Methodologies and approaches for chemical risk assessment in the area of food

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OUTLINE

1. Data for chemical RA: what do we need?
2. Data: new types, new sources, new approaches
3. Evidence, significance and uncertainties

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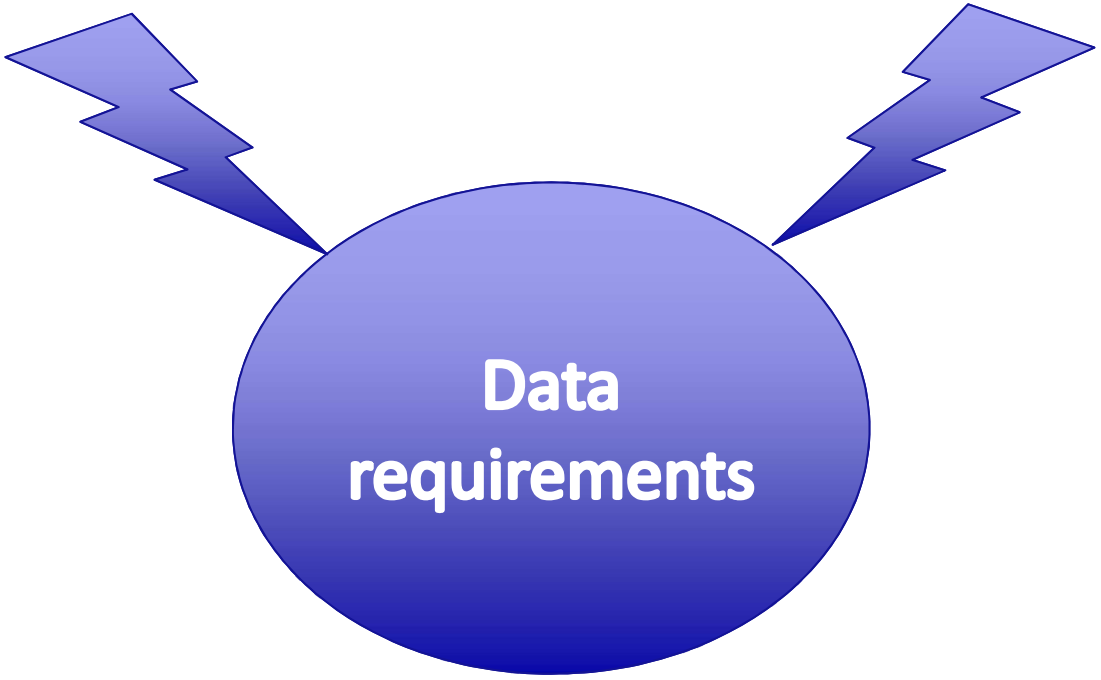
2. Data: new types, new sources, new approaches
3. Evidence, significance and uncertainties

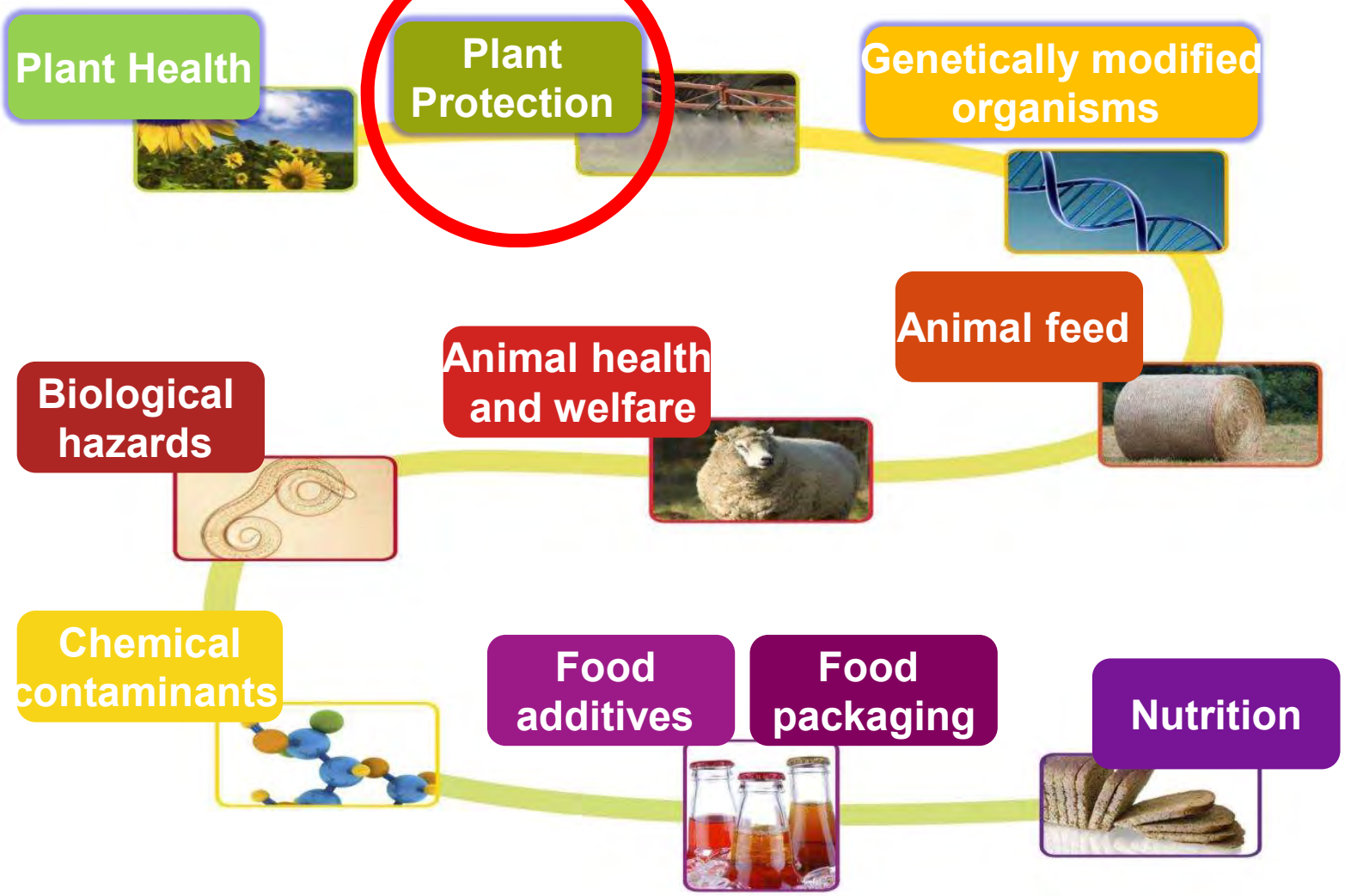
DATA: WHAT DO WE NEED?

