Investigating Combined Effects of Multiple Chemicals to support Risk Assessment—EFSA Activities

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OUTLINE

- Risk assessment of combined exposure to multiple chemicals: Principles
- Examples and Recommendations
- Multiple Chemicals: Investigating Toxicokinetics and Toxicity
- Future Perspectives
- Conclusion and Recommendations
Risk Assessment of combined exposure to multiple chemicals: Principles

EFSA’s Role in Risk Analysis

Methodology Codex Alimentarius:
- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk characterisation

EFSA
- Independent Risk Assessment
- Risk communication

Scientific Risk Assessment
Risk Management
Risk Communication

Risk Manager
- EC
- EU Parliament
- Member States
- Council
FROM QUESTION TO ANSWER

European Commission
European Parliament
Member States
EFSA ("self mandate")

Terms of reference
Background

Question?

Opinion

Risk Assessment

Consumer
Media
Industry
Professionals

Risk Communication

HUMAN RISK ASSESSMENT: CHEMICALS IN FOOD

Exposure assessment

Hazard Assessment

Exposure data (Concentration in food)
Food consumption

Uncertainty Factor

Toxicokinetics / Toxicodynamics

Benchmark Dose/ NOAEL

Health-based guidance value

MOE
ADI / TDI
ARfD

Risk Characterisation
METHODOLOGIES

Problem formulation
- What to consider prior to a risk assessment (RA)
- Relevance of 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 (Physiologically-based toxicokinetic models)

Risk characterisation/uncertainty analysis
- Combine exposure and hazard assessment-sum risk estimates
- Discuss uncertainties for each step (exposure, hazard, risk...)
DATA AVAILABILITY AND METHODOLOGIES

Assess Data Quality

Whole Mixture
- Mixture of Concern
- Sufficiently Similar Mixture
- Group of Similar Mixtures

Compounds
- Toxically Similar
- Toxically Independent
- Interactions

Mixture RfD/C Slope Factor
- Comparative Potency
- Environmental Transformation
- Hazard Index
- Relative Potency Factors
- Response Addition
- Interactions Hazard Index

Compare and Identify Preferred Risk Assessment, integrate Summary with Uncertainty Discussion


WHOLE MIXTURE APPROACH

Whole Mixture Data Available

Whole Mixture of Concern
- Toxico logical Evaluations
- Derive RfDx/RCx; Slope Factors

Sufficiently Similar Mixture
- Epidemiologic Evaluations

Exposure Assessment of Whole Mixture/Similar mixture
- Hazard Quotient; Risk Estimate
- Epidemiological Risk Measures

Modified from US-EPA (2007)
COMPONENT-BASED APPROACH

- Component Data Available
  - Toxicological data for each Component
  - Toxicological data for similar components
  - Partial toxicological data for similar and independent Components
  - Toxicological data for independent Components

- Component Exposure Assessment
  - Component Data Available
  - Dose Addition
    - Available Interactions Data
    - Relative Potency Factors
    - PB-TK Modelling
  - Response Addition
    - Integrated Additivity Method

- Component Exposure Assessment
  - Risk estimates, HI
  - BINWoE Interaction Profiles
  - Interaction Based HI
  - Cumulative HI
  - Relative Potency Factors
  - Internal Dose HI
  - Internal Dose Based Risk Estimates
  - HQ

WHO FRAMEWORKS

**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: Simple semi-quantitative estimates of exposure
- Tier 1: Generic exposure scenarios using conservative estimates
- Tier 2: Refined exposure assessment using measured data
- Tier 3: Probabilistic exposure estimates

**Tiered Hazard Assessments**
- Tier 0: Detailed dose addition for all components
- Tier 1: Refined potency based on individual POD
- Tier 2: More refined potency (RF) and grouping based on NOA
- Tier 3: PBPK or BBDR: probabilistic estimates of risk

Yes, no further action required
- Is the margin of exposure adequate?

No, continue with iterative refinement as needed (i.e. more complex exposure & hazard models)
EFSA’S HAZARD ASSESSMENT OF PESTICIDES

Identify Cumulative Assessment Group

Refine definition of Common effect

Tier 1: ADI, ARID (For Common Effect)

Tier 2: Adjusted ADI, ARID (For Common Effect)

Tier 3a: Relative Potency Factors
NOAEL-derived

Tier 3b: Relative Potency Factors
BMDL-derived

Modified from EFSA (2009)

Examples and Recommendations
SCIENTIFIC OPINIONS - HUMAN RISK ASSESSMENT (I)

- **EFSA 7th Scientific Colloquium (2007)**
  - “Cumulative risk assessment of pesticides to human health, the way forward.”

