Toxikologiska rådet

Toxikologiska rådet är ett expert- och samrådsorgan som verkar för ett bättre hälso- och miljöskydd. Rådet består av representanter för myndigheter samt enskilda forskare och är knutet till Kemikalieinspektionen.

Combination Effects in Ecotoxicology and Toxicology - Science and Regulation

A seminar and poster presentation in English, organized by The Swedish Toxicological Council in co-operation with the Swedish Research Council Formas, 17 November 2009, Stockholm

Seminar Report

Dr Malik Altahir, Scientific Secretary for the Toxicological Council.
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Prof. Hans Blanck, Member of the Toxicological Council,
Plant& Environmental Sci., University of Gothenburg.

Prof. Agneta Oskarsson, Member of the Toxicological Council,
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Foreword

Chemical risk assessment is generally made for each chemical alone, in spite of the fact that the actual exposure situation is far more complex. Tens of thousands of chemicals are in regular use - each with its own life-cycle and fate properties. In addition, the chemical diversity of the environment is further increased by the multitude of degradation or reaction products formed in industrial and environmental processes as well as after metabolism in various organisms. By evaluating the risk from each chemical separately we may under-estimate the total risk of chemical contamination. This is not necessarily due to exceptional synergistic interactions, but to regular and predictable combination effects. At present there is an emerging political awareness of this so-called “cocktail effect”.

Science provides several approaches to estimate mixture toxicity experimentally, and to predict mixture toxicity from the toxicities of the mixture components. Such models and predictions can be developed to useful instruments for risk assessment of chemical mixtures. Cost-efficient ways of making these evaluations are urgently needed. The purpose of the seminar is to describe the present scientific and regulatory state of the art, and to discuss further needs to develop a scientifically sound and yet practical approach to control combination effects of chemicals both in the environment and for human health.

Förord

Riskbedömning görs i allmänhet för varje enskild kemikalie, trots att den faktiska exponeringssituationen är långt mer komplex. Tiotusentals kemikalier används dagligen – var och en med sin livscykel och slutegenskaper. Dessutom ökar antalet kemikalier i miljön genom att nedbrytnings- eller reaktionsprodukter bildas. Detta sker både i industriella processer och i miljön, t.ex. efter metabolism i olika organismer. Genom att bedöma risken med varje enskild kemikalie kan vi underskatta den totala risken för kemisk förorening. Detta beror inte nödvändigtvis på ovanliga synergistiska interaktioner, utan snarare på normala och förutsägbara kombinationseffekter. För närvarande finns en tilltagande politisk medvetenhet om denna så kallade cocktaileffekt.

Det finns flera vetenskapliga tillvägagångssätt för att bedöma blandningars toxicitet experimentellt, och för att förutsäga blandningens toxicitet utifrån toxiciteten av blandningens komponenter. Sådana modeller och metoder kan utvecklas till användbara instrument för riskbedömning av kemiska blandningar. Kostnadseffektiva sätt att göra sådana bedömningar behövs skyndsamt. Syftet med seminariet är att beskriva det nuvarande vetenskapliga och lagstiftningsmässiga läget och att diskutera ytterligare behov att utveckla vetenskapligt hållbara men ändå praktiska sätt att kontrollera kemikaliers kombinationseffekter både i miljön och på människans hälsa.

