Future perspectives on Mixture Risk Assessment at EFSA

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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…)

SCIENTIFIC OPINIONS-HUMAN RISK ASSESSMENT (I)

- 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.

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
  - 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

-SCIENTIFIC OPINIONS –ECOLOGICAL RISK ASSESSMENT-

- Panel on Plant Protection Products and their Residues

  - EFSA (2012) Science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)

  - 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

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

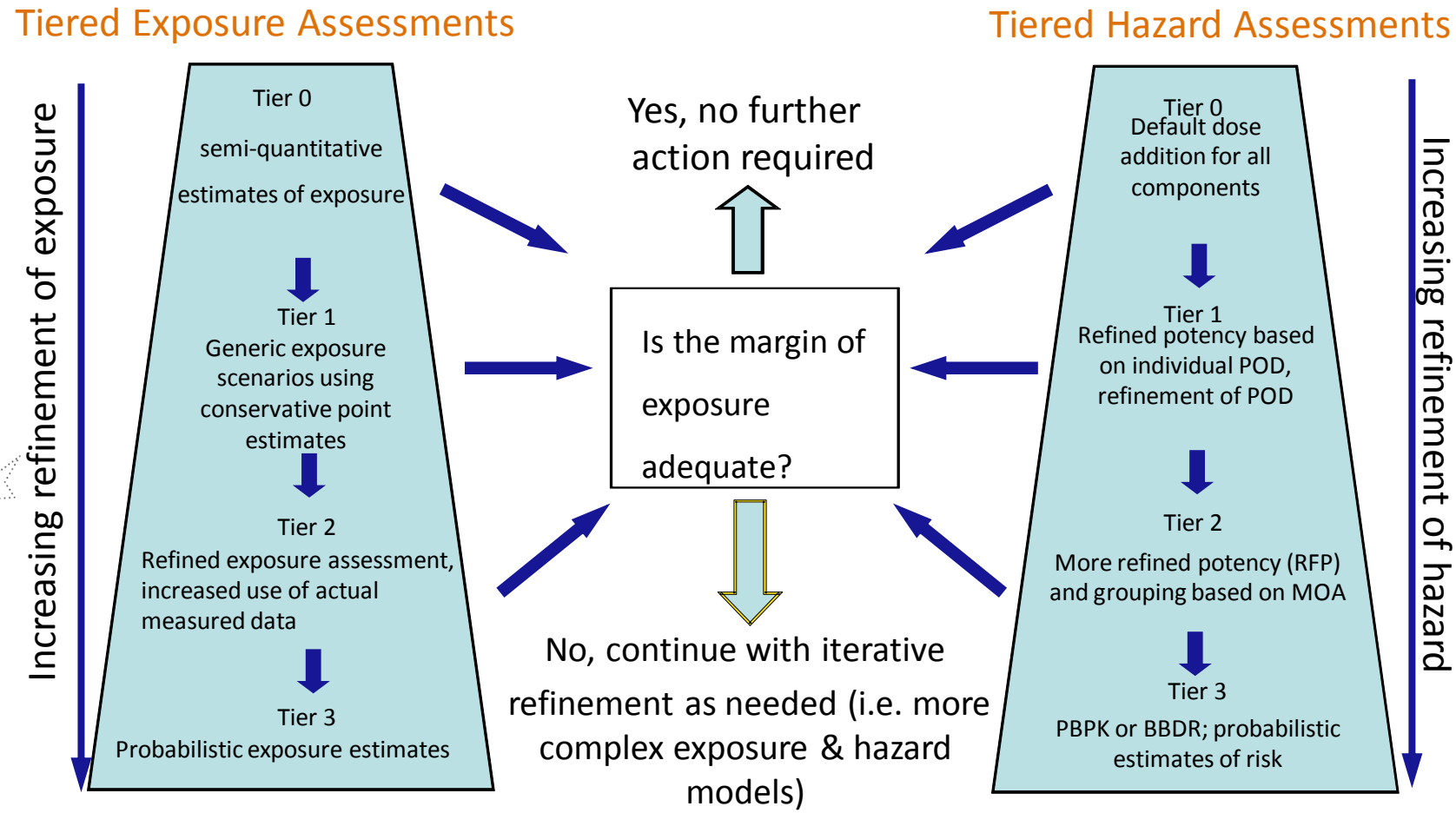
ABSTRACT

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.

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)

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/95thpercentiles)

- **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**

RECOMMENDATIONS: 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**
  - ✓ Sum stats TK TD interactions of chemical mixtures for human RA: summary statistics *in vitro*/*in vivo* data humans/test species for chemicals of relevance to EFSA- published 2015
  - ✓ Summary stats toxicity mixtures animal and ecology-June 2015.

EXTERNAL SCIENTIFIC REPORT

LASER ANALYTICA

APPROVED: 8 December 2014

PUBLISHED: 31 March 20

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 t human risk assessment of combined exposure to multiple compounds. A number of activities ha already been undertaken by EFSA, notably in the fields of pesticides and contaminants. This proje aimed at providing quantitative information on combined effects of multiple compounds to suppo evidence-based hazard assessment. The first step was to record and collect, using extensive literatu searches/systematic review methods, Pharmaco/Toxicokinetic (PK/TK) and Pharmac dynamic/Toxicodynamic (PD/TD) information on potential interactions between selected compoun *In vivo* PD/TD and *in vitro* and *in vivo* PK/TK data were collected mostly for binary mixtures pharmaceuticals (substrates of major routes of metabolism and known inhibitors/inducers) and ma classes of regulated compounds and contaminants relevant to food safety. All data were th consolidated via meta-analyses to quantify magnitudes of interaction and their inter-individ variability for both TK and TD dimensions. Overall, this report illustrates application of systematic da collection for both human TK and TD aspects of multiple compounds to quantify magnitudes metabolic and toxicological interactions respectively. Further analyses are recommended to integra such magnitude of interaction and variability data in human hazard assessment of multi compounds. © LASER Analytica, 2015

EXTERNAL SCIENTIFIC REPORT

APPROVED: 24 July 2015

PUBLISHED

Data collection on Combined Toxicity of Multip Chemicals for Animal Health and Ecological Ri Assessment

LASER Analytica

Nadia Quignot, Audrey Grech, Billy Amzal

Abstract

There is an increasing need to develop harmonised frameworks and methods for the risk of combined exposure to multiple chemicals. A number of activities have already been un EFSA, notably in the fields of pesticides and contaminants. This project aims at se reviewing, collecting and synthesizing the published data on combined effects of multiple more than 50 species of veterinary and ecological relevance, using extensive literature se taxonomical hierarchy for the literature searches was very wide ranging from bact invertebrates and vertebrates (amphibians, reptiles, birds, cats, cattle, deers, goats, ho pigs, sheep, fishes, spiders, ephemeroptera, hymenoptera, diptera, earthworms, Ca elegans, molluscs, mites, crustacea, odonata, orthoptera, collembola, coleoptera, blat cyanobacteria, proteobacteria, fungi). *In vivo* toxicity data were extracted from 199 representing 3,074 individual studies on pesticides, environmental contaminants, products, hormones, metals, mycotoxins and pharmaceuticals. The magnitudes of

