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

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www.efsa.europa.eu

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## OUTLINE

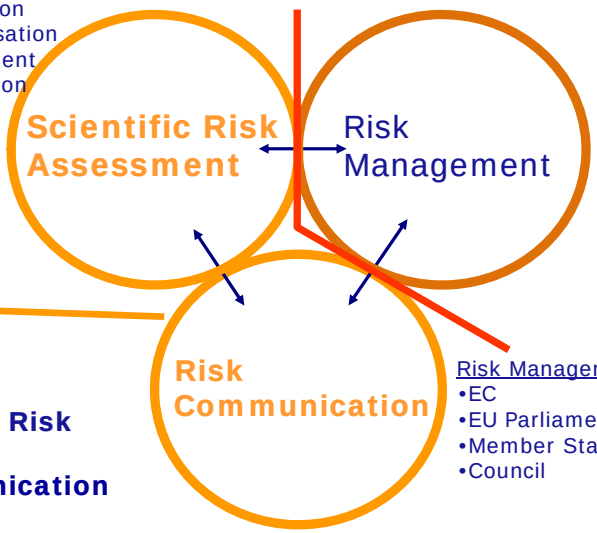
- Risk assessment of combined exposure to multiple chemicals: Principles
- Examples and Recommendations
- Multiple Chemicals: Investigating Toxicokinetics and Toxicity
- Future Perspectives
- Conclusion and Recommendations

# Risk Assessment of combined exposure to multiple chemicals: Principles

## EFSA's Role in Risk Analysis

Methodology Codex Alimentarius:

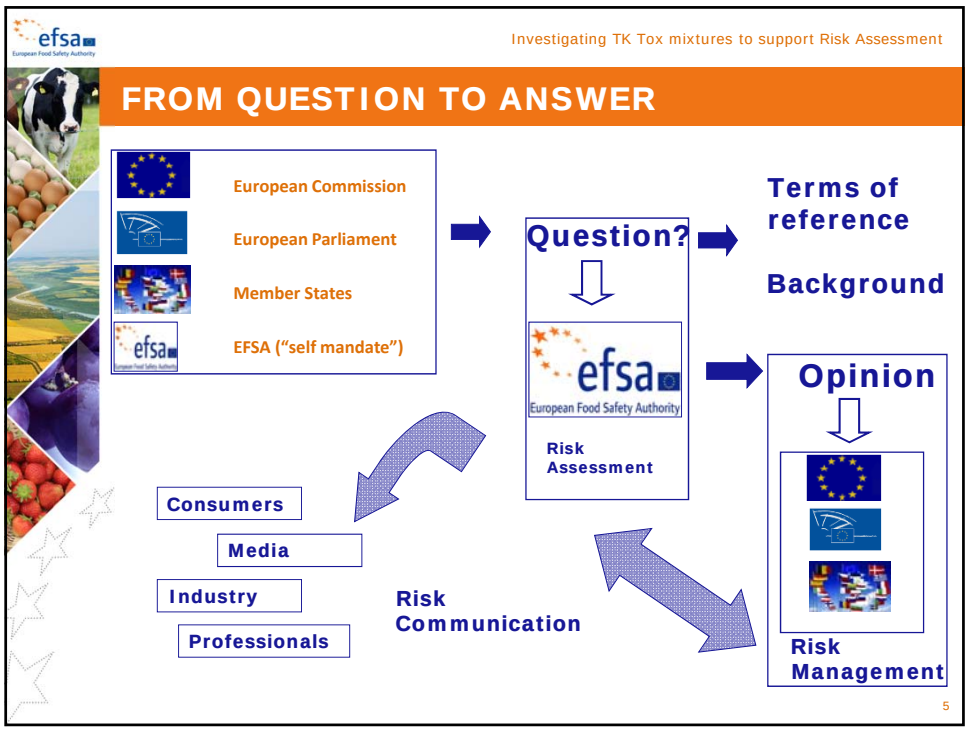
- Hazard identification
- Hazard characterisation
- Exposure assessment
- Risk characterisation




