SICUREZZA ALIMENTARE:
STATO DELL'ARTE E
PROSPETTIVE

Venerdì 24 marzo 2023
Sala Italia, Castel dell'Ovo - Napoli

ISTITUTO SUPERIORE DI SANITÀ
DIPARTIMENTO
AMBIENTE E SALUTE

ORDINE DEI DOTTORI AGRONOMI E DEI DOTTORI FORESTALI DELLA PROVINCIA DI NAPOLI
Ministero della Giustizia

COMUNE DI NAPOLI

CONSIGLIO DELL'ORDINE NAZIONALE DEI DOTTORI AGRONOMI E DEI DOTTORI FORESTALI
Ministero della Giustizia

Evoluzione degli aspetti tossicologici ed influenza sulla Regolamentazione dei Prodotti Fitosanitari

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I prodotti fitosanitari non possono essere commercializzati o utilizzati se non prima autorizzati.

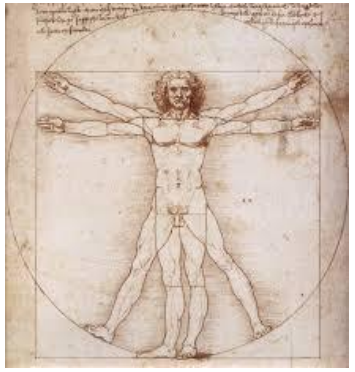
Si usa un sistema a **due livelli** in cui **l'EFSA** valuta con un processo peer review le **sostanze attive** contenute nei prodotti fitosanitari e gli **Stati membri** valutano e autorizzano i *prodotti fitosanitari* a livello nazionale.

- EU legislation and EFSA guidance documents detail how to compile dossiers for submission and the information and studies required for the evaluation

The **toxicological data-package** included:

✓Toxicity studies in vivo with rat, mice, dogs, rabbits

✓ADME (Absorption, Distribution, Metabolism and Excretion) studies in vivo metabolism at least in the rat…..



Aspetto qualitativo

HAZARD IDENTIFICATION:
What might harm you? What kind of effects are caused by the contaminant?

- Identification of adverse health effects
  - Human epidemiological data
  - Animal-based toxicological studies
  - *In vitro* toxicology data
  - Structure-activity consideration

Il sistema di classificazione ed etichettatura (CLP) è basato esclusivamente sul PERICOLO

Rappresenta quindi una caratteristica qualitativa della sostanza o di un prodotto, indipendentemente dal livello di esposizione

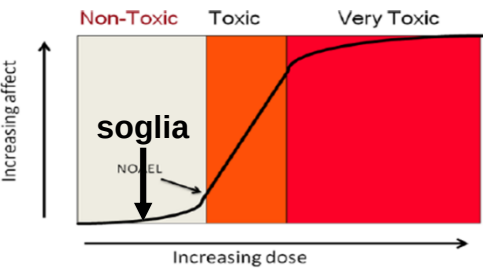
EXPOSURE ASSESSMENT: What is population that might be exposed to the contaminant? What are the routes, magnitude, duration and timing of the doses that people might receive?

- Levels of substance in food and diet
- Amounts of food consumed
- Intake in special population groups
- Intake in individuals
  - Max/min, regularly/occasionally

Aspetto quantitativo

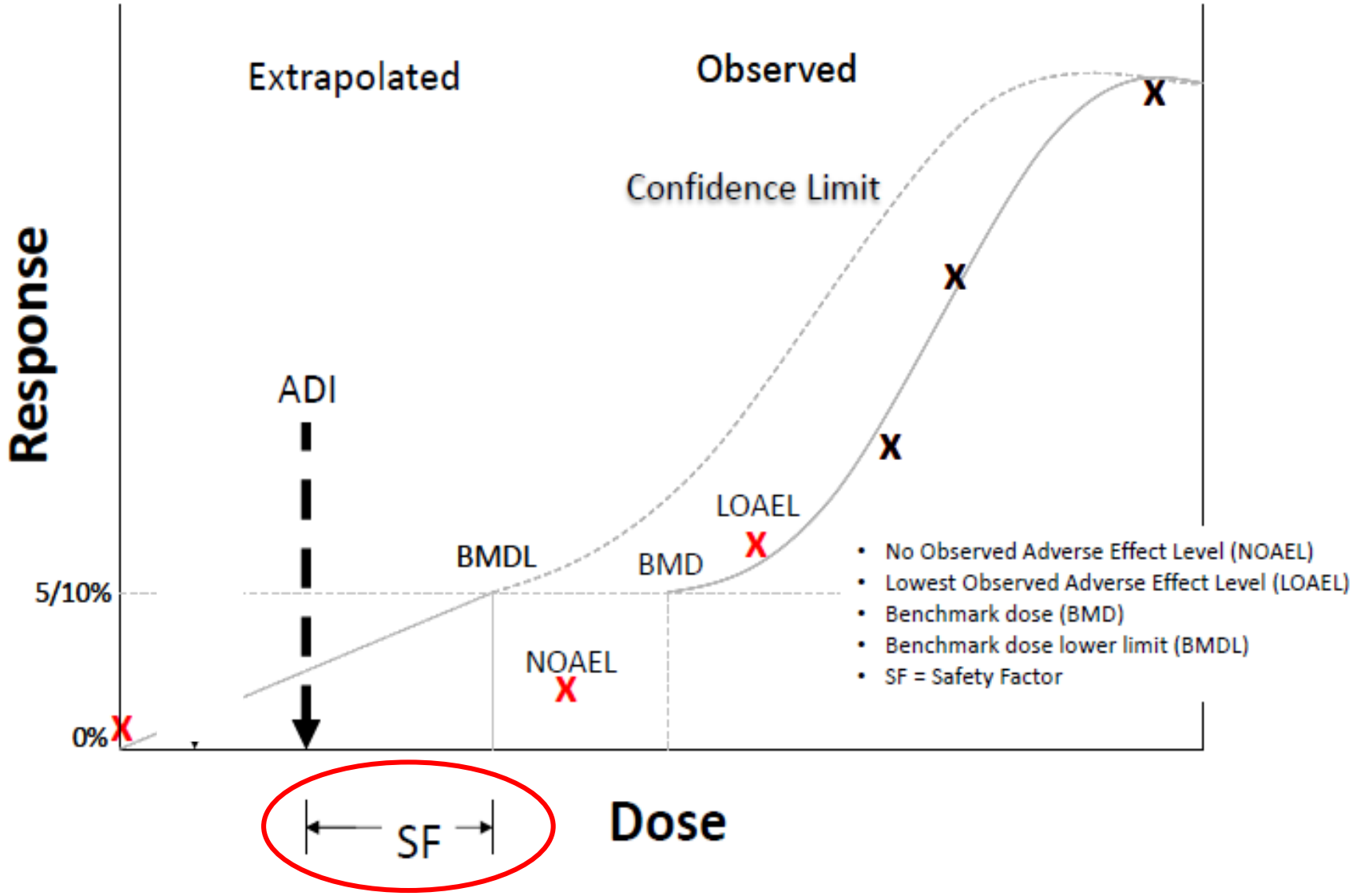
DOSE-RESPONSE RELATIONSHIP:
Which is the magnitude of health effects At different exposures?