OpenFoodTox: EFSA’s Open Source Hazards Database

DATA/EVIDENCE AVAILABLE IN CHEMICAL RA

Tier	Exposure Assessment		Hazard identification		Hazard characterisation		Risk Characterisation
	Occurence	**Consumption**	**TK**	**TD**	**TK**	**TD**	
0	Semi-Q	Default values	No data	No data	*in silico* Read across	Default values TTC Read across *In silico* Default UF	e.g. Default values Qualitative
1	Point estimates	Point estimates in food categories	*In silico* Limited data Semi-Q	*In silico* Limited data Read across	*in silico* Basic TK Read across	*in silico* Read across NOAEL Default UF	e.g. Semi-quantitative
2	Measured data	Measured in some food categories	Dossier data Qttve	Dossier Data	*in silico* ADME data	NOAEL/ BMDL Default *in silico* UF	e.g. Quantitative Deterministic/ Probabilistic
3	Large measured dataset	Full patterns - food categories	Dossier and/or lit. (*in vitro*, *in vivo*)	Data in dossier and/or lit. (*in vitro*, OMICs, epi)	MoA/AOP, Epi data, PB-PK model, BBDR, BMDL Chemical specific adjustment factor (CSAF)		e.g. Quantitative Full probabilistic

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)

11,818 risk assessment summaries

4,185 Substances (chemical identifiers including SMILES)