EFSA

- **Independent Risk Assessment**
- **Risk communication**

Risk Manager
- EC
- EU Parliament
- Member States
- Council


Investigating TK Tox mixtures to support Risk Assessment
FROM QUESTION TO ANSWER
European Commission
European Parliament
Member States
EFSA ("self mandate")
Question?
Risk Assessment
Terms of reference
Background
Opinion
Risk Management
Consumers
Media
Industry
Professionals
Risk Communication
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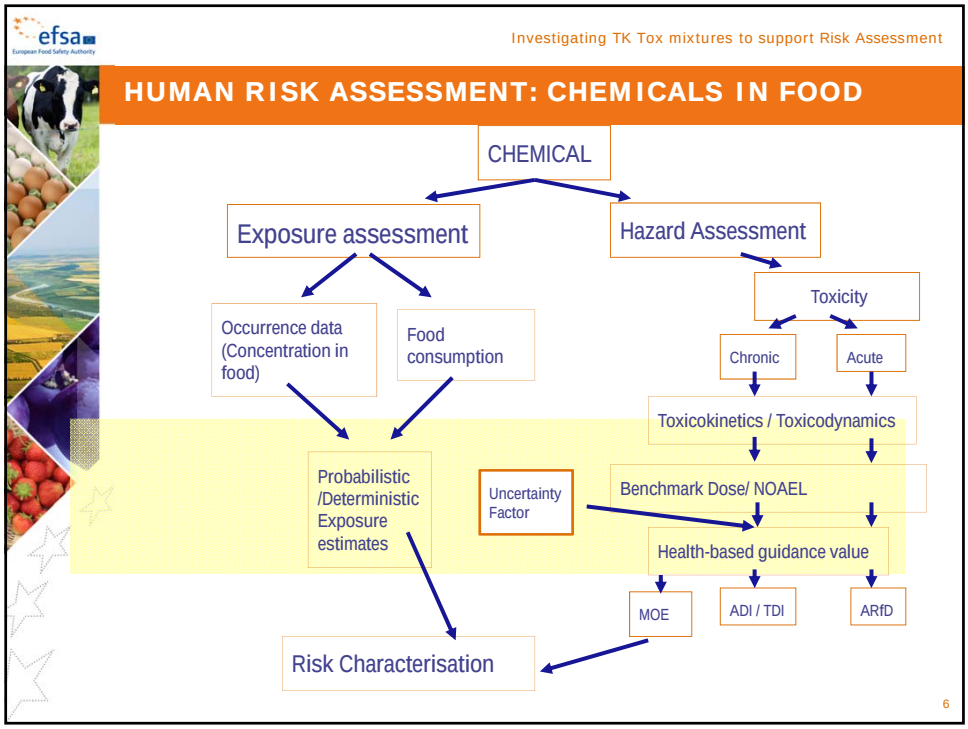
Investigating TK Tox mixtures to support Risk Assessment
HUMAN RISK ASSESSMENT: CHEMICALS IN FOOD
CHEMICAL
Exposure assessment
Hazard Assessment
Occurrence data (Concentration in food)
Food consumption
Toxicity
Chronic
Acute
Toxicokinetics / Toxicodynamics
Probabilistic /Deterministic Exposure estimates
Uncertainty Factor
Benchmark Dose/ NOAEL
Health-based guidance value
MOE
ADI / TDI
ARfD
Risk Characterisation
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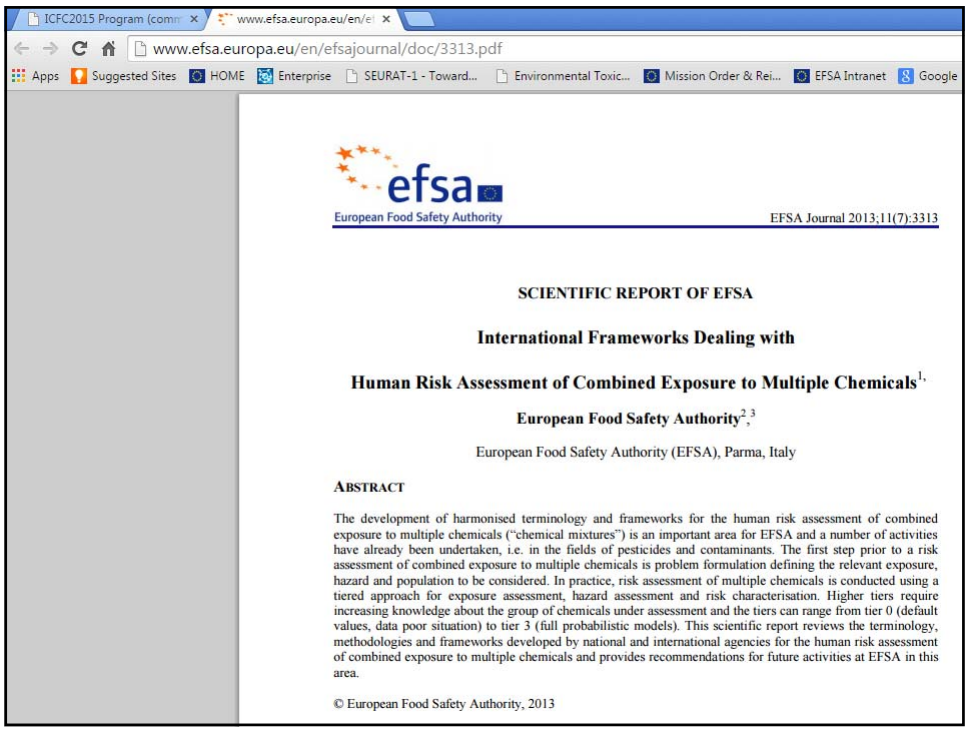