Seminar Programme

09:00	**Welcome and brief scoping** Ms. Ethel Forsberg, Director-General, the Swedish Chemicals Agency
09:10	**Combined Effects of Chemicals – an introduction** Prof. Göran Bengtsson, Ecology, Lund University
09:20	**Combination Effects in Ecotoxicology- cornerstones and development** Dr Thomas Backhaus, Plant & Environmental Sci., University of Gothenburg
09:50	**Combination Effects in Toxicology- Studies in Rats** Dr Sofie Christiansen, Department of Toxicology and Risk Assessment National Food Institute, Technical University of Denmark
10:20	**Coffee and posters**
11:00	**Chemical Diversity in Man and the Environment** Prof. Åke Bergman, Environmental Chemistry, Stockholm University
11:20	**Comparative Regulatory Perspectives on Mixture Toxicity** Dr Thomas Backhaus, Plant & Environmental Sci., University of Gothenburg
11:50	**Lunch and poster exhibition**
13:10	**EFSA's PPR Opinion** Prof. Alan Boobis, Health Toxicology, Imperial College London
13:30	**Novel Methods for Estimation of Combination Effects – a challenge for future regulation of chemicals** Research Director Hans Løkke, Terrestrial Ecology, Aarhus University
13:50	**Scientific and Regulatory Aspects Associated with the Toxic Equivalency Concept for Mixture of Dioxin Like Compounds** Prof. Helen Håkansson, Inst of Environmental Medicine, Karolinska Institutet
14:10	**Probabilistic Cumulative Risk Assessment. An Example.** Dr Elsa Nielsen, Department of Toxicology and Risk Assessment National Food Institute, Technical University of Denmark
14:30	**Chemical-Industry Perspectives** Dr Mick Hamer, Syngenta
15:00	**Coffee and poster exhibition**
15:20–16.00	**Discussion: What Development of Science and Regulation Should We Aim For?** Prof. Göran Bengtsson, Ecology, Lund University

Summaries of Seminar Presentations

Combined Effects of Chemicals - an introduction

Prof. Göran Bengtsson, Section of Chemical Ecology, Department of Ecology, Lund University, SE-223 62 Lund, Sweden. Ecology, Lund University

Approaches to risk assessment

- Risk analysis of specific single threats–risk for injury to specific characteristics of organism/ecosystem, e.g. fecundity, survival, crop yield

➚
Source ➔ **Characteristics**
➘

- Vulnerability analysis –risk of injury to system (group, unit) by various threats, e.g. plant species, forest ecosystem, groundwater aquifer

➘
Sources ➔ **System**
➚

Multiple threats –the scope

Different threats to populations and communities can occur associated or disassociated and act alone or in combination, in an additive or synergistic mode. Anthropogenic threats to species often co-occur, but also e.g. predators, competitors, and parasites act in combination to reinforce their effect on a species. Not only different toxic chemicals combine as threats to organisms but also chemicals in combination with predators, competitors, parasites, and environmental factors, such as temperature and salinity.

Multiple threats: Predator and chemical

- Conventional short-term (4-d) LC50 toxicity test showed no toxic effect
- Low chemical concentration (3-4 % of LC504-d) killed 60-90 % of tadpoles in slightly longer exposure time (10 d)
- With chemical cues from a predator, survival declined from 40 to 3 % at lowest chemical concentration
-

Multiple threats: Predator, parasite and chemical

- The crustacean *Daphnia magna* was exposed to a predator (kairomones from larvae of *Chaoborus crystallinus*), a parasite (obligate endoparasite *Pasteuria ramosa),* and chemical (carbaryl; nominal 5 µg/L, measured 0.5-0.7 µg/L)
- Delayed maturation by each single threat
- Additive effect by combinations of threats, predicted by model of independent action

Multiple threats: Chemical 1, chemical 2,…and chemical *n*

The Ecotoxicology of Chemical Mixtures- Cornerstones and recent developments

Dr Thomas Backhaus, Plant& Environmental Sci., University of Gothenburg, P.O. Box 461, SE 405 30 Gothenburg, Sweden

- Mixture toxicity concepts: Dissimilarly acting substances, Independent Action (IA), and Similarly acting substances, Concentration Addition (CA) predict the joint action of chemicals.
- IA and CA are extremly powerful on various levels of biological complexity (from receptor binding assays to ecological communities). Strong deviations are the exception from the rule.
- No effect concentration (NOEC) are no true zero effect levels: Below the NOEC toxicity may be absent or may be present but undetected, due to a limited sensitivity of the experimental protocol.
- Empirical knowledge on the joint action of multi- component mixtures at low-effect concentrations of the individual constitutents is scarce.
- Empirical knowledge on the quantitative deviation from conceptually expected mixture toxicities is scarce. How often and in which scenarios do interactions occur? Do synergistic or antagonistic interactions predominate?
- **Mixtures have to be considered in chemical risk assessments, both for humans health and for the environment.**