- Legislation
- EFSA guidance on data requirements

- Application domain
- Use levels



SCIENTIFIC FIELDS



Legal considerations: example of the pesticides

II

(Non-legislative acts)

REGULATIONS

COMMISSION REGULATION (EU) No 283/2013

of 1 March 2013

setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

(Text with EEA relevance)

INTRODUCTION

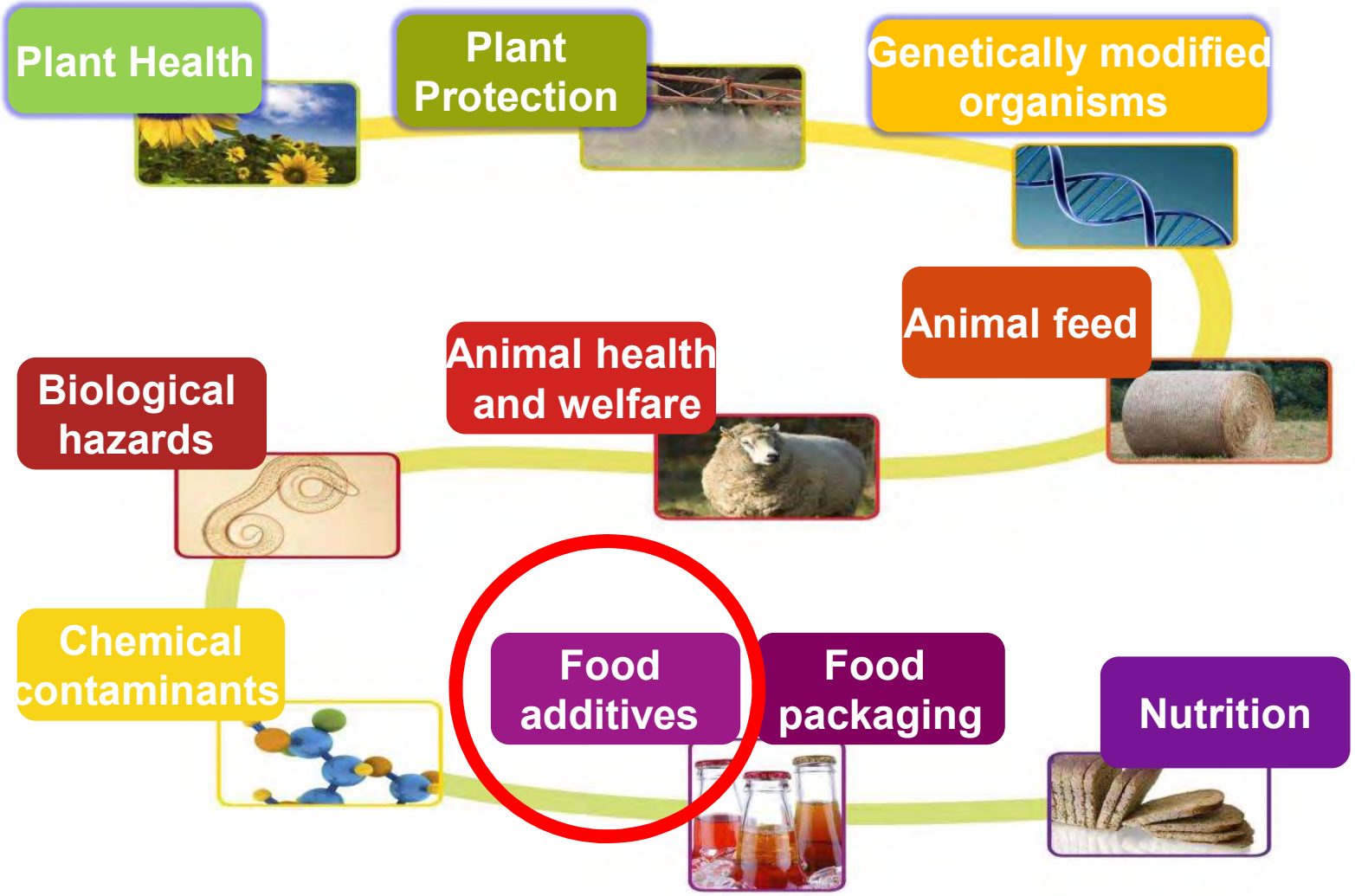
Information to be submitted, its generation and its presentation

1. The information submitted shall meet the following requirements.

1.1. The information shall be sufficient to evaluate the foreseeable risks, whether immediate or delayed, which the active substance may entail for humans, including vulnerable groups, animals and the environment and contain at least the information and results of the studies referred to in this Annex.

SECTION 5.	***Toxicological and metabolism studies***	5.2.4.	Skin irritation	5.6.	Reproductive toxicity
	Introduction	5.2.5.	Eye irritation	5.6.1.	Generational studies
5.1.	Studies on absorption, distribution, metabolism and excretion in mammals	5.2.6.	Skin sensitisation	5.6.2.	Developmental toxicity studies
5.1.1.	Absorption, distribution, metabolism and excretion after exposure by oral route	5.2.7.	Phototoxicity	5.7.	Neurotoxicity studies
5.1.2.	Absorption, distribution, metabolism and excretion after exposure by other routes	5.3.	Short-term toxicity	5.7.1.	Neurotoxicity studies in rodents
5.2.	Acute toxicity	5.3.1.	Oral 28-day study	5.7.2.	Delayed polyneuropathy studies
5.2.1.	Oral	5.3.2.	Oral 90-day study	5.8.	Other toxicological studies
5.2.2.	Dermal	5.3.3.	Other routes	5.8.1.	Toxicity studies of metabolites
5.2.3.	Inhalation	5.4.	Genotoxicity testing		
		5.4.1.	*In vitro* studies		
		5.4.2.	*In vivo* studies in somatic cells		
		5.4.3.	*In vivo* studies in germ cells		
		5.5.	Long-term toxicity and carcinogenicity		

SCIENTIFIC FIELDS



FOOD ADDITIVES: EFSA GUIDANCE

REGULATION (EC) No 1333/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 16 December 2008

on food additives

(Text with EEA relevance)

(7) Food additives should be approved and used only if they fulfil the criteria laid down in this Regulation. Food additives must be safe when used, there must be a technological need for their use, and their use must not mislead the consumer and must be of benefit to the consumer. Mis-

EFSA Journal 2012;10(7):2760

SCIENTIFIC OPINION

Guidance for submission for food additive evaluations[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

FOOD ADDITIVES: EFSA GUIDANCE FOR STUDY REQUIREMENTS

TIER 1*

- **Absorption**
- **Genotoxicity**

- in vitro testing
- genotoxic impurities

- **Extended 90-day tox study**

- repro endpoints
- endocrine activity
- other (immune, neuro)

Triggers for Tier 2

- *Absorption/Systemic exposure*
- *GI toxicity*
- *Subchronic toxicity*
- *(+ve) in vitro genetox*

* Minimal dataset - Applicable to all compounds

TIER 2

- **ADME**
- **Genotoxicity**
  - in vivo testing
- **Chronic/Carc**
  - stand-alone or combined
- **Reproductive**

1. EOGRT study (rat - various endpoints)
   - repro & developmental
   - immunotoxicity, neurotoxicity
2. Pre-natal developmental tox (rabbit)

Triggers for Tier 3

- *Bioaccumulation/slow excretion*
- *(+ve) in vivo genetox*
- *Chronic/carc*
- *EOGRTS toxicity*
- *Developmental toxicity (rabbit)*

Approach to Tier 3 studies

- *Consideration of all available data*
- *Case-by-case approach*
- *Equivocal findings*

TIER 3**

- Toxicokinetics
  - PBK modelling, volunteer studies
- Carcinogenicity
  - 2nd species (?)
- Repro
  - F2 generation
  - developmental immuno- & neurotox
- Specialised studies
  - immunotox, neurotox, endocrine

** Additional studies - Only if triggered

DATA: WHAT DO WE NEED?