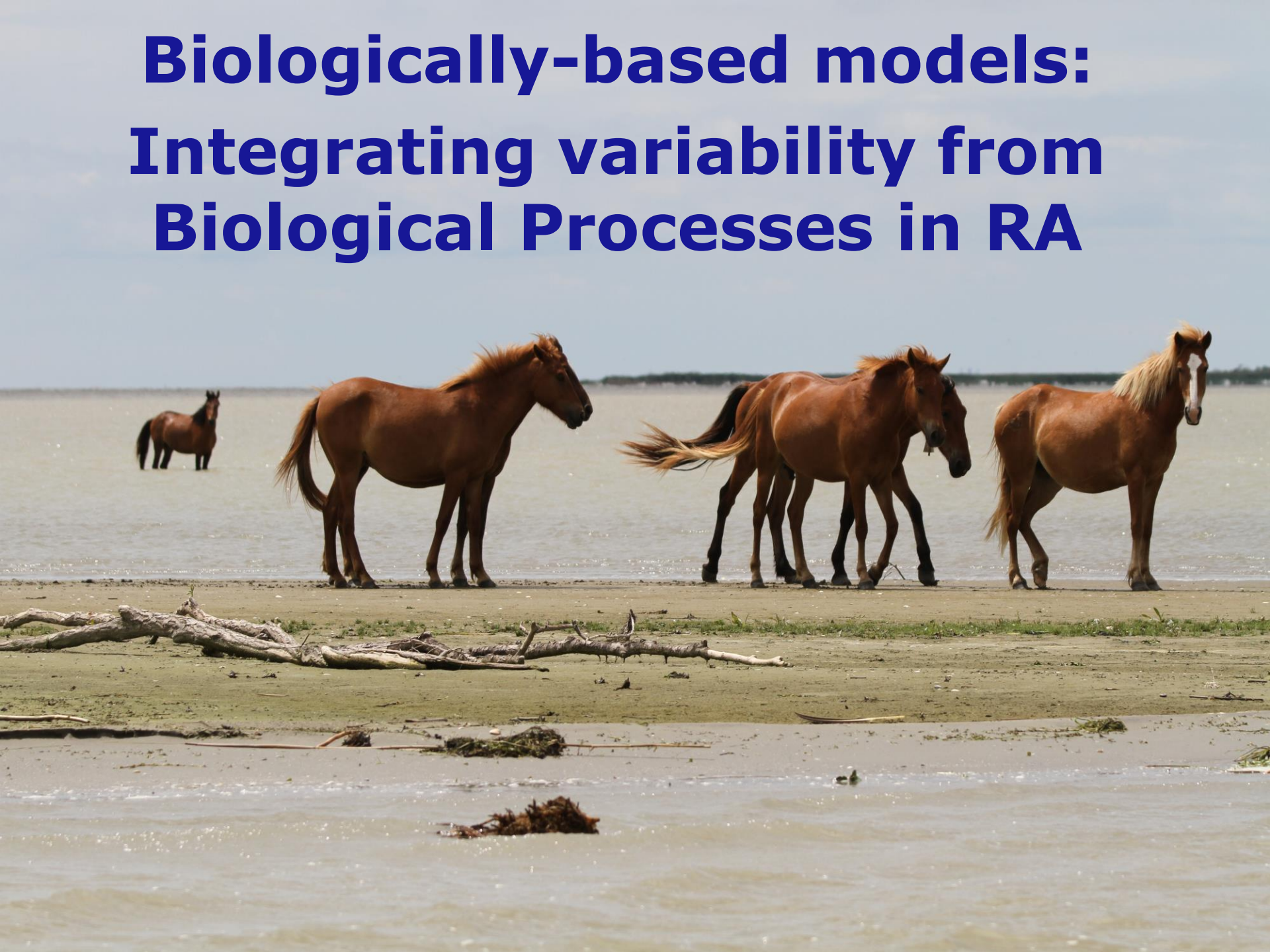
8,400 Toxicological endpoint studies

133 Positive genotoxicity studies

OPENFOODTOX AND *IN SILICO* TOOLS

- 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



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**

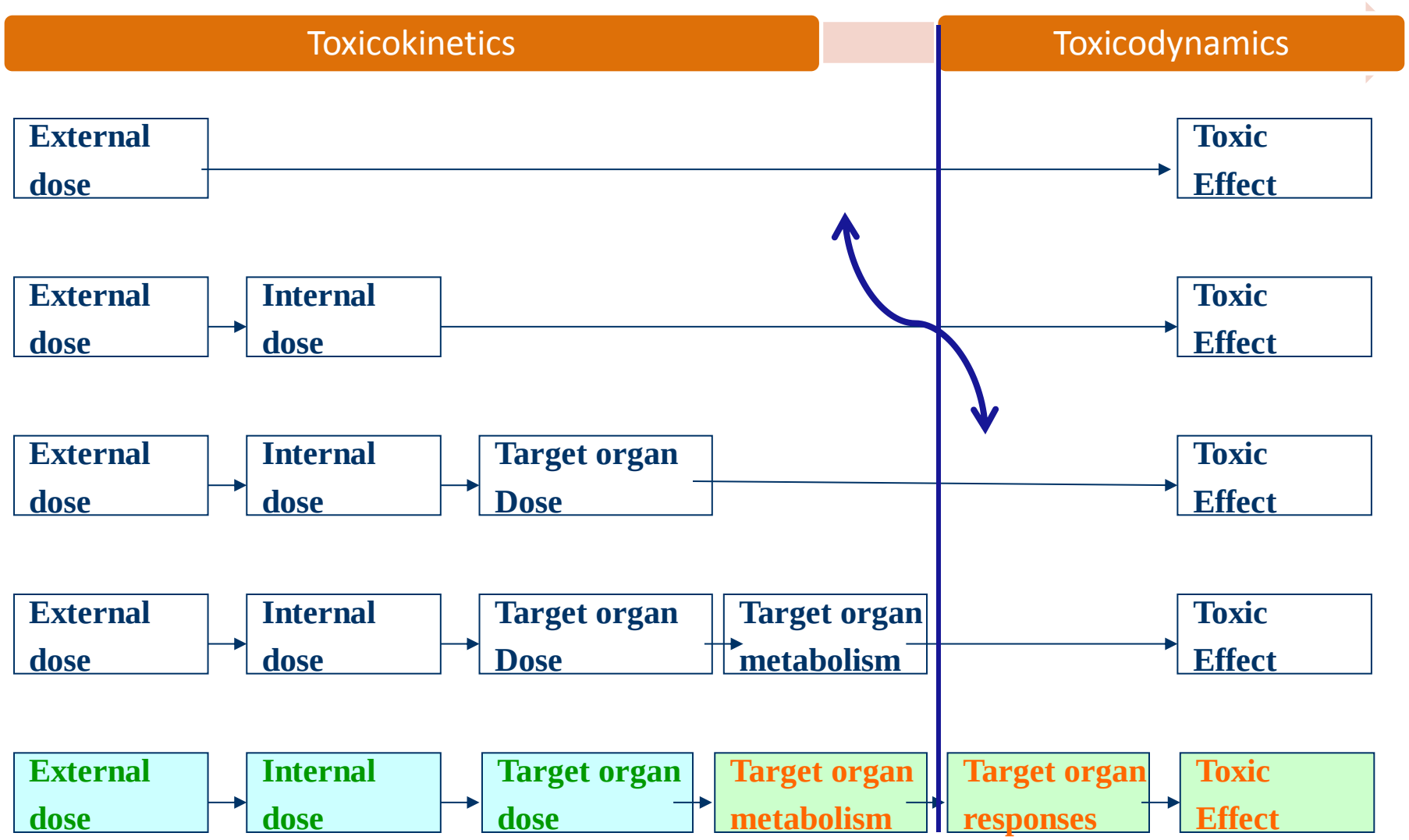
ABSTRACT

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.

KEY WORDS

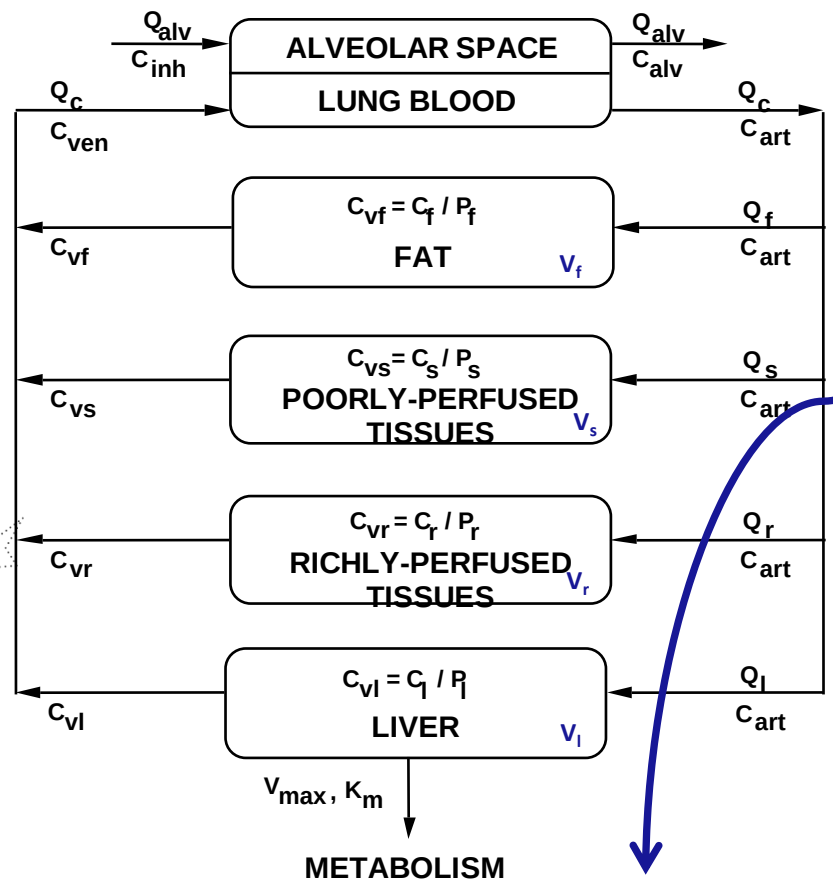
mode of action, adverse outcome pathway, integrated testing strategy, physiologically-based models, *in silico*, OMICs

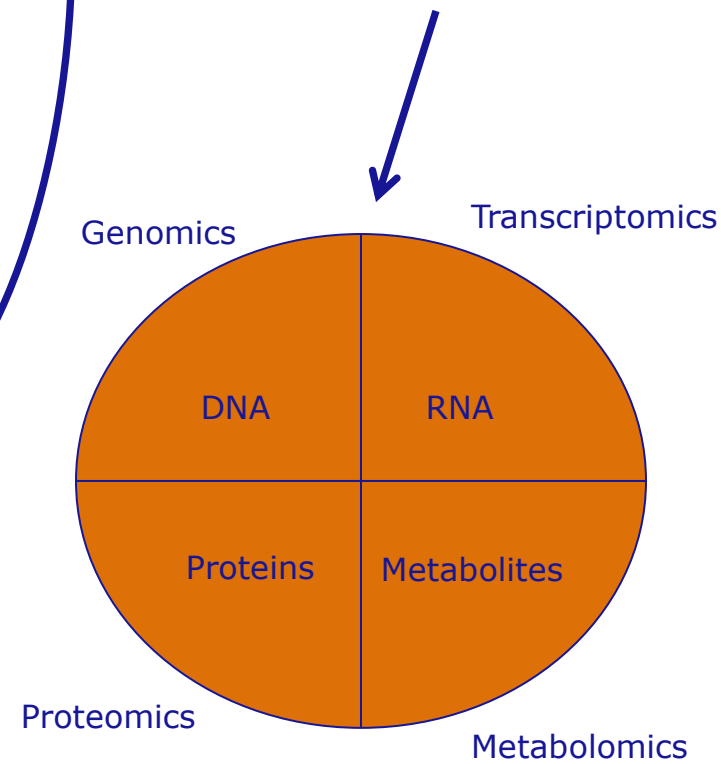
-Levels of Knowledge, Toxicokinetic and Toxicodynamic processes



