Future perspectives on Mixture Risk Assessment at EFSA

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Scientific Committee and Emerging Risks Unit
European Food Safety Authority

Scientific Committee Plenary Meeting
16th-18th February 2016
Past Activities on Chemical Mixtures in EFSA
METHODOLOGIES

- **Problem formulation**
  - What to consider prior to a risk assessment (RA)
  - Relevance exposure/co-exposure/hazard/toxicity/population exposed

- **Exposure Assessment**
  - Depending on data availability/ purpose of RA: Tiered Approach
  - From default values to full probabilistic models

- **Hazard Assessment**
  - Depending on data availability/ purpose of RA: Tiered Approach
  - Whole mixture approach/component-based approach
  - Default values-probabilistic models: e.g. PB-TK / PB-TK-TD models

- **Risk characterisation/uncertainty analysis**
  - Combine exposure and hazard assessment-sum risk estimates
  - Discuss uncertainties for each step (exposure, hazard, risk...)
Panel on Plant Protection Products and their Residues


EFSA (2009) Risk assessment Cumulative Effects- Triazole fungicides

EFSA (2013)
1. Identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile.
2. Relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food.
Panel on Contaminants in the Food Chain

- EFSA (2008) Polycyclic Aromatic Hydrocarbons in Food
- EFSA (2009) TEF approach-Non-ortho polybrominated biphenyls
- Marine biotoxins –Saxitoxin Group Pectenotoxin Group
- EFSA (2011) Whole mixture approach applied to Mineral Oil Saturated Hydrocarbons
- EFSA (2012) dose addition approach- Pyrrolizidine and Ergot alkaloids
Panel on Plant Protection Products and their Residues


- Methodologies to deal with mixture toxicity/synergistic effects of pesticides in honey bees, bumble bees and solitary bees

- **Key Recommendations**
  - Need dose responses: lethal/sub-lethal effects in adults/larvae to predict magnitude of interactions for honey bees, solitary bees and bumble bees- Lab work performed on 6 compounds and 6 mixtures for 3 species-publication summer 2016.
  - Include multiple stressors in risk assessment (e.g. chemicals and bee diseases): MUST-BEE project on-going 2015-2019
SCIENTIFIC REPORT OF EFSA

International Frameworks Dealing with

Human Risk Assessment of Combined Exposure to Multiple Chemicals

European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The development of harmonised terminology and frameworks for the human risk assessment of combined exposure to multiple chemicals ("chemical mixtures") is an important area for EFSA and a number of activities have already been undertaken, i.e. in the fields of pesticides and contaminants. The first step prior to a risk assessment of combined exposure to multiple chemicals is problem formulation defining the relevant exposure, hazard and population to be considered. In practice, risk assessment of multiple chemicals is conducted using a tiered approach for exposure assessment, hazard assessment and risk characterisation. Higher tiers require increasing knowledge about the group of chemicals under assessment and the tiers can range from tier 0 (default values, data poor situation) to tier 3 (full probabilistic models). This scientific report reviews the terminology, methodologies and frameworks developed by national and international agencies for the human risk assessment of combined exposure to multiple chemicals and provides recommendations for future activities at EFSA in this area.

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**Problem Formulation**

Nature of exposure? Is exposure likely? Co-exposure within a relevant timeframe?

Rationale for considering compounds in an assessment group?

### Tiered Exposure Assessments

- **Tier 0**: Semi-quantitative estimates of exposure
  - **Tier 1**: Generic exposure scenarios using conservative point estimates
  - **Tier 2**: Refined exposure assessment, increased use of actual measured data
  - **Tier 3**: Probabilistic exposure estimates

### Tiered Hazard Assessments

- **Tier 0**: Default dose addition for all components
  - **Tier 1**: Refined potency based on individual POD, refinement of POD
  - **Tier 2**: More refined potency (RFP) and grouping based on MOA
  - **Tier 3**: PBPK or BBDR; probabilistic estimates of risk

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**Is the margin of exposure adequate?**

Yes, no further action required

No, continue with iterative refinement as needed (i.e. more complex exposure & hazard models)

