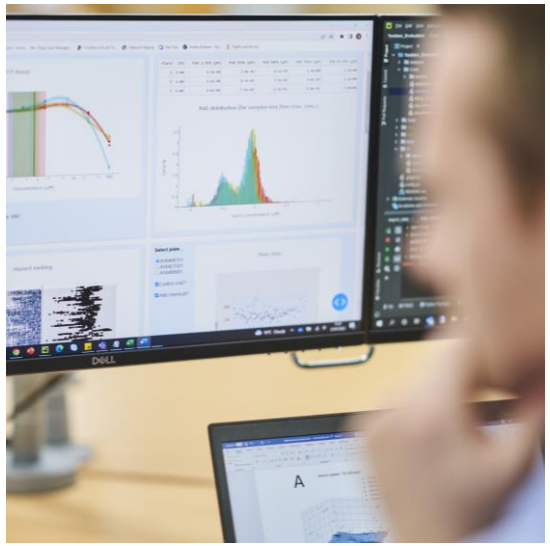
Application of New Approach Methodologies (NAMs) in Next Generation Risk Assessment (NGRA) for food safety

在食品安全中应用下一代风险评估里的新途径方法

Dawei Tang (汤大为), Ans Punt and Paul Hepburn

Unilever Safety and Environmental Assurance Centre



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Unilever at a glance

Established over 100 years ago, we are one of the world's largest consumer goods companies. We are known for our great brands and our belief that doing business the right way drives superior performance.

190
countries
where our products are sold

4.4 million
retail stores
served by distributors in top 10 emerging markets

€59.6 billion
turnover in 2023
with 58% in emerging markets

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Unilever's Safety & Environmental Assurance Centre (SEAC)
联合利华安全与环境保障中心

SEAC is Unilever's global centre of excellence in Safety & Sustainability Sciences (安全和可持续发展科学), part of R&D's Safety, Environment & Regulatory Sciences Capability.

Diverse, multi-disciplinary team of ~150 scientists based at Colworth, UK; ~70 miles north of London

Highly collaborative (广泛合作), working with over 70 academic, industry, government & NGO partners worldwide

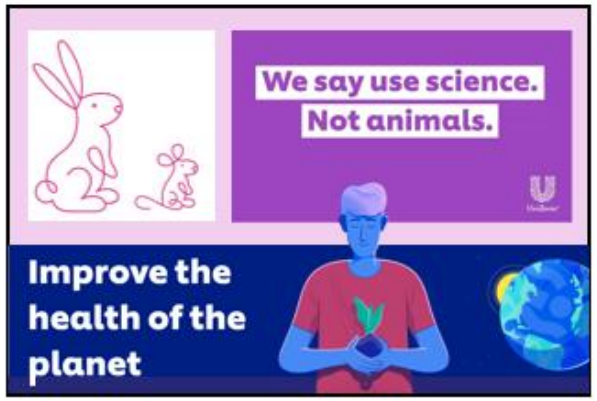
Business Group R&D

Global Teams

Business Units

'One R&D' Centre

- **Safety, Environment & Regulatory**
- Digital & Partnerships
- Sustainable Packaging



Team SEAC's purpose is to protect people & the environment (保护人和环境) by ensuring:

1. Unilever's products & innovations are **Safe & Sustainable by Design without animal testing** (不使用动物测试，从设计上保证安全和可持续性)
2. Our scientists & capabilities are **industry-leading with high business impact via Unilever's Products & Brands**
3. Safety & Env. Sustainability **policies & regulations are based on modern science**

Much of our strength lies in our shared Values – to be an **inclusive, supportive** & **collaborative** Team that is **pioneering, transparent** & **high-performing** with a strong focus on **learning** & **wellbeing**.

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Team SEAC's purpose is to protect people & the environment

SEAC is a diverse, multi-disciplinary team of ~150 scientists covering:

- Cell Biology
- Chemistry
- Computational Modelling
- Environmental Safety
- Environmental Sustainability
- Exposure Science
- Informatics & Data Science
- Mathematics
- Microbiology
- Molecular Biology
- Process Safety
- Statistics
- Toxicology

20+ Nationalities
15+ Languages

- Deploy expertise on higher risk business projects
- Collaborate with leading external research teams to develop & apply new capability
- Leverage science & global networks for consumer trust & freedom to operate

Safety Risk Assessments
- Consumers, Workers, Environment

Life Cycle Assessments
- Environmental Impacts

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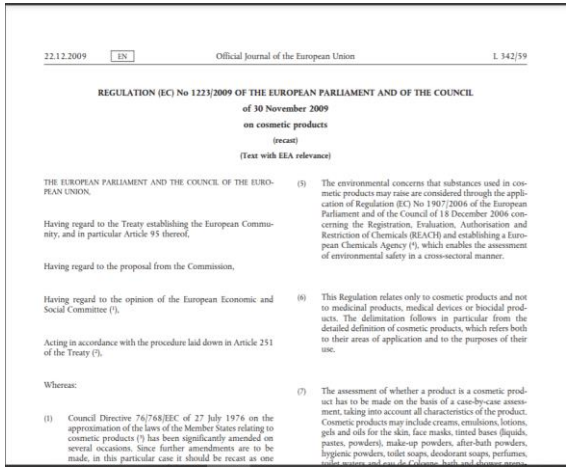
The need for non-animal safety assessments (非动物安全评估的需求)

Societal Attitudes/Consumer Preference (社会和消费者的态度)

Human Relevance (人体相关性)



22.12.2009 EN Official Journal of the European Union L 342/59

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 30 November 2009
on cosmetic products
(recast)
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee (1),

Acting in accordance with the procedure laid down in Article 251 of the Treaty (2),

Whereas:

(1) Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (3) has been significantly amended on several occasions. Since further amendments are to be made, in this particular case it should be recast as one

(5) The environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency (4), which enables the assessment of environmental safety in a cross-sectoral manner.