-Biologically-Based models and OMICs-

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

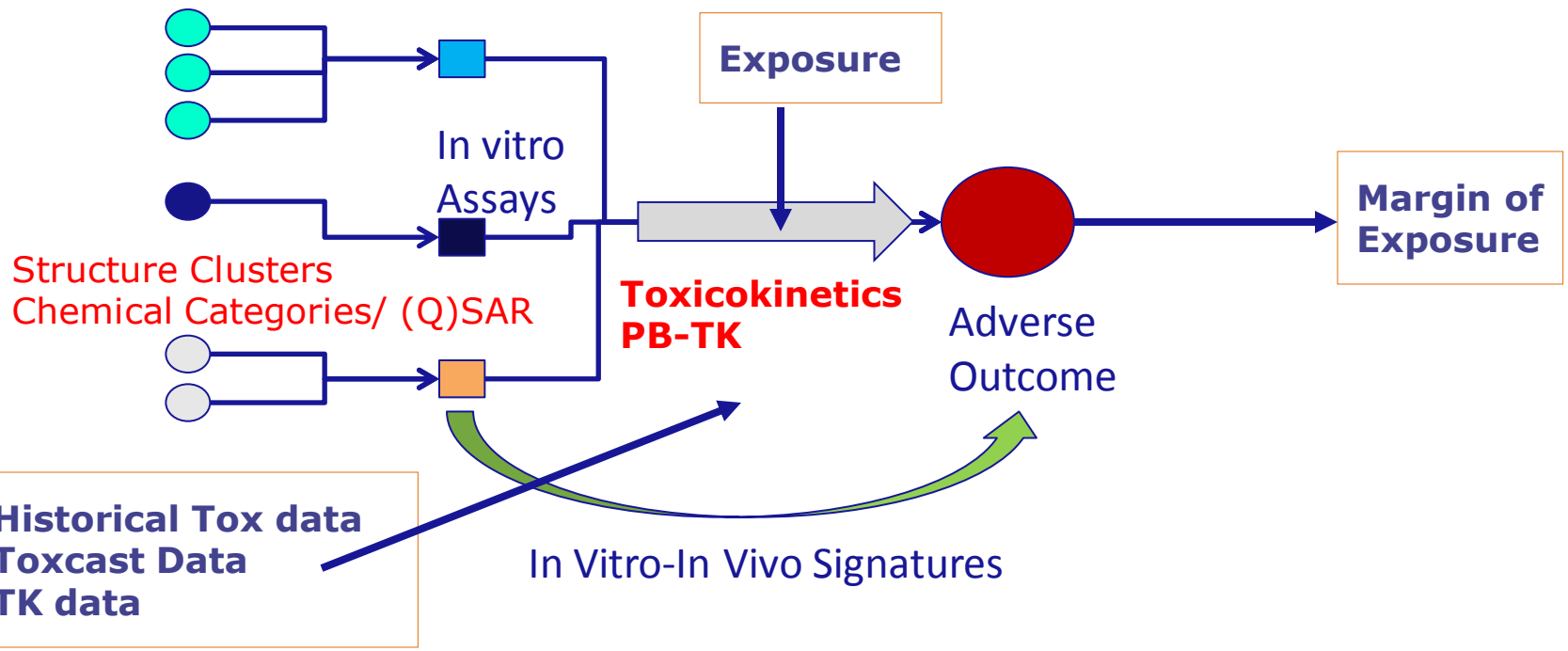




PB-TK models

OMICs

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, proetin 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

Wetmore 2014 population variability HTS subpopulations dosimetry.pdf - Adobe Reader

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TOXICOLOGICAL SCIENCES 2014
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Advance Access publication August 21, 2014

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-

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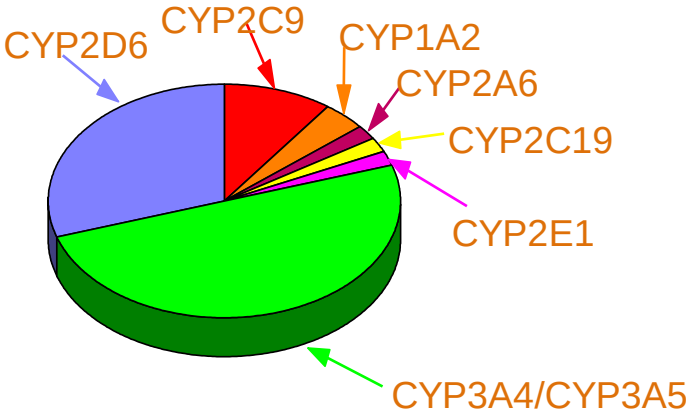
MAJOR METABOLIC/EXCRETION ROUTES IN HUMANS

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

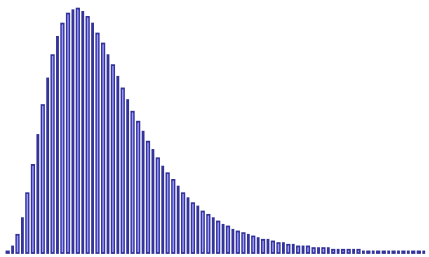
Transporters

Phase 0- Uptake transporters:
e.g OATPs, OCTs.

Phase III-Efflux pumps:
e.g ABCs (P-glycoproteins and MRPs)

Renal excretion

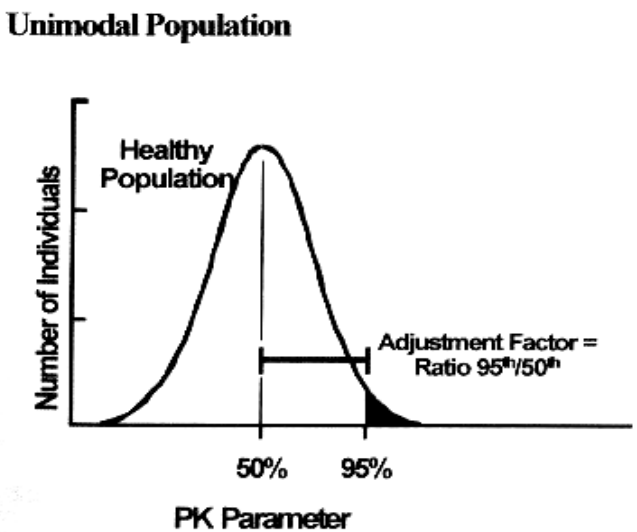
-HUMAN VARIABILITY IN TOXICOKINETICS -

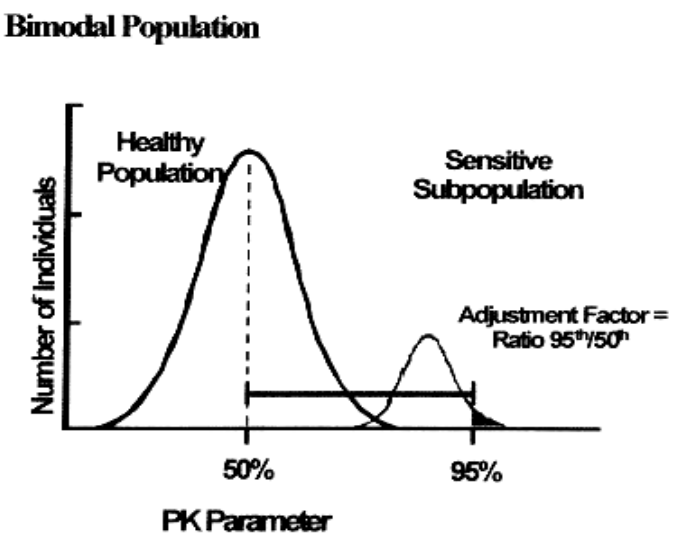


From pharmaceutical database human variability in TK available for many drugs /enzyme isoforms in different subgroups of the population.