Combination Effects in Toxicology-Studies in Rats

Dr Sofie Christiansen, Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark

- Endocrine system send clear messages. Endocrine disrupters send the wrong message at the wrong time to the wrong place.
- Endpoints in the studies:
Nipple retention (NR) , Anogenital distance (AGD), Pup growth, Semen quality, Histopathology, Hormone-levels and gene expression, Development of breast tissue, Malformations of external repro. Organs, Behaviour.
- Procedure for the studies:
1)Dose-response for single chemicals, 2)Calculation of additivity expectations, 3) Mixture study, dose-response, 4)Compare observed combination effects with predicted response
- Conclusions:
Mixture effects at NOAEL levels: Yes!
Can mixture effects of anti-androgens be predicted based on dose- additivity expectations?
-Similar mechanism: Yes
-Dissimilar mechanism: AGD, NR + organ weights: Yes
Dysgenesis, Malformations: No – synergy!
- **Human risk assessment based on the NOAELs for single anti-androgens underestimate the risk for adverse effects**

Chemical Diversity in Man and the Environment- How to approach the task of cocktail effects by clever selections of chemicals in mixtures for testing?

Prof. Åke Bergman, Environmental Chemistry, Stockholm University, SE-106 91 STOCKHOLM, Sweden

- ***"Chemicals" or Chemicals:*** PCB as a technical product, or as an individual, PCB congener or PCB congeners retained in humans.
- ***Areas of application and environmental distribution:*** Pigments, Flame retardants, Softeners, Detergents, Pharmaceuticals, Insecticides, Herbicides, Fungicides, Cutting fluids, Antioxidants, Filling agents, Dye agents, Surfactants, Detergents, Cosmetics, Bactericides, And much more ….
- ***Abiotic transformation products***
- ***Metabolites***
- ***External and Internal exposure scenarios:*** A question of mixture assessments of Persistent chemicals (POPs) and/or Semi-Persistent chemicals and/or Pseudo-Persistent chemicals

  Persistent chemicals (POPs)

  Based on characteristics:

  Commercial mixtures or individual compounds

  Based on occurrence:

in wildlife	**in humans**
herring or salmon file	mother's milk
chicken	human blood
pilot whale blubber	
seal meat	
Approximate number*	Approximate number*
100-200 individual POPs plus metabolites	50-100 individual POPs
150-500	100-200

  Semi-Persistent chemicals: Chemicals with high persistency (abiotic characteristics) and "no" bioaccumulativity (biological parameter).

  Number of semi-persistent chemicals: Very high

  Examples: phthalate esters, phosphate esters, many pharmaceuticals, pesticides (esters, amides, carbamates, …). With a huge number of metabolites, incl. reactive intermediates!

  Pseudo-Persistent chemicals: Chemicals with short biological half-lives but with continuous external exposure, possibly at high levels.

  Limited number of chemicals: Examples: PAHs, TBBPA, "Parabens" (4-hydroxy-bezoic acid esters

- ***Potential solutions:***
  Studies of chemicals within a class
  Studies of artificial mixtures, prep. from ARS
  Improved methods for isolating bio-mixtures of POPs

Regulatory Perspectives on Mixture Toxicity

Dr Thomas Backhaus, Plant& Environmental Sci., University of Gothenburg, P.O. Box 461, SE 405 30 Gothenburg, Sweden

Are mixtures considered in European regulations?