www.efsa.europa.eu/en/efsajournal/doc/3313.pdf

European Food Safety Authority

EFSA Journal 2013;11(7):3313

SCIENTIFIC REPORT OF EFSA

**International Frameworks Dealing with**

**Human Risk Assessment of Combined Exposure to Multiple Chemicals**[1,]

**European Food Safety Authority**[2,3]

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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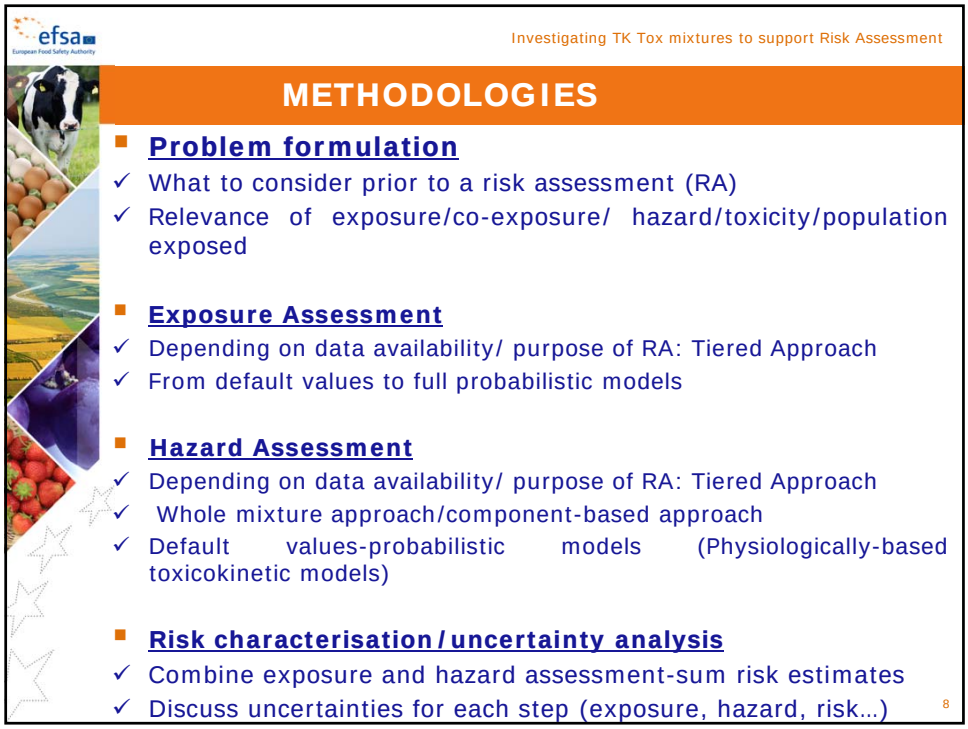
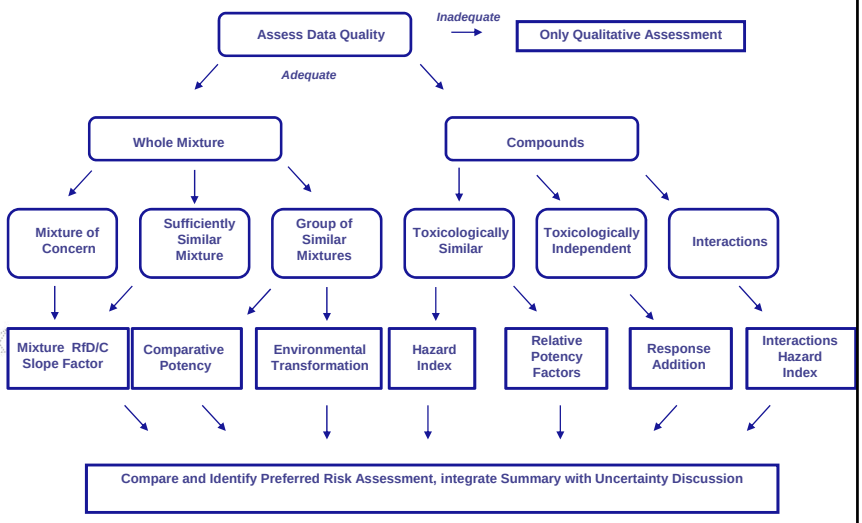