- **Panel on Plant Protection Products and their Residues**

- **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.

SCIENTIFIC OPINIONS - HUMAN RISK ASSESSMENT (II)

- **Panel on Contaminants in the Food Chain**
  - EFSA (2008) Polycyclic Aromatic Hydrocarbons in Food
  - EFSA (2009) TEF approach - Non-ortho polybrominated biphenyls
  - EFSA (2009) TEF approach - Marine biotoxins – Saxitoxin Group
  - EFSA (2009) TEF approach - Marine biotoxins – Pectenotoxin Group
  - EFSA (2011) Whole mixture approach applied to Mineral Oil Saturated Hydrocarbons

- **EFSA (2012)**
  - dose addition approach - Pyrrolizidine alkaloids
  - dose addition approach - 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
  - Dose responses for lethal/sub-lethal effects in adults/larvae to predict magnitude of interactions for honey bees, solitary bees and bumble bees - Lab work on 6 mixtures on-going 2014-
  - Id interaction molecular basis: realistic environmental exposure
  - Include multiple stressors in risk assessment (e.g. chemicals and bee diseases): MUST-BEE project ongoing 2015

Terminology

Develop consistent approaches across different EU legislations

- Harmonised Terminology
  - Pesticides EC/ 396/2005: cumulative risk assessment versus WHO: combined exposure to multiple chemicals
  - Difficulty: meanings differ between human and ecological risk assessment e.g. bioavailability

- Harmonised Methodologies
  - Human Risk assessment
    - Regulated compounds versus contaminants
  - Animal Health risk assessment and Ecological risk assessment

- Future Methods for Risk Assessment
  - Multiple chemicals combined with other stressors (biological, physical)
**PROBLEM FORMULATION**

Optimise process of problem formulation to identify chemicals of priority prior to embark on risk assessment of multiple chemicals

- **Exposure-based problem formulation**
  - Id chemicals in food/feed with sign human exposure (occurrence in commodities, biomonitoring and/or sources of exposure /sub-groups population exposed (adults, children, high consumers...))

- **Hazard-based problem formulation**
  - Id chemicals in food safety using toxicological/epidemiological criteria (e.g. persistence and bioaccumulation, high toxicity, severity of effects, evidence for interactions)...

- **Integration of Exposure and Hazard-based problem formulation**
  - Provide guidance taking into account differences in legal frameworks e.g. regulated substances and contaminants (intentional versus coincidental mixtures)

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**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**
HAZARD 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 using Systematic Review**
  - Summary stats toxicity mixtures animal and ecology-June 2015

RISK CHARACTERISATION/UNCERTAINTY ANALYSIS

Harmonisation of methodologies for risk characterisation and uncertainty analysis

- **Guidance Document**
  - Guidance to perform uncertainty analysis for exposure assessment available.
  - Recent guidance for probabilistic exposure assessment for pesticides including multiple chemicals and uncertainty analysis
  - Guidance on uncertainty analysis for steps of RA due June 2015
  - Guidance for uncertainty analysis on hazard assessment/risk characterisation for combined exposure to multiple chemicals of value
  - Such a guidance will support the activities for problem formulation, exposure assessment and hazard assessment
MIXTURE TOX DATA COLLECTION

- **Metabolic interactions: Toxicokinetic Data**
  ✓ Parameters (clearances/AUC, Cmax, half life...) and statistical estimates for single compounds and binary/complex mixtures.
  ✓ Data for inhibitors and inducers of specific metabolic pathways (Cytochrome P-450, glucuronidation...)

- **Synergistic effects: Toxicodynamic Data**
  ✓ Collect parameters (toxicity endpoints...)/ statistical estimates for single compounds and binary/complex mixtures.
  ✓ Collect data for synergistic effects

- **Use quantitative data as basis to develop OECD template**
  ✓ Develop data model fitting OECD harmonised templates (OHT)
  ✓ Identify picklists/ columns/fields

Multiple Chemicals: Investigating Toxicokinetics and Toxicity
Levels of Knowledge, Toxicokinetic and Toxicodynamic processes

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**Toxicokinetics**

- External dose
- Internal dose
- Target organ dose
- Target organ metabolism

**Toxicodynamics**

- Toxic effect
- Toxic effect
- Toxic effect
- Toxic effect
- Toxic effect
- Toxic effect
**MOA AND ADVERSE OUTCOME PATHWAY - TOXICOKINETICS?**