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Modified from WHO (2011)
RECOMMENDATIONS: EXPOSURE ASSESSMENT

- **Occurrence Data collection for multiple priority chemicals in food samples**
  - Monitoring/ total diet studies for priority chemicals id using either exposure/hazard-based criteria, susceptible populations, legislation (MRLs)
  - Multi-agency collaboration gather exposure data via other routes
  - Investigation co-occurrence multiple substances in individual food samples and correlations of co-occurrence for acute/chronic exposure (mean/95th percentiles)

- **Develop case /training sets comparing deterministic vs probabilistic methods**
  - Characterise dietary exposure for chemicals of priority: occurrence data and existing/other databases/tools (EFSA Databases, total diet studies, monitoring...).
  - Methods/guidelines adapted to co-occurrence of chemicals and need of exposure assessment (left-censored data, acute/chronic exposure, regulated versus contaminants)

- **Develop methods for aggregate exposure assessment**
Support harmonisation of Hazard assessment methodologies

- **Scientific basis for whole mixture approach**
  - Methods whole mixtures (WM): large fraction un-id chemicals
  - Evidence (stat/chem/tox) for similar WM as surrogates for others

- **Scientific basis for setting assessment groups**
  - Explore criteria for settings Assessment groups (AGs) e.g. MOA (TK and TD aspects (interspecies diff/ human variability))
  - Methods to set AGs-criteria/WoE for different scenarios (common, unknown, different MOA...)

- **Data Collection TK/TD**
  - Summary stats toxicity mixtures animal and ecology-June 2015.
EXTERNAL SCIENTIFIC REPORT

APPROVED: 8 December 2014

PUBLISHED: 31 March 2015

Data collection on toxicokinetic and toxicodynamic interactions of chemical mixtures for human risk assessment

LASER Analytica
Nadia Quignot, Camille Béchaux, Billy Amzal

Abstract

There is an increasing need to develop harmonised terminology, approaches and frameworks for the human risk assessment of combined exposure to multiple compounds. A number of activities have already been undertaken by EFSA, notably in the fields of pesticides and contaminants. This project aimed at providing quantitative information on combined effects of multiple compounds to support evidence-based hazard assessment. The first step was to record and collect, using extensive literature searches/systematic review methods, Pharmaco/Toxicokinetic (PK/TK) and Pharmacodynamic/Toxicodynamic (PD/TD) information on potential interactions between selected compounds. In vivo PD/TD and in vitro and in vivo PK/TK data were collected mostly for binary mixtures of pharmaceuticals (substrates of major routes of metabolism and known inhibitors/inducers) and major classes of regulated compounds and contaminants relevant to food safety. All data were then consolidated via meta-analyses to quantify magnitudes of interaction and their inter-individual variability for both TK and TD dimensions. Overall, this report illustrates application of systematic data collection for both human TK and TD aspects of multiple compounds to quantify magnitudes of metabolic and toxicological interactions respectively. Further analyses are recommended to integrate such magnitude of interaction and variability data in human hazard assessment of multiple compounds. © LASER Analytica, 2015.
EXTERNAL SCIENTIFIC REPORT

APPROVED: 24 July 2015

Data collection on Combined Toxicity of Multiple Chemicals for Animal Health and Ecological Risk Assessment

LASER Analytica

Nadia Quignot, Audrey Grech, Billy Amzal

Abstract

There is an increasing need to develop harmonised frameworks and methods for the risk assessment of combined exposure to multiple chemicals. A number of activities have already been undertaken by EFSA, notably in the fields of pesticides and contaminants. This project aims at selecting, reviewing, collecting and synthesizing the published data on combined effects of multiple chemicals targeting more than 50 species of veterinary and ecological relevance, using extensive literature searches and species taxonomical hierarchy for the literature searches was very wide ranging from bacteria, fungi, to invertebrates and vertebrates (amphibians, reptiles, birds, cats, cattle, deer, goats, horses, pigs, sheep, fishes, spiders, ephemeroida, hymenoptera, diptera, earthworms, Caenorhabditis elegans, molluscs, mites, crustacea, odonata, orthoptera, collembola, coleoptera, blattaria, cyanobacteria, proteobacteria, fungi). In vivo toxicity data were extracted from 199 studies representing 3,074 individual studies on pesticides, environmental contaminants, industrial products, hormones, metals, mycotoxins and pharmaceuticals. The magnitudes of combined toxicity were investigated for 2,944 studies representing 3,065 individual studies.
OpenFoodTox: EFSA’s Open Source Hazards Database
# DATA/EVIDENCE AVAILABLE IN CHEMICAL RA