1) *Complex substances and chemical products:*

- **UVCB:** Substances of unknown or variable composition, e.g. petroleum products
  MCS: Multi-constituent substances, i.e. a "substance", in which more than one main constituent is present in a concentration > 10% and < 80% (w/w). The result of a chemical reaction, e.g. an isomeric mixture of xylene
  Chemical products, Preparations: Result of a deliberate mixing of "pure" ingredients, e.g. pesticide products.
- Covered by substance-specific regulations and directives, such as e.g.
  REACH (Regulation (EC) No 1907/2006); GHS (Globally Harmonized System of Classification and Labelling of Chemicals)/CLP within REACH (Regulation (EC) No 1272/2008); Biocide Directive (98/8/EC) and Pesticide Directive (Council Directive 91/414/EEC)
- Approaches
  Direct testing of the mixture
  Concentration Addition
  Summation Method

2) *Mixtures originating from a common process (emission based)*

- -IPPC: Emission Limit Values (ELVs) for dioxins and furans emitted from waste incineration plants.
  -Wastewater discharge permits.
- Approaches
  -Direct testing of the mixture of concern (e.g. the wastewater)
  -CA in the form of Toxicity Equivalency Factors, TEFs)

3) *Mixtures found in a particular compartment or organism (immission based perspective)*

- -Rarely considered in European Regulations
  -Within the scope of the Water Framework Directive (Directive 2000/60/EC)
  -Ongoing EFSA activities to establish Maximum Residue Levels of pesticide mixtures in food (Regulation (EC) No 396/2005

The Forthcoming New Legislation on Plant Protection Products (Common Position (EC) No 25/2008 adopted by the EP on 13 January 2009)

Article 4
Active substances and plant protection products

- shall not have any harmful effects on human health, **taking into account known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available,...**
- shall not have any unacceptable effect on the environment
  For 1st and 2nd reading in the European Parliament, the Committee on the Environment, Public Health and Food Safety recommended to amend this environmental requirement also by...,
  taking into account cumulative and synergistic effects and all relevant exposure routes to organisms in the environment; methods to assess such effects will be presented by the Authority. This initiative was unsuccessful.

Which of the two assessment and prediction concepts, dose addition or independent action, should be utilized in practice?

The correct one.	→	CA, IA, advanced modeling, case-by-case
The cautious one	→	CA
The one that can be applied using existing toxicity data.	→	CA

Which chemicals should be subjected to mixtures risk assessment?

- Those that co-occur in time and space
- Those that affect the same (eco)toxicological
- Role of Modes and Mechanisms of Action?

Application in practice?

- a dedicated, trans-sectorial European guideline
- Learn from existing guidelines (US EPA, WHO) and adapt them to the European situation
- Tiered approach

Critical knowledge gaps

- Exposure: Which compounds? Number of compounds?
- Relevant compounds
- Synergisms, Antagonisms: Involved compounds? How often? How severe?

EFSAS´s PPR Opinion

Prof. Alan R Boobis, The Faculty of Medicine **,** Imperial College London
Hammersmith Campus, London W12 0NN, UK

Default assumptions in cumulative risk assessment

- At low levels of exposure (< respective NOAELs), there is a need to consider only dose additivity and only for compounds that share a common mode of action
- A case by case approach is needed to consider whether there is biological plausibility for synergy at these exposure levels.
- Maximum deviation from effect predicted on the basis of dose additivity?
- In all other instances, assume independent action, so that combined exposure will have no effect

Proposed tiered approach for cumulative risk assessment

Tiered exposure assessment **Tiered hazard assessment**
-Actual exposure scenario or
-Maximum Residue Limit(MRL) setting exposure scenario

↓ ↓

Risk assessment
Interative process

Is the margin of exposure adequate?

↓ ↓

YES
No further action required

No
Continue by going to a higher tier in either exposure assessment, hazard assessment or both and subsequently refine the risk assessment

Some lessons learned

Simplification of the tiered approach

- Refine the Cumulative Assessment Group (CAG) to the extent possible early in the assessment. If the CAG is based on broad criteria, and the assessment fails to give adequate reassurance, this could be a trigger further research
- Restrict each exposure scenario to two tiers, one deterministic and one probabilistic

The method of calculation of the "background exposure" in the deterministic tiers requires further validation

Handling of non-detects in the deterministic and the probabilistic approaches needs to be resolved

Next steps

- The basis for and establishment of relevant CAGs, on a European level
- Confirmation that both the deterministic and probabilistic approaches for cumulative exposure assessment provide appropriate levels of protection
- Completion of further guidance on appropriate methodologies for exposure assessment

Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe/ Integrating environmental and human toxicology – NoMiracle

Dr Hans Løkke, National Environmental Institute, Aarhus University, Denmark

Benefits of integration of ecotoxicology and human toxicology
• Efficiency – less duplication of effort
• Scientific Quality – the best science from ecologists and health scientists is used
• Completeness – unconventional and indirect exposures and effects are revealed
• Sentinels – nonhuman organisms are more exposed and often more sensitive

THE INFINITY OF MIXTURE TOXICITY
• Testing every possible mixture combination is impractical and unfeasible
• There is need for models that can accurately predict mixture toxicity from single compound data
•There is a need for intelligent strategies
•Focus should be on the receptor (individuals/ecosystems)

Mixture toxicity and toxicokinetics
_ Present: Toxicokinetic methods are not used for assessing mixture toxicity
_ New: Integration of toxicological and toxicokinetic methods
Achievements by use of the Dynamic Energy Budget (DEB) method:

- Improved mechanistic understanding
- Preliminary models for assessment of the modulation of mixture toxicity and multiple stressors

Mixture toxicity with conventional models
_ Present: The independent action (IA) and the concentration addition model (CA)
_ New: Methods for assessing the probability of deviations from the IA and CA models
Achievements:

- Establishment of a large database of new high quality data
- Procedure for assessing deviations from IA and CA

Combined effects of natural stressors and chemicals

– Tools to predict the likelihood of synergism: Development of new methods for comparative risk assessment by integration of mixture toxicity and natural stressors (toxic stress, pathogens, drought, temperature etc)

Screening with cell lines

• Better knowledge about multiple stressor effects on immunotoxicity was obtained
• We recommend to use reporter cells for screening since they can be most easily adapted to higher throughput formats compared to other methods
• Reporter cell lines are well in agreement with ELISA data
• Cell lines may significantly underestimate effects on primary cells
• Extrapolation from in vitro to in vivo requires experimental work
• Suitable tools are still needed to analyse complex mixture
data in immunotoxicity

Can wildlife and human exposure models be improved by learning from each other?

• There are differences in the
- effect level endpoints,
- stressors,
- exposure routes and media taken into account,
- simulation of receptor's activities in space.
• Human exposure models might be improved by
- modelling human behaviour as emergent from individual traits and interacting with the environment,
- including non-chemical stressors that might interact with chemical stressors.
• Wildlife exposure models might be improved
- using data on activity patterns,
- addressing all the relevant exposure routes

The Dynamic Energy Budget (DEB) approach for prediction of mixture toxicity from single compound data and QSAR data

• A process-based approach, focusing on the biological entity in a world of multiple stressors rather than on "the chemical"
• One framework for all endpoints over time –time-dependent measurements needed in test guidelines
• Ready to tackle complex mixtures by prediction from QSAR data
• More work is needed to validate predicted interactions and extrapolations

Exposure, effect and Multiple Risk Assessment Tools (see complete list at http://nomiracle.jrc.ec.europa.eu/)

• Individual-based risk assessment models
– Eco-SpaCE
– Respiratory health of a mobile person
• Analysis of Landscape and Climate Parameters for Continental scale

Assessment of the fate of pollutants (ALPaCA)
• Multimedia Assessment of Pollutant Pathways in Europe (MAPPE)
• Nested box model that estimates spatially variable concentrations in Europe
• Worst Case Definition (WCD) Model
• Uncertainty and variability tool
• Novel probabilistic assessment factors
• Vulnerability
– Tool for ecological vulnerability analysis
– Vulnerability index for surface water ecosystems

Scientific and regulatory aspects associated with the TEQ-concept for dioxin-like compounds

*Prof. Helen Håkansson***,** Institute of Environmental Medicine, Karolinska Institutet, Karolinska Institutet SE-171 77 Stockholm, Sweden

What is the TEQ-concept?