- Quantification of adverse health effects
  - Dose-response for critical effect
  - Selection of critical data
  - Mode/mechanism of action
  - Kinetic variability
  - Dynamic variability



Per le sostanze chimiche senza potenziale genotossico esiste una **dose soglia** al di sotto della quale non ci sono effetti avversi (PoD per HBGV)

RISK CHARACTERISATION :
What is the **probability** of experiencing an health effect in the exposed population at that level of exposure?


Extrapolated
Observed
Confidence Limit
Response
ADI
BMDL
BMD
LOAEL
NOAEL
5/10%
0%
SF
Dose
• No Observed Adverse Effect Level (NOAEL)
• Lowest Observed Adverse Effect Level (LOAEL)
• Benchmark dose (BMD)
• Benchmark dose lower limit (BMDL)
• SF = Safety Factor

Current safety testing methods

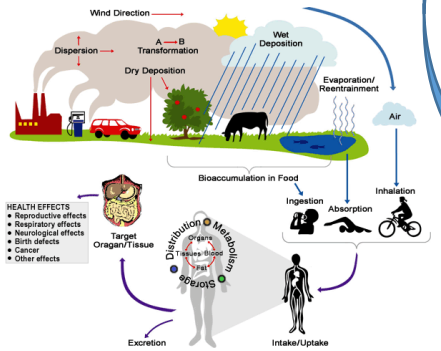
In Vitro

In Vivo

Toxicological profile

vs

Exposure



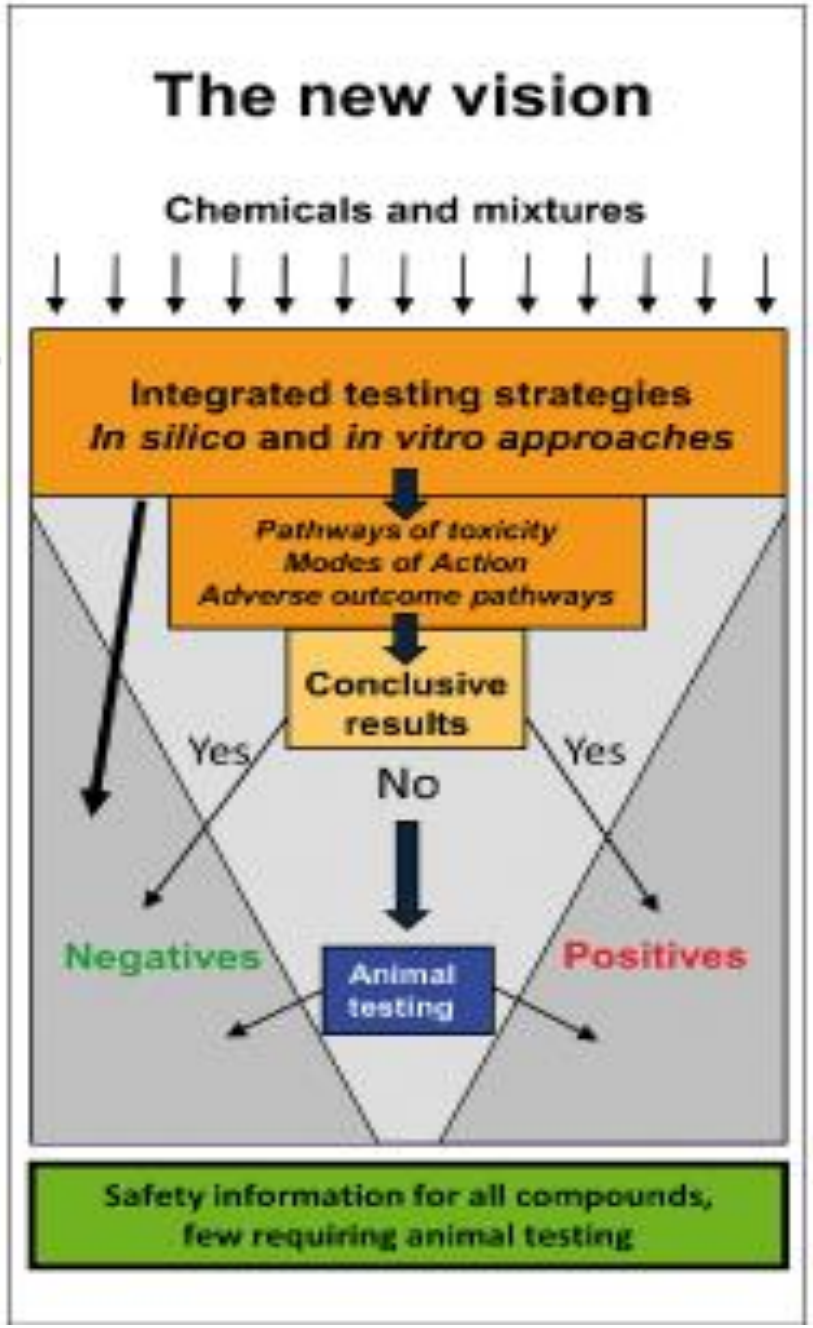
Risk assessment

The present RA paradigm generally focuses on hazard identification and characterisation as first steps.