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OPENFOODTOX: EFSA'S CHEMICAL HAZARDS DATABASE

- **Catalogue of EFSA’s chemical toxicity data since creation**
  - Contaminants (Human and Animal health)
  - Vitamins and minerals (Human health) (NDA),
  - Food additives and Nutrient Sources, Food contact materials, Flavourings and processing aids (Human Health)
  - Feed Additives (Human and Animal Health, Ecotoxicology)
  - Pesticides (Human and Animal health, Ecotoxicology)

- **Easy Reference and Crisis**
  - One reference DB Chemical Hazards: Search easily and efficiently
  - Crisis: Quick and Easy access to all EFSA’s Hazard Data

- **International Harmonisation**
  - Use OECD Harmonised Templates (OHT) for data model (ECHA/OECD) compatible with IUCLID/ECHA-OECD QSAR toolbox
  - Search compounds by name, CAS number on e-chem portal
  - Generate data sheet as summary of hazard id and charact (June 2016)
WHAT DOES OPENFOODTOX CONTAIN?

- **Chemical Information**
  Information on chemical nomenclature (EU nomenclature, IUPAC, CAS...), trade name, chemical group/panel (i.e. pesticide), chemical use (i.e. fungicide), chemical structure (i.e triazoles, organophosphates...).

- **Document descriptors**
  Information on EFSA’s opinion for the specific chemical or group of chemicals. Info from EFSA ‘s RAW system (question number, mandate, number), link to the document

- **Toxicity Endpoint/ Hazard identification**
  Information on critical toxicity study using OECD picklists when possible (species, dose, target organ...)

- **Critical study to demonstrate genotoxicity status**
  Providing essential information of critical genotoxicity study when assessed

- **Hazard /Risk characterisation**
  Information for health based guidance values (ADI/TDI) uncertainty factors...
CONTENT

1,479 Scientific outputs (metadata + DOI)

4,185 Substances (chemical identifiers including SMILES)

8,400 Toxicological endpoint studies

11,818 risk assessment summaries

133 Positive genotoxicity studies
Explore Case studies to develop *in silico* tools e.g. QSAR

• Explore use of metabolism and TK data and tox

• Combined toxicity mixtures (dose addition assumption/synergistic etc...)

• Tools for RA mixtures with little data (e.g emerging contaminants –new mycotoxins etc..)
Biologically-based models: Integrating variability from Biological Processes in RA
SCIENTIFIC REPORT OF EFSA

Modern methodologies and tools for human hazard assessment of chemicals

European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 11 July 2014, replaces the earlier version published on 24 April 2014

ABSTRACT

This scientific report provides a review of modern methodologies and tools to depict toxicokinetic and toxicodynamic processes and their application for the human hazard assessment of chemicals. The application of these methods is illustrated with examples drawn from the literature and international efforts in the field. First, the concepts of mode of action/adverse outcome pathway are discussed together with their associated terminology and recent international developments dealing with human hazard assessment of chemicals. Then modern methodologies and tools are presented including in vitro systems, physiologically-based models, in silico tools and OMIC’s technologies at the level of DNA/RNA (transcriptomics), proteins (proteomics) and the whole metabolome (metabolomics). Future perspectives for the potential applications of these modern methodologies and tools in the context of prioritisation of chemicals, integrated test strategies and the future of risk assessment are discussed. The report concludes with recommendations for future work and research formulated from consultations of EFSA staff, expert Panels and other international organisations.