High use – high exposure

Low use – low exposure

Data require-ments

2-Year carcinogenicity
Chronic toxicity
Reproductive/ developmental toxicity

Sub-chronic toxicity
Full ADME

Genotoxicity
ADME

Example: Food contact materials

- The higher the 'migration' into food, the greater the amount of data is required

Migration (mg/kg food)	< 0.05	0.05 - 5	5-60
Genotoxicity	+	+	+
90-day study		+	+
Data on accumulation		+	+
ADME			+
Reproduction study			+
Developmental studies			+
Long term study			+

FULL SET OF TOXICITY DATA

- Means 1,500-3,000 laboratory animals (cost €30-40 million)

Ethical considerations

But as risk assessors we need

- **more data**
  - more endpoints
- **better data**
  - more robust data
  - more data points
  - less uncertainty
  - to allow better predictions

CHALLENGES IN FOOD SAFETY RISK ASSESSMENT

- Not all chemicals can be tested!
  - Single chemicals: known impurities and contaminants
  - Complex and undefined chemicals: what to test?
  - Unknown chemicals: e.g. NIAS in food contact materials.
  - Metabolites of chemicals: e.g. pesticide metabolites.
  - Mixtures of chemicals: which mixture to test?
- 'Unorthodox chemicals'
  - Endocrine active substances

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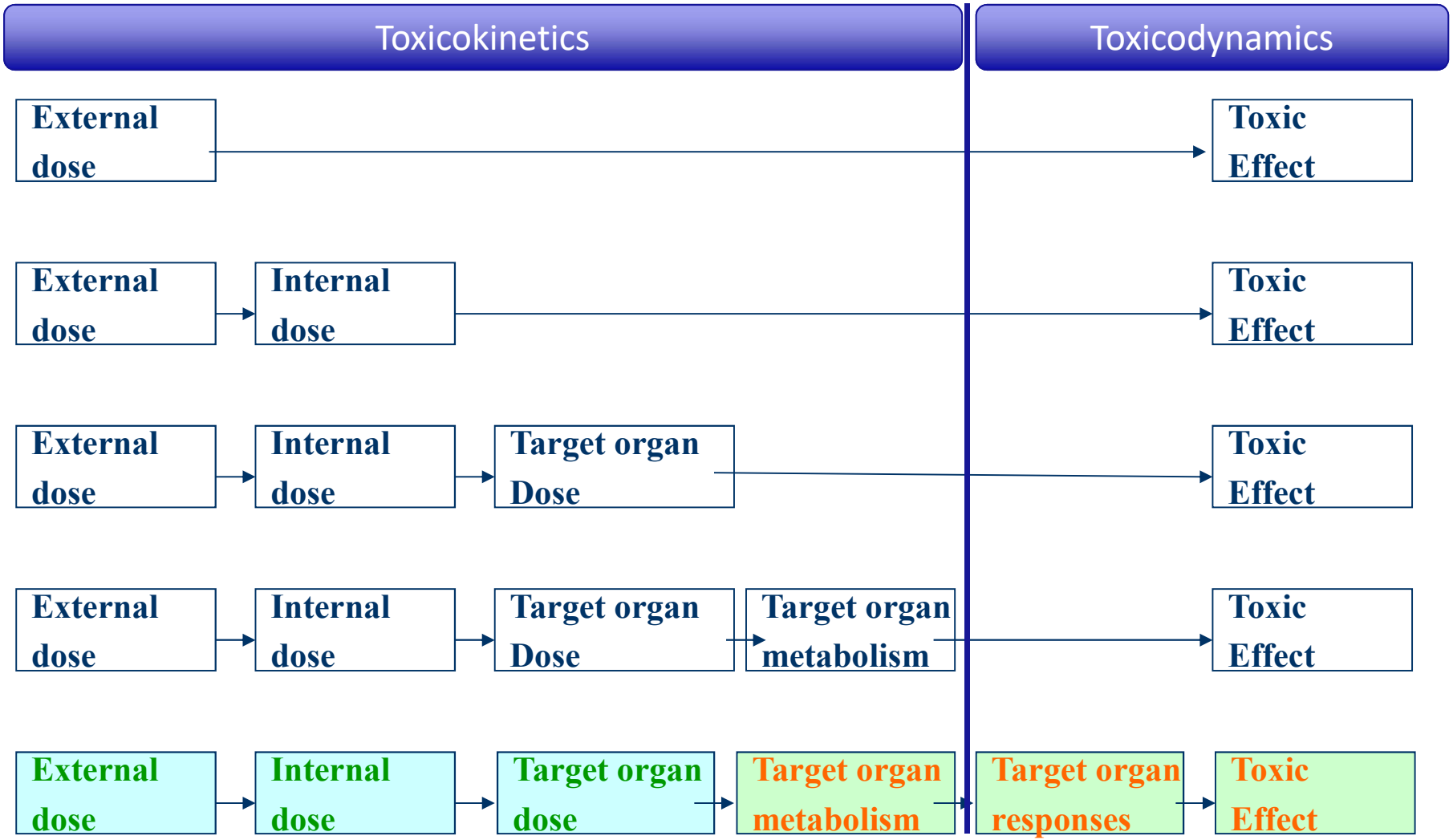
EFSA's initiatives in

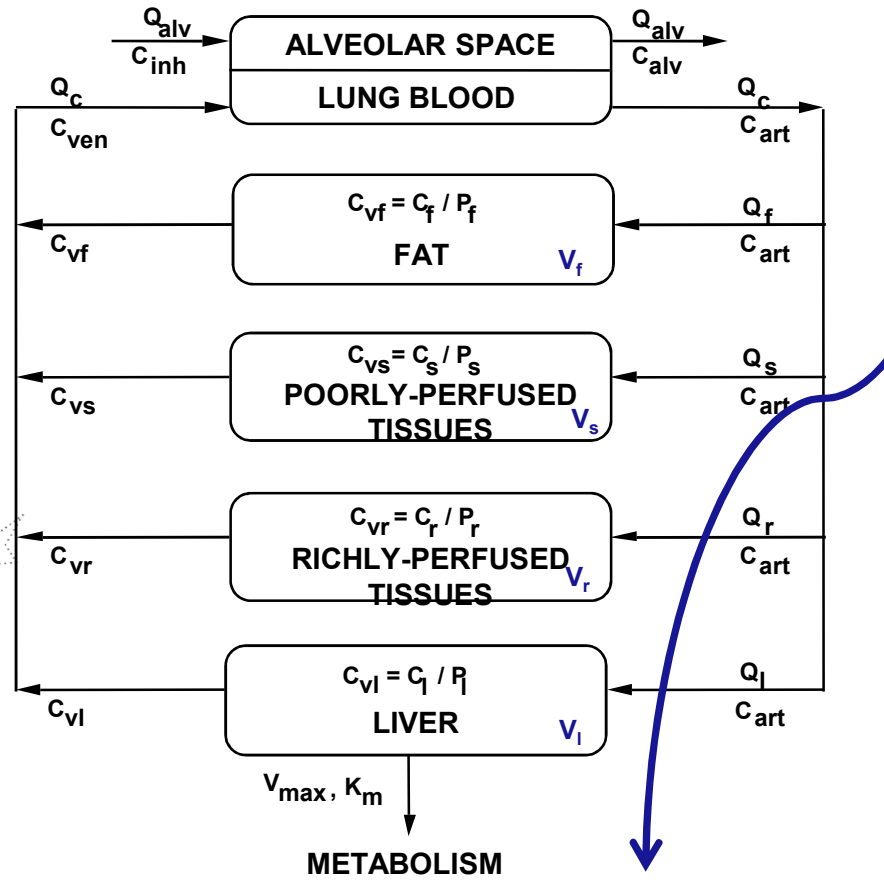
Methodologies and approaches for chemical risk assessment in the area of food

