred to radiation protection
- Timing
  - Look at suitable reports from NoMiracle and their availability (March 2009)
  - End report: Dec 2010
- No Progress since last time

MS-effect modelling course
Preliminary programme

	Day 1	Day 2	Day 3
Morning session	Opening and registration **Thomas Backhaus** (Göteborg University, Sweden) - Experimental design - Introduction to reference models (concentration addition and independent action) and to their strengths and sensitivities	**Claus Svendsen** (CEH, UK) Testing for deviations from reference models using surface design or isoboles	**Stefan Van Dongen** (University of Antwerp, Belgium) - Best-fit method for concentration-response curves - Statistical testing of deviations from reference models (including calculation of confidence belts)
Afternoon session	**Thomas Backhaus** continued **Calculus session** (**Nathalie Vanhoudt, Nele Horemans**, SCK•CEN, Belgium): prediction of mixture effect from single concentration-response curve	**Nina Cedergreen** (University of Copenhagen, Denmark) ***to be confirmed*** - Use of mixture toxicity within REACH and Water framework directive - Can the choice of endpoint lead to contradictory results	**Stefan Van Dongen** continued **Calculus session** continued Closing remarks

MS-effect modelling course

- Organised by SCK•CEN
- Suggestion: May 2010
- Possibly some funding by IUR for foreign attendee(s)
- If 20 participants: ~250 EUR/participant
  - (travel and accomodation teachers, small fee for lecturers, course material, rent of auditoria, lunches; no SCK salaries!)

Concentration addition

- Sham experiment:

0.5TU + 0.5TU = 1TU

0.1TU + 0.9TU = 1TU

- Functional relation between single substance TU and mixture TU!
- Similarly acting compounds!

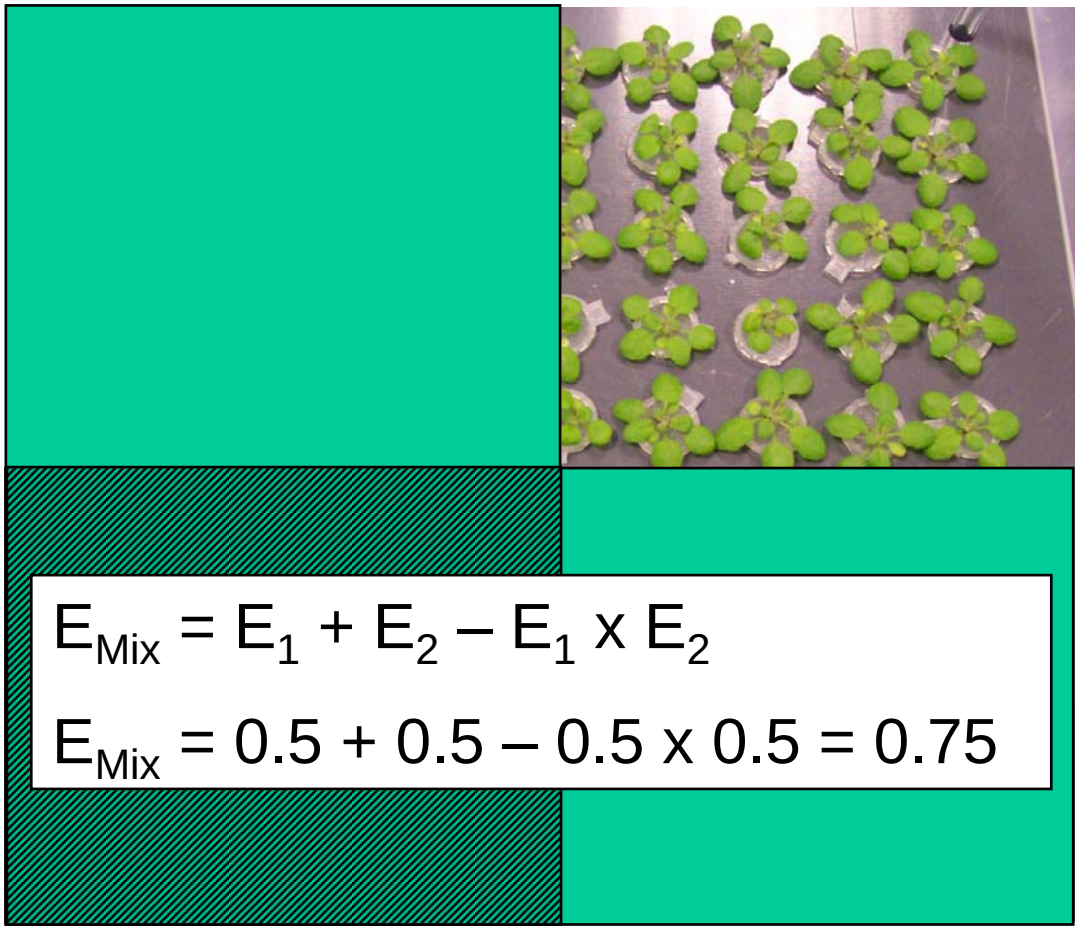
Independent Action (IA)

- Dissimilarly acting chemicals
- Assumption 1: toxicity each chemical is not influenced by presence other chemicals
- Assumption 2: all chemicals affect same biological endpoint
- Same effect via different pathways

Independent Action for Binary Mixture

Substance 1:

50% effect



$$E_{Mix} = E_1 + E_2 - E_1 \times E_2$$

$$E_{Mix} = 0.5 + 0.5 - 0.5 \times 0.5 = 0.75$$

Substance 2:

50% effect