There is a demand for changing the basis of RA, giving more focus on

1) modes of action and TK (mechanistic approach)
2) a progressive reduction of tests using laboratory animals
3) exposure driven process

Towards the Tox21 and the EU SC document on New challenges for RA (2013)



TESTING STRATEGIES IN AN ANIMAL FREE ENVIRONMENT (IATA)

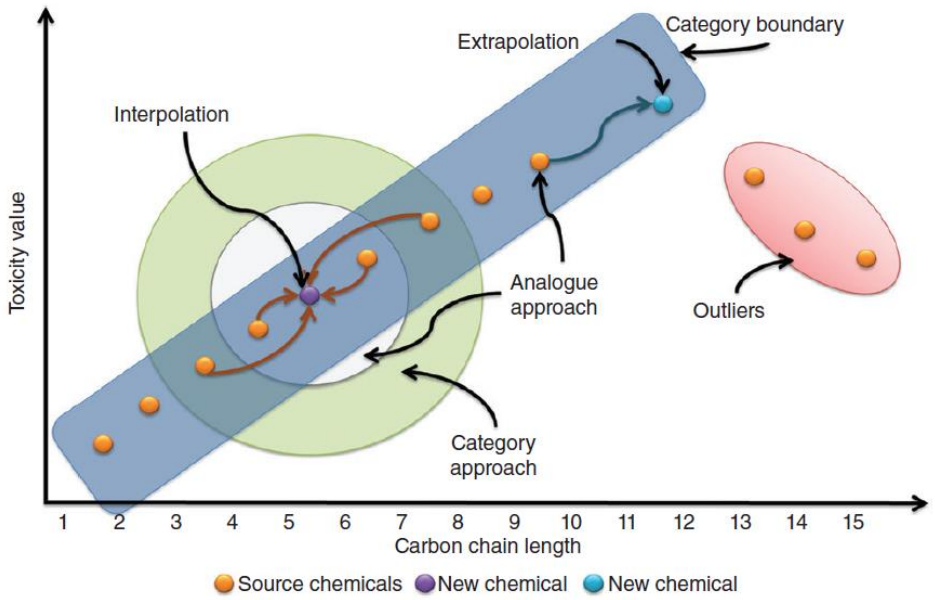
- ✓ Tutte le informazioni disponibili devono essere considerate e pesate per rilevanza, affidabilità, qualità (**Weight of Evidence** approach)
- ✓ E' possible applicare per ottenere 'supporting information' i cosidetti **non testing methods:**
  1. Read across (usato spesso per i metaboliti)
  2. In silico methods (es: OECD toolbox for *structural alert of genotoxicity*)
  3. Threshold of Toxicological Concern (TTC) usata ad es. per 'groundwater metabolites'

In silico approach or 'non testing methods': an example

Read Across : If information on structurally similar chemicals are available it is possible to apply the read across principle

The toxicological profile of chemical A is known (source chemical), scant info available for chemical B

If you can support with in silico analysis (e.g. SAR Structure activity relationship) the structural similarity, or with 'bridging studies' (both in vitro and in vivo) the similarity of A and B toxicological profile, the read across principle can be applied.



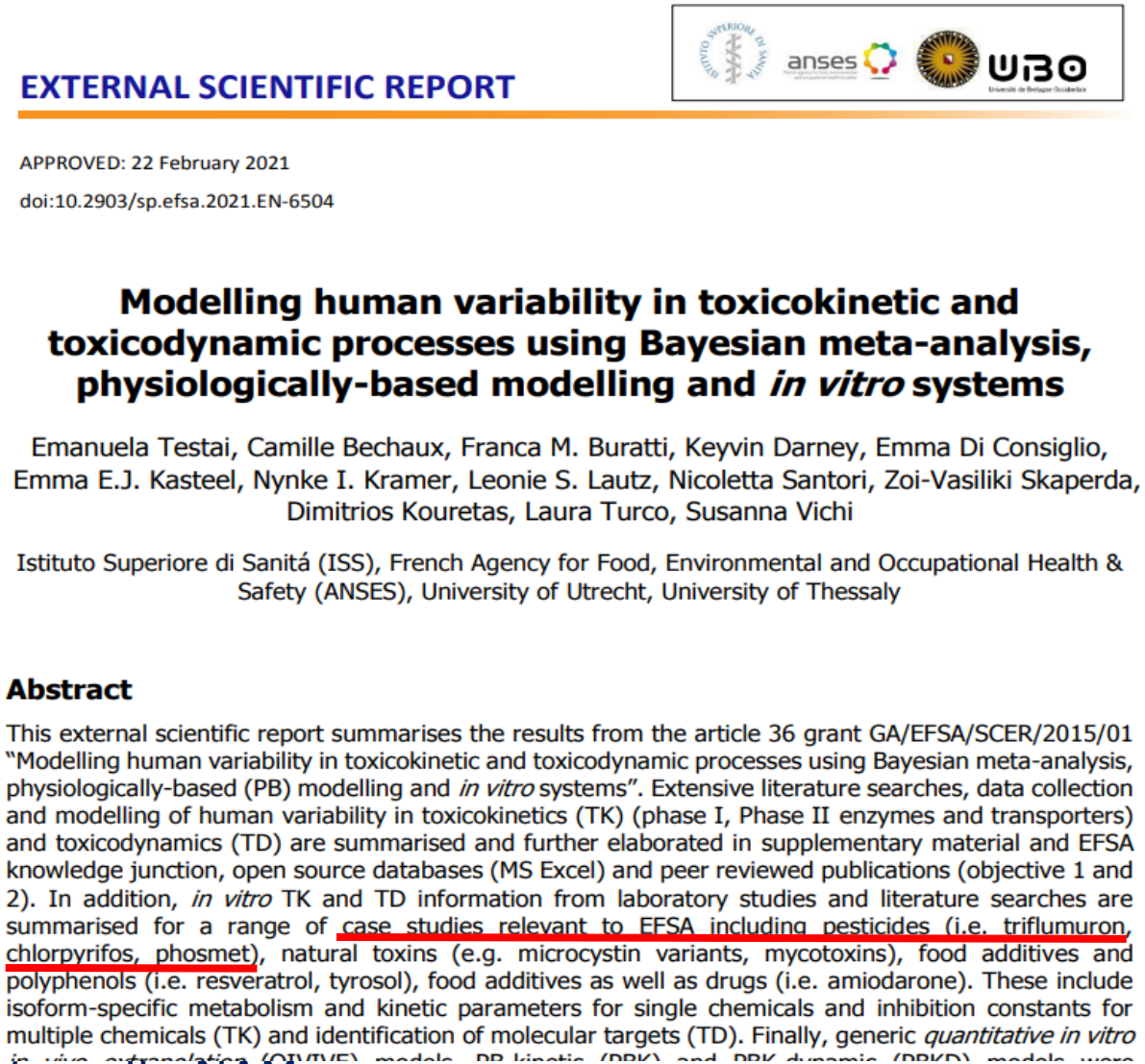
ECHA: Pratical Guidance 6
http://echa.europa.eu/documents/10162/13655/pg_report_readacross_it.pdf
http://echa.europa.eu/support/grouping-of-substances-and-read-across