Improved understanding from toxicokinetic and toxicodynamic processes

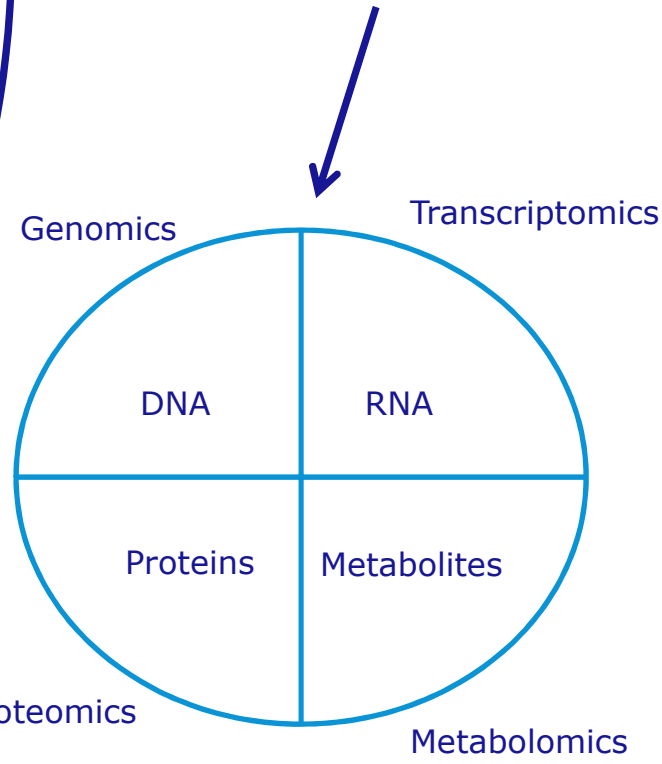


BIOLOGICALLY BASED MODELS AND OMICs

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



PB-TK models



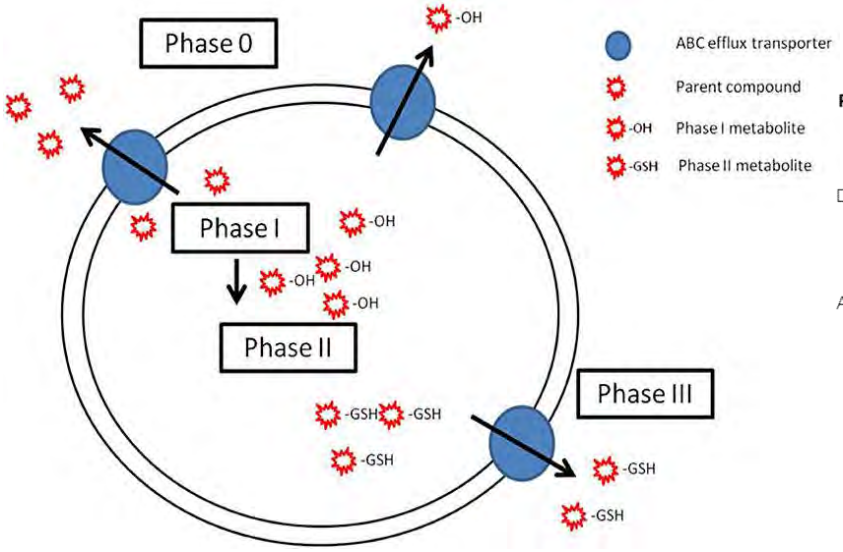
OMICs

OPEN SOURCE TK MODELS: DATA AND MODELS

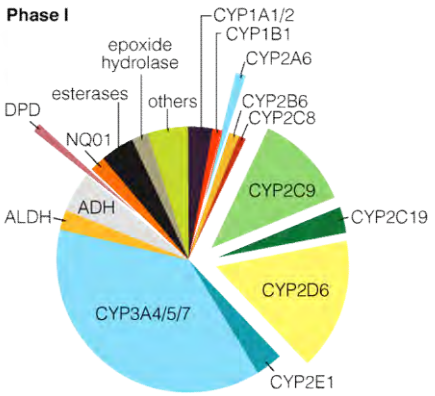
- ✓ Collection data physiological and biological parameters: calibration of TK tools
  - Body weight, variability enzymes expression gut/liver etc.
  - Human Variability metabolism and excretion (CYP2C9, CYP2C19, UGTs, Renal excretion) using Pharmaceutical DB
  - TK tools from one compartment to multi compartment/PB-PK e.g. blood/liver/gut/kidney
  - Case studies 10 compounds relevant to food and feed safety combining TK and TD: regulated, contaminants
- ✓ In the future: Open TK tools in R
- ✓ In parallel, TK tools for veterinary species (cow, pig, cat, chicken) and ERA (zebrafish, trout, earth worm)

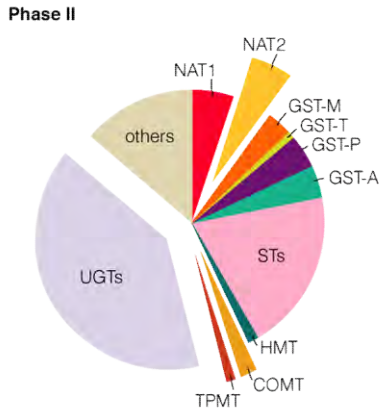
Human variability in toxicokinetics

MAJOR METABOLIC/EXCRETION ROUTES IN HUMANS

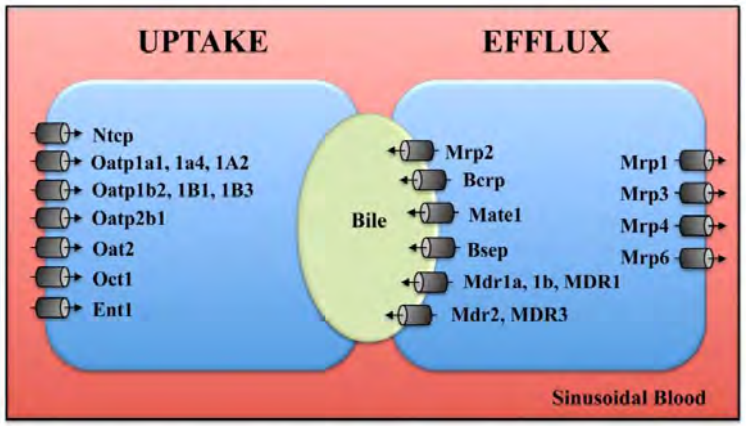


Source: Ferreira et al. Front. Physiol. (2014)

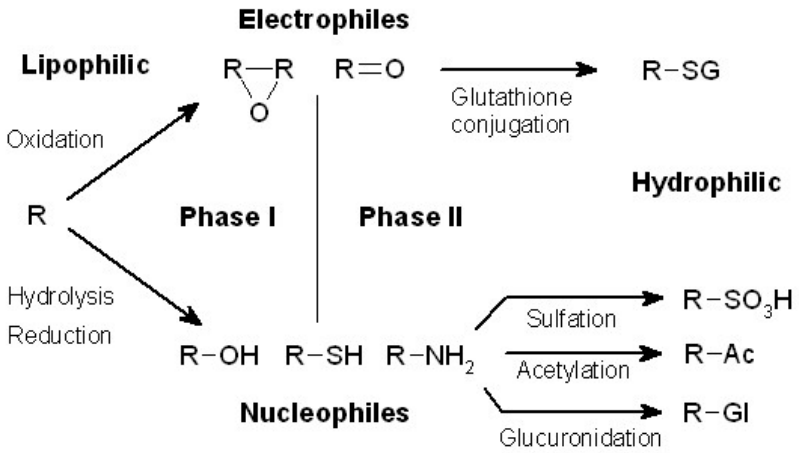




Source: Evans&Relling, Science (1999)