- The toxic equivalency (TEQ) concept is a **regulatory tool** based on the idea that the toxicity of compounds, which share a common mechanism of action, can be added.
- The concept takes into consideration that individual compounds may differ in toxic potency in a way that can be compensated for by assigning **TEF-values** to individual compounds.
- The TEF-value for an individual compound represents an integrated judgement of different biological end-points, where more weight is given to the end-points that are of concern in the **risk assessment** process

What is a dioxin-like compound?
PCDD - polychlorinated dibenzo-*p*-dioxins
2,3,7,8-TCDD (N = 7/75)
PCDF – polychlorinated dibensofurans
(N =10/135)
PCB – polychlorinated biphenyls
(N = 12/209)

Toxicological profile: From experiments to human disease?
Until the early 1990íes

- Cancer (non genotoxic)
- Reproduction
- Immune system

1990ies – today

- Early (foetal) development
- Neurobehavioral alterations
- Endocrine disruption
    - → Estrogenic/Anti-estrogenic
    - → Thyroid hormones
    - → Retinoid system
    - → More???

Today – Future?
Disease of developmental origin?

- Obesity
- Diabetes
- Cardiovascular diseases
- Bone and tooth abnormalities
- Hearing problems
- More???

Need of regular revision process

- Compilation, evaluation and integration of new data
- Identification of data gaps and efforts to cover those
- Revisit of TEF-scheme inclusion criteria
- Revisit of TEQ-concept criteria

Probabilistic cumulative risk assessment- An Example: Anti-androgenic substances

Dr Elsa Nielsen, Department of Toxicology and Risk Assessment
National Food Institute, Technical University of Denmark

Probabilistic integrated risk assessment- SAFE FOODS Approach
Quantitative risk assessment of combined exposure

- Probabilisticriskmodelling
  Exposure to pesticides, mycotoxins, natural toxins
  Health effects
  Integrated model
- Evaluate uncertainties(separatefrom variability)
- Criteria for compararativeriskassessment
- Evaluation ofcombined exposuretocontaminantsandnatural toxins

Why use an integrated probabilistic approach?

- To include individual variability in exposure
- To include individual variability in dose descriptor
- To include uncertainty in the assessment
- To create a refined prediction of the risk
- To give the possibility of protection

Probabilistic exposure assessment- Simulate large number of individuals with:
Individual consumption patterns
Individual residue concentrations in their food
Individual processing factors

Probabilistic hazard characterisation

- Select appropriate dose-response model
- Select critical effect size (CES)

- Determine critical effect dose (CED) (average animal)
- Extrapolate to human
- Generate distribution of individual CED (ICED)

ICED = CED_{animal}/Assessment Factor (AF)

Anti-androgenic substances
Androgens: Key regulators of male sexual differentiation during pre- and early post-natal development
Antiandrogens: Substances that counteract the androgen action at some stage in this period and thereby affect the male reproductive system
Effects on the male reproductive system following in uteroexposure in animals:

- Reduced anogenital distance (AGD)
- Retained nipples
- Reproductive organ weights changes, histopathology
- Malformations of external genitalia e.g. hypospadias, vaginal pouch, cleft phallus etc.

An example: Anti-androgenic pesticides
With exposure and effect data available
Vinclozolin: AR-antagonist
Procymidone: AR-antagonist
Prochloraz: AR-antagonist, inhibition of enzymes in the steroid synthesis, AhR-agonist mm.

Effect data:
In uteroexposure study in rats performed at the National Food Institute, DTU (Laier et al. 2005, Metzdorff et al. 2007, Hass et al. 2007)

Exposure data:
Dutch residue database (2002-2003)
Dutch food consumption survey (1997/1998)

Conclusion:
- Probabilistic cumulative assessment to three anti- androgenic substances was performed by using the IPRA model with the relative potency factor approach.
- Cumulative exposure to vinclozolin, procymidone and prochloraz is not likely to be of concern for the reproductive development of male foetuses due to in uteroexposure with the residue levels found in fruit and vegetables in Holland (2002-2003).
- Other anti-androgenic substances should be included

Combination Effects in Ecotoxicology and Toxicology- Science and Regulation: An Industry Perspective

Dr Mick Hamer, Environmental Safety Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK

Where are we now ?
How to predict potential effects from environmental mixtures of pesticides & other chemical stressors ?