OECD: GUIDANCE ON GROUPING OF CHEMICALS, SECOND EDITION Series on Testing & Assessment No. 194 (2014)
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en

EFSA : WG on grouping on pesticides and for mixtures

TESTING STRATEGIES IN AN ANIMAL FREE ENVIRONMENT (IATA)

- ✓ Tutte le inform … nce approach)
- ✓ E' possible app … on testing methods:
  1. Read acros…
  2. In silico me…)
  3. Threshold … ater metabolites'



EXTERNAL SCIENTIFIC REPORT

APPROVED: 22 February 2021

doi:10.2903/sp.efsa.2021.EN-6504

Modelling human variability in toxicokinetic and toxicodynamic processes using Bayesian meta-analysis, physiologically-based modelling and *in vitro* systems

Emanuela Testai, Camille Bechaux, Franca M. Buratti, Keyvin Darney, Emma Di Consiglio, Emma E.J. Kasteel, Nynke I. Kramer, Leonie S. Lautz, Nicoletta Santori, Zoi-Vasiliki Skaperda, Dimitrios Kouretas, Laura Turco, Susanna Vichi

Istituto Superiore di Sanitá (ISS), French Agency for Food, Environmental and Occupational Health & Safety (ANSES), University of Utrecht, University of Thessaly

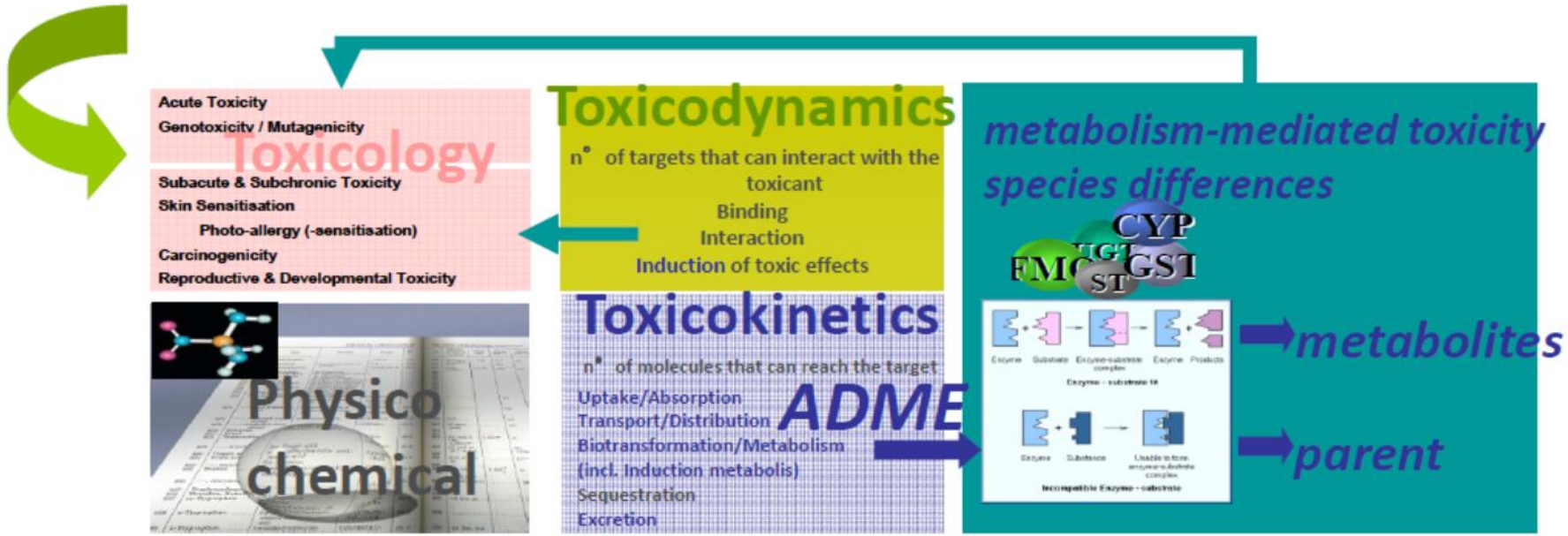
Abstract

This external scientific report summarises the results from the article 36 grant GA/EFSA/SCER/2015/01 "Modelling human variability in toxicokinetic and toxicodynamic processes using Bayesian meta-analysis, physiologically-based (PB) modelling and *in vitro* systems". Extensive literature searches, data collection and modelling of human variability in toxicokinetics (TK) (phase I, Phase II enzymes and transporters) and toxicodynamics (TD) are summarised and further elaborated in supplementary material and EFSA knowledge junction, open source databases (MS Excel) and peer reviewed publications (objective 1 and 2). In addition, *in vitro* TK and TD information from laboratory studies and literature searches are summarised for a range of case studies relevant to EFSA including pesticides (i.e. triflumuron, chlorpyrifos, phosmet), natural toxins (e.g. microcystin variants, mycotoxins), food additives and polyphenols (i.e. resveratrol, tyrosol), food additives as well as drugs (i.e. amiodarone). These include isoform-specific metabolism and kinetic parameters for single chemicals and inhibition constants for multiple chemicals (TK) and identification of molecular targets (TD). Finally, generic *quantitative in vitro*