(6) This Regulation relates only to cosmetic products and not to medicinal products, medical devices or biocidal products. The delimitation follows in particular from the detailed definition of cosmetic products, which refers both to their areas of application and to the purposes of their use.

(7) The assessment of whether a product is a cosmetic product has to be made on the basis of a case-by-case assessment, taking into account all characteristics of the product. Cosmetic products may include creams, emulsions, lotions, gels and oils for the skin, face masks, tinted bases (liquids, pastes, powders), make-up powders, after-bath powders, hygienic powders, toilet soaps, deodorant soaps, perfumes,

Regulatory Changes (e.g. Cosmetics Regulation) (法规变化)

Archives of Toxicology (2023) 97:3075–3083
https://doi.org/10.1007/s00204-023-03601-5

REGULATORY TOXICOLOGY

Check for updates

Analysis of health concerns not addressed by REACH for low tonnage chemicals and opportunities for new approach methodology

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Resource/time constraints (时间资源的限制)

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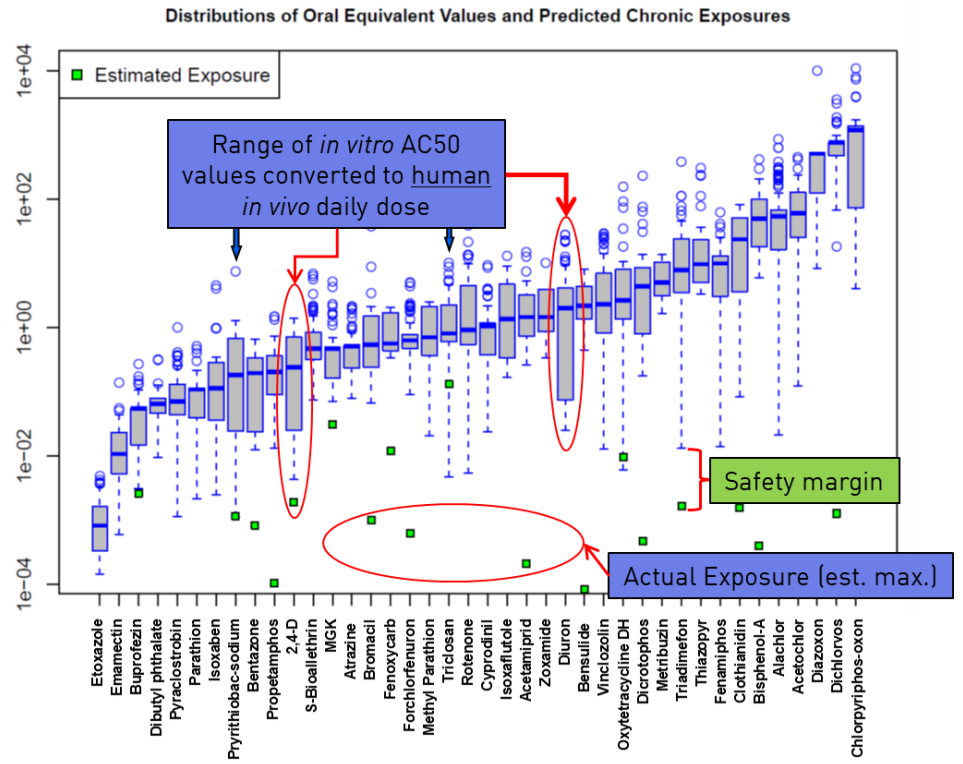
Shift towards next generation risk assessment (向下一代风险评估的转变)



Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

$$BER = \frac{Lowest\ bioactivity\ POD}{Internal\ in\ vivo\ exposure\ (Cmax)}(uM)$$

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NGRA and New Approach Methodologies (NAMs)

NGRA: "An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm to consumers"

Dent *et al* ., (2018) *Comp Tox* 7:20-26

In vitro
Experiments with human cells or tissues (人源细胞和组织)

In silico
Experiments based on computer models (计算机模型)

NGRA uses a combination of NAMs that are directly relevant to humans (和人体直接相关的新途径方法)