- Consider potential for effects (additivity worst-case), dose extrapolation, additivity below NOAEL (threshold effects), clarify common MoAs
- Need to consider exposure, pesticide registration worst-case edge of field, ensure co-occurrence in time and space to avoid over conservative approach

Uncertainties need clarification through targeted research programmes – NoMiracle, CEFIC LRI

Where are we now ?
Pesticide registration in EU, still based on single ais

- Some authorities (e.g. KemI , Ctgb, AFSSA) have requested assessment for combination effects for multiple ai products , particularly where there is no testing
- EFSA (2008, Appendix 4) for birds and mammals
  -additivity for acute effects
  -do not recommend for sublethal and reproduction at present
  -in principle possible to apply for same biological endpoints
  -evidence for mixture effects from NOEC addition, to be expected for same MoA (eg Kortenkamp 2007 – endocrine)

Water Framework Directive

- Chemical monitoring, ecological status in water bodies across EU
- Predicted No Effect Concentrations for individual chemicals, but how to consider prospective risk assessments for all chemical mixtures (can't add PNECs unless demonstrated same biological endpoint, MoA)
- May be able to predict toxicity of mixtures in water, but can't predict the mixture
- If water quality is perceived to be below good ecological status, can use mixture toxicity theory to determine if combination of chemicals has potential to cause effects

Cumulative risk assessment – human safety ((Paul Parsons AGCHEM Forum, 2009)

- Group compounds based on common toxicological MoA
- Understanding of nature of interaction, dose addition, response addition, interaction
- Toxic effects relative to human health
- Understanding of exposure

- Evaluation of relative potency within a CAG – additional data may be required (BMD, are DR curves parallel at low doses?)
- Use of toxicogenomic data to define common pathways - under consideration by US EPA (SAP meeting 2010)
- Toxicology data generated by regulators (e.g. EPA NHEERL) and academia in addition to core regulatory data from industry – need to ensure consolidated approach and criteria for data acceptability and data interpretation
- Avoid compounding effect of multiple conservative assumptions
- How to deal with residues below LOD/LOQ?
- Risk management - what levels of risk are unacceptable?
- How to prioritise compounds for evaluation?

Future Needs

- Better understanding of effects
  -Dose response – extrapolation from effect concentrations to low dose effects, threshold concentrations
  -MoA, additivity, independence
- Better characterisation of exposure
  Probabilistic, not cumulative worst-case, how to deal with LOD/LOQ
- Understanding of the uncertainties
  Risk management , do not be over-precautionary
- Involve all stakeholders – industry can contribute its data and experience

Discussion: What Development of Science and Regulation Should We Aim For?

Prof. Göran Bengtsson, Ecology, Lund University

Questions waiting for answers

- How much complexity on chemical mixtures do regulators/producers want to know about to make quantitative risk analysis/assessment?
- What is the first realistic steps of a regulatory approach considering combination effects?
- Optimal (for whom?) strategy for selecting mixtures of threats for testing - tiered approaches including screening of worst cases, etc?
- Are we ready to give up guideline values for single chemicals and replace with probabilities of co-occurences and multiple effects?
- What research strategy will be most efficient/demanding – concentrate on refining details of toxicity (new endpoints, higher sensitivity, mechanistic understanding) or accepting variance in general predicitive models?
- Can real exposure data be substituted by environmental criteria and mixture toxicity assessment?
- Is it synergy only – or the predictable combination effects that are of concern?