Integrare l'uso … caratterizzazione del rischio basata s… d'azione):

- ❖ comprensior… tossicità e cancerogeni… **omics** data, high throughput …) and Adverse Outcome Pathways (**A**…
- ❖ uso dei dati … **a dose interna** (in-silico tools, enhan… TD) modelling, quantitative …
- ❖ riduzione de… ando **strategie integrate d**… ssessment and Testing Strategies) basate sulle NAM.
- ❖ identificazione di gruppi di popolazione sensibili attraverso la comprensione di **differenze inter-individuali**

ADME systems as essential parts of IATA's for systemic toxicity



Metabolism/Biotransformation: *need for reliable, relevant, easily accessible human metabolic competent test systems*

PBTK (Phisiologically-Based ToxicoKinetic) *modelling is currently regarded as the most adequate approach to simulate the fate of compounds in the human body*

If in preclinical tox species there is lower or no exposure to a metabolite which is formed in humans (disproportionate or unique human metabolite), toxicity is not adequately assessed

The relevance of having **toxicity data in animal** models with **dissimilar metabolic profile** to those found in **humans** shall be addressed (human relevance of findings in a WoE approach)

Whenever such metabolic information is available, should be taken into consideration for study design and risk assessment and for understanding the MoA

The EU Legal framework (Reg EU 283/2013, OJ: L93/22)

5.1.1 ADME after exposure by oral route
Comparative in vitro metabolism studies *shall be performed on animal species to be used in pivotal studies and on* ***human*** *material (microsomes or intact cell systems) in order to determine the* ***relevance of the toxicological animal data*** *and to* ***guide in the interpretation of findings and in further definition of the testing strategy***

An **explanation** shall be given or **further tests** shall be carried out where a **metabolite is detected in vitro in human material** and not in the tested animal species.

5.5. Long term toxicity and carcinogenicity
- If comparative metabolism data indicate that either rat or mouse is an inappropriate model for human cancer risk assessment, an alternative species shall be considered

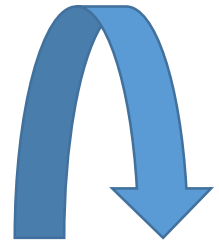
http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32013R0283

efsa
European Food Safety Authority
Committed *since 2002* to ensuring that Europe's food is safe

How to perform such kind of studies?

No OECD test guideline is available

High variability in data submission to EFSA



EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 10 November 2021

doi: 10.2903/j.efsa.2021.6970

Scientific Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) on testing and interpretation of comparative *in vitro* metabolism studies

EFSA Panel on Plant Protection Products and their Residues (EFSA PPR Panel), Antonio F Hernandez-Jerez, Paulien Adriaanse, Annette Aldrich, Philippe Berny, Tamara Coja, Sabine Duquesne, Andreas Focks, Marina Marinovich, Maurice Millet, Olavi Pelkonen, Silvia Pieper, Aaldrik Tiktak, Christopher J Topping, Anneli Widenfalk, Martin Wilks, Gerrit Wolterink, Ursula Gundert-Remy, Jochem Louisse, Serge Rudaz, Emanuela Testai, Alfonso Lostia, Jean-Lou Dorne and Juan Manuel Parra Morte

Abstract

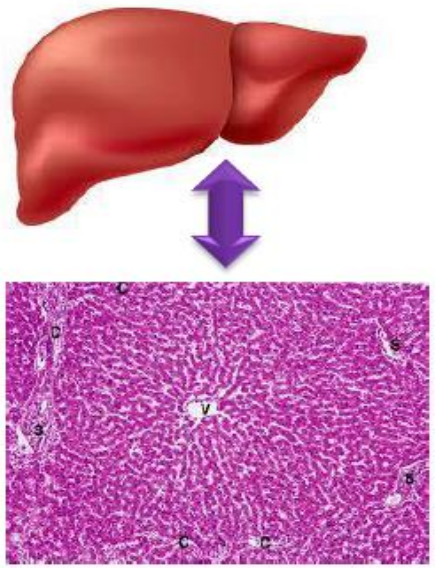
EFSA asked the Panel on Plant Protection Products and their residues to deliver a Scientific Opinion on testing and interpretation of comparative *in vitro* metabolism studies for both new active substances and existing ones. The main aim of comparative *in vitro* metabolism studies of pesticide active substances is to evaluate whether all significant metabolites formed in the human *in vitro* test system, as a surrogate of the *in vivo* situation, are also present at comparable level in animal species tested in toxicological studies and, therefore, if their potential toxicity has been appropriately covered by animal studies. The studies may also help to decide which animal model, with regard to a particular compound, is the most relevant for humans. In the experimental strategy, primary hepatocytes in suspension or culture are recommended since hepatocytes are considered the most representative *in vitro* system for prediction of *in vivo* metabolites. The experimental design of $3 \times 3 \times 3$ (concentrations, time points, technical replicates, on pooled hepatocytes) will maximise the chance to identify unique (UHM) and disproportionate (DHM) human metabolites. When DHM and UHM are being assessed, test item-related radioactivity recovery and metabolite profile are the most important

1. Definition of the term "**unique human metabolite**" (UHM).

UHM= a metabolite not detectable in in vitro incubations with test material from other test species or in vivo laboratory species

2. Definition of **disproportionate metabolite (DHM)**.

DHM= any metabolite that is present at a quantity higher than four times at any sampling time point in human hepatocytes as compared with other species The factor of 4 is derived from the TK interspecies subfactor of the default uncertainty value of 10 to account for animal to human differences in TKs as defined by IPCS (2005).

