PARMA SUMMER SCHOOL

28 – 30 SEPTEMBER 2021, Parma

Food Safety Aspects of Integrated Food Systems

Harmonised methodologies for the risk assessment of combined exposure to multiple chemicals: Principles and Applications

Jean Lou CM Dorne - EFSA

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The "Cocktail Effect"

Limoncello

\+

Bargnolino

\+

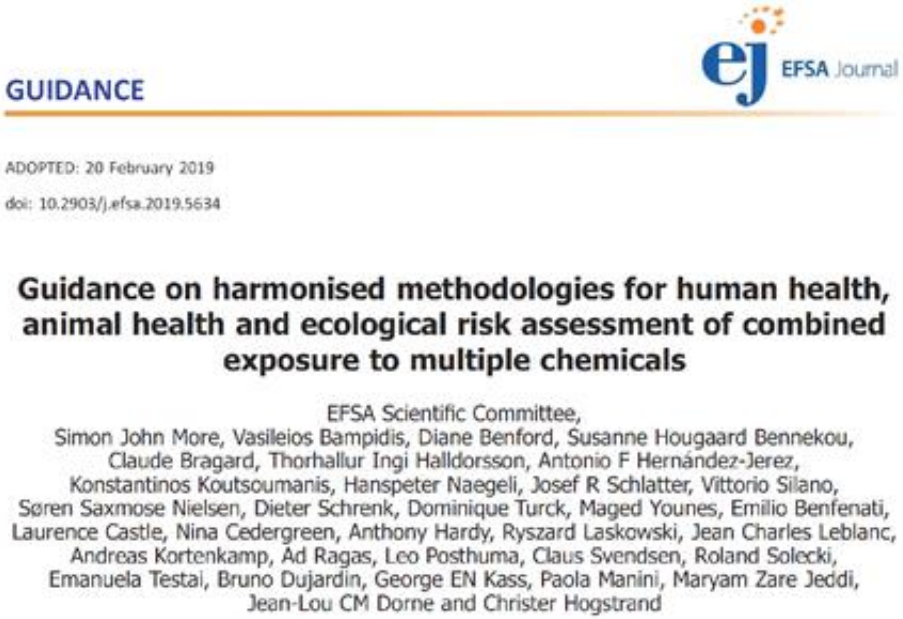
Nocino

Hazardous mixture

Be Careful With This One !

=

MIXTOX Guidance Document (2019)



GUIDANCE

EFSA Journal

ADOPTED: 20 February 2019
doi: 10.2903/j.efsa.2019.5634

Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Vasileios Bampidis, Diane Benford, Susanne Hougaard Bennekou, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Konstantinos Koutsoumanis, Hanspeter Naegeli, Josef R Schlatter, Vittorio Silano, Søren Saxmose Nielsen, Dieter Schrenk, Dominique Turck, Maged Younes, Emilio Benfenati, Laurence Castle, Nina Cedergreen, Anthony Hardy, Ryszard Laskowski, Jean Charles Leblanc, Andreas Kortenkamp, Ad Ragas, Leo Posthuma, Claus Svendsen, Roland Solecki, Emanuela Testai, Bruno Dujardin, George EN Kass, Paola Manini, Maryam Zare Jeddi, Jean-Lou CM Dorne and Christer Hogstrand

- **Harmonised Guidance**
  - **Whole Mixture approach**
  - **Component-based approach**
  - Include interactions