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NGRA in practice

https://youtu.be/5Z2S8MnKp7g

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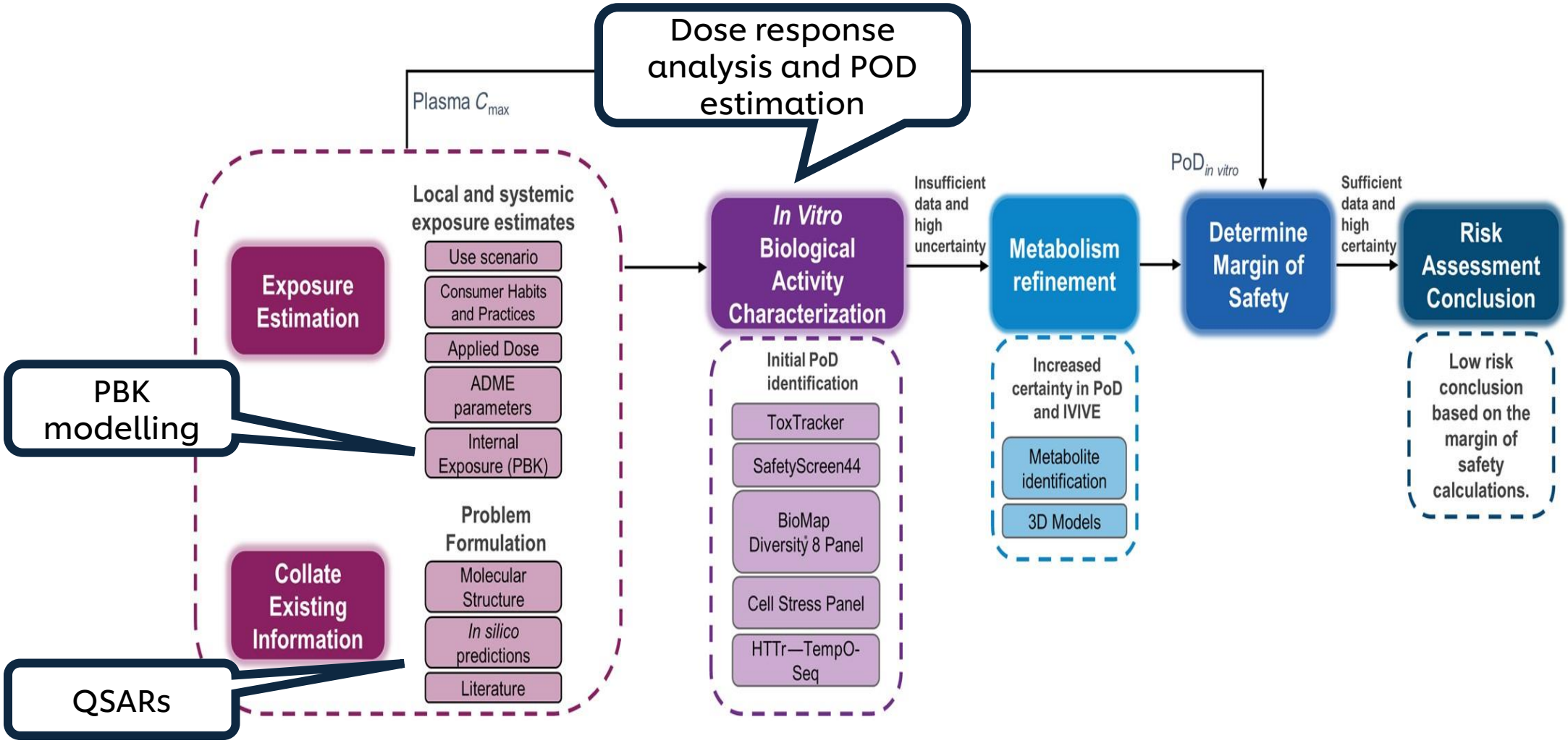
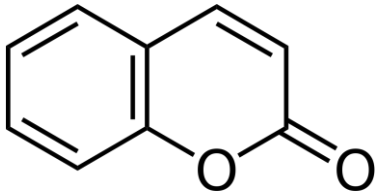
From Principles to Application



Baltazar, *et al.* Tox.Sci 2020

Example exposure scenarios



香豆素

Coumarin (flavouring and fragrance, naturally present in e.g. cinnamon)

Use Scenario	**Exposure route**	**Risk classification**
Dietary intake, 4.1 mg/day	Oral	Low risk

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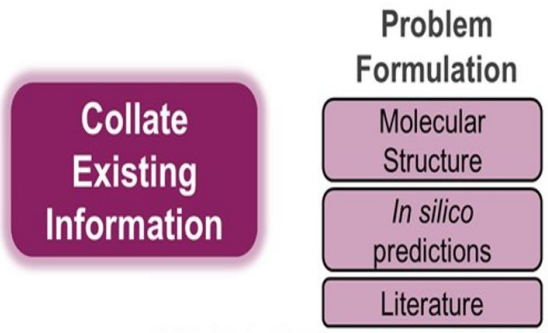
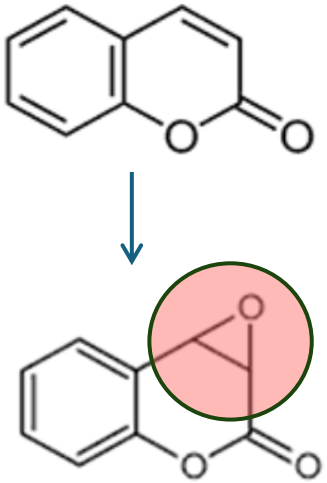
Collation of existing information: in silico predictions





In silico tools (ToxTree, MIE ATLAS, OECD toolbox, Meteor) predicted

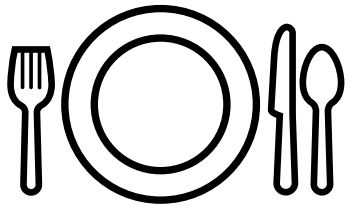
- Protein binding- MIE for induction of skin sensitisation
- DNA binding alert – MIE for genotoxicity
- Reactive metabolites (e.g. epoxide formation)- alerts both genotoxicity and skin sensitisation
- No binding alerts for the 39 targets in MIE atlas (e.g. nuclear receptors, enzymes, transporters)

Allen, Timothy EH, et al. "Using 2D structural alerts to define chemical categories for molecular initiating events." Toxicol. Sci. 165.1 (2018): 213-223.