## METHODOLOGIES

- **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

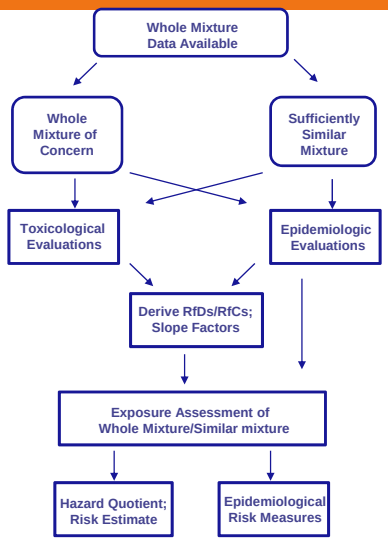



US-EPA (2000, 2007)





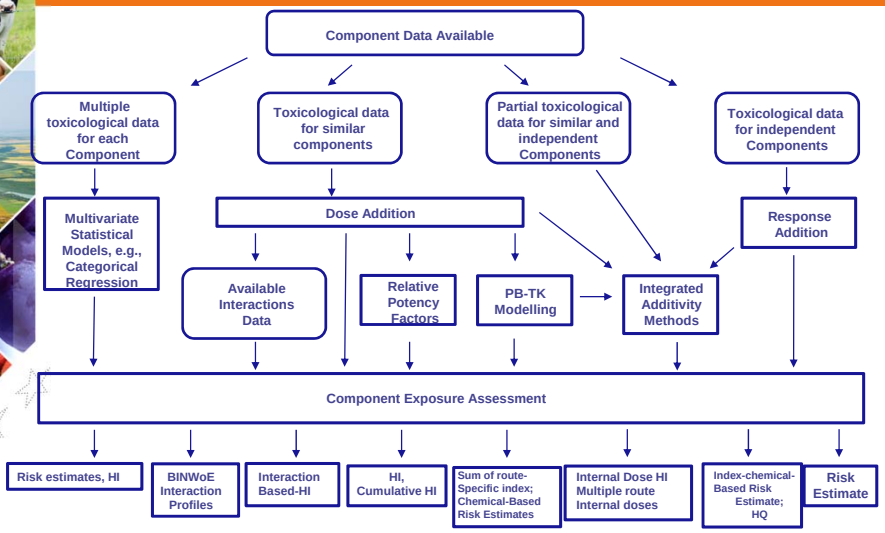
## WHOLE MIXTURE APPROACH




Modified from US-EPA (2007)

RfDs: Reference doses, RfCs: Reference concentrations

## COMPONENT-BASED APPROACH




PB-PK modelling; Physiologically-based pharmacokinetic modelling; HI: Hazard Index; BINWoE: Binary Weight of evidence; Interaction-based HI: Interaction-based Hazard Index; cumulative HI: cumulative Hazard Index; Internal Dose HI: Internal Dose Hazard Index; HQ: Hazard Quotients

Modified from US-EPA (2007)

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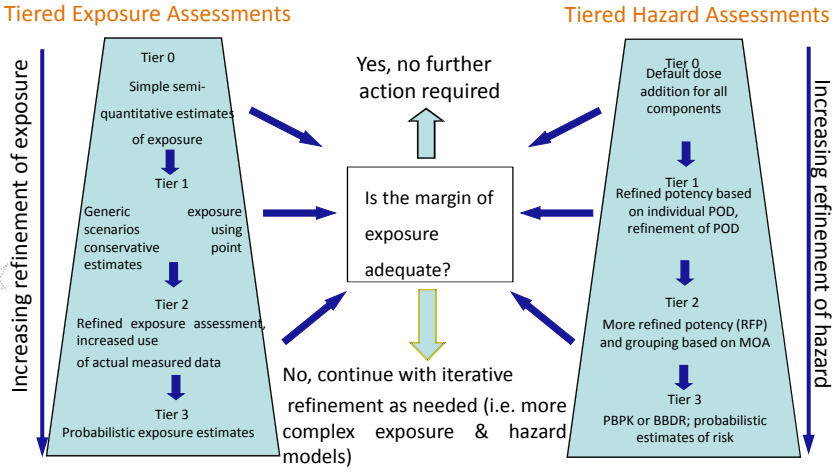
Investigating TK Tox mixtures to support Risk Assessment