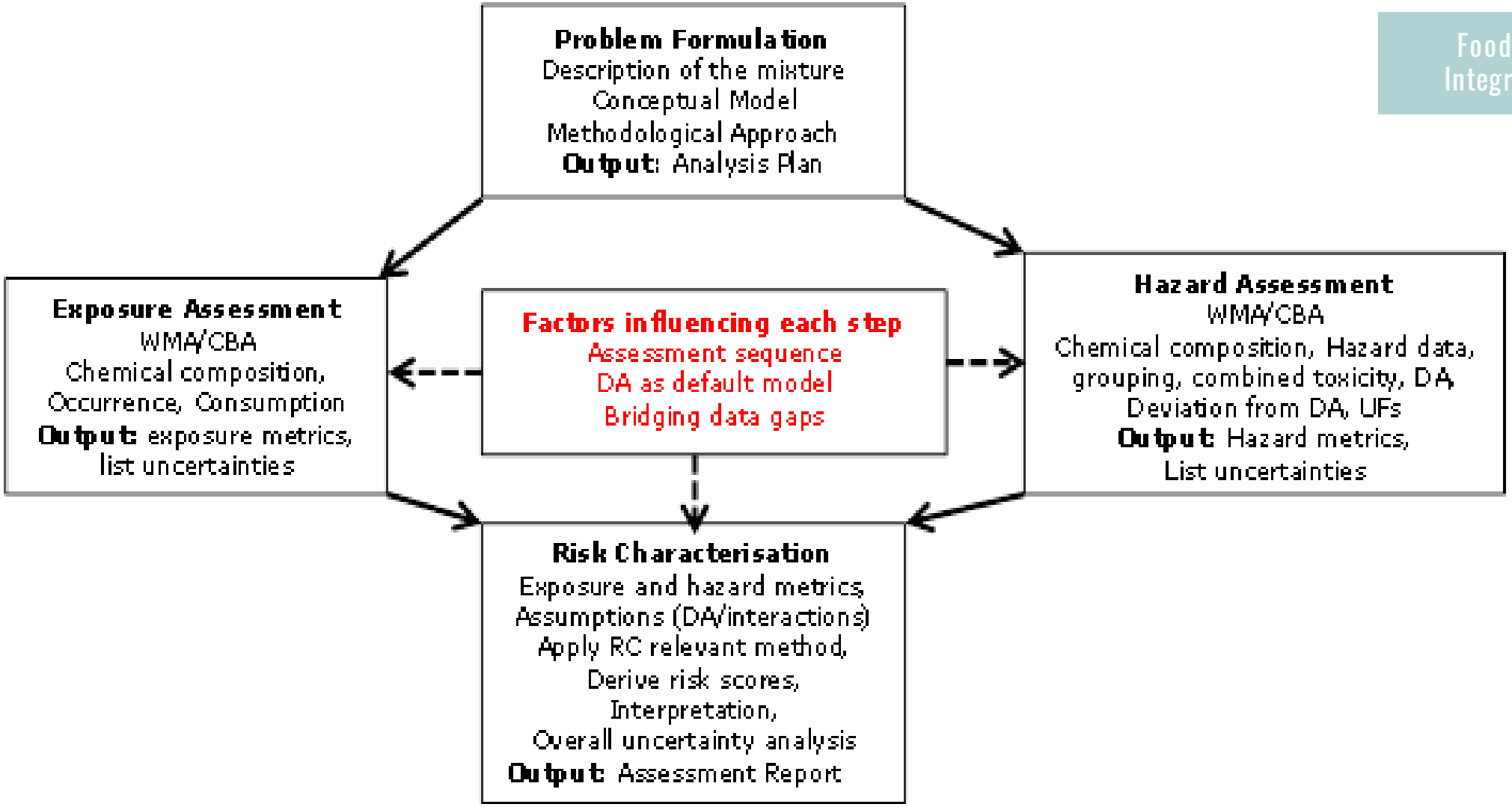
Problem Formulation

Exposure and Hazard Assessment

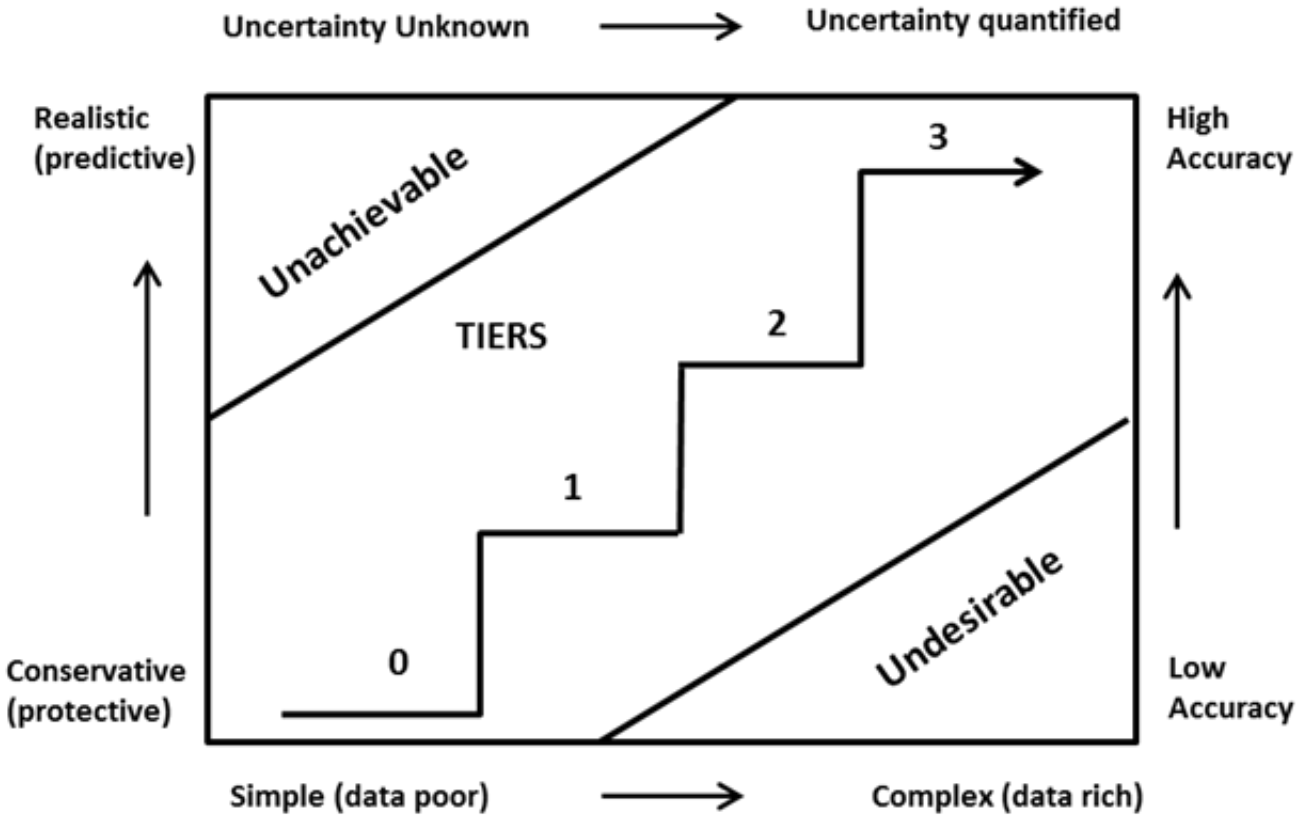
Risk Characterisation

- **Reporting Table**
  - Human Health, Animal Health
  - Environment

Harmonised Framework

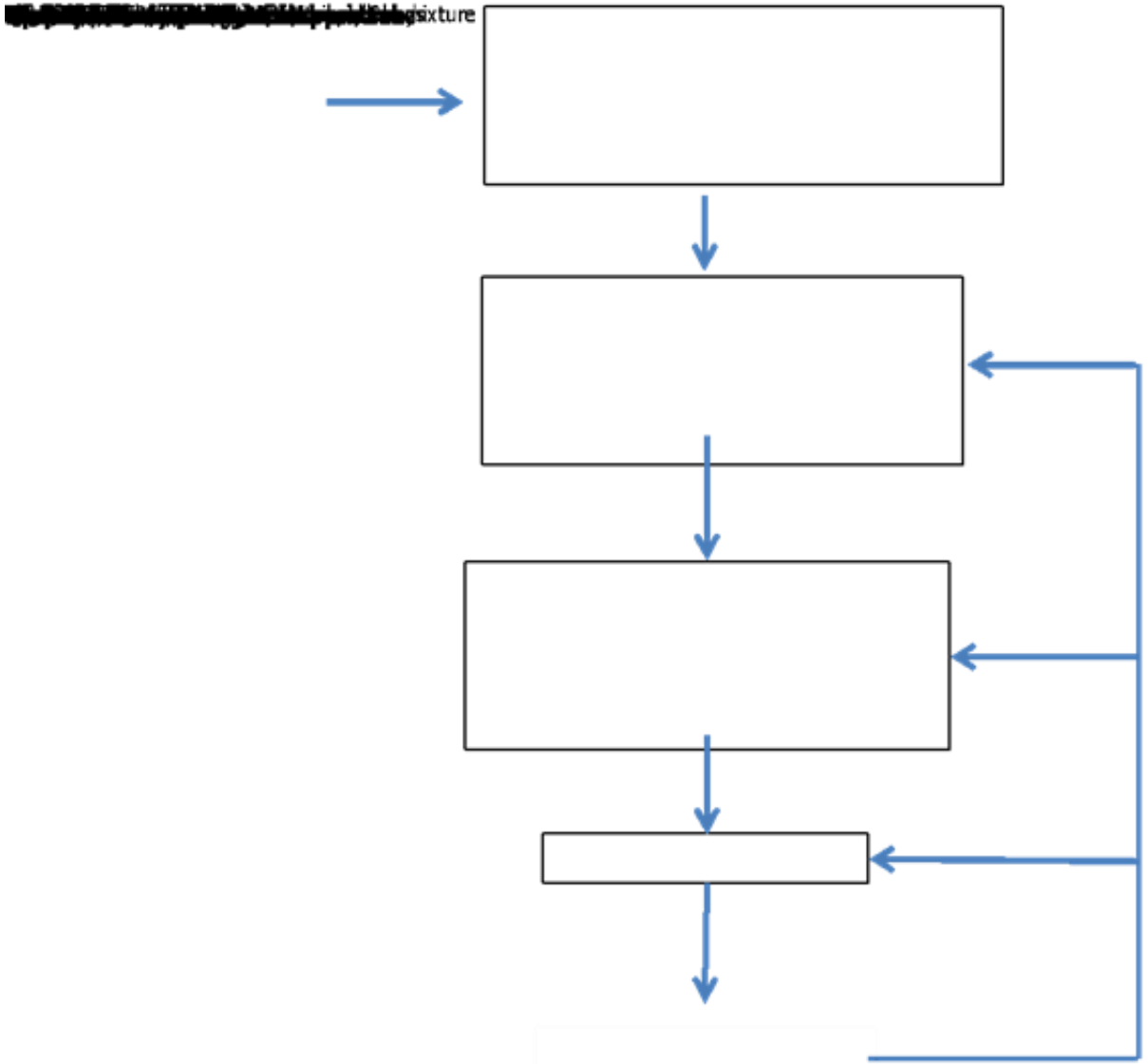


Tiering Principles



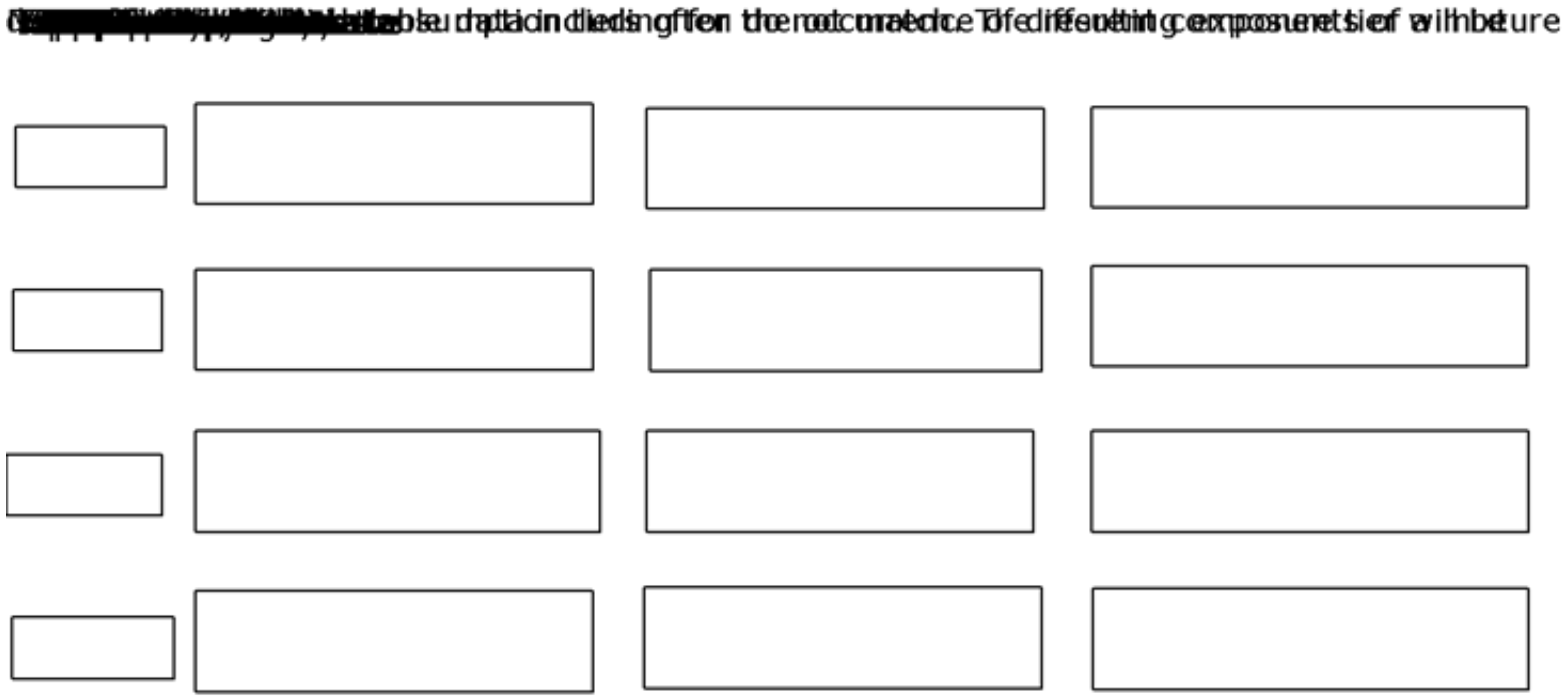
Relationships between tiers, data availability, uncertainty, accuracy and outcome of a risk assessment. Solomon et al. (2006) and MIXTOX GD.