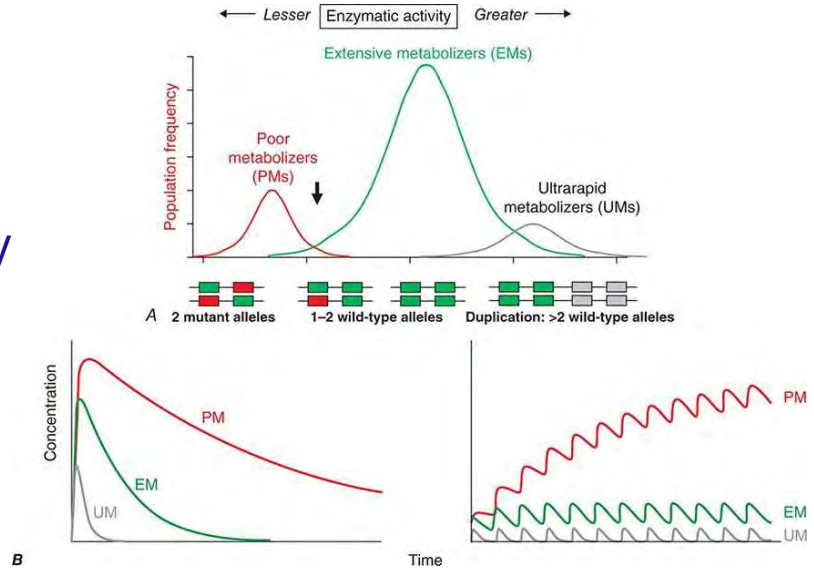
Source: Moscovitz&Aleksunes, Int. J. Mol. Sci. (2013)



By TimVickers - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=4549628

HUMAN VARIABILITY IN TK: ongoing work in food safety

CYP2D6 activity

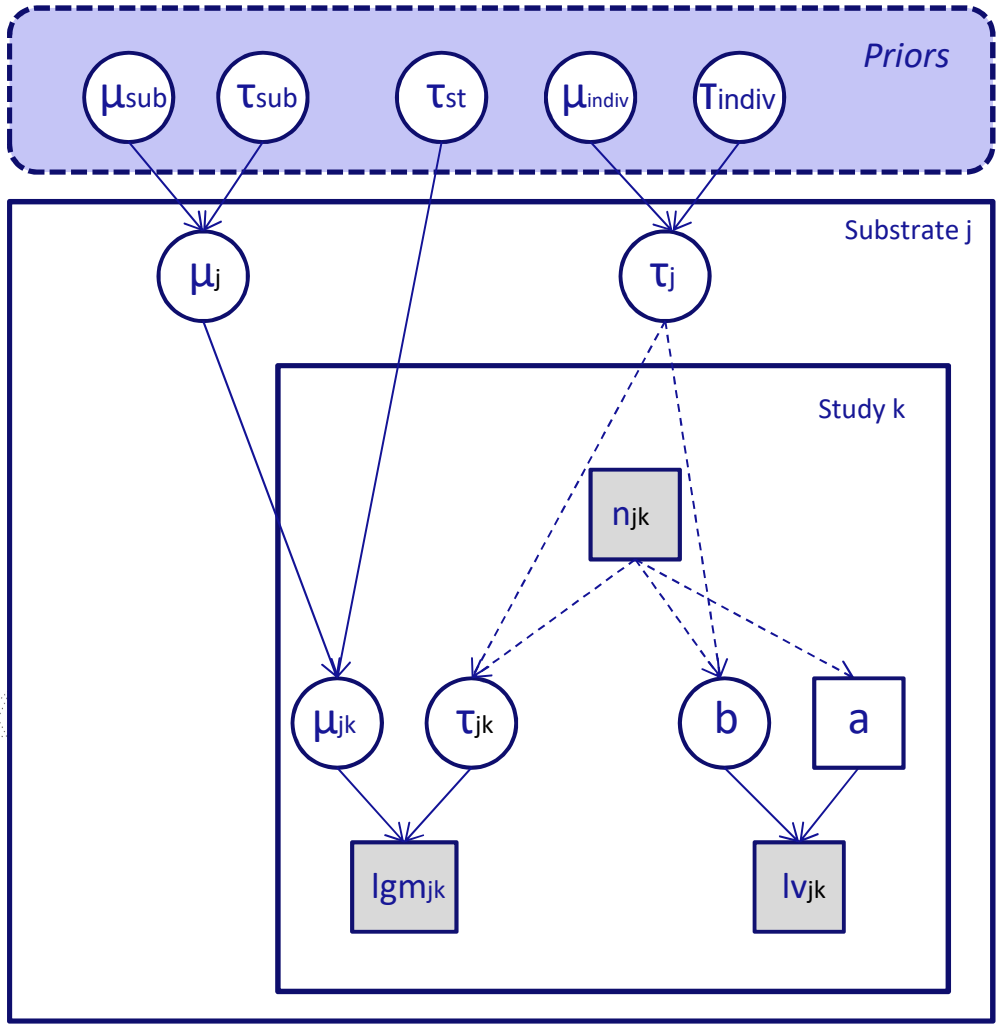


Source: Dahl et al. JPET (1995)

From pharmaceutical databases and compounds relevant to food safety,

- ✓ Identify Phase 0, I, II, III isoforms *in vitro*, excretion data etc.
- ✓ PK parameters of acute and chronic exposure: Meta-analysis
- ✓ Human variability distributions - isoform specific for different subgroups of the population

BAYESIAN META-ANALYSIS OF TK DATA



Amzal et Dorne, 2008

3 levels:inter-study, inter-substrate and inter-individual variability

$$\tau_j \sim Normal(\mu_{ind}, \frac{1}{\tau_{ind}})$$

$$\mu_j \sim Normal(\mu_{sub}, \frac{1}{\tau_{sub}})$$

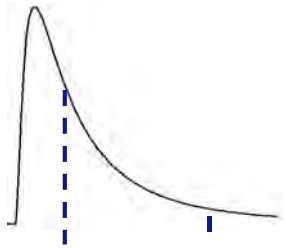
$$\mu_{jk} \sim Normal(\mu_j, \frac{1}{\tau_{st}})$$

$$\lg m_{jk} \sim Normal(\mu_{jk}, \frac{1}{n_{jk}\tau_j})$$

$$lv_{jk} \sim \frac{1}{n_{jk}\tau_j} Chi^2(n_{jk}-1)$$

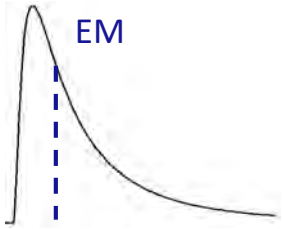
COMBINING POLYMORPHISM DATA EXTENSIVE AND POOR METABOLISERS (EMS AND PMS) CYP2D6