List of posters presented at the seminar

Chronic toxicity of human pharmaceuticals to bacterial communities- evaluation of the mixture toxicity of sulfamethoxazole and trimethoprim
Henrik Johansson, University of Gothenburg, Gothenburg, Sweden

Combined exposure to endocrine disrupting pesticides impairs parturition and causes pup mortality in rats
Christiansen Sofie, Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark

Developmental neurobehavioural effects after co-exposure to gamma-radiation and methyl mercury of neonatal brain development in mice during a critical phase.
Per Eriksson, University of Uppsala, Uppsala, Sweden

Effects of xenobiotics on PXR-CYP3A signaling in rainbow trout
Malin Celander, University of Gothenburg, Gothenburg, Sweden

Effects of PSII inhibitors on the succession of natural microalgalcommunities is predictable by concentration addition
Tobias Porsbring, University of Gothenburg, Gothenburg, Sweden

Enhanced developmental neurobehavioural defects in mice neonatally co-exposed to an ortho-substututed PCB (PCB153), co-planar PCB (PCB 126) or abrominated flame retardant (PBDE 99) in addition to methylmercury
Per Eriksson, University of Uppsala, Uppsala, Sweden

Highly brominated diphenyl ethers (PBDE 209) interact with the perflourooctanic acid (PFOA) during neonatal brain development to enhance developmental eurobehavioural defects
Per Eriksson, University of Uppsala, Uppsala, Sweden

Interactions in pharmaceutical mixtures - a challange for predictive mixture ecotoxicology?
Sara Brosché, University of Gothenburg, Gothenburg, Sweden

Interactions of pharmaceuticals on biomarker
Malin Celander, University of Gothenburg, Gothenburg, Sweden

Interaction study of Bromkal 70-5 DE and Cereclor 70L in the rat
Katrin Lundstedt-Enkel, University of Uppsala, Uppsala, Sweden

Mixture effects of three imidazole fungicides on adrenal hormone secretion in the human adrenocortical cell line H295R
Erik Ullerås, Swedish University of Agricultural Sciences, Uppsala, Sweden.

New roles for the Ah-receptor and CYP1A1
Agneta Rannug, Karolinska institutet, Sweden

Physiological levels of 17-beta-estradiol potentiates PCB126-induced changes in human endothelial cells
Hélen Andersson, University of Uppsala, Uppsala, Sweden

Polybrominated flame retardants in eggs from Swedish white-tailed sea eagles (*Haliaeetus albicilla*)
Ulrika Nordlöf, University of Stockholm, Sweden

Probabilistic cumulative risk assessment of anti-androgenic pesticides in food
A.K. Müller1, S. Bosgra2, P.E. Boon3, H. van der Voet3,4, E. Nielsen1, O. Ladefoged1

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2 Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands
3 RIKILT - Institute of Food Safety, Wageningen University and Research centre, Wageningen, the Netherlands
4 Biometris, Wageningen University and Research centre, Wageningen, the Netherlands

Research activities/competence of the Biotest-group at ITM, Stockholm University
Britta Eklund, University of Stockholm, Sweden

Variations in ecology and biology leads to interesting differences in contaminant patterns
in salmon (*Salmo salar*)
Katrin Lundstedt-Enkel, University of Uppsala, Uppsala, Sweden

List of participants in the seminar

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Maria Andersson	Swedish Chemicals Agency
Helen Andersson	Uppsala University
Yvonne Andersson	Swedish Chemicals Agency
Lisa Anfält	Ministry of the Environment
Karin Artursson	Swedish Veterinary Institute
Henrik Appelgren	Swedish Chemicals Agency
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Thomas Backhaus	Gothenburg University
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Elin Boalt	The Swedish Museum of Natural History
Alan Boobis	Imperial College London
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Hans Borg	Stockholm University
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Sofie Christiansen	Technical university of Denmark
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Roger Corner	Miljöförvaltningen, Stockholm
Susana Cristobal	Stockholm University
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Britta Eklund	ITM, Stockholm University
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Ulla Eriksson	ITM, Stockholm University
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Stefan Gabring	Swedish Chemicals Agency
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Hanna Gustafsson	Swedish Chemicals Agency
Nicklas Gustavsson	The Swedish Museum of Natural History
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Mick Hamer	Syngenta
Tove Hammarberg	The Swedish Environmental Protection Agency
Karin Hanze	Swedish Chemicals Agency
Anne-Marie von Hofsten	Swedish Chemicals Agency
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Maria Linderoth	Swedish Chemicals Agency
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Katarina Lundberg	Swedish Chemicals Agency
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Pierre Nord	Cederroth International AB

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