Problem Formulation



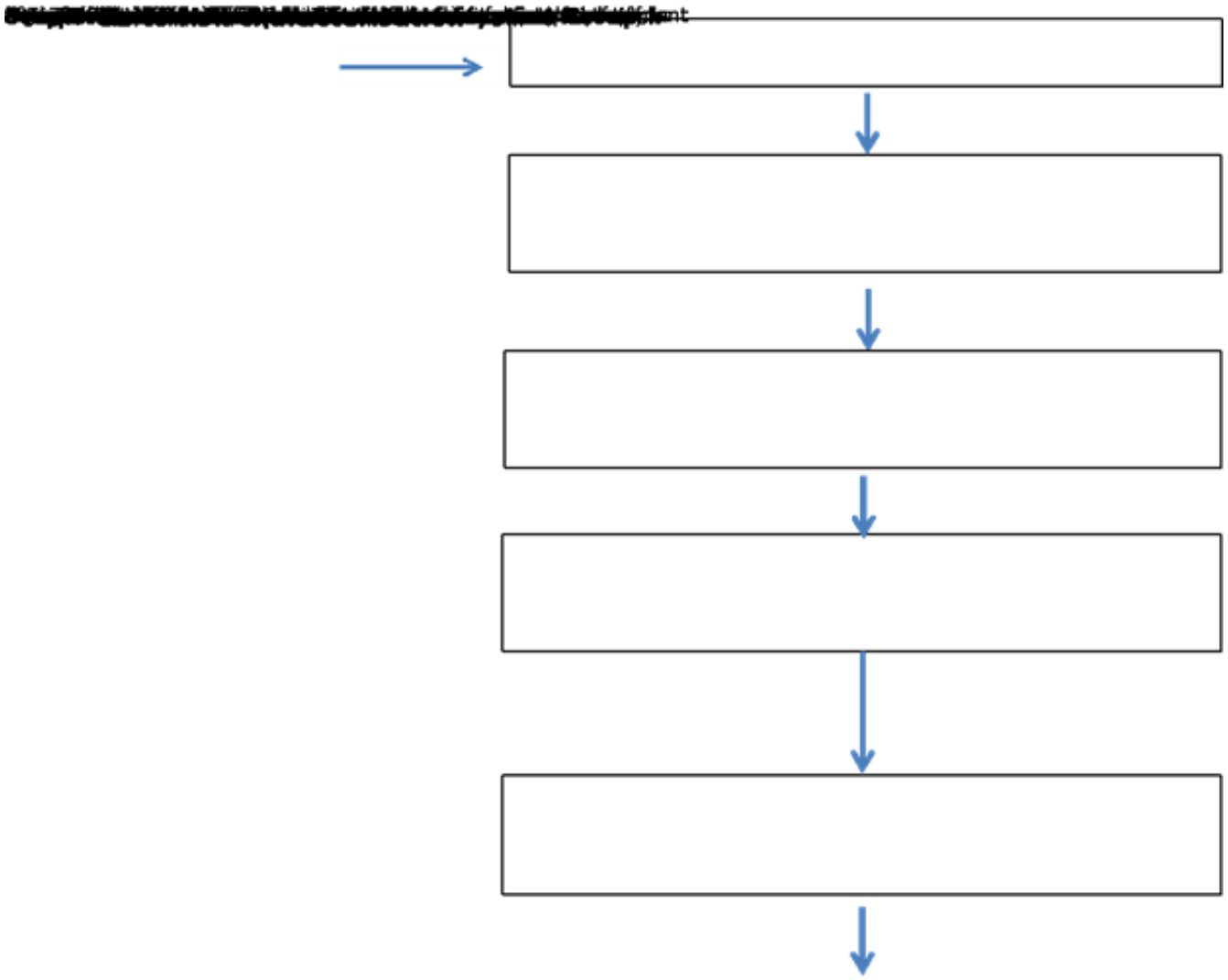
- **What is the question, species to assess and the multiple chemicals "mixture" ?**
- **How do we deal with it for risk assessment (RA) ?**
- **Plan to perform the RA**

Exposure Assessment



- **Amount of each chemical in food**
- **How much food is consumed ?**
- **Combine the two together for each chemical**

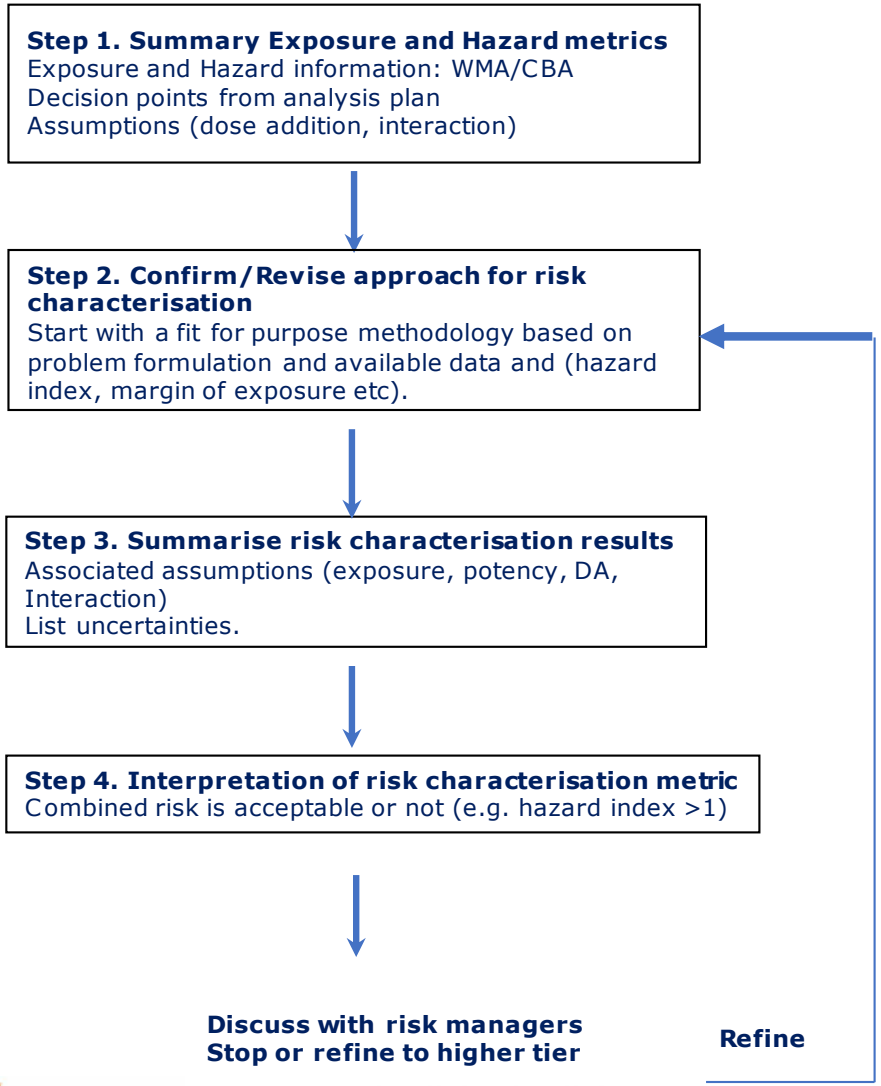
Hazard Assessment



- How toxic is each chemical ?
- Get the toxicity values for each chemical
- Do they interact and become more toxic together ?

Risk Characterisation

Risk characterisation
Human/Sub-population(s)
Farm/Companion Animals
Environmental Specie(s)
Ecosystem(s)



- **Combine exposure and toxicity data for all chemicals to get risk values**
- **Is there a health or environmental concern ?**
- **If No, stop**
- **If yes , What is next ?**

Refine or discuss with risk managers to reduce exposure ?

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Reporting Table

Problem formulation	***Description of the mixture***	Simple or complex mixture, Composition, Data availability for components or whole mixture
	Conceptual Model	Question/Terms of reference, Source, exposure pathways, Species/sub-population, Regulatory framework, Other ?
	Methodology	Overview of available data Whole mixture or component-based approach or a mixture of the two. Assessment group, Other ?
	Analysis Plan	
Exposure assessment	***Characterisation Whole Mixture*** ***Components Assessment group***	
	Summary Occurrence (concentration) data	
	Summary exposure	Assumptions, Exposure metrics
Hazard Assessment	***Mixture Composition WMA/CBA***	
	Reference points ***Reference values***	
	Summary Hazard metrics	Assumptions combined toxicity (DA, RA), hazard metrics
		Uncertainties
Risk Characterisation	***Summary Exposure and hazard metrics***	
	Risk characterisation Approach	
	Summary Risk Metrics	Associated Assumptions (DA, RA, interactions), Risk metrics
		Uncertainties

Implementing MIXTOX in Practice

PARMA SUMMER SCHOOL

Food Safety Aspects of Integrated Food Systems

TECHNICAL REPORT

efsa European Food Safety Authority Supporting Publications

APPROVED: 10 December 2019

doi:10.2903/sp.efsa.2020.EN-1759

Human risk assessment of multiple chemicals using component-based approaches: A horizontal perspective

European Food Safety Authority (EFSA),

Jean Lou CM Dorne, Amélie Crépet, Jan Dirk te Biesebeek, Kyriaki Machera, and Christer Hogstrand

TECHNICAL REPORT

efsa European Food Safety Authority Supporting Publications

APPROVED: 10 December 2019

doi:10.2903/sp.efsa.2020.EN-1760

Animal Health Risk assessment of multiple chemicals in essential oils for farm animals

European Food Safety Authority (EFSA),
Jean Lou CM Dorne, Paola Manini and Christer Hogstrand

efsa European Food Safety Authority

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MYCHIF: Mycotoxin Mixtures

EXTERNAL SCIENTIFIC REPORT

APPROVED: 4 December 2019
doi: 10.2903/sp.efsa.2020.EN-1757

Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach:

MYCHIF

Author(s)

Paola Battilani, Roberta Palumbo, Paola Giorni, Chiara Dall'Asta, Luca Dellafiora, Athanasios Gkrillas, Piero Toscano, Alfonso Crisci, Carlo Brera, Barbara De Santis, Rosaria Rosanna Cammarano, Maurella Della Seta, Katrina Campbell, Chris Elliot, Armando Venancio, Nelson Lima, Ana Gonçalves, Chloe Terciolo, Isabelle P Oswald

microorganisms MDPI

Article

Occurrence and Co-Occurrence of Mycotoxins in Cereal-Based Feed and Food

Roberta Palumbo [1], Alfonso Crisci [2], Armando Venâncio [3], José Cortiñas Abrahantes [4], Jean-Lou Dorne [4], Paola Battilani [1,*] and Piero Toscano [2]

- **Exposure: Co-occurrence and consumption patterns**
- **Hazard : Collect hazard data for each mycotoxin using EFSA OpenFoodTox.**
- **Risk Characterisation: Combined Margin of Exposure for humans and farm animals**

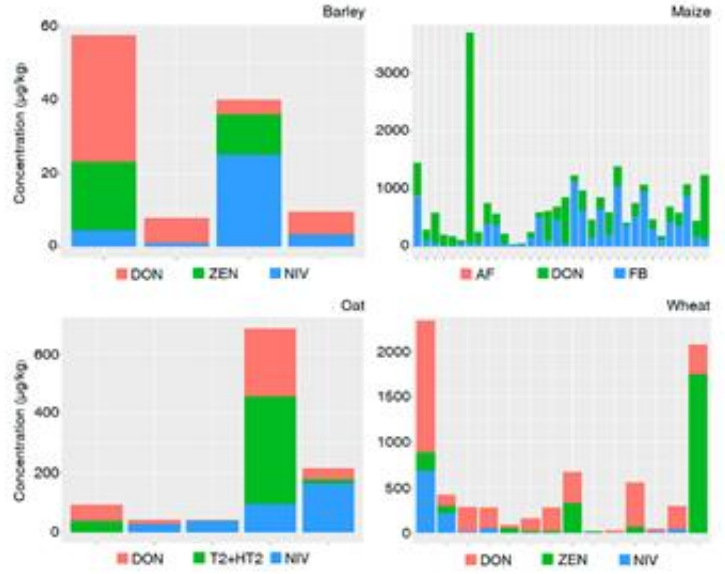

Figure 7. Concentrations of each co-occurring mycotoxin for barley, maize, oat, and wheat.