From applied dose to internal concentrations (从外暴露到内暴露)

External dose

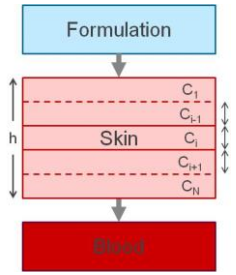
- Route of exposure
- Consumer use (Habits &Practices)
- Applied dose (external concentration)



ADME parameters

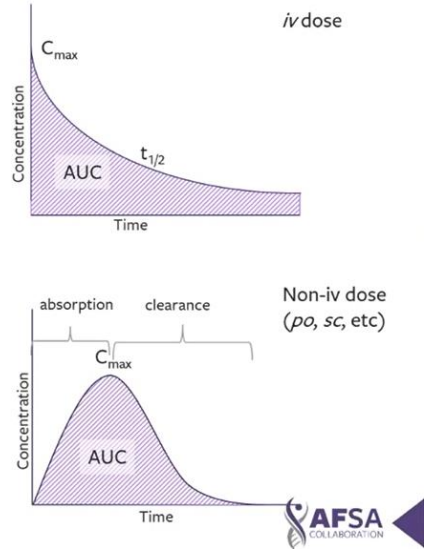
Absorption
Distribution
Metabolism
Elimination

- Oral absorption
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- Blood:plasma ratio



Kinetic profile of chemical

Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



Images from: AFSA training module "Dosimetry (Internal Exposure)",2022

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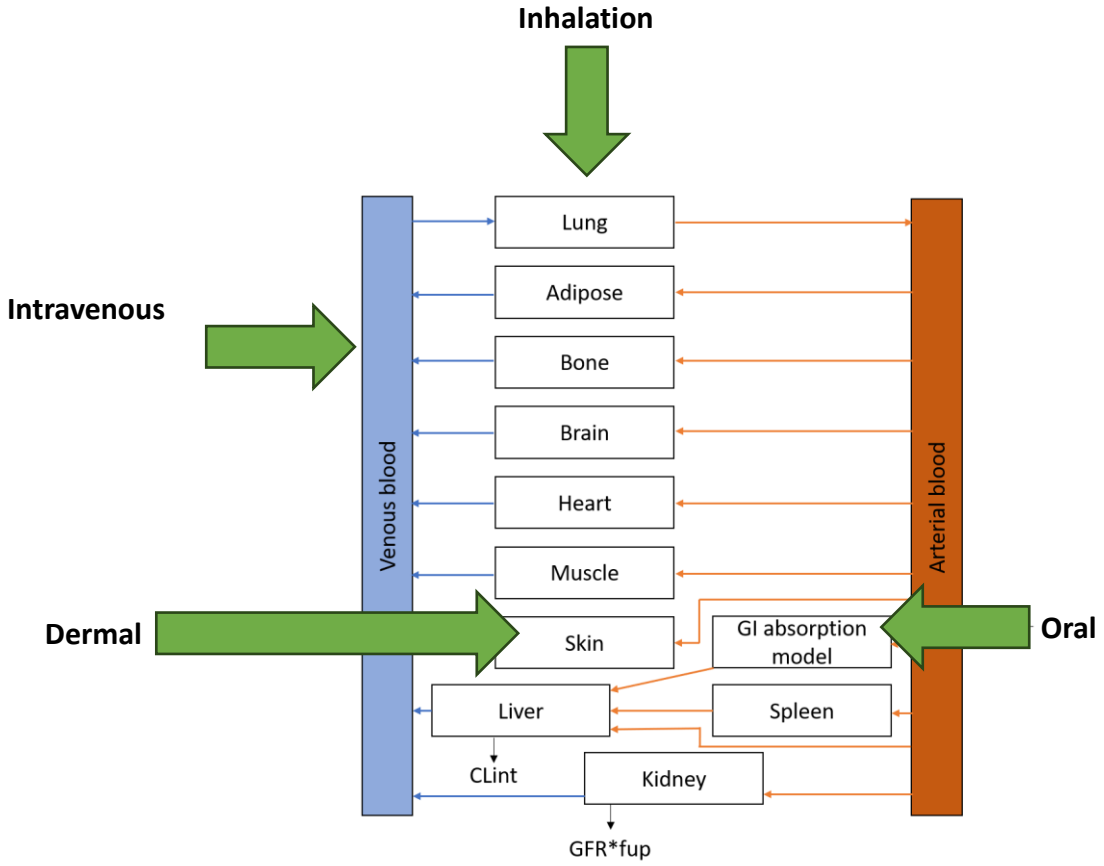
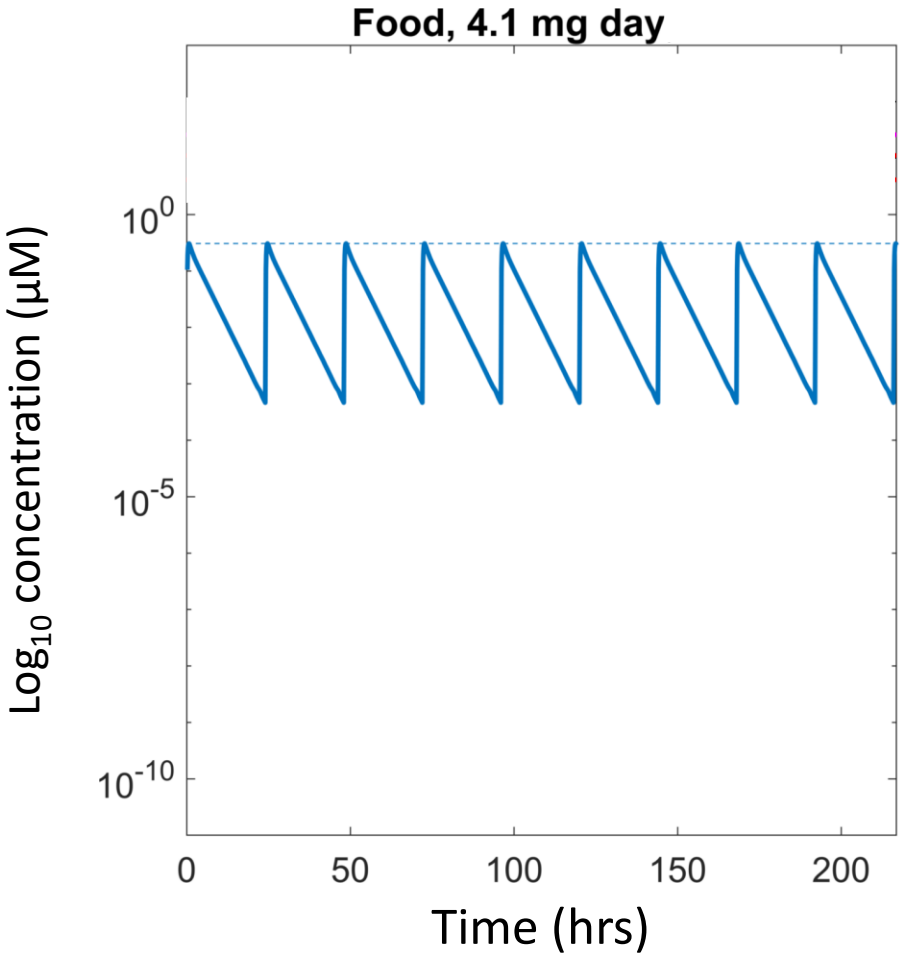
Exposure estimation: PBK modelling (基于药代动力学的建模)