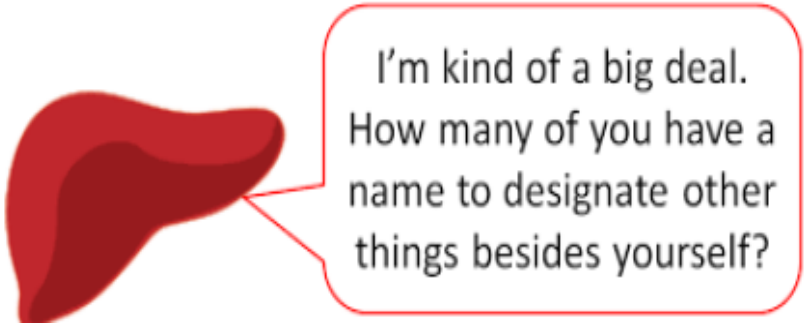


3. The most appropriate metabolising **test system** according to what we know about the specific active substance, analysing the **advantages and disadvantages** of using them

4. The **species to be included**

5. The minimum **criteria for the conduction, acceptance and interpretation** of the studies

6. The most appropriate **analytical method(s)**



The opinion focuses on **in vitro methodologies and study design related to liver** metabolism with the aim to identify UHM and DHM because the liver is considered the organ with the highest metabolic capacity compared to other organs and tissues

Depending on the existing information on the test item, **extrahepatic tissue metabolism** needs consideration as well. If organs other than the liver are studied, the same principles as indicated for the liver apply to identify UHM and DHM.

The study design and experimental strategy outlined in the opinion intend to **optimise the detection of potential UHM and allow the evaluation of DHM**.

SITOX

21° Congresso Nazionale

Società Italiana di Tossicologia

Pericolo, rischio e rapporto rischio-beneficio

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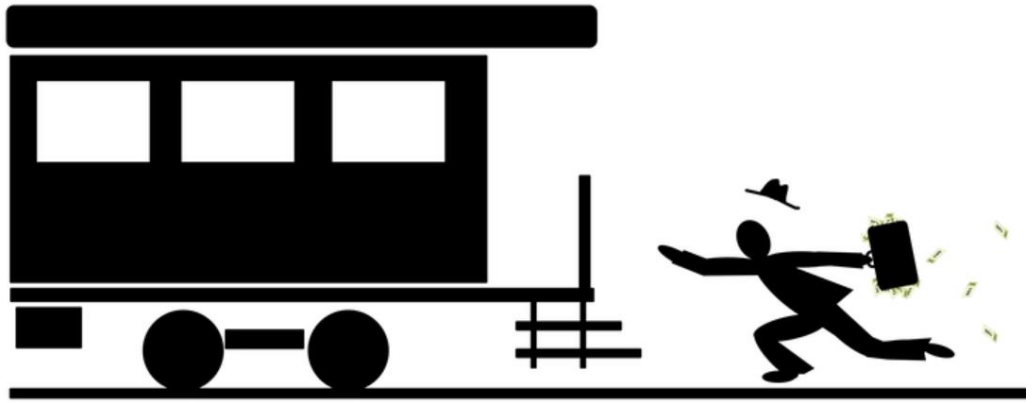
BOLOGNA
20-22 Febbraio 2023



Mercoledì 22 febbraio 2023

SALA GARISENDA - PROGRAMMA NON ECM

9.00-11.00 **SIMPOSIO**
PROSPETTIVE PER L'APPLICAZIONE DI NAMS (*NEW APPROACH METHODOLOGIES*) PER LA VALUTAZIONE DEL PERICOLO E DEL RISCHIO
Moderatori: Marco Corvaro (Roma), Marina Marinovich (Milano)

9.00-9.20 La strategia EFSA per l'implementazione dei NAMs nella valutazione del rischio
Maria Chiara Astuto (Parma)

9.20-9.40 Implementazione pratica dei NAMs: l'esempio dell'*Integrated Approaches to Testing And Assessment* (IATA) per la *Developmental Neurotoxicity* (DNT)
Andrea Terron (Parma)

9.40-10.00 Ruolo dei NAMs nel progetto HESI TEA (*Transformation of Evaluation of Agrochemicals*)
Marco Corvaro (Roma)

10.00-10.15 Messa a punto di un NAM per gli inibitori dell'enzima 4-idrossifenilpiruvato diossigenasi (HPPDi)
Giovanna Semino (Sophia Antipolis- Francia)

10.15-10.30 NAMs per gli agrofarmaci microbici: fattibilità e prospettive future
Sara Lamperti (Varese)

10.30-11.00 Panel discussion
Discussant: Arianna Giusti (Bruxelles), Emanuela Testai (Roma)