## WHO FRAMEWORKS

***Problem Formulation***

*Nature of exposure? Is exposure likely? Co-exposure within a relevant timeframe?*
*Rationale for considering compounds in an assessment group?*




Modified from WHO (2011)

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## EFSA'S HAZARD ASSESSMENT OF PESTICIDES

Identify Cumulative Assessment Group

↓

Refine definition of Common effect

↓

Tier 1 : ADI, ARfD (For Common Effect)

↓

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

↓ Tier 3a : Relative Potency Factors NOAEL-derived

↓ Tier 3b : Relative Potency Factors BMDL-derived

Modified from EFSA (2009)

## 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 (2008) suitability of existing methodologies assessing cumulative and synergistic risks from pesticides to human health to set MRLs (Regulation (EC) 396/2005).
  - 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.



Investigating TK Tox mixtures to support Risk Assessment

## 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
  - EFSA (2012) dose addition approach-Ergot alkaloids

## SCIENTIFIC OPINIONS –ENVIRONMENTAL RISK ASSESSMENT

- Panel on Plant Protection Products and their Residues

➢ EFSA (2013) Science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)

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

o 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

o **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

o **Harmonised Methodologies**

✓ Human Risk assessment

Regulated compounds versus contaminants

✓ Animal Health risk assessment and Ecological risk assessment

o **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)





## 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/95$^{th}$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 TK TD interactions of chemical mixtures for human RA: summary statistics *in vitro*/*in vivo* data humans/test species for 100 chemicals of relevance to EFSA- published 2015
  - ✓ 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

European Food Safety Authority

EFSA Journal 2014;12(4):3638

**SCIENTIFIC REPORT OF EFSA**

**Modern methodologies and tools for human hazard assessment of chemicals**[1]

**European Food Safety Authority**[2,3]

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 OMICs 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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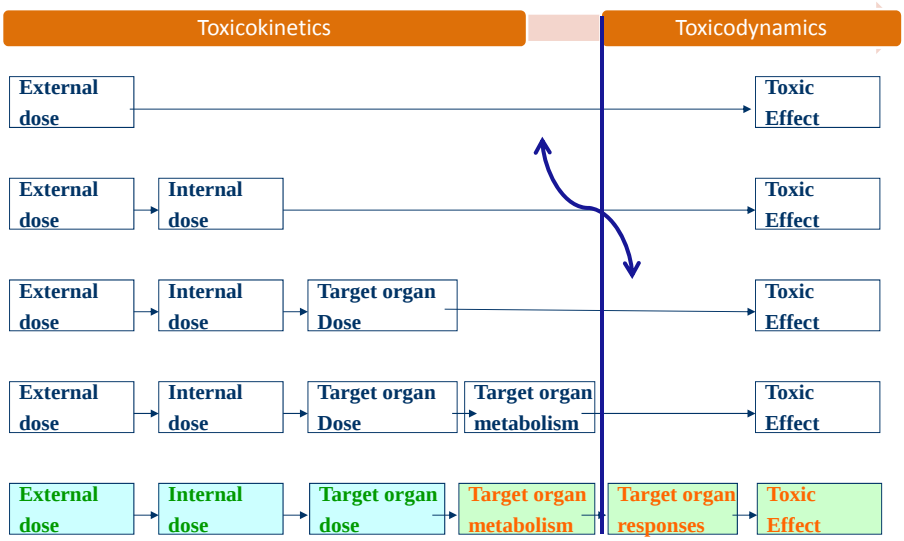
**KEY WORDS**

mode of action, adverse outcome pathway, integrated testing strategy, physiologically-based models, *in silico*, OMICs