- Different regions of the body (e.g. organs) are divided into separate compartments
- Connection between compartments reflects physiology
- Distribution of substances between compartments are governed by biophysical processes such as diffusion, perfusion, active transport etc
- Different exposure routes (dermal, oral, inhalation, intravenous) can be captured within the model.

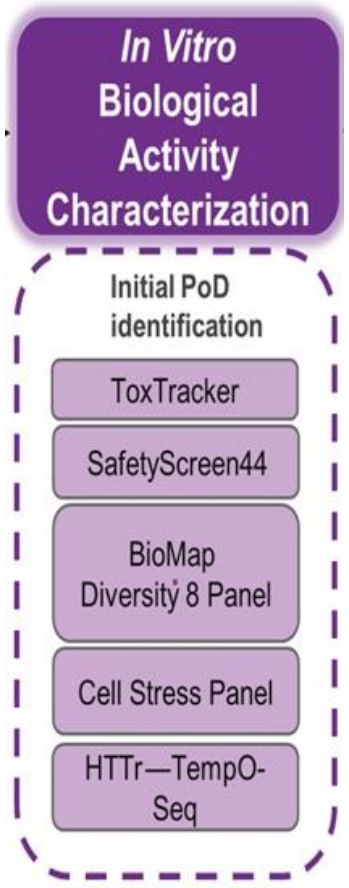
Physiologically based kinetic (PBK) models are used to simulate the behaviour of a chemical in the body for a given exposure scenario.

Exposure estimation: PBK modelling outcome (模型结果)

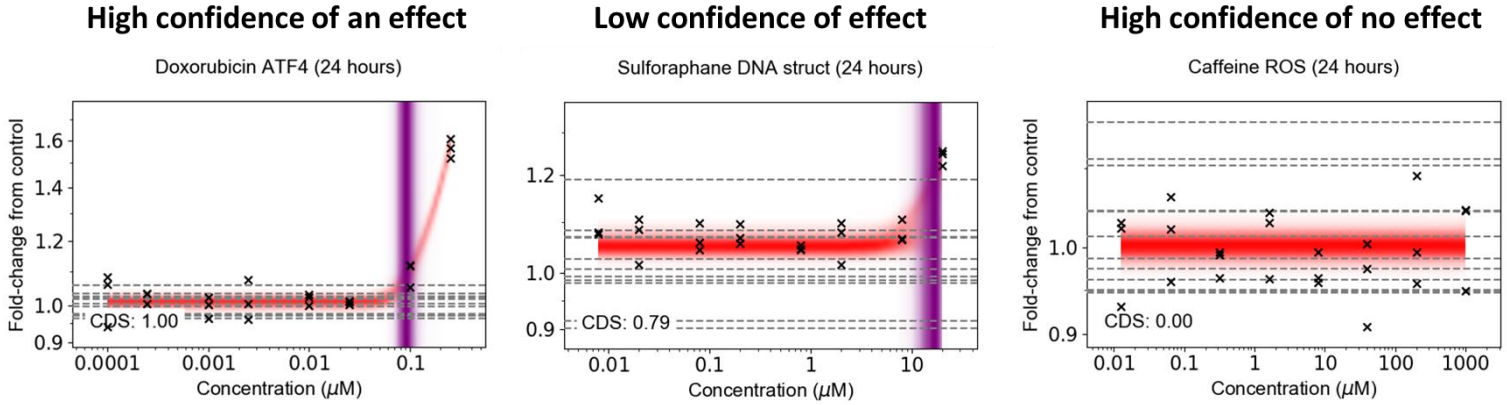


PBK-model predicted maximum concentration (C_{max})