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MIXTOX2

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Draft Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee, Simon John More, Vasileios Bampidis, Diane Benford, Claude Bragard, Antonio Hernandez-Jerez, Susanne Hougaard Bennekou, Thorhallur Ingi Halldorsson, Konstantinos Panagiotis Koutsoumanis, Kyriaki Machera, Hanspeter Naegeli, Søren Saxmose Nielsen, Josef Rudolf Schlatter, Dieter Schrenk, Vittorio Silano, Dominique Turck, Maged Younes, Emilio Benfenati, Amélie Crépet, Jan Dirk Te Biesebeek, Emanuela Testai, Bruno Dujardin, Jean Lou C M Dorne, Christer Hogstrand

DRAFT

Background

- **EFSA request for scientific opinion on criteria for grouping chemicals for Human RA Mixture**

- **Setting cumulative assessment groups for human RA of pesticides as requested by DG-SANTE and relevance to EFSA Panels:**

- **CONTAM: Grouping contaminants** (PFAs, brominated FR etc)
- **FEEDAP: Mixture RA of essential oils/botanicals**
- **FAF: smoke flavourings and grouping.** Include chemical properties (e.g structure, class, functional group etc). Use ECHA read across GD and OECD QSAR toolbox.
- **Overall support all panels dealing with chemical RA**

Terms of Reference

- **Guidance Document on scientific criteria for grouping chemicals into assessment groups**

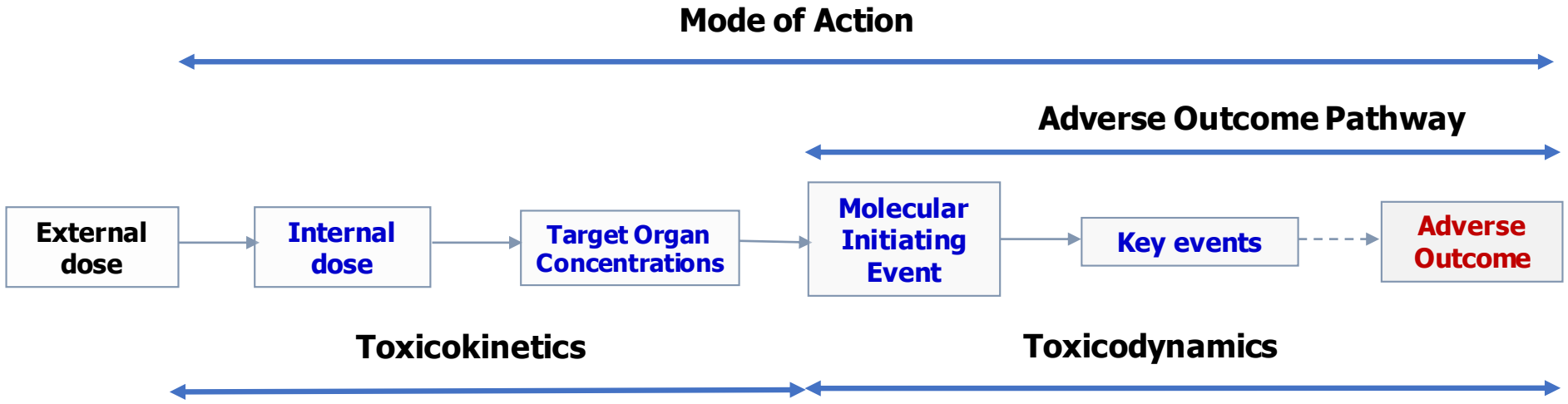
- Scientific principles and relevant cross-cutting guidance
- Context of risk assessment (priorities, urgent, pre- and post-market)
- Tiering and fit for purpose scenarios consider available data
- Prioritisation approaches: Risk-based and exposure-driven
- Relevant EFSA areas and international activities
- Harmonisation, avoid duplication
- Publication for public consultation (PC)

Content

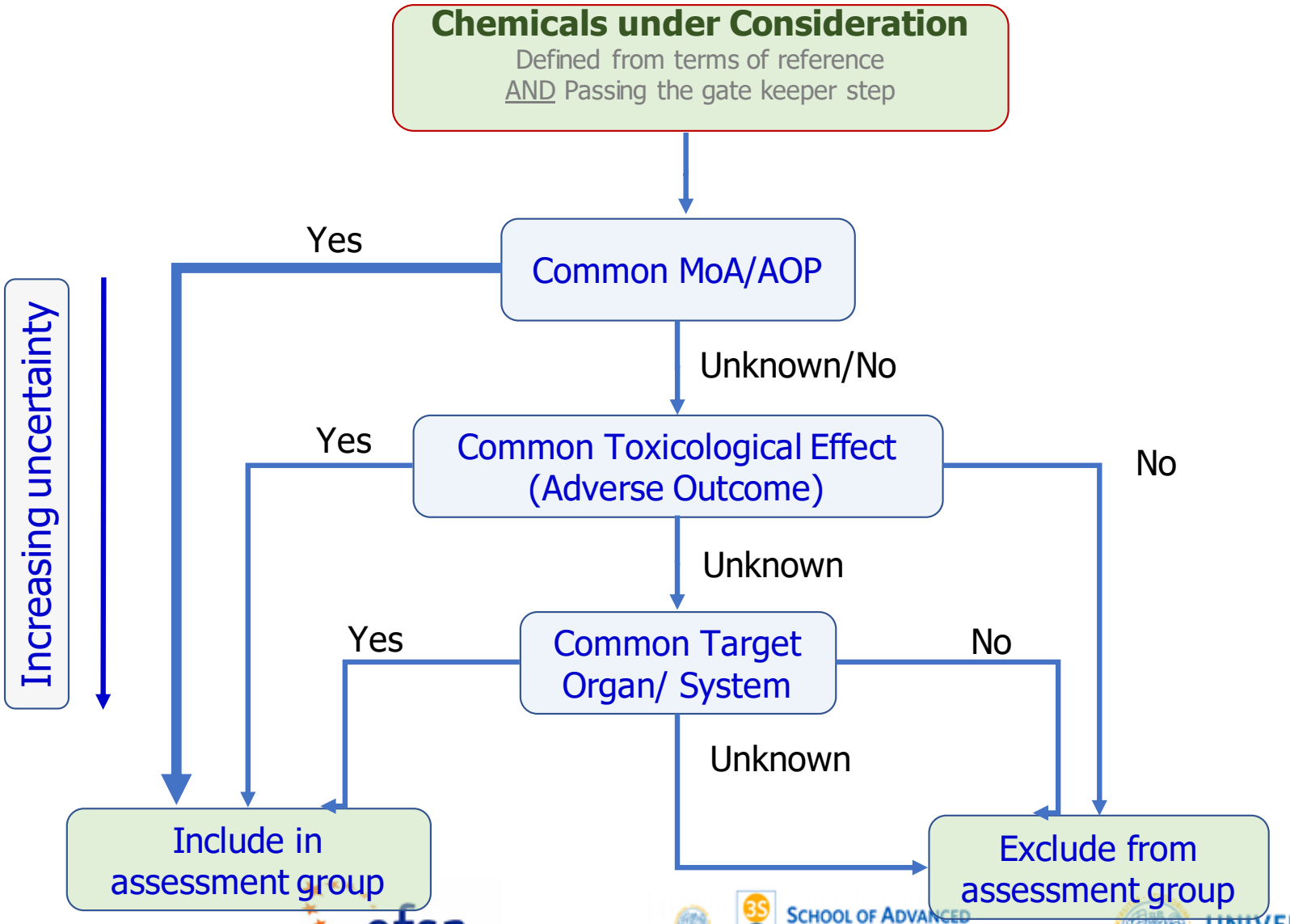
- **Introduction: Background, Terms of Reference/ Interpretation**
- **General principles: Problem formulation and Grouping**
- **Hazard-driven criteria**
- **Prioritisation methods: Risk-based and exposure-driven**
- **Recommendations**

- Annexes
- Annex A: Glossary
- Annex B: Generic WoE methodology for grouping using hazard-driven criteria
- Annex C: Prioritisation method for grouping pesticides (risk metrics for single chemicals)
- Annex D: Prioritisation method: grouping contaminants in breast milk (combined exposure metrics)