## -Levels of Knowledge, Toxicokinetic and Toxicodynamic processes



## -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**

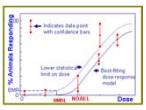
**Adverse Outcome Pathway**





## DERIVING HEALTH-BASED GUIDANCE VALUES

**ADI/TDI (mg/kg/day) = NOAEL or BMDL (mg/kg) / 100**



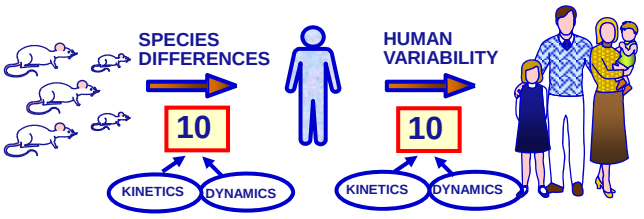

**Chronic exposure**

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

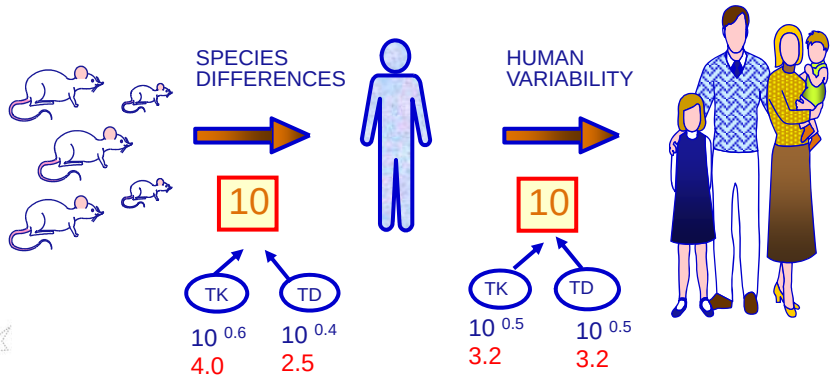
**Acute exposure**

- **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)

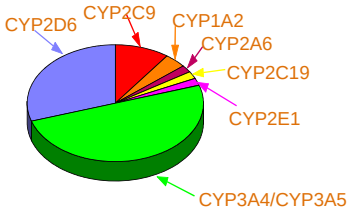
**-Categorical uncertainty factors e.g for humanTK pathway-related UF** (Dorne and Renwick, 2005)





## MAJOR METABOLIC/EXCRETION ROUTES IN HUMANS

Phase I enzymes
Cytochrome P-450,
ADH, Esterases…




Phase II enzymes
Conjugation reactions

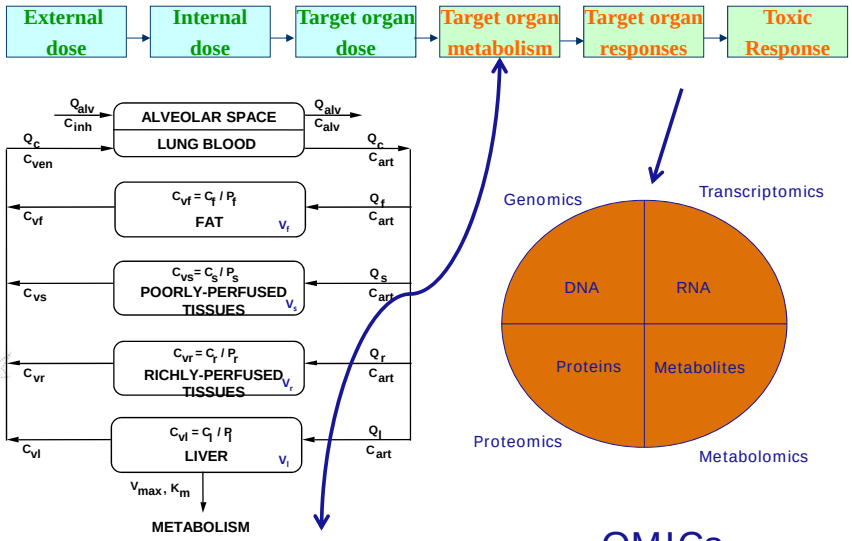
UDP-Glucuronyltransferases,
Sulphotransferases
Glutathione-s-transferases
Methyl-transferases
N-acetyltransferases
Amino acid conjugation