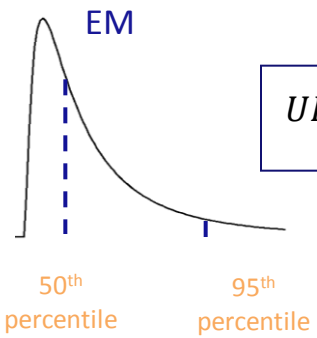
Rationale for meta-analysis of TK data to derive metabolism variability distributions

Can be combined with *in vitro* data and used in QIVIVE

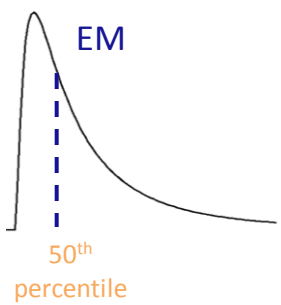


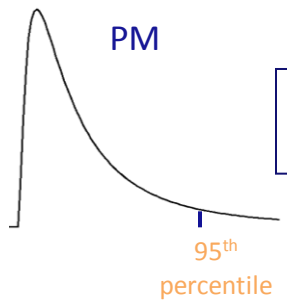


Combining Polymorphism data Extensive and Poor Metabolisers (EMs and PMs) : CYP2D6 Example



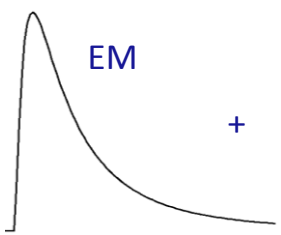
$$UF_{EM} = \frac{p95_{EM}}{p50_{EM}}$$



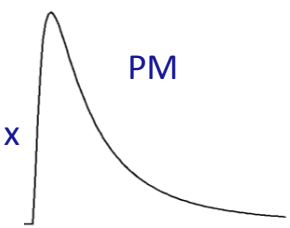


$$UF_{PM} = \frac{p95_{PM}}{p50_{EM}}$$

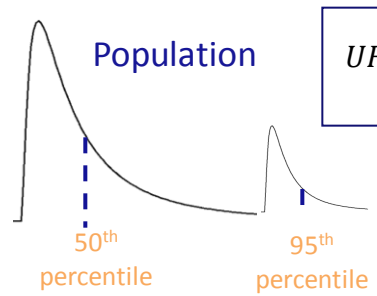