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KEY WORDS

mode of action, adverse outcome pathway, integrated testing strategy, physiologically-based models, in silico, OMICS
Levels of Knowledge, Toxicokinetic and Toxicodynamic processes

- Toxicokinetics
  - External dose
  - Internal dose
- Toxicodynamics
  - Target organ dose
  - Target organ metabolism
  - Target organ responses
Biologically-Based models and OMICs

**External dose** → **Internal dose** → **Target organ dose** → **Target organ metabolism** → **Target organ responses** → **Toxic Response**

**External dose**

**Internal dose**

**Target organ dose**

**Target organ metabolism**

**Target organ responses**

**Toxic Response**

**Genomics**

**Transcriptomics**

**DNA**

**RNA**

**Proteins**

**Metabolites**

**Proteomics**

**Metabolomics**

**PB-TK models**

**OMICs**

**Future Mixture RA EFSA**
INTEGRATED TESTING STRATEGIES

**Toxicokinetics**

*In vitro* measurements id isoforms phase I, II, transporters in human cells
Consequences of metabolism id of toxic moiety(ies)
TK parameters (Vm, Km, Clint, CLm, protein binding, free/bound fractions)
Measure inhibition/induction on Clearances in vitro and/or metabolic CL isof
Use human Variability in TK from historical databases and software
Incorporating Population Variability and Susceptible Subpopulations into Dosimetry for High-Throughput Toxicity Testing

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2Present address: United States Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, NC 27711.

Received May 8, 2014; accepted August 9, 2014

Momentum is growing worldwide to use in vitro high-throughput screening (HTS) to evaluate human health effects of chemicals. However, the integration of dosimetry into HTS assays and incorporation of population variability will be essential for molecular targets and pathways provides an efficient, economical, and humane alternative to the current use of high-dose animal-based studies. Although these attributes make HTS desirable within a toxicity testing framework, several key considerations need to be addressed before it can be realistically con-
**MAJOR METABOLIC/EXCRETION ROUTES IN HUMANS**

**Phase I enzymes**
Cytochrome P-450, ADH, Esterases...

**Phase II enzymes**
Conjugation reactions
- UDP-Glucuronyltransferases
- Sulphotransferases
- Glutathione-s-transferases
- Methyl-transferases
- N-acetyltransferases
- Amino acid conjugation

**Transporters**
- Phase 0- Uptake transporters: e.g OATPs, OCTs.
- Phase III-Efflux pumps: e.g ABCs (P-glycoproteins and MRPs)

**Renal excretion**
HUMAN VARIABILITY IN TOXICOKINETICS

From pharmaceutical database human variability in TK available for many drugs /enzyme isoforms in different subgroups of the population.

Rationale for meta-analysis of TK data to derive metabolism variability distributions

Can be combined with in vitro data and used in QIVIVE
Combining Polymorphism data
Extensive and Poor Metabolisers
(EMs and PMs):
CYP2D6 Example

\[ UF_{EM} = \frac{p_{95EM}}{p_{50EM}} \]

\[ UF_{PM} = \frac{p_{95PM}}{p_{50EM}} \]

\[ UF_{POP} = \frac{p_{95POP}}{p_{50POP}} \]
Pathway-related uncertainty Factors

Chemical

Metabolism/Pharmacokinetics

In vitro and/or in vivo data on pathway of metabolism

Single pathway

Multiple pathways

Pathway-related variability

Prediction of kinetic variability (Monte Carlo model)

Probabilistic distribution

Modelled Chemical Specific Adjustment factor

In vivo chemical specific data or Physiologically-based pharmacokinetic model available

Unknown

Chemical-specific adjustment factor (CSAF)
TK AND MULTIPLE CHEMICALS: DATA AND MODELS

- Integrating TK in Human, animal, environmental RA

✓ Objective 1: Review model/ Tools in each area human, animal, Env RA

✓ Objective 2: Collect physiological/ biological parameters
  - Develop TK tools and models for single compounds (from simple tools to generic PB-PK models).
  - Case studies 10 compounds relevant to food and feed safety combining TK and TD

✓ Objective 3: Develop TK tools and models for multiple chemicals (from simple tools to generic PB-PK models).
  - Case studies 10 compounds relevant to food/feed safety
DEB MODELS

Quantitative theory for metabolic organisation from ‘first principles’
– time, energy and mass balance

Life-cycle of the individual
– links levels of organisation: molecule → ecosystems

Kooijman (2010)
What are DEB MODELS?