Mode of Action and Adverse Outcome Pathways

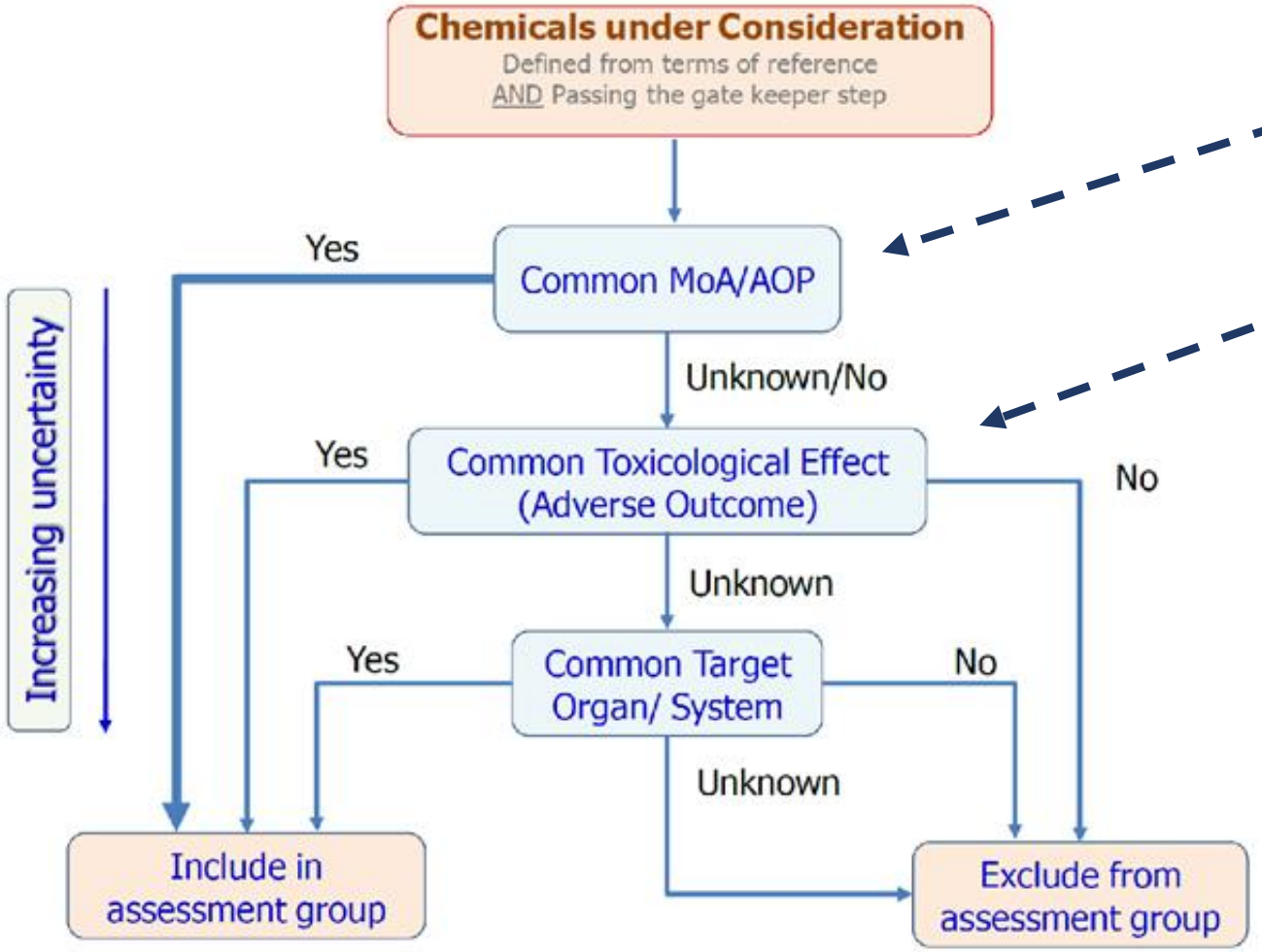


Hazard-Driven Criteria for groupin chemicals into assessment groups



- **Top-down hierarchical process**
- **Gold standard Common MoA/AOP for grouping into assessment group**
- **Then move to common toxicity or target organ**
- **If not grouping can use also *in silico/structure etc***

Implementation of the grouping criteria for pesticide residues



Acetylcholinesterase inhibition

Hypothyroidism
Functional alterations of the motor division of the NS
Craniofacial malformations

SCIENTIFIC REPORT

ADOPTED: 20 March 2020

doi: 10.2903/j.efsa.2020.6087

Cumulative dietary risk characterisation of pesticides have acute effects on the nervous system

European Food Safety Authority (EFSA),
Peter S Craig, Bruno Dujardin, Andy Hart, Antonio F Hernández-Jer
Susanne Hougaard Bennekou, Carsten Kneuer, Bernadette Ossendorp, Ragn
Gerrit Wolterink and Luc Mohimont

Abstract

A retrospective acute cumulative risk assessment of dietary exposure to pesticide resi
by an uncertainty analysis based on expert knowledge elicitation, was conducted for tw
nervous system: brain and/or erythrocyte acetylcholinesterase inhibition, and functio
the motor division. The pesticides considered in this assessment were identified and
the scientific report on the establishment of cumulative assessment groups of pes
effects on the nervous system. Cumulative exposure assessments were conducted thro
modeling by EFSA and the Dutch National Institute for Public Health and the Envi
using two different software tools and reported separately. These exposure as
monitoring data collected by Member States under their official pesticide monitoring
2014, 2015 and 2016 and individual consumption data from 10 populations of
different countries and different age groups. This report completes the characterisatio
risk, taking account of the available data and the uncertainties involved. For
populations, it is concluded with varying degrees of certainty that cumulative exposu
that have the acute effects on the nervous system mentioned above does not exceed t
regulatory consideration established by risk managers.

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of European Food Safety Authority.

Keywords: pesticide residues, nervous system, cumulative risk assessment, probab
expert knowledge elicitation

Requestor: EFSA

Question number: EFSA-Q-2018-00345

Correspondence: pesticides.MRL@efsa.europa.eu

SCIENTIFIC REPORT

APPROVED: 20 March 2020

doi: 10.2903/j.efsa.2020.6088

Cumulative dietary risk characterisation of pesticides have chronic effects on the thyroid

European Food Safety Authority (EFSA),
Peter S Craig, Bruno Dujardin, Andy Hart, Antonio F Hernandez-Jerez
Susanne Hougaard Bennekou, Carsten Kneuer, Bernadette Ossendorp, Ragnor
Gerrit Wolterink and Luc Mohimont

Abstract

A retrospective chronic cumulative risk assessment of dietary exposure to pesticide resid
by an uncertainty analysis based on expert knowledge elicitation, was conducted for two
thyroid, hypothyroidism and parafollicular cell (C-cell) hypertrophy, hyperplasia and
pesticides considered in this assessment were identified and characterised in the scie
the establishment of cumulative assessment groups of pesticides for their effects o
Cumulative exposure assessments were conducted through probabilistic modelling by
Dutch National Institute for Public Health and the Environment (RIVM) using two diff
tools and reported separately. These exposure assessments used monitoring data collec
States under their official pesticide monitoring programmes in 2014, 2015 and 2016
consumption data from 10 populations of consumers from different countries and differe
This report completes the characterisation of cumulative risk, taking account of the ava
the uncertainties involved. For each of the 10 populations, it is concluded with varyi
certainty that cumulative exposure to pesticides that have the chronic effects on the thy
above does not exceed the threshold for regulatory consideration established by risk ma

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of European Food Safety Authority.

Keywords: pesticide residues, thyroid, cumulative risk assessment, probabilistic mo
knowledge elicitation

Requestor: EFSA

Question number: EFSA-Q-2018-00346

Correspondence: pesticides.mrl@efsa.europa.eu

SCIENTIFIC REPORT

EFSA Journal

APPROVED: 21 December 2020

doi: 10.2903/j.efsa.2021.6392

Cumulative dietary risk assessment of chronic acetylcholinesterase inhibition by residues of pesticides

European Food Safety Authority (EFSA),
Maria Anastassiadou, Judy Choi, Tamara Coja, Bruno Dujardin, Andy Hart,
Antonio F Hernandez-Jerrez, Samira Jarrah, Alfonso Lostia, Kyriaki Machera, Iris Mangas,
Alexandra Mienne, Marloes Schepens, Anneli Widenfalk and Luc Mohimont

Abstract

A retrospective cumulative risk assessment of dietary exposure to pesticide residues was conducted for chronic inhibition of acetylcholinesterase. The pesticides considered in this assessment were identified and characterised in a previous scientific report on the establishment of cumulative assessment groups of pesticides for their effects on the nervous system. The exposure assessments used monitoring data collected by Member States under their official pesticide monitoring programmes in 2016, 2017 and 2018, and individual food consumption data from 10 populations of consumers from different countries and from different age groups. Exposure estimates were obtained by means of a two-dimensional probabilistic model, which was implemented in SAS® software. The characterisation of cumulative risk was supported by an uncertainty analysis based on expert knowledge elicitation. For each of the 10 populations, it is concluded with varying degrees of certainty that cumulative exposure to pesticides contributing to the chronic inhibition of acetylcholinesterase does not exceed the threshold for regulatory consideration established by risk managers.