EM

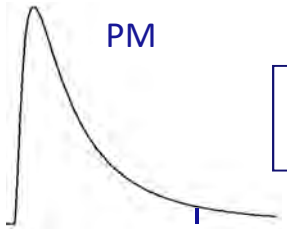


$$UF_{EM} = \frac{p95_{EM}}{p50_{EM}}$$

EM

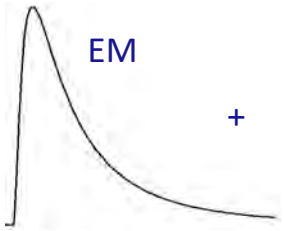


PM

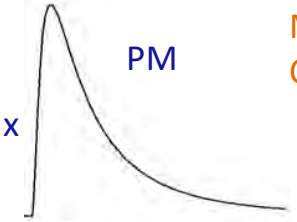


$$UF_{PM} = \frac{p95_{PM}}{p50_{EM}}$$

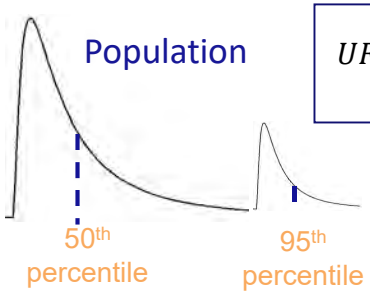
(1-α) EM x



+ α PM x

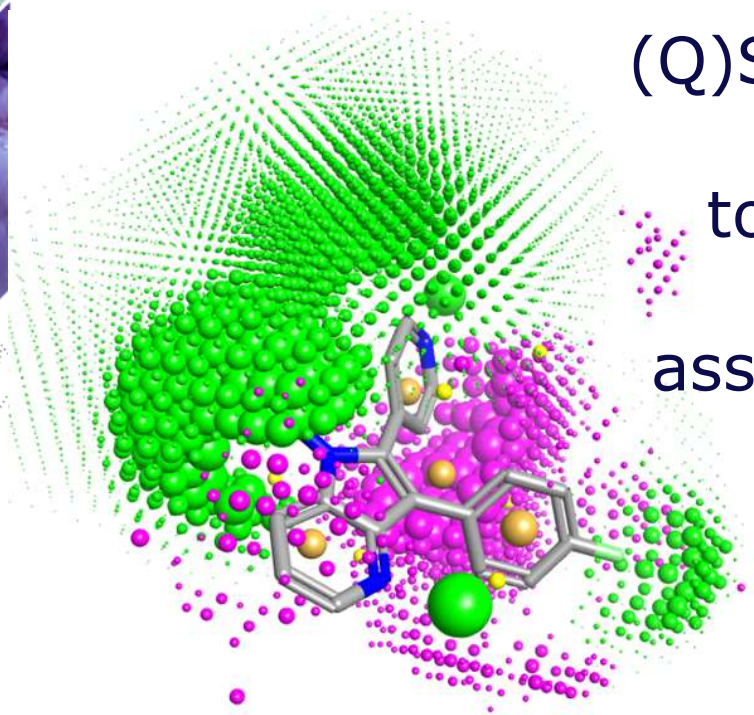


Monte-Carlo →



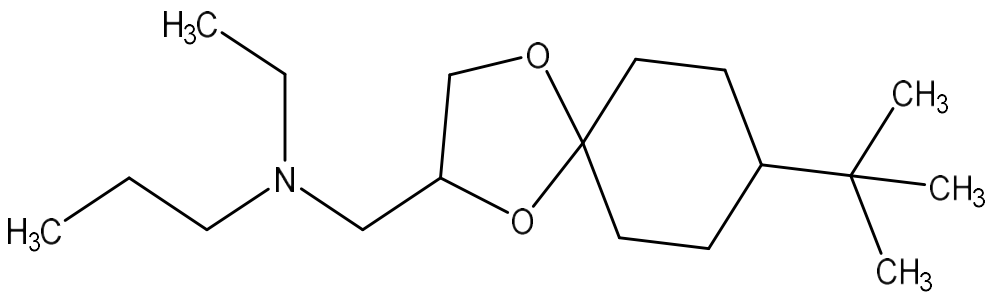
$$UF_{pop} = \frac{p95_{pop}}{p50_{pop}}$$

Data generation through non-testing approaches
Example: Pesticide metabolites



(Q)SAR and read-across for the assessment of toxicologically relevant metabolites in the assessment of dietary risk

SPIROXAMINE

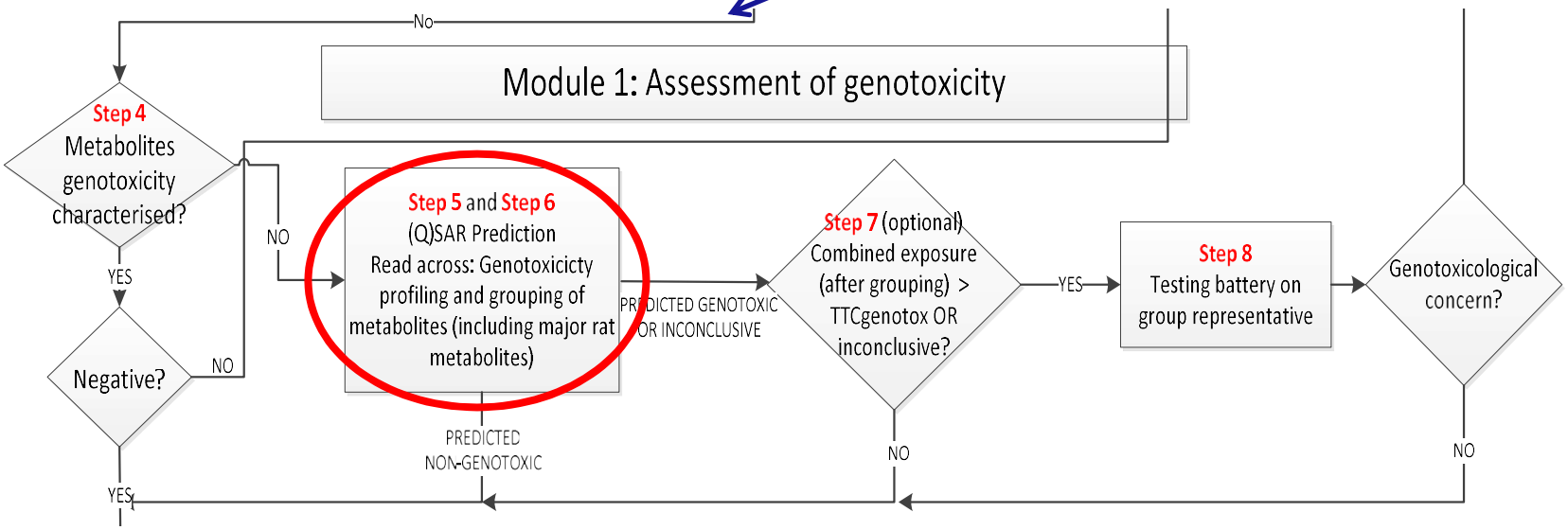


EXAMPLE

A systemic fungicide used to control common fungal diseases on cereals and fruit

EXAMPLE - SPIROXAMINE

- 43 metabolites: 15 conjugated metabolites (glycosides, glucuronides) – toxicological assessment covered by their aglycons
- Exclusion of metabolites of no concern – None
- Metabolites classified as genotoxic - None



STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

- Gene mutation:

  CAESAR Mutagenicity Model (http://www.vega-qsar.eu/)

  OASIS AMES Mutagenicity model (http://oasis-lmc.org/)

- Chromosomal alterations

  Toxtree in vivo micronucleus model(http://toxtree.sourceforge.net/)

  OASIS Chromosomal Aberration model (http://oasis-lmc.org/)

STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

		CAESAR prediction of gene mutation (Applicability Domain)	OASIS prediction of gene mutation (Applicability Domain)	Rule based model for prediction of in vivo CA (Toxtree) (no Applicability Domain evaluation is available)	OASIS prediction of CA (Applicability Domain)
M01	Desethyl	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M02	Despropyl	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M04	N-formyl-desethyl	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M05	Hydroxyl	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M07	Hydroxy acid	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M08	8-hydroxy acid	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M09	Hydroxy-despropyl	Positive (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M10	Hydroxy-N-oxide	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M11	Desethyl acid	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M12	Despropyl acid	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M13	Cyclohexanol	Negative (In)	Negative (In)	Negative	Negative (out)
M14	Diol	Negative (In)	Negative (In)	Negative	Negative (In)
M15	Ketone	Negative (Could be out)	Negative (In)	Negative	Negative (out)
M16	Hydroxy-ketone	Negative (In)	Negative (In)	Negative	Negative (out)
M25	Sulfate	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M26	Desethyl-sulfate	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M27	Despropyl-sulfate	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M28	Aminodiol	Negative (In)	Negative (In)	Positive alert for CA	Negative (In)
M29	Aminodiol-N-oxide	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)
M30	Desethyl-aminodiol	Negative (Could be out)	Negative (In)	Positive alert for CA	Negative (out)
M31	Despropyl-aminodiol	Negative (In)	Negative (In)	Positive alert for CA	Negative (out)
M35	Docosanoic acid ester	Negative (Could be out)	Negative (In)	Negative	Negative (out)
M36	Tetracosanoic acid ester	Negative (Could be out)	Negative (In)	Negative	Negative (out)
M37	Cyclohexenol	Negative (In)	Negative (out)	Negative	Positive (In)
M38	N-formyl-despropyl	Negative (Could be out)	Negative (out)	Positive alert for CA	Negative (out)
M41	Hydroxy-desethyl	Negative (Out)	Negative (out)	Positive alert for CA	Negative (out)

STEP 5: (Q)SAR PREDICTION OF GENOTOXICITY

- 20 metabolites are predicted as positive (potentially genotoxic) from at least one of the models.
- 6 metabolites are predicted as negative from all models.
- All metabolites are moved to the next step – **read across analysis**.