EFSA ROADMAP FOR ACTION ON NAMS

EXTERNAL SCIENTIFIC REPORT

APPROVED: 2 May 2022

doi:10.2903/sp.efsa.2022.EN-7341

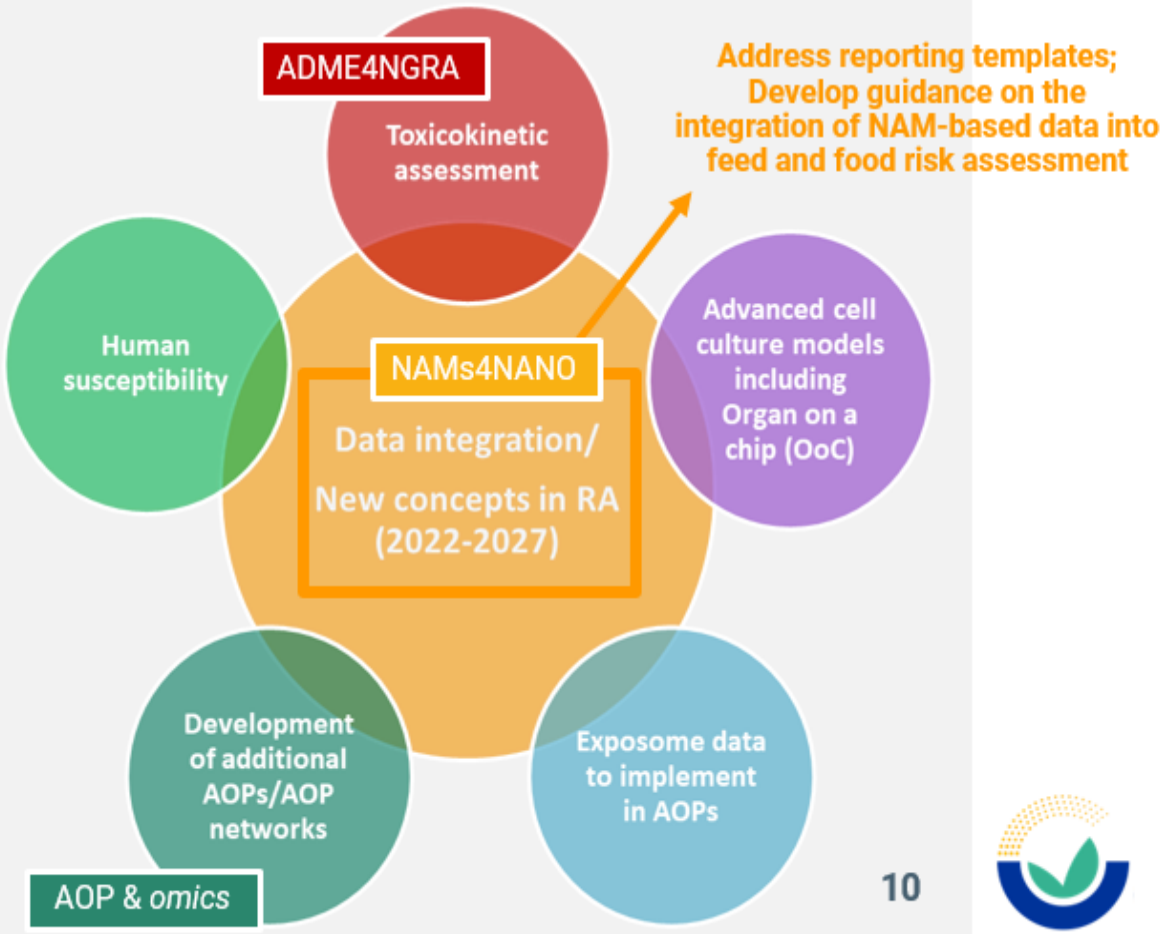
Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment

Sylvia E. Escher[1], Falko Partosch[1], Sebastian Konzok[1], Paul Jennings[2], Mirjam Luijten[3], Anne Kienhuis[3], Victoria de Leeuw[3], Rosmarie Reuss[4], Katrina-Magdalena Lindemann[4], Susanne Hougaard Bennekou[5]

[1] Fraunhofer ITEM, [2] Vrije Universiteit Amsterdam, [3] National Institute for Public Health and the Environment, [4] Eura AG, [5] The National Food Institute Denmark

Aim: to propose potential **EFSA priorities regarding the incorporation of NAMs** into regulatory hazard and risk characterisations of chemicals in food and feed

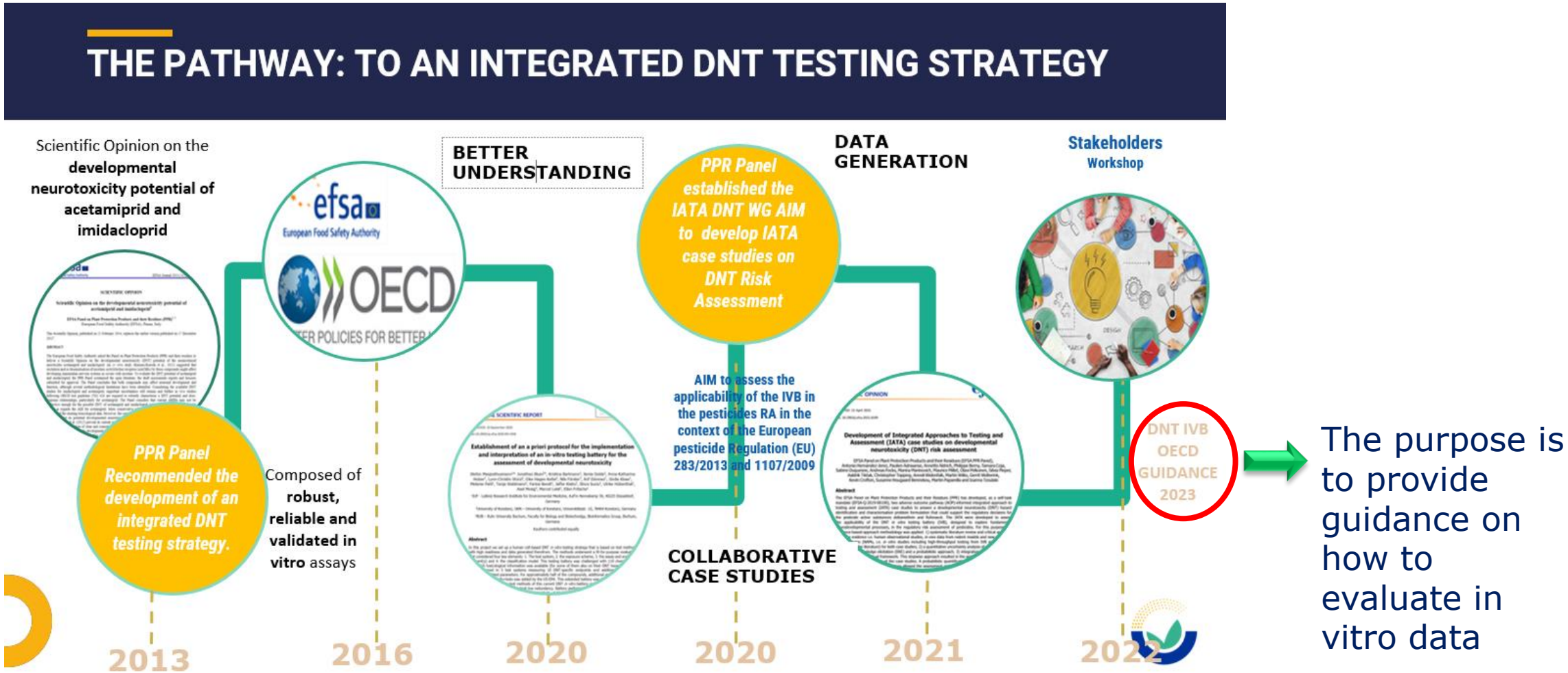
Result: six prioritised areas



Dalla presentazione dr. Maria Chiara Astuto –EFSA al 21°Congresso SITOX, Bologna 2023