Chemical Exposure

- Toxicokinetics
- Chemical properties

Molecular target
- Receptor/Ligand interaction
- DNA binding
- Protein oxidation

Cellular response
- Gene activation
- Protein synthesis
- Altered signaling

Tissue/Organ
- Altered Physiology/
- Disrupted homeostasis

Individual/Organism
- Lethal effects
- Sub-lethal effects

Population
- Structure/Recruitment/Extinction

**Toxicity Pathway**

**Adverse Outcome Pathway**

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**DERIVING HEALTH-BASED GUIDANCE VALUES**

\[
\text{ADI/TDI (mg/kg/day)} = \frac{\text{NOAEL or BMDL (mg/kg)}}{100}
\]

**Chronic Exposure**
- Acceptable Daily Intake (ADI) - regulated
- Tolerable Weekly Intake - contaminants

**Acute Exposure**
- Acute Reference Dose (ARfD)
- NOAEL: No observed-Adverse-Effect-Level-BMDL: Benchmark Dose

100-fold uncertainty factor (UF)
REFINING UNCERTAINTY FACTORS IN HUMAN RA

Options to replace default uncertainty Factors (UF)
- **Chemical specific adjustment factors** using physiologically-based models (PB-TK/PB-TK-TD) (WHO, 2006)
- **Categorical uncertainty factors e.g for humanTK pathway-related UF** (Dorne and Renwick, 2005)

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
-Biologically-Based models and OMICS-

Investigating TK Tox mixtures to support Risk Assessment

PB-TK models

OMICs

- HUMAN VARIABILITY IN TOXICOKINETICS AND UF -

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 pathway-related distributions.

Uncertainty Factors

Can be made available online and allows for future refinement.
**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)
  - Default uncertainty factor (3.16)
  - Probabilistic distribution

**Modelled Chemical Specific Adjustment factor**

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**TK AND INTEGRATED TESTING STRATEGIES**

- **In Vitro Id isoforms phase I, II, transporters.**
- **Consequences of metabolism id of toxic moiety(ies)**
- **TK parameters (Vm,Km, Clint, Fu).**
- **Use human Variability in TK from historical databases and software IVIVE and QIVIVE**

**Exposure**

**Margin of Exposure**

**Adverse Outcome**

**TK/Tox Data Read-across...**

**In Vitro-In Vivo Signatures**

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**Investigating TK Tox mixtures to support Risk Assessment**
Investigating TK Tox mixtures to support Risk Assessment

TK IN RISK ASSESSMENT: RECOMMENDATIONS

- **Need Basic TK data**
  - Improve *in vitro* methods to measure TK parameters (TOX21, ECVAM...)
  - Human relevance Test species (e.g. evolution TK/ADME/enzymes...)
  - Integrate species, taxa differences/human variability in ADME
  - Mixtures: scientific basis to set Assessment Groups using TK criteria

- **Guidance on use of PB-TK models in Risk Assessment**
  - TK and TD models; *in vivo* assays/*in vitro*/IVIVE/*in silico* (QSAR..)
  - Tiered approaches/different contexts (data poor/rich, prioritisation, mixture)

- **Develop prototype physiologically-based models**
  - Need for databases providing critical parameters to build models
  - To refine uncertainty factors (categorical or chemical specific)
  - From simple models to full PB-TK-TD: context dependent
Future Perspectives
**TK IN RISK ASSESSMENT: EFSA'S STRATEGY**

- **Integrating TK in Human, animal, environmental RA**
  - Contract September 2014-2017
  - **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 and feed safety combining TK and TD

**CONCLUSIONS AND RECOMMENDATIONS**

Harmonisation of methodologies for risk to multiple chemicals
All activities presented and summary report of colloquium as basis for working group of SC

- **Guidance Document with EFSA scientific Committee**
  - Harmonisation human/ ecological RA multiple chemicals (Start 2016)
  - Combine evidence of interactions for RA (TK/TD)
  - Use case studies of relevance to food safety (e.g. contaminants)

- **Mechanistic data: potential synergies with EUROMIX**
  - Use Tiered approaches depend time, data, resources/contexts (data poor/ rich, prioritisation, mixtures)
  - Use relevant *in vitro*/IVIVE/*in silico* (QSAR..)/Historical *in vivo*
  - Link external dose-internal dose (generic PB-PK models)
  - Harmonise use of mechanistic data in human and ecological RA
Many Thanks

Questions?

Discussion