In vitro bioactivity characterization: defining in vitro PODs

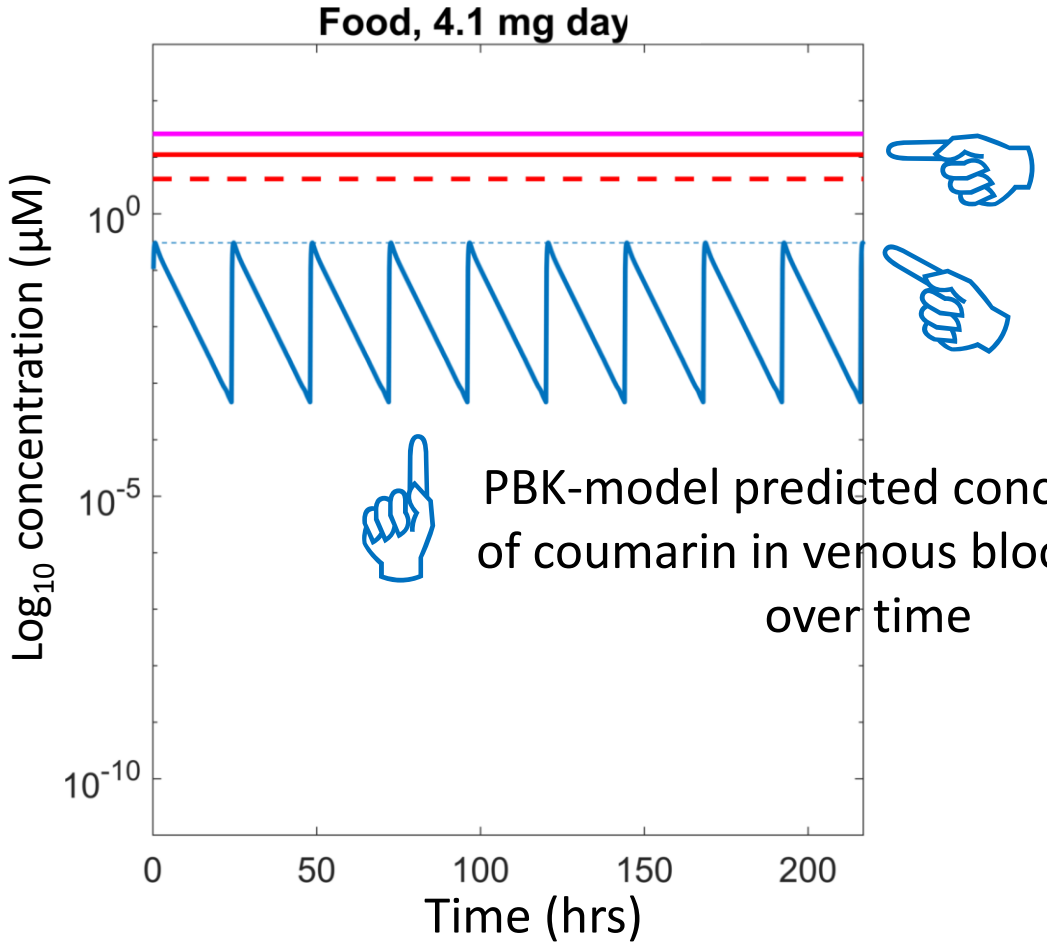


- POD definition: "The point on a dose–response curve established from experimental data used to derive a safe level" [source: EFSA]
- PODs can be estimated using mathematical models that fit data from concentration or dose response data.
- NGRA involves generating potentially 10,000s of concentration response data sets.
- In addition to the POD, an important metric when looking across multiple datasets is the confidence score on e.g., whether a response is truly a chemical dependent effect.



Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Example: exposure to coumarin through oral dietary intake (BER>1)



Points of Departure from in vitro cell assays, measured in µM

PBK-model predicted maximum concentration (C_{max})

PBK-model predicted concentration of coumarin in venous blood plasma over time

PODs:

- In vitro pharmacological profiling (MAO-A)
- High throughput transcriptomics (HepG2)
- High throughput transcriptomics (MCF-7)

Acceptable BER?

Conceptually, with the following assumptions a BER>1 indicates a low risk of adverse effects in consumers following use of the product:

a) The in vitro measures of bioactivity provide appropriate biological coverage

b) There is confidence that the test systems are at least as sensitive to perturbation as human cells in vivo

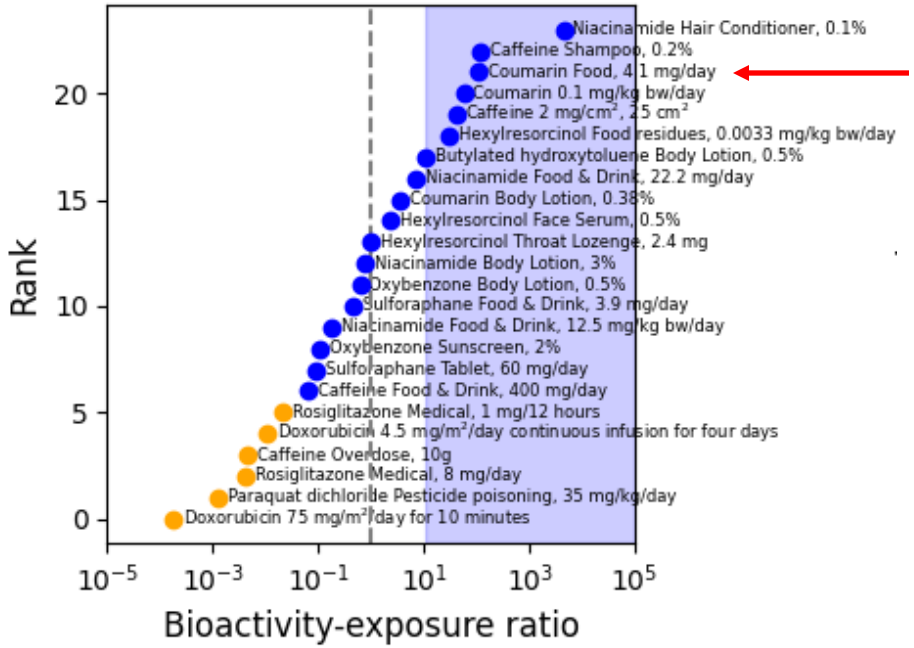
c) The exposure estimate is conservative for the exposed population