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-



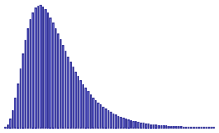

PB-TK models

OMICs





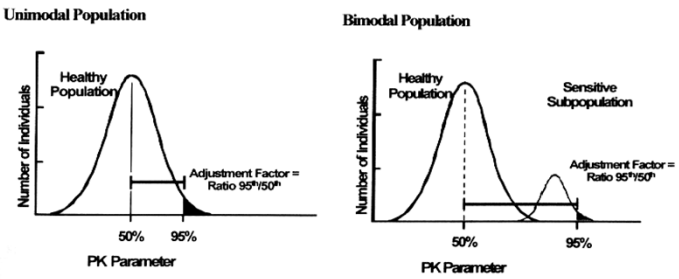
## -HUMAN VARIABILITY IN TOXICOKINETICS AND UF-



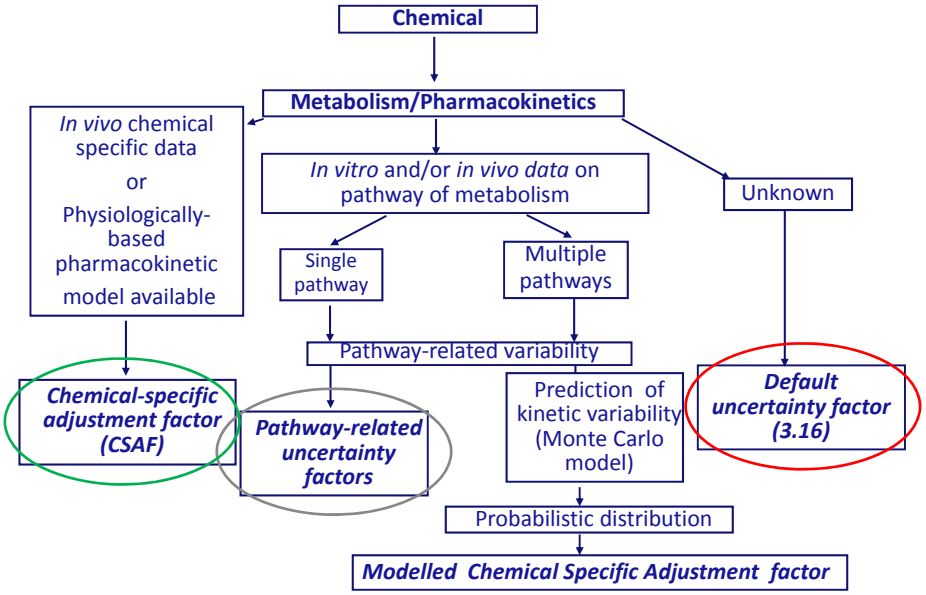
From pharmaceutical database human variability in TK available for many drugs /enzyme isoforms in different subgroups of the population.

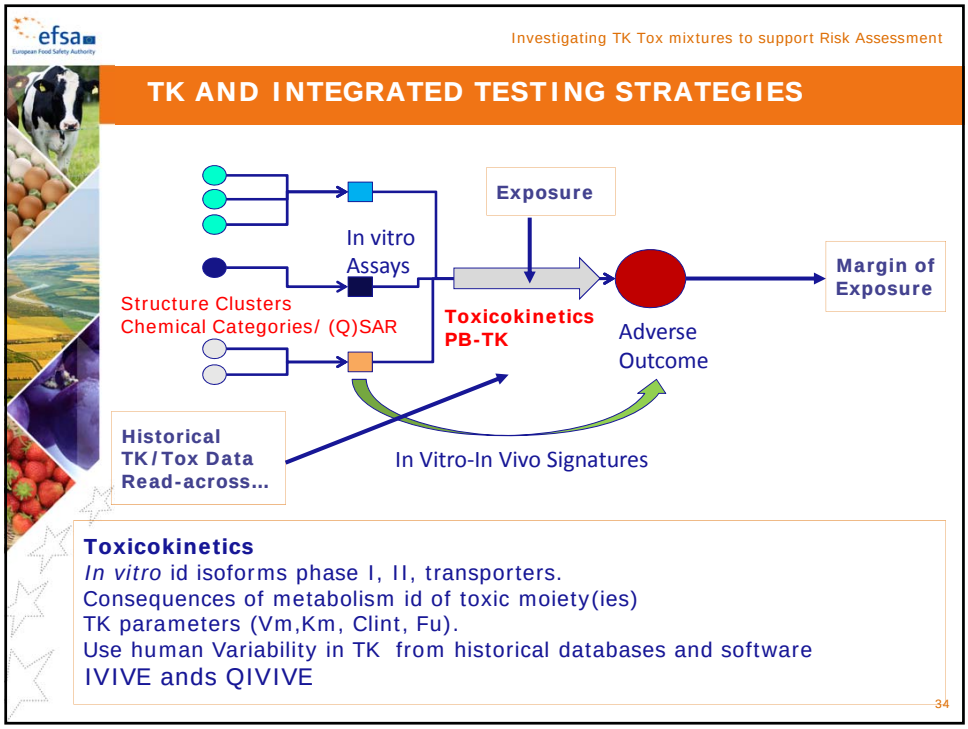
Rationale for meta-analysis of TK data to derive pathway-related distributions.
Uncertainty Factors