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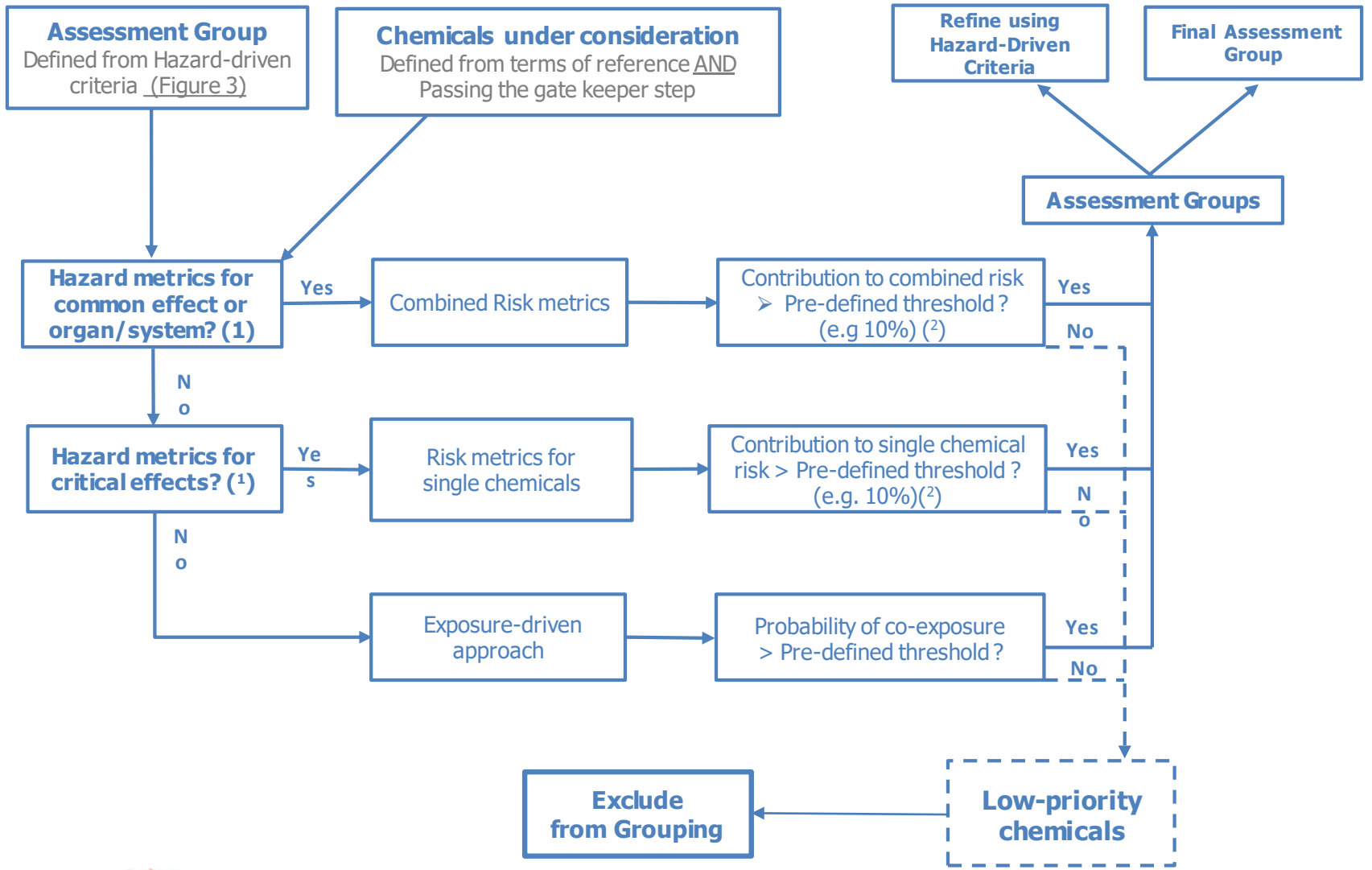
Keywords: cumulative risk assessment, pesticide residues, acetylcholinesterase inhibition, probabilistic modelling, knowledge elicitation