STEP 6: READ ACROSS

Endpoint

- Well defined endpoint
  - Gene mutation and chromosomal aberrations

Similarity

- Well defined and justified similarity
  - molecular initiating events – covalent binding to DNA and/or proteins
  - evaluation of the influence of the rest part of the molecule

Data

- High quality of the data used
  - Data for the source substance (parent and/or metabolite) - Commission Regulation (EU) No 283/2013

STEP 6: READ ACROSS

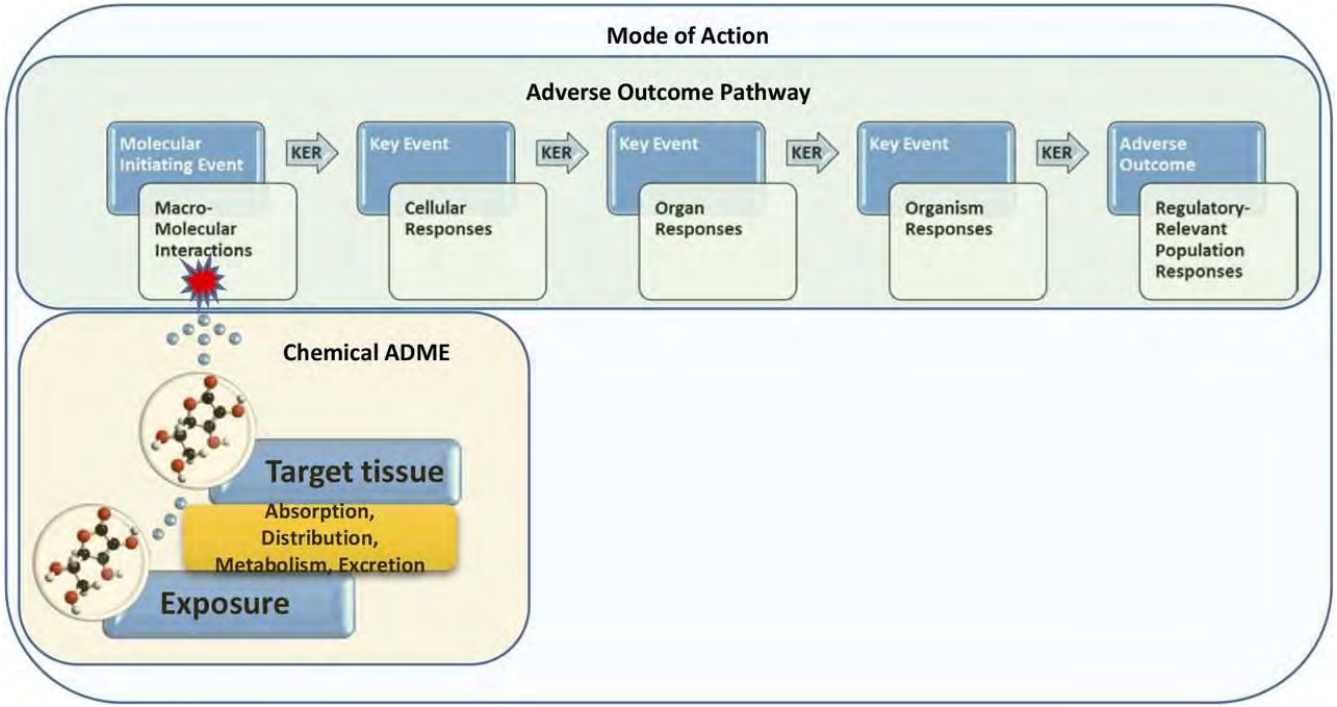
- OECD Toolbox:
  - DNA binding by OASIS
  - DNA binding by OECD
  - Protein binding by OASIS
  - Protein binding by OECD
  - DNA alerts for AMES, MN and CA by OASIS
  - In vitro mutagenicity (AMES test) alerts by ISS
  - In vivo mutagenicity (Micronucleus) alerts by ISS
  - Protein binding alerts for Chromosomal aberrations by OASIS

  - Organic functional groups

(Q)SAR PREDICTION AND READ ACROSS - CONCLUSIONS

- 4 metabolites negative by all (Q)SAR models and no new alerts were identified by read across: no concern for genotoxicity.
- 6 metabolites where genotoxicity concern cannot be excluded (+ve (Q)SAR predictions and/or read across considerations): exposure assessment and comparison against TTC (step 8) and/or testing (step 9)
- 12 metabolites predicted as potential genotoxicants by (Q)SAR models, BUT as a result of read across: no concern for genotoxicity.

Adverse Outcome Pathways and Mode of Action Analysis



Edwards et al. JPET (2016)

OUTLINE

1. Data for chemical RA: what do we need?
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GUIDANCE ON UNCERTAINTY IN RISK ASSESSMENT

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: dd mmmm yyyy PUBLISHED: dd mmmm yyyy AMENDED: dd mmmm yyyy

doi:10.2903/j.efsa.20YY.NNNN

Revised Draft for Internal Testing

Guidance on Uncertainty in EFSA Scientific Assessment

EFSA Scientific Committee[1, 2]

European Food Safety Authority (EFSA), Parma, Italy

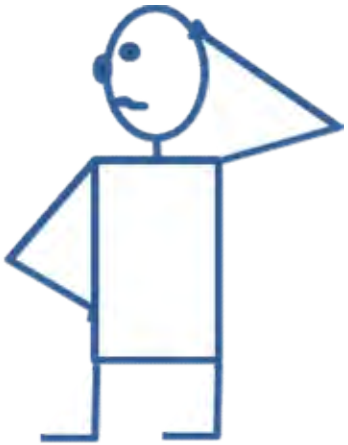
GUIDANCE ON UNCERTAINTY IN RISK ASSESSMENT

- To provide guidance on how to characterise, document and explain all types of uncertainty arising in EFSA's scientific assessments.
- Public consultation on draft guidance
- Updated draft guidance published for trial phase (including external volunteers)
- Panel and Risk Manager training + Testing phase: Mar 2016 – May 2017
- Integrate lessons learnt
- Expected adoption: End 2017

(work started in Sept 2012)

"Risk is measurable uncertainty"

"Uncertainty is unmeasurable risk"

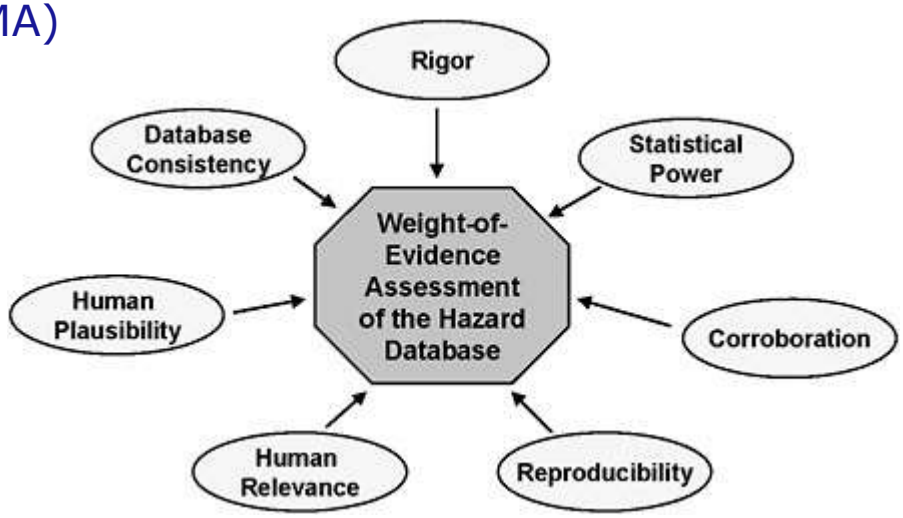


The Weight of Evidence

Weight of evidence is a measure of evidence on one side of an issue as compared with the evidence on the other side of the issue, or to measure the evidence on multiple issues.