What about a larger subset of chemicals? (Part 1):

Selection of chemicals and exposure scenario

- Chemicals with well-defined human exposures
- Traditional safety assessment available

Chemical	Exposure scenario	Risk classification
Oxybenzone	**2 scenarios:** 0.5%; 2% sunscreen	Low risk
Caffeine	**2 scenarios:** 0.2% shampoo & coffee oral consumption 50 mg	Low risk
Caffeine	10g – fatal case reports	High risk
Coumarin	**3 scenarios:** 4 mg/d oral consumption; 1.6% body lotion (dermal); TDI 0.1 mg/kg oral	Low risk
Hexylresorcinol	**3 scenarios:** Food residues (3.3 ug/kg); 0.4% face cream; throat lozenge 2.4 mg	Low risk
BHT	Body lotion 0.5%	Low risk
Sulforaphane	**2 scenarios:** Tablet 60 mg/day; food 4.1-9.2 mg/day	Low risk
Niacinamide	**4 scenarios:** oral 12.5-22 mg/kg; dermal 3% body lotion and 0.1 % hair condition	Low risk
Doxorubicin	75 mg/m2 IV bolus 10 min; 21 days cycles; 8 cycles	High risk
Rosiglitazone	8 mg oral tablet	High risk
Paraquat	Accidental ingestion 35 mg/kg	High risk



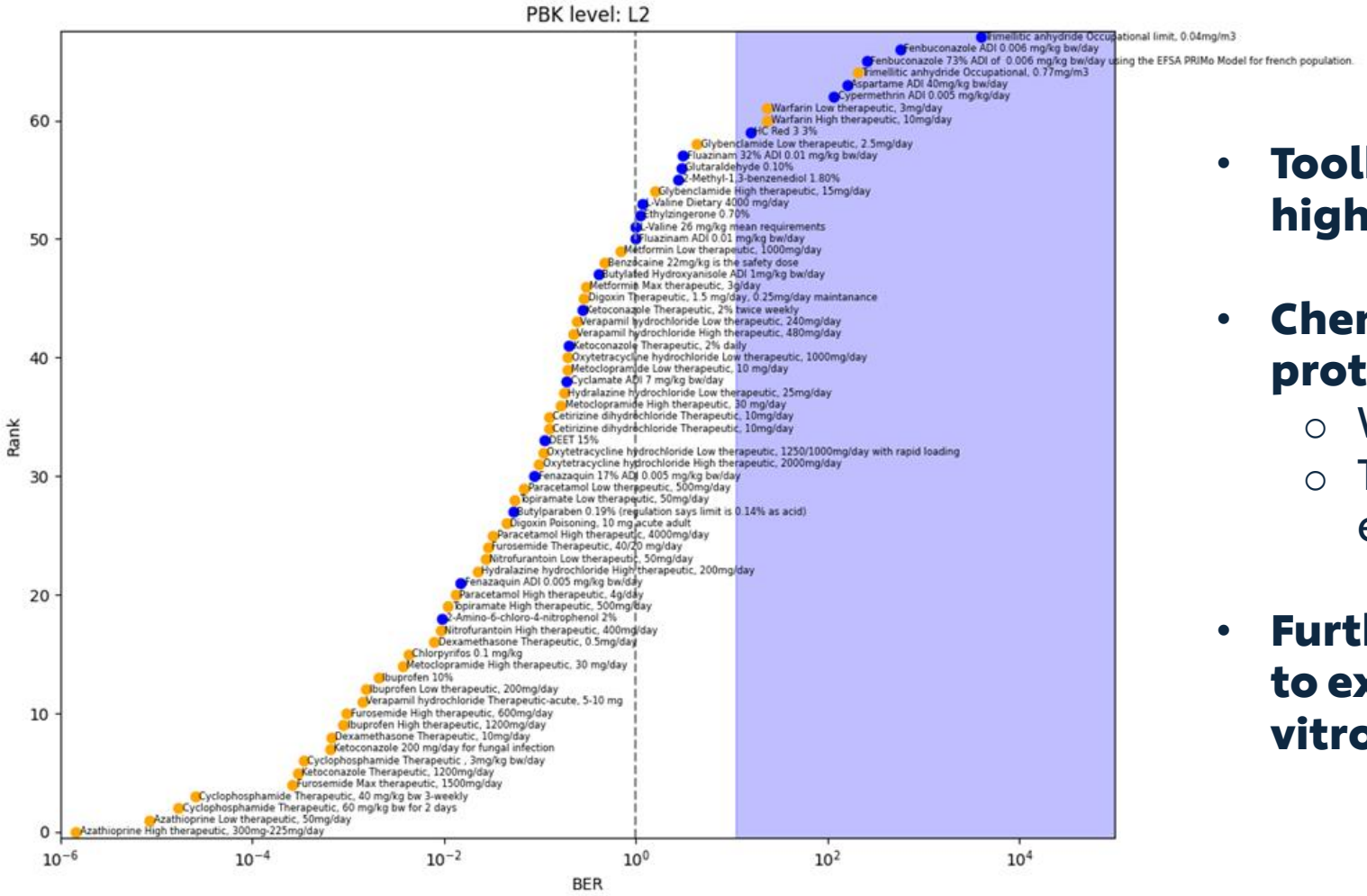
BER=lowest POD/Plasma Cmax
Blue: low risk chemical-exposure scenario
Yellow: high risk chemical-exposure scenario