Requestor: EFSA

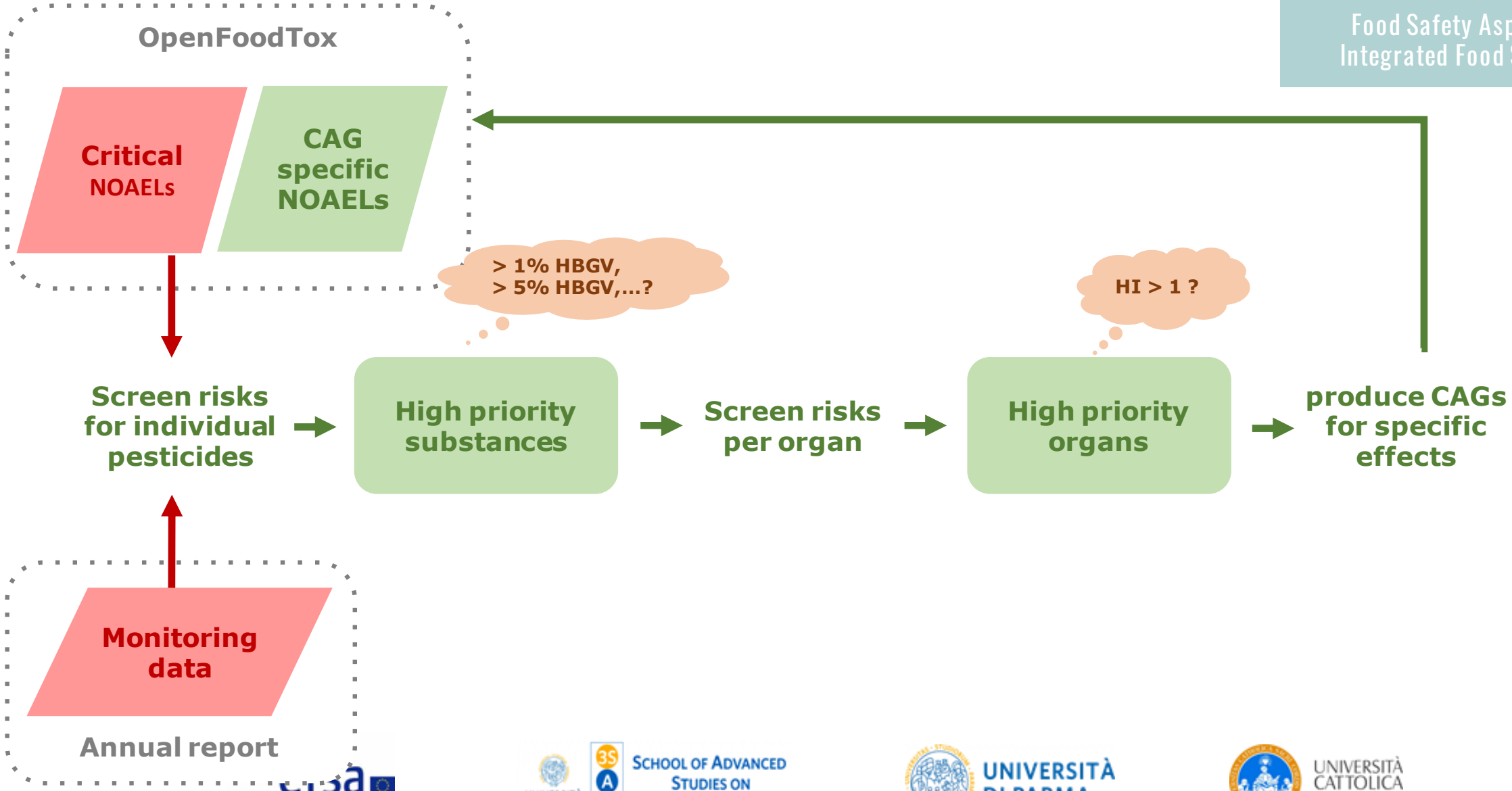
Question number: EFSA-Q-2020-00411

Correspondence: pesticides.mrl@efsa.europa.eu

Prioritisation Methods

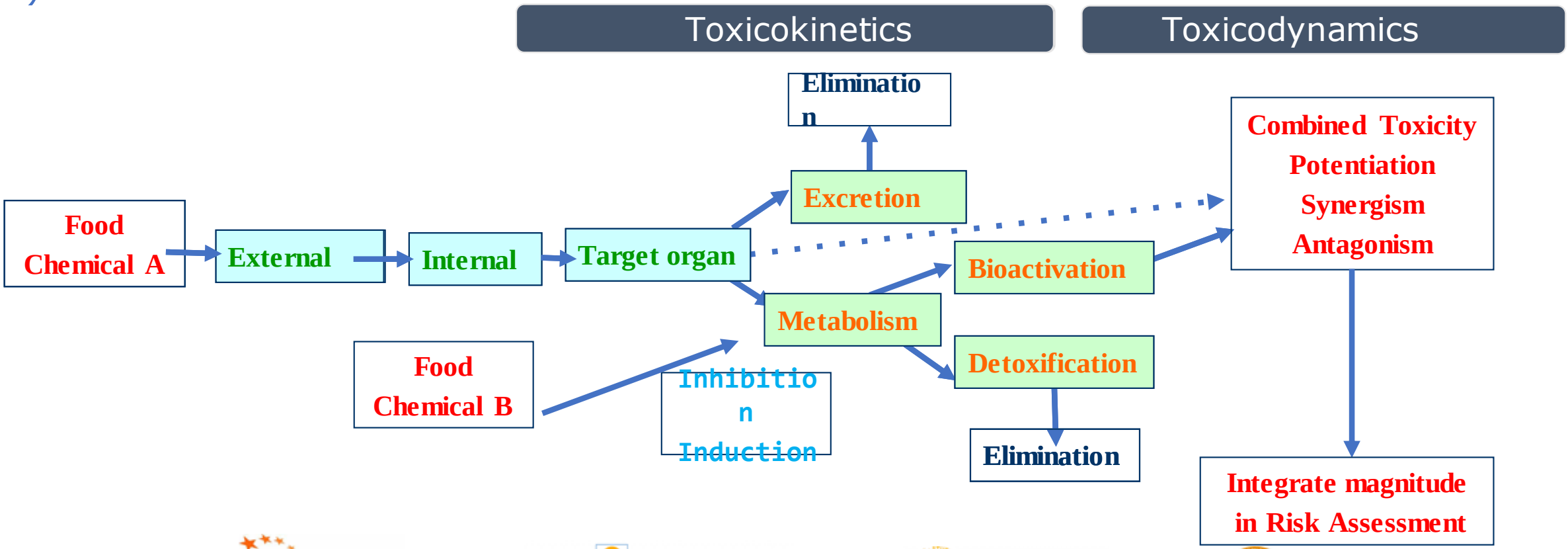


Prioritisation Method for pesticides

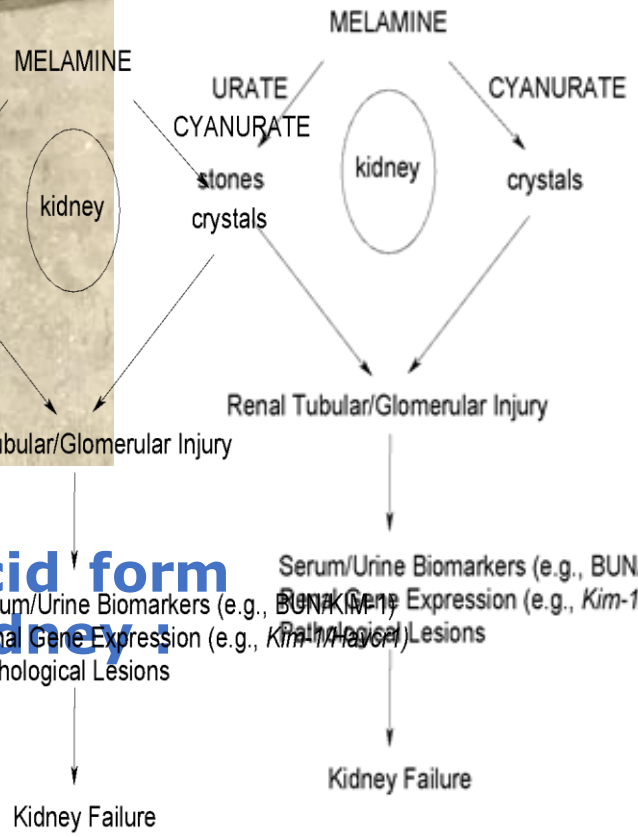


Example of Open Source Models

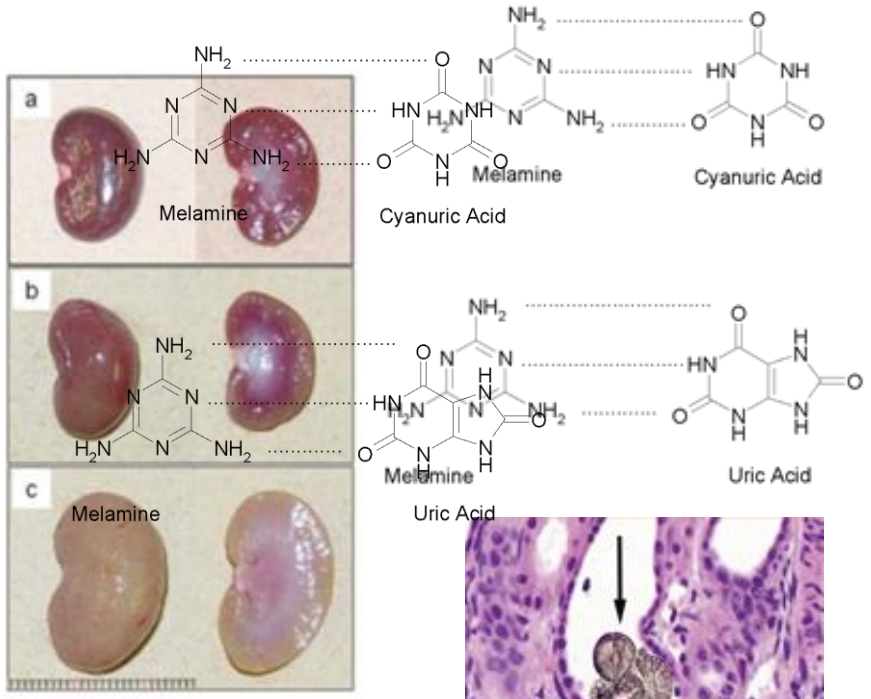
- **Mixture Toxicity** can involve **interactions**
  - **Check** interactions for the RA **if occurs at current levels of exposure**
  - **Integrate in risk characterisation** (extra uncertainty factor, biological-based model etc)



Melamine and Cyanuric acid



Melamine and Cyanuric Acid form a covalent complex in Kidney : Synergistic toxicity



Kobayashi et al. (2010)
Scanning electron microscope x 7000 and x 14000
A control Day 7
B Melamine
C Melamine+Cyanuric acid

Melamine and Cyanuric acid in Fish

Toxicology and Applied Pharmacology 370 (2019) 184–195

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Investigating the interaction between melamine and cyanuric acid using a Physiologically-Based Toxicokinetic model in rainbow trout

Cleo Tebby[a], Céline Brochot[a], Jean-Lou Dorne[b], Rémy Beaudouin[a,c,*]

[a] Institut National de l'Environnement Industriel et des Risques (INERIS), Models for Ecotoxicology and Toxicology Unit, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France
[b] European Food Safety Authority (EFSA), Scientific Committee and Emerging Risks Unit, Via Carlo Magno 1A, 43126 Parma, Italy
[c] Institut National de l'Environnement Industriel et des Risques (INERIS), UMR-I 02 SEBIO, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France

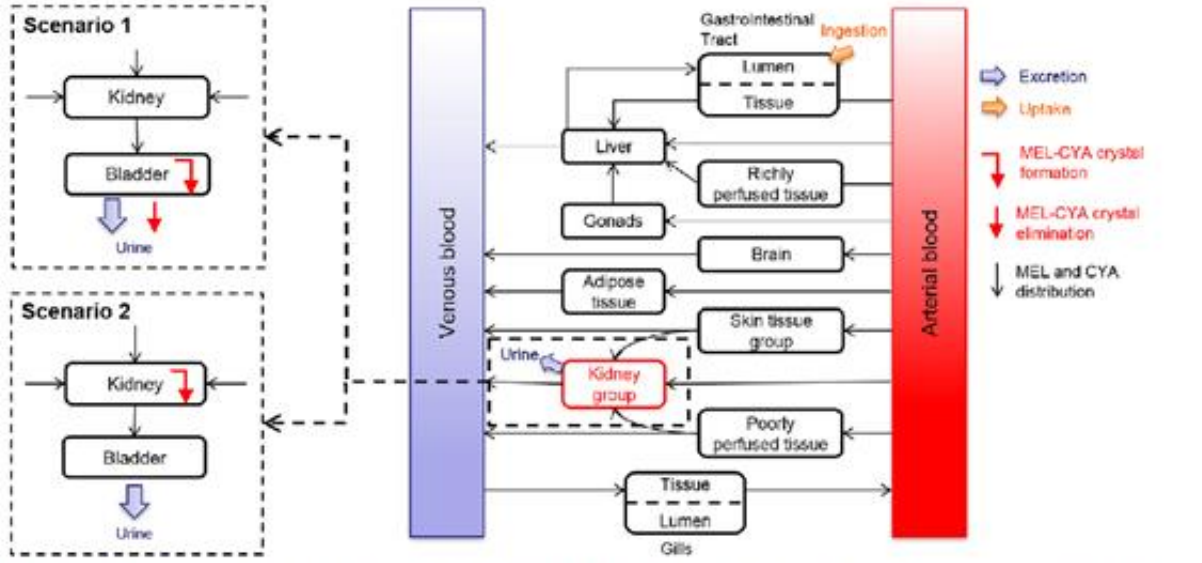


Fig. 1. Schematic description of the PBTK model for rainbow trout (Grech et al., 2019) adapted to MEL and CYA, with two scenarios of crystal formation.

Grape Fruit Juice (GJF) and Saint John' s Wort (SJW)

Archives of Toxicology
https://doi.org/10.1007/s00204-018-2325-6

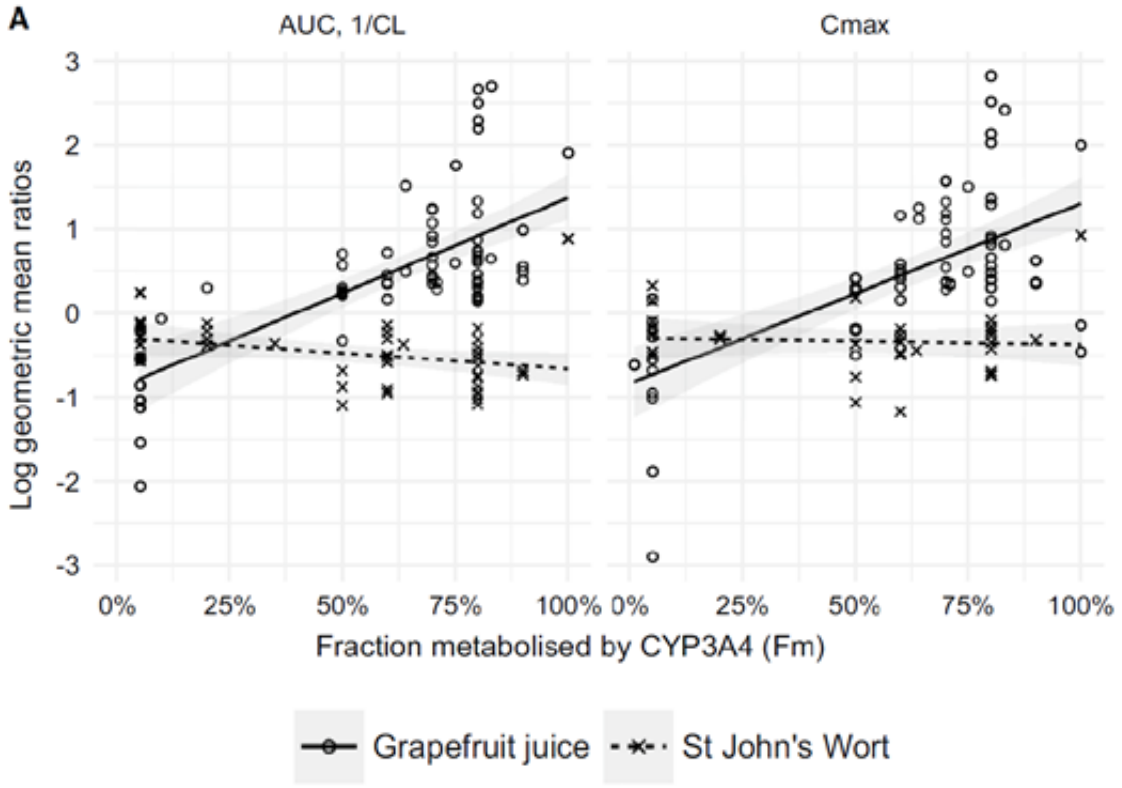
TOXICOGENOMICS

The Yin–Yang of CYP3A4: a Bayesian meta-analysis to quantify inhibition and induction of CYP3A4 metabolism in humans and refine uncertainty factors for mixture risk assessment

Nadia Quignot[1] · Witold Wiecek[2] · Billy Amzal[1] · Jean-Lou Dorne[3]

GFJ : Competitive inhibition of CYP3A4
SJW: Induction of CYP3A4

-Derive Uncertainty Factors from human clinical trials on drugs
-Changes in kinetic parameters as basis for mixture RA for CYP3A4 inhibitors/inducers