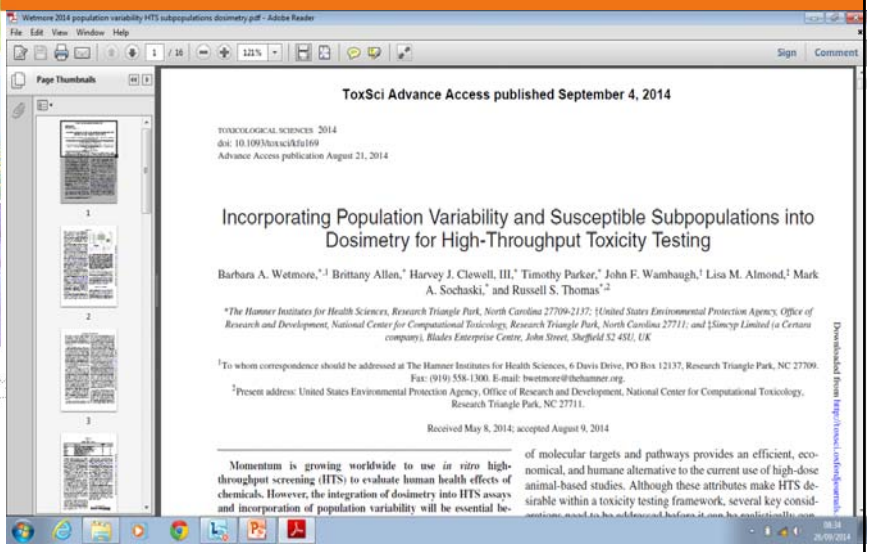
Can be made available online and allows for future refinement



## -Pathway-related uncertainty Factors-





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Incorporating Population Variability and Susceptible Subpopulations into Dosimetry for High-Throughput Toxicity Testing

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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 be-

of 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 consid-

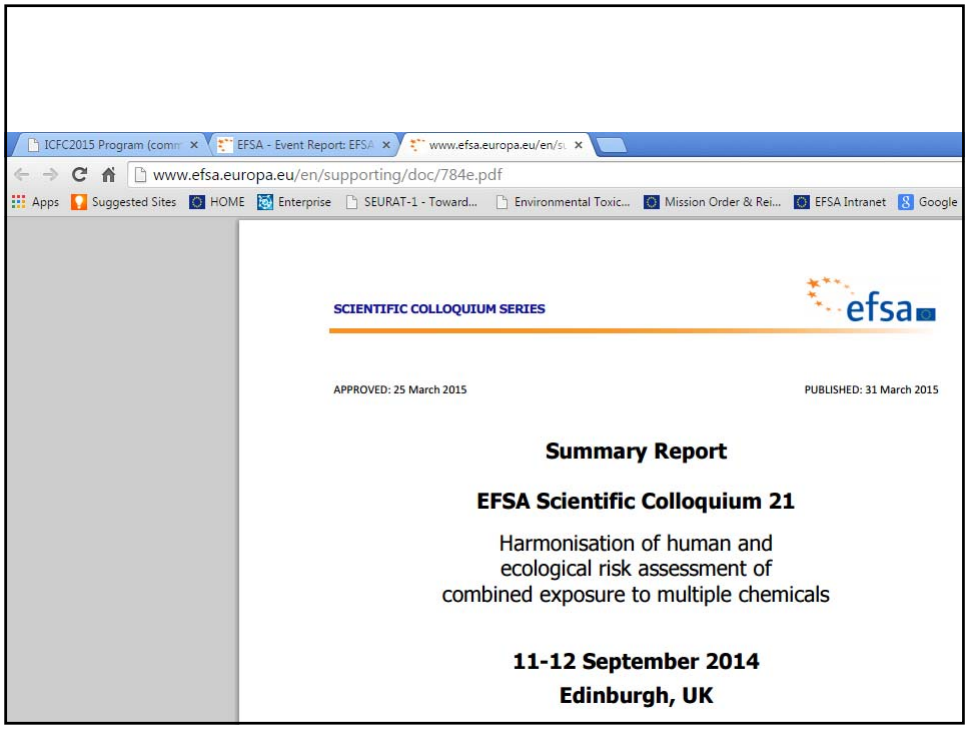


Investigating TK Tox mixtures to support Risk Assessment

## 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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Enterprise
SEURAT-1 - Toward...
Environmental Toxic...
Mission Order & Rei...
EFSA Intranet
Google
SCIENTIFIC COLLOQUIUM SERIES
efsa
APPROVED: 25 March 2015
PUBLISHED: 31 March 2015
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

## 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 Commitee**

✓ 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