(1-α) EM x



+ α PM x

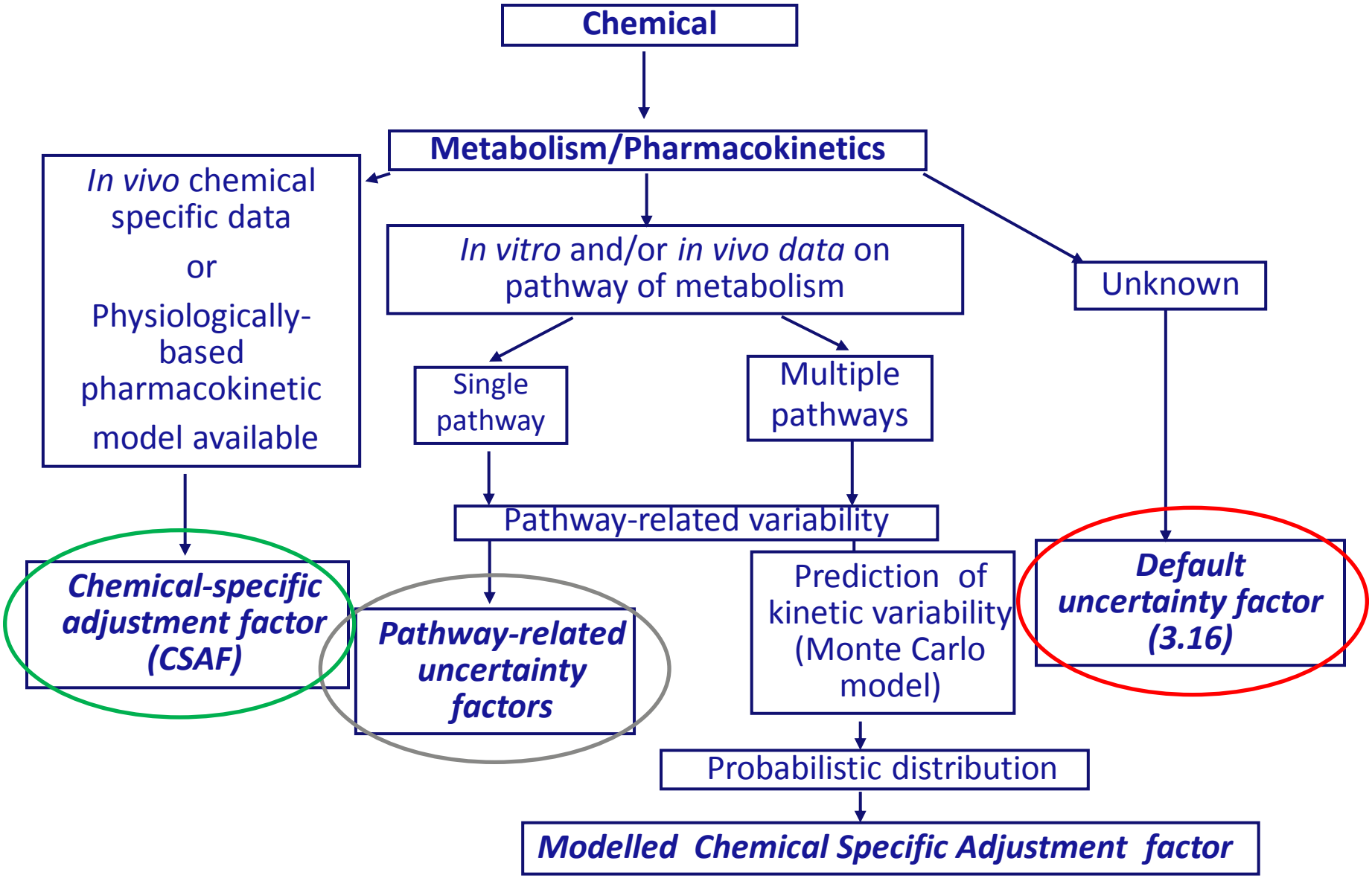


Monte-Carlo →



$$UF_{pop} = \frac{p95_{pop}}{p50_{pop}}$$

-Pathway-related uncertainty Factors-



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

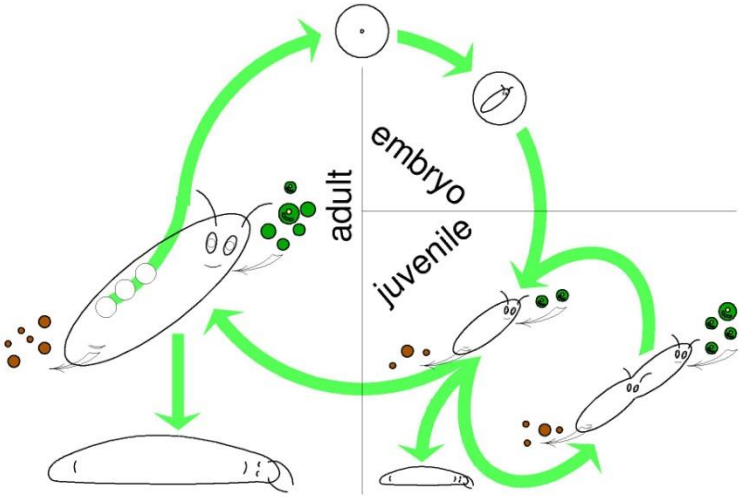
DEB MODELS

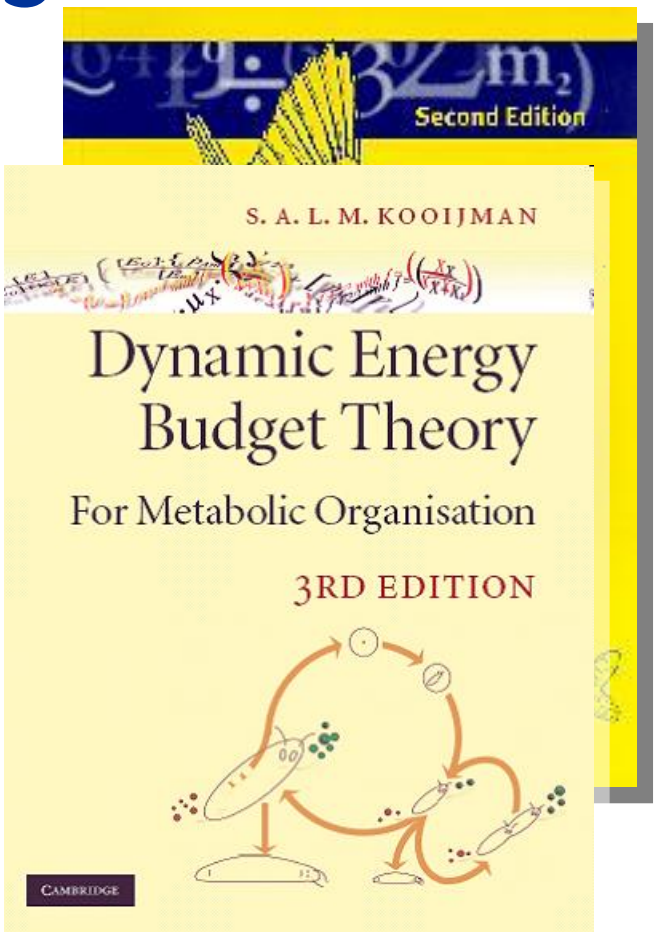
Quantitative theory for metabolic organisation from 'first principles'

- time, energy and mass balance

Life-cycle of the individual

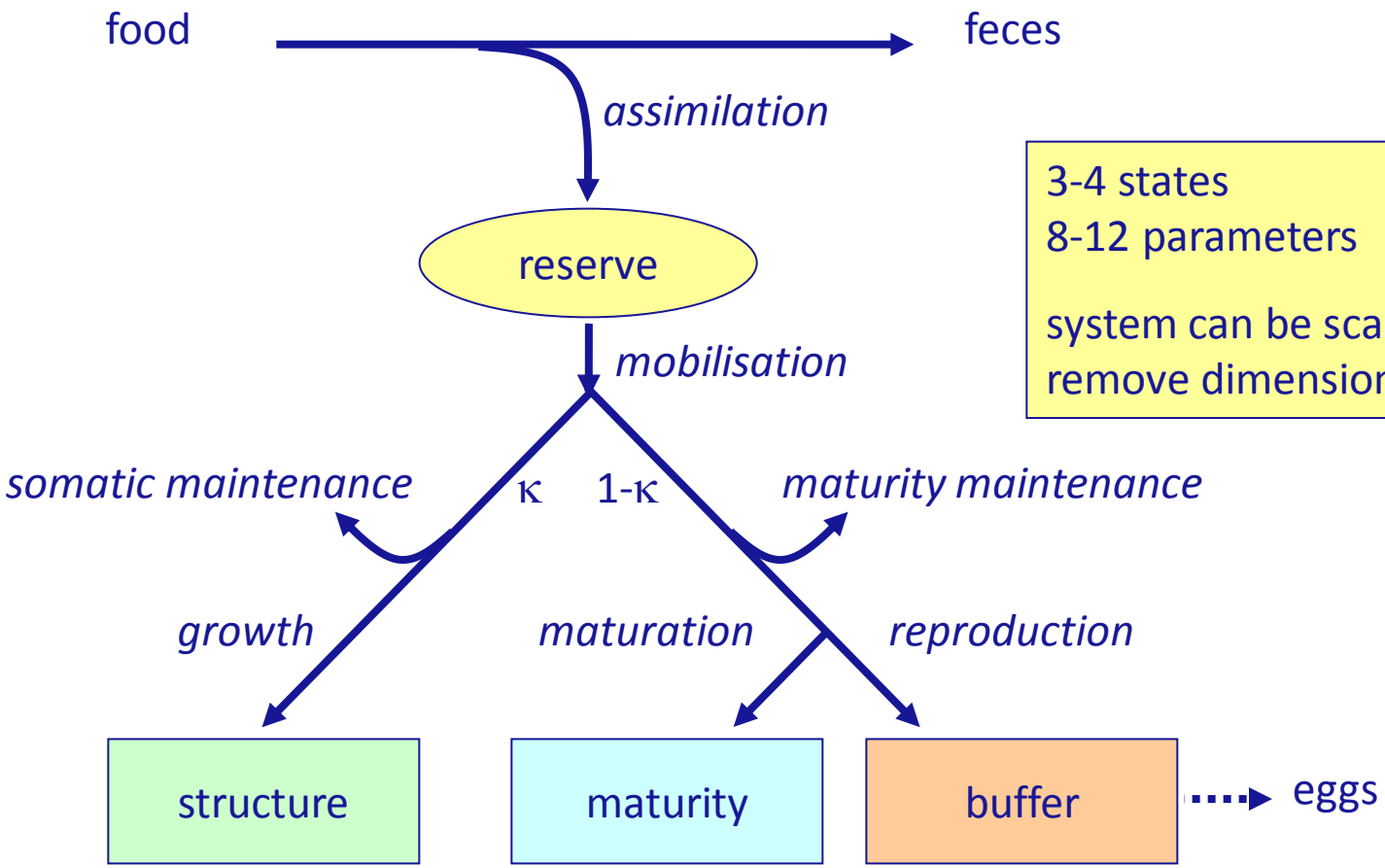
- links levels of organisation: molecule → ecosystems