Dealing with Interactions: Combined toxicity in Bees

Contents lists available at ScienceDirect
Environment International
journal homepage: www.elsevier.com/locate/envint

Investigating combined toxicity of binary mixtures in bees: Meta-analysis of laboratory tests, modelling, mechanistic basis and implications for risk assessment

Edoardo Carnesecchi[a,b], Claus Svendsen[c], Stefano Lasagni[d], Audrey Grech[e], Nadia Quignot[f], Billy Amzal[f], Cosimo Toma[b], Simone Tosi[g], Agnes Rortais[h], Jose Cortinas-Abrahantes[h], Ettore Capri[i], Nynke Kramer[a], Emilio Benfenati[b], David Spurgeon[c], Gilles Guillot[j], Jean Lou Christian Michel Dorne[h,k,*]

Acute contact Toxicity

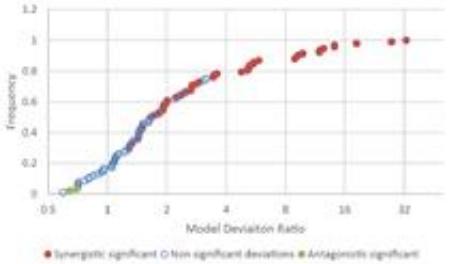
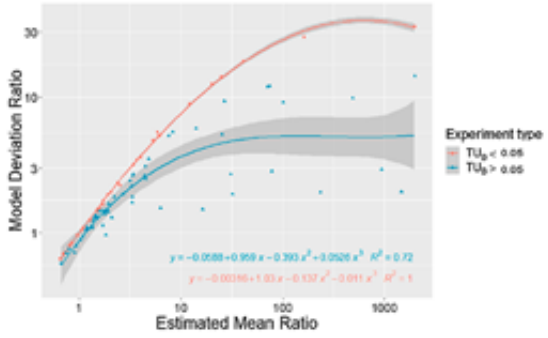
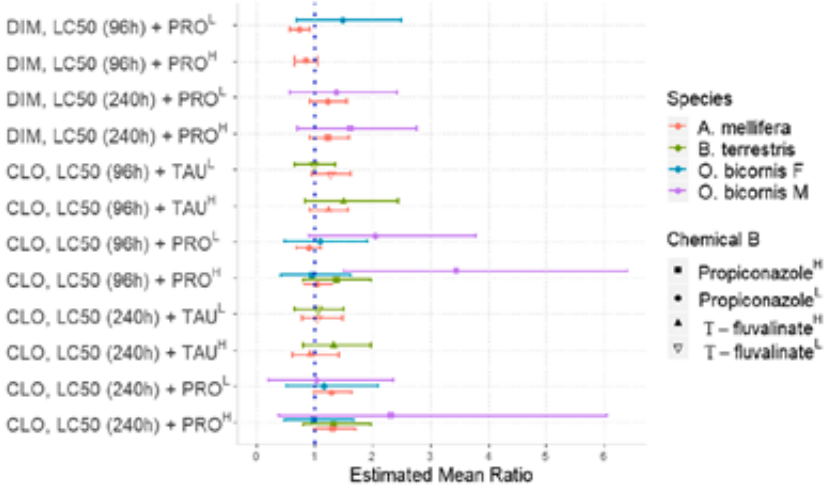


Fig. 11. Cumulated frequency of Model Deviation Ratio. MDR for statistically significant studies resulting from the meta-analysis of acute contact toxicity studies on honey bees (Iwasa et al., 2004; Johnson et al., 2013, 2006, 2009; Ellis et al., 1997). MDR > 1.25 represents "synergistic" interactions, 0.83 < MDR < 1.25 represents "additive" effects; MDR < 0.83 represents "antagonistic" interactions.



Chronic oral Toxicity



- **Interactions/Synergism in bees**

Mostly due to inhibition of CYP metabolism

- Limited data (oral) magnitude interactions acute contact toxicity > acute oral/chronic oral
- Addressing (co)-exposure dimension
- Mortality as common metrics for risk characterisation (starting point)

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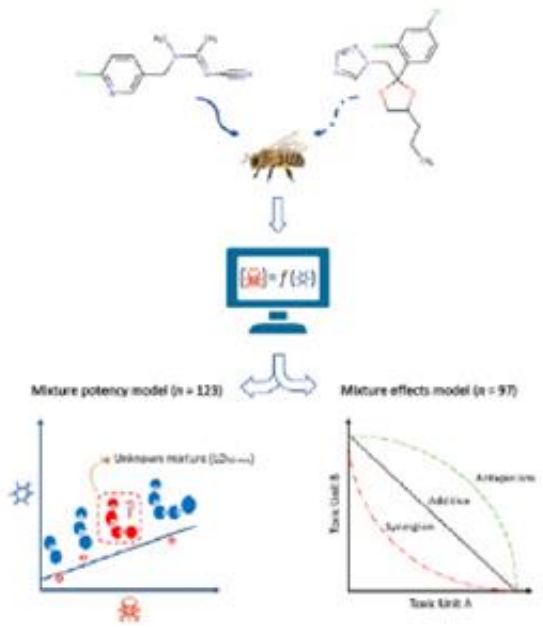
Dealing with Interactions: QSAR Development

Contents lists available at ScienceDirect
Science of the Total Environment
journal homepage: www.elsevier.com/locate/scitotenv

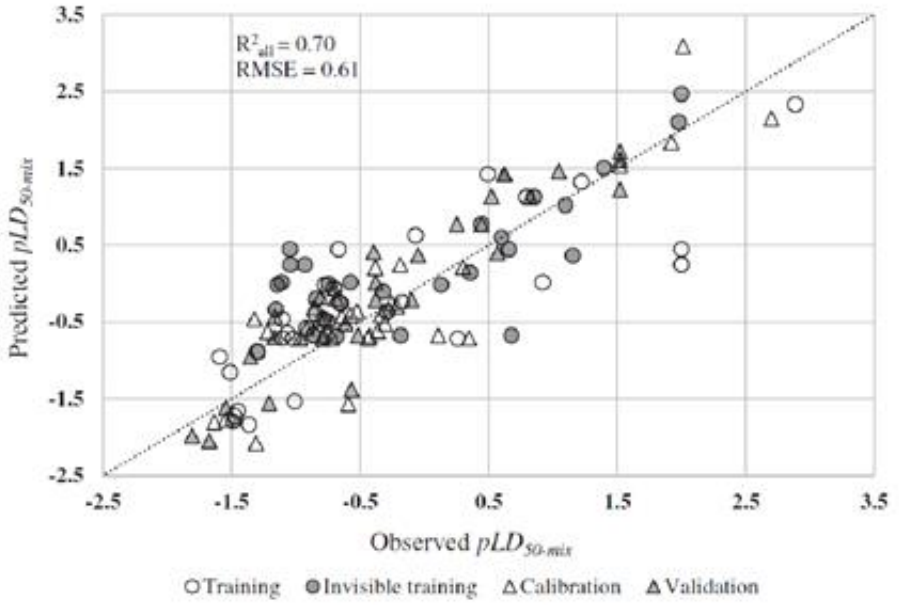
Predicting acute contact toxicity of organic binary mixtures in honey bees (*A. mellifera*) through innovative QSAR models

Edoardo Carnesecchi [a,b,*], Andrey A. Toropov [a], Alla P. Toropova [a], Nynke Kramer [b], Claus Svendsen [c], Jean Lou Dorne [d], Emilio Benfenati [a]



- **QSAR models predicting nature of combined toxicity and binary mixture toxicity**

(Acute contact toxicity)



Conclusions and Future perspectives

- **MIXTOX** provides **Harmonised GD for RA of multiple chemicals**

Incl. **Frameworks for each RA step** and **reporting Table as summary of results**

- **Scientific criteria for grouping: Hazard-driven criteria and prioritisation methods** Public consultation Draft Guidance: May-July 2021-Publication: Dec 2021

- **Future work in this area includes**
- *New Approach Methodologies* for RA of multiple chemicals incl.QSARs
- Dealing with interactions
- Physiologically-based models and other biologically-based models
- Environmental RA: Multiple stressors for bees (MUST-B opinion-May 2021)

Thank You !! Questions ?

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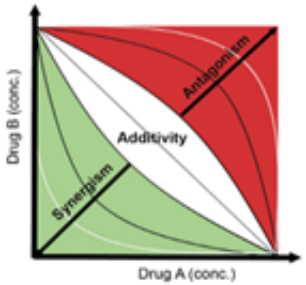
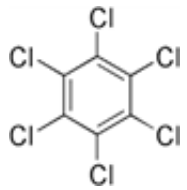
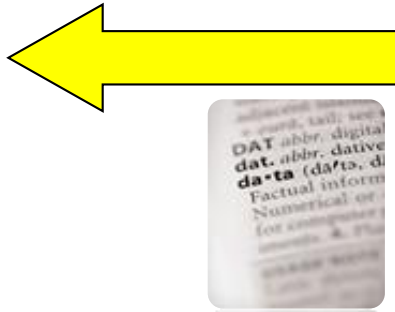
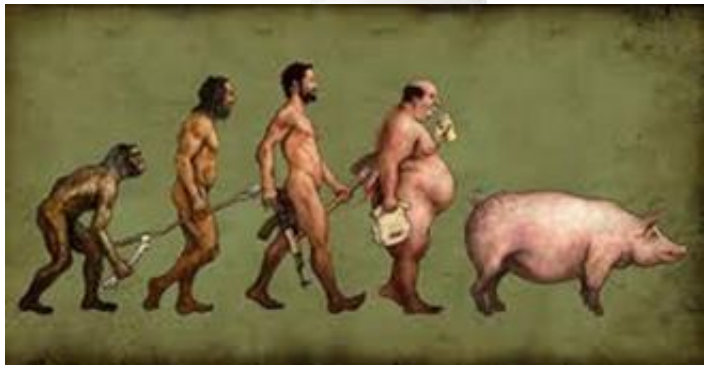


Ecological







Human

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