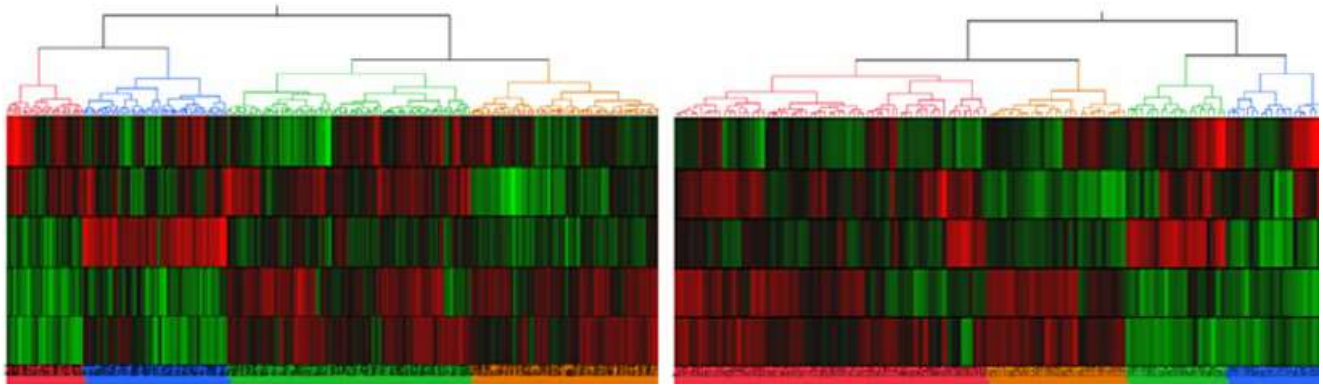
GUIDANCE ON THE WEIGHT OF EVIDENCE APPROACH

- To provide guidance on the use of the WoE in scientific risk assessment using qualitative and quantitative approaches
- Case studies for various areas under EFSA's remit to be annexed
- Timelines: public consultation in 2016, adoption Sept 2017
- In addition to Panel representation and specific experts in the WG:
  - Sister Agencies (ECHA, EMA)
  - Hearing expert (NTP, US)



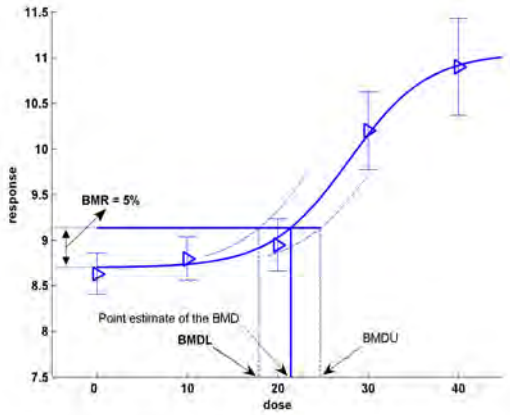
BIOLOGICAL RELEVANCE

- ToR: to provide generic issues and criteria to consider when deciding whether an observed effect is of biological relevance for the assessment
  - Definitions and concepts, such as adverse, adaptive, harm, homeostasis, biological threshold, etc.
- All EFSA areas of activity to be considered
- Public consultation in 1st quarter 2017
- Adoption: mid-2017



UPDATE of the Guidance on Benchmark dose approach

- Ongoing revision of BMD 2009 guidance, after 6 years of implementation




GUIDANCE DOCUMENT

EFSA Journal

ADOPTED: PUBLISHED: dd mmmm yyyy AMENDED: dd mmmm yyyy

doi:10.2903/j.efsa.20YY.NNNN

Use of the benchmark dose approach in risk assessment

EFSA Scientific Committee

- Public consultation: Summer 2016
- Adoption: November 2016

Substances in Food for Infants below 16 Weeks of Age

- **Background:** Mandate from EC for a guidance on food additives, contaminants, pesticide residues and food contact materials in food destined for infants <16 weeks.
- Population where health-based guidance values (ADI, TDI, ARfD) do not apply.
- Considered a very sensitive population due to immaturity of organs.

- **Timeframe**
  - Public consultation: End 2016
  - Adoption: April 2017

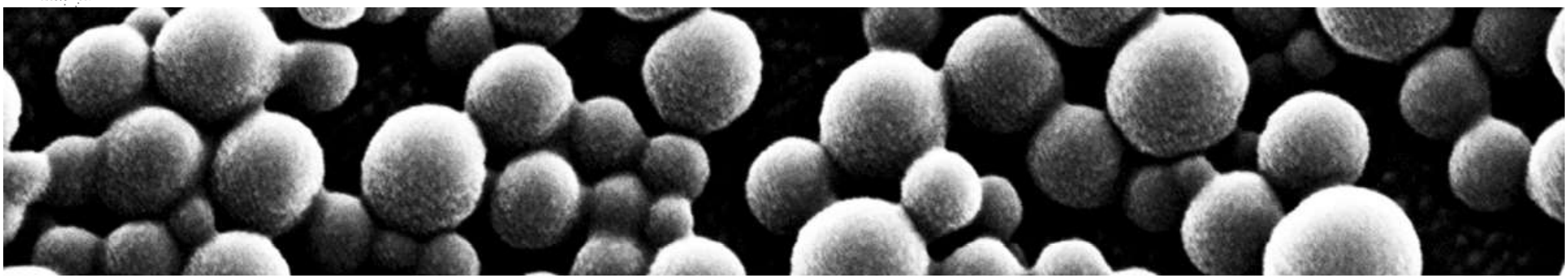
EFSA WORK ON BOTANICALS - NEXT CHALLENGES

- Clarify what should be an appropriate composition characterisation of a botanical preparation
- Botanical preparations = complex chemical mixtures
- Compendium of Botanicals version N° 3

  Timeframe:
  - intermediate draft published July 2016
  - Final report: mid 2018

NANOMATERIALS

- Future developments are expected
  - Nano-encapsulates and nano-composites in applications such as novel foods, food/feed additives, biocides, food contact materials, but especially as pesticides
- Need to update the 2011 SC Nanomaterial Guidance to stay aligned with
  - Scientific innovations
  - Legal requirements
- To take into account the general extensions needed to cover also nanopesticides and nanoformulations, food contact materials, food and feed additives and novel foods
- Update of the physico-chemical property measurements and the other data needed for food/feed assessment.
- A second guidance document to be produced on the environmental risk assessment for nanoparticles used in the food chain



RISK ASSESSMENT OF CHEMICAL MIXTURES

- Complex problem due to
  - Type of mixture (intentional, coincidental)
  - Exposure (combined, single or multiple routes)
  - Whole mixture approach versus component-based approach?
  - Quantitative assessment versus qualitative assessment
- Scientific event held in 2015
- Development of guidance on the harmonisation of human risk assessment and environmental risk assessment to combined exposure to 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

THANK YOU

ANY QUESTIONS?