Kooijman (2010)

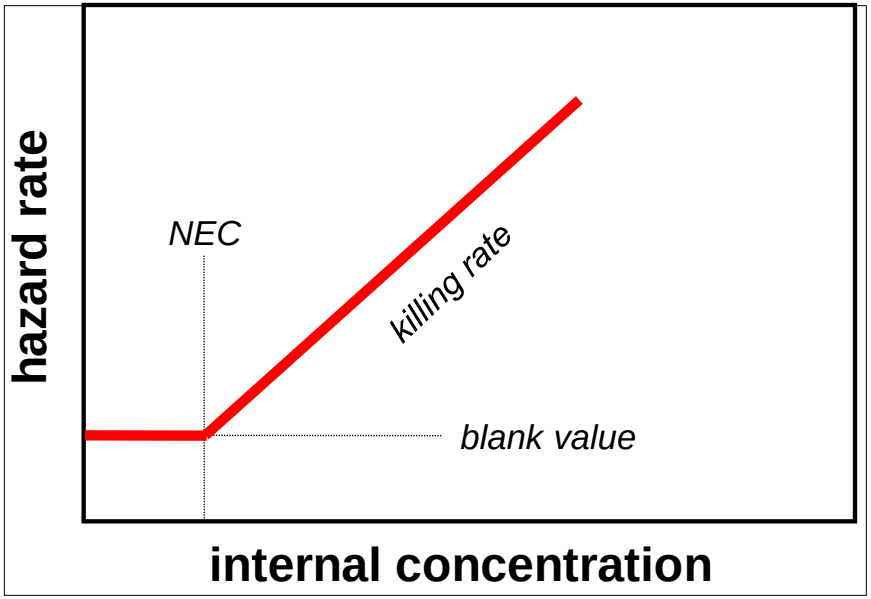
What are DEB MODELS ?

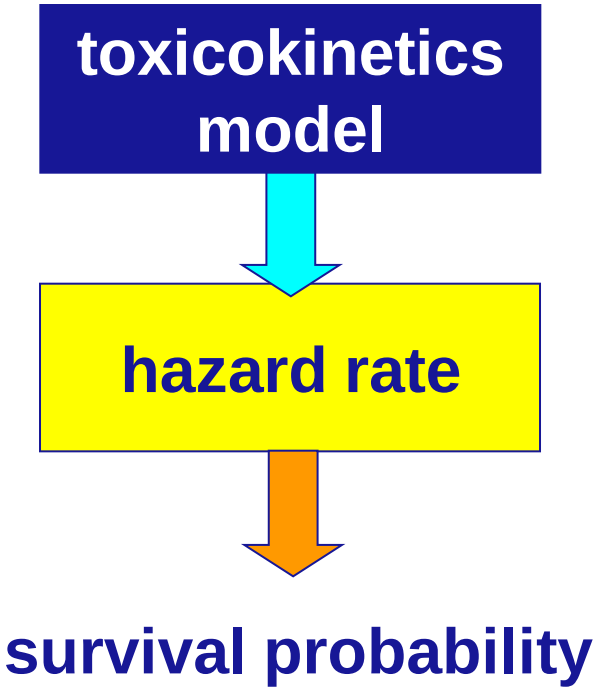


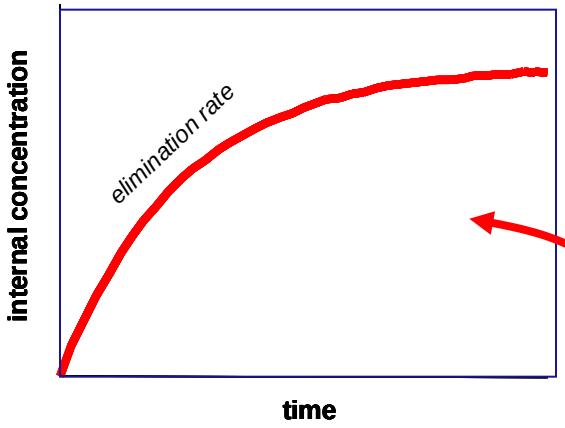
3-4 states
8-12 parameters

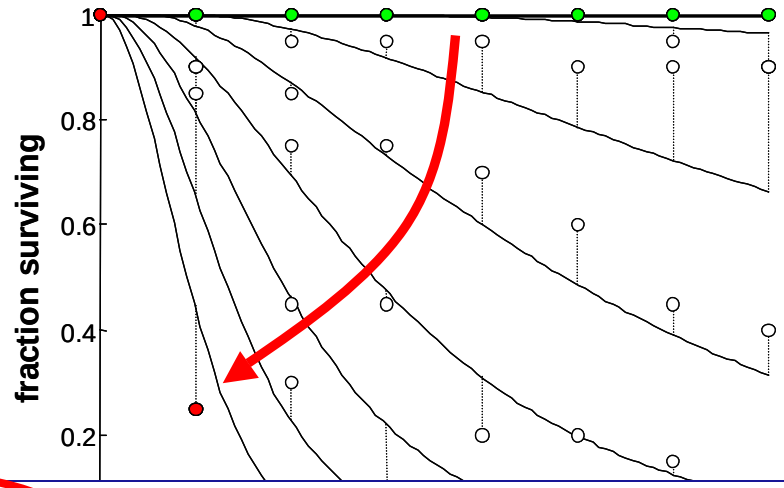
system can be scaled to remove dimension ‘energy’

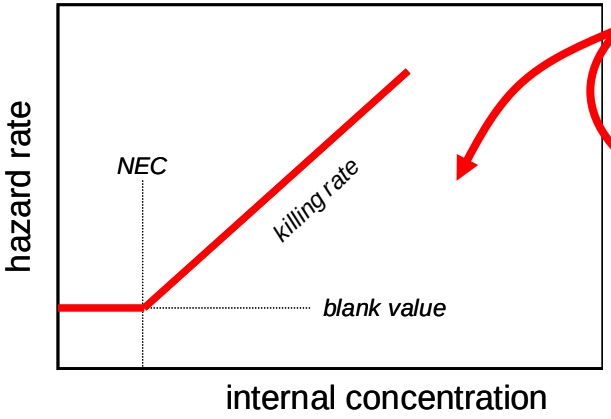
- Chemical affects the ***probability*** to die
  - hazard modelling



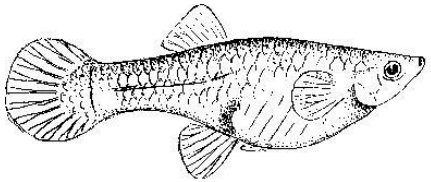








Elimination rate	0.73	d^{-1}
Blank hazard rate	0.0064	d^{-1}
NEC	2.8 (2.1-3.1)	µg/L
Killing rate	0.031	L/(µg d)