- Food $\rightarrow$ Assimilation $\rightarrow$ Reserve $\rightarrow$ Mobilisation $\rightarrow$ Growth $\rightarrow$ Structure
- Reserve $\rightarrow$ Maturation $\rightarrow$ Maturity
- Reserve $\rightarrow$ Reproduction $\rightarrow$ Eggs

- Somatic maintenance
- Maturity maintenance

- 3-4 states
- 8-12 parameters
- System can be scaled to remove dimension ‘energy’
Chemical affects the *probability* to die

- hazard modelling

![Graph showing hazard rate vs. internal concentration]

- **hazard rate**
- **internal concentration**
  - **NEC**
  - **killing rate**
  - **blank value**

![Diagram of toxicokinetics model]

- **toxicokinetics model**
- **hazard rate**
- **survival probability**
Elimination rate: 0.73 d\(^{-1}\)
Blank hazard rate: 0.0064 d\(^{-1}\)
NEC: 2.8 (2.1-3.1) µg/L
Killing rate: 0.031 L/(µg d)
Objective 1: Review DEB models (Dec 2015)

Objective 2: Collect physiological/biological parameters - calibration of models single compounds incl DEB (Jan 2016-April 2017)
- Develop generic/specific models for aquatic and terrestrial organisms for single compounds-Endocrine case study

Objective 3: Develop tools and models for multiple chemicals (Jan 2016-Jan 2018).

All tools in R and as Open Sources on EFSA website
INTEGRATED RA METHODOLOGIES FOR MIXTURES: MYCOTOXINS: FROM SYNTHESIS TO EFFECTS ON ORGANISMS

- **Objective 1:** Extensive literature searches and structured data collection on biochemical, genetic and environmental variables and impact on mycotoxin production

- **Objective 2:** Extensive literature searches and structured data collection on realistic occurrence of mycotoxin mixtures, TK and combined toxicity in animals and humans

- **Objective 3:** An integrated approach to the risk assessment of mycotoxin mixtures using modelling

Combine environmental variables, TK, toxicity data for RA using whole food chain approach (from environment to internal dose incl. carry over in farm animals and toxicity) plus comparative approach to mycotoxin toxicity in vertebrates.
Harmonisation of Human and Ecological Risk Assessment of multiple Chemicals
Summary Report

EFSA Scientific Colloquium 21

Harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals

11-12 September 2014
Edinburgh, UK

European Food Safety Authority
-TERMS OF REFERENCE-

- SC to develop GD: Harmonisation of HRA and ERA combined exposure to multiple chemicals.
- High relevance to panels dealing with chemical RA
- Develop frameworks using tiered approaches for relevant steps i.e. pb formulation, hazard id/ charact, exposure assessment, risk characterisation/uncertainty analysis
- Tiered approaches, circumstances under which harmonisation may not be possible discussed.
- Case studies for human/ ecological RA: annex -fit for purpose
- Take into account EFSA, European, international activities.
- EFSA’s initiative on Transparency and Engagement in RA (TERA): Publication Consultation on Terms of Reference
CONCLUSIONS AND TIMELINES

- Mixture as priority topic for previous and current SC (Sep-Dec 2015)
- Harmonisation of methodologies for human and Ecological RA risk to multiple chemicals – start spring 2016 (May-June)
- Public consultation on ToR optional
- WG expertise balanced between human RA and ERA
- Activities/summary report colloquium as support material for WG
- Use Tiered approaches – International-WHO, EPA (time, data, resources/ contexts (data poor/ data rich, prioritisation etc...))
- Scientific reports to bring case studies in Human and Eco area
  Incl. harmonised use of mechanistic data
-SUMMARY-

Which exposure is right?

Drug B (conc.)

Additivity

Antagonism

Drug A (conc.)

Mixed hazards

Ecological

Human

METHODS

40
THANK YOU