AOP informed IATA case studies for DNT risk assessment

The goal is to assess any pesticide for DNT using an integrated approach (IATA) and minimize the request for DNT in vivo Guidelines studies (high costs, time and resource consuming, uncertainties in the interpretation, species differences)



The purpose is to provide guidance on how to evaluate in vitro data

Figura dalla presentazione dr. Andrea Terron–EFSA al 21°Congresso SITOX, Bologna 2023

Ruolo dei NAMs nel Progetto HESI TEA

Health and Environmental Sciences Institute

Transforming the Evaluation of AgroChemicals IT'S TEATIME! (JAN 2021)

Project Vision, Structure, Mission and Objectives

Transformed evaluation of agrochemicals for globally sustainable agriculture

Harmonized, integrated, and sustainable fit-for-safety testing of agrochemicals to inform hazard and risk assessment

Create a roadmap that

- ⇨ Transforms the evaluation of agrochemicals
- ⇨ Better reflects current and emerging science
- ⇨ Accounts for current and emerging evidence requirements for agrochemicals

HESI Collaborative effort

- Multisectoral
- Multidisciplinary
- International

Dalla presentazione dr. Marco Corvaro –Corteva al 21°Congresso SITOX, Bologna 2023

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

Abstract

This Guidance document describes harmonised risk assessment methodologies for combined exposure to multiple chemicals for all relevant areas within EFSA's remit, i.e. human health, animal health and ecological areas. First, a short review of the key terms, scientific basis for combined exposure risk assessment and approaches to assessing (eco)toxicology is given, including existing frameworks for these risk assessments. This background was evaluated, resulting in a harmonised framework for risk assessment of combined exposure to multiple chemicals. The framework is based on the risk assessment steps (problem formulation, exposure assessment, hazard identification and characterisation, and risk characterisation including uncertainty analysis), with tiered and stepwise approaches for both whole mixture approaches and component-based approaches. Specific

GUIDANCE

EFSA Journal

ADOPTED: 17 November 2021

doi: 10.2903/j.efsa.2021.7033

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, Claude Lambré, 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 CM Dorne and Christer Hogstrand

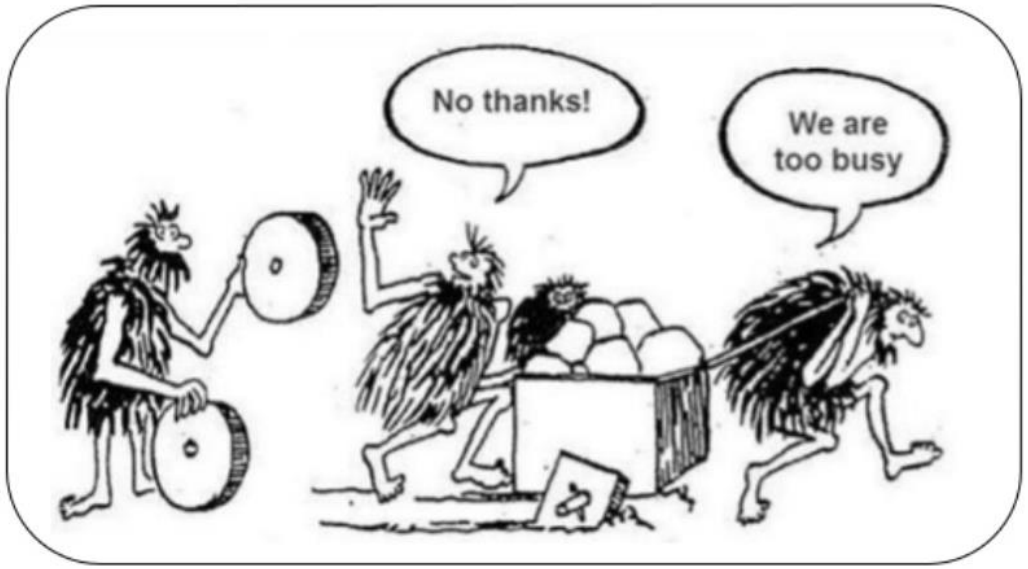
Abstract

This guidance document provides harmonised and flexible methodologies to apply scientific criteria and prioritisation methods for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. In the context of EFSA's risk assessments, the problem formulation step defines the chemicals to be assessed in the terms of reference usually through regulatory criteria often set by risk managers based on legislative requirements. Scientific criteria such as hazard-driven criteria can be used to group these chemicals into assessment groups. In this guidance document, a framework is proposed to apply hazard-driven criteria for grouping of chemicals into assessment groups using mechanistic information on toxicity as the gold standard where available (i.e. common mode of action or adverse outcome pathway) through a structured weight of evidence approach. However, when such mechanistic data are not available, grouping may be performed using a common adverse outcome. Toxicokinetic data can also be useful for grouping, particularly when metabolism information is available for a class of compounds and common toxicologically relevant

Le persone, gli animali e l'ambiente possono essere esposti **a molteplici sostanze chimiche** provenienti da una varietà di fonti. L'EFSA ha già sviluppato alcuni approcci per valutare l'esposizione combinata a più pesticidi (uomo e api). Sono in via di sviluppo nuovi approcci e strumenti per armonizzare il modo in cui valutiamo i rischi per l'uomo e l'ambiente derivanti dall'esposizione combinata a più sostanze chimiche nella catena alimentare: "miscele chimiche" e i loro effetti, a volte chiamati "effetti cocktail".

Ottobre 2022: L'EFSA pubblica una valutazione dei rischi per le donne in gravidanza derivanti dall'esposizione alimentare cumulativa a residui di pesticidi che hanno effetti acuti sullo sviluppo degli embrioni. La valutazione è stata condotta per due tipi di malformazioni craniofacciali: alterazioni dovute ad uno sviluppo scheletrico anormale e alterazioni dei tessuti molli della testa e difetti del tubo neurale. La conclusione è che, seppur con diversi gradi di certezza, l'esposizione è al di sotto della soglia che fa scattare l'azione regolamentare.

GRAZIE!



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