DYNAMIC ENERGY BUDGET MODELS FOR TERRESTRIAL AND AQUATIC ORGANISMS

- ✓ 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

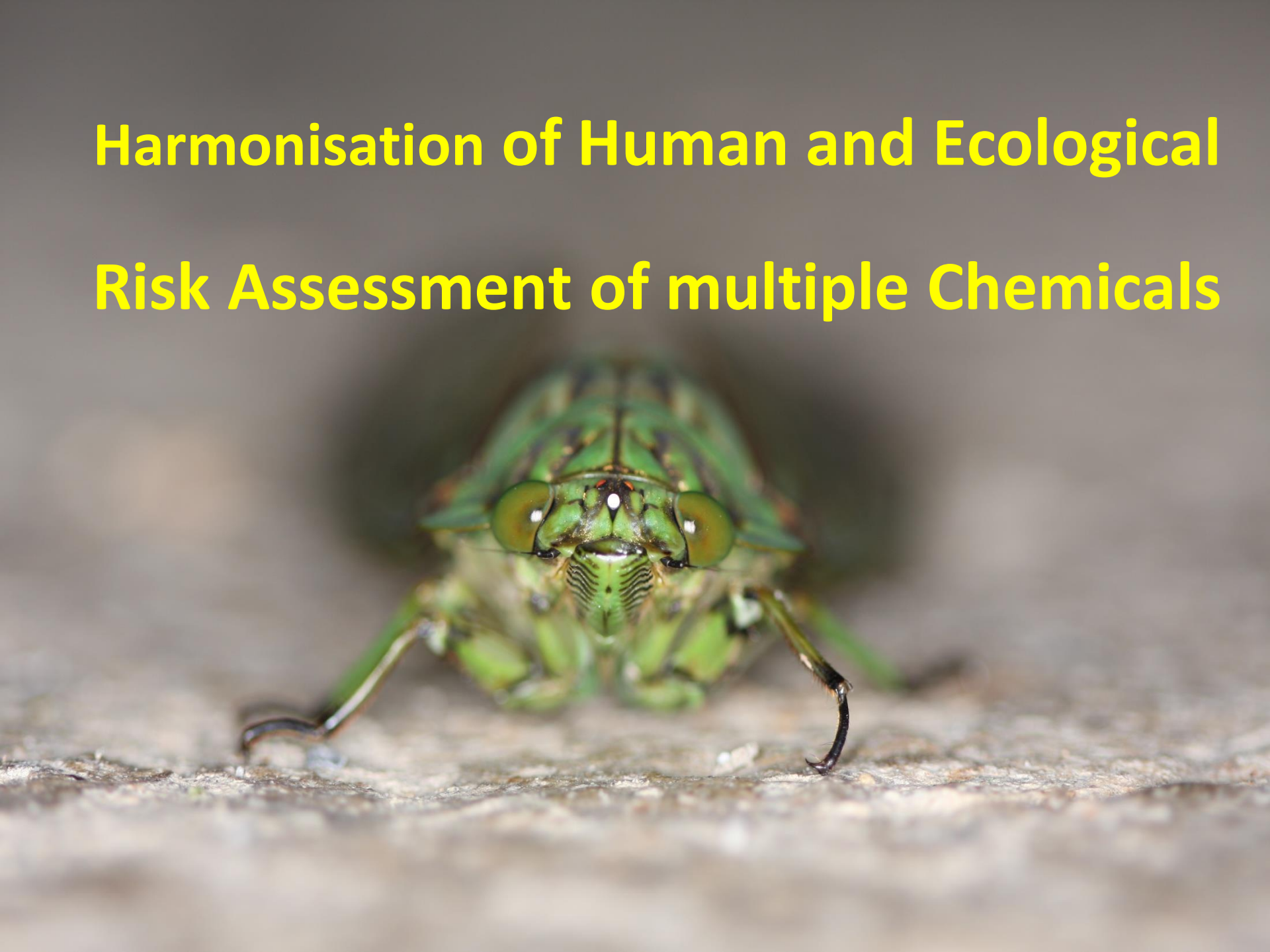
- Objective 1: **Extensive literature searches and structured data collection on biochemical, genetic and environmental variables and impact on mycotoxin production**

- 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



SCIENTIFIC COLLOQUIUM SERIES

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

European Food Safety Authority

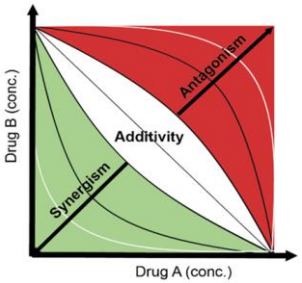
-TERMS OF REFERENCE-

- 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

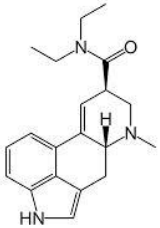
- Mixture as priority topic for previous and current SC (Sep-Dec 2015)
- Harmonisation of methodologies for human and Ecological RA risk to multiple chemicals –start spring 2016 (May-June)
- Public consultation on ToR optional
- WG expertise balanced between human RA and ERA
- Activities/summary report 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

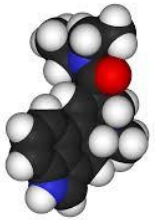
-SUMMARY-

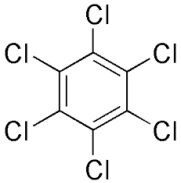


Ecological

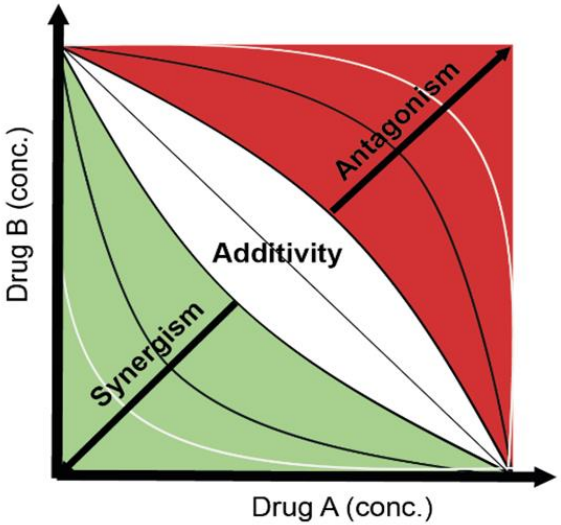
Human

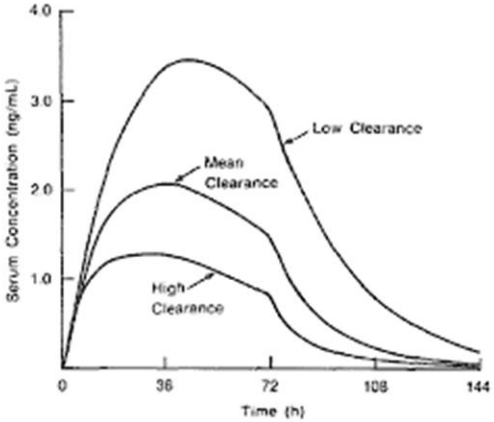






THANK YOU


Antagonism
Additivity
Synergism
Drug B (conc.)
Drug A (conc.)


4.0
3.0
2.0
1.0
Serum Concentration (ng/mL)
Low Clearance
Mean Clearance
High Clearance
0
36
72
108
144
Time (h)