Blue shaded region BER> 11

10 chemicals – 25 exposure scenarios

Middleton AM et al (2022). Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicological Sciences, 189:124-147.

NAM Systemic toolbox remains protective (>90%) when 38 additional chemicals and 70 exposure scenarios were tested (Part 2):



- **Toolbox not protective for 3/46 of the high-risk exposure scenarios**
- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure
- **Further research is being performed to explore additional relevant in vitro assays to be added the toolbox.**

Cable et al (2024) – in preparation

The NEW Gold Standard (新的金标准)

Was:

- Rodents
- Pathology
- High-dose apical endpoints
- No adverse effect level
- Uncertainty factors

Is Now:

- Human focused
- Broad-based NAMs
- Bespoke new NAMs
- Exposure led (PBK)
- **Bioactivity not pathology**
- **Protection not prediction**
- Underpinned by Computational modelling

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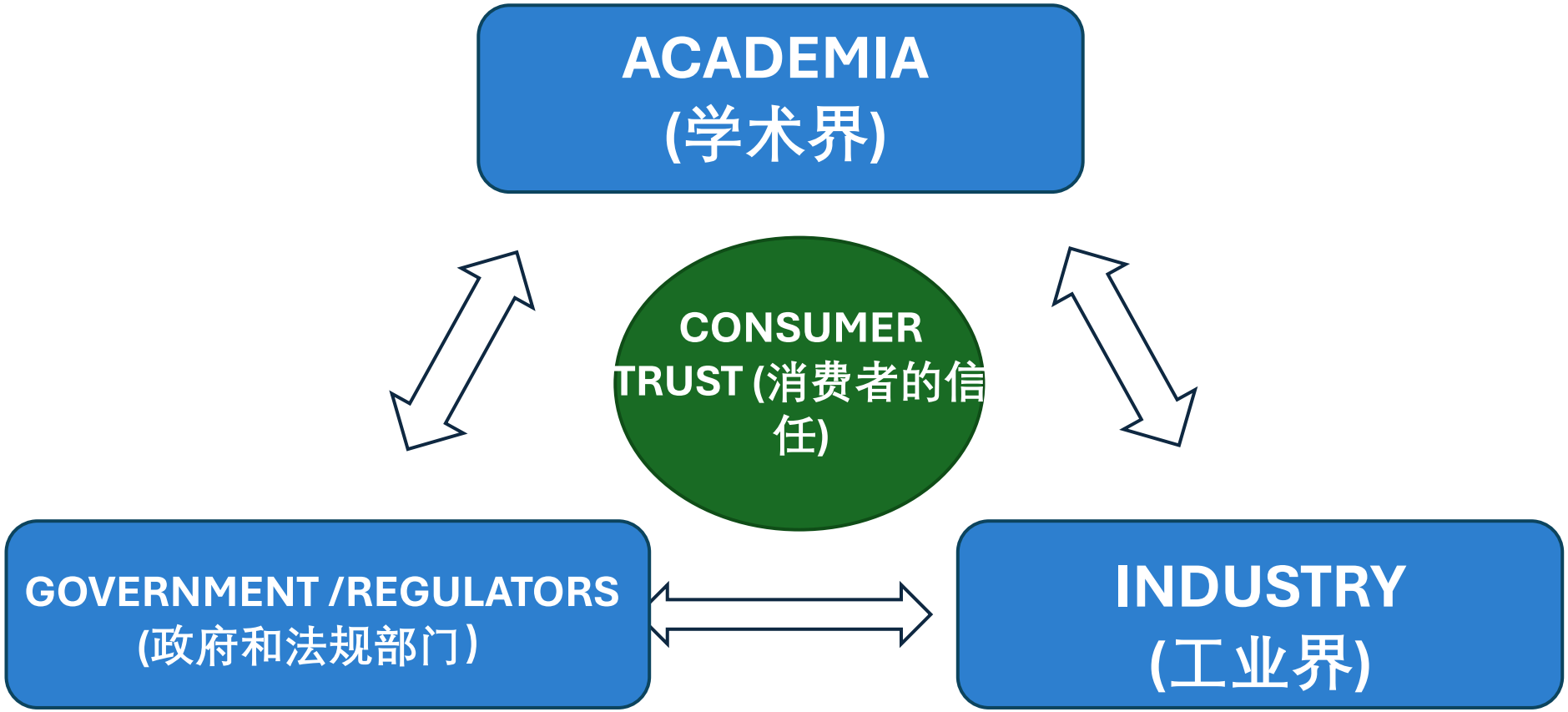
Conclusions

- Use of tiered, exposure-led approaches allows safety decisions to be made for systemic effects without animal test data
- An application of the approaches in food safety risk assessment is demonstrated here
- More work is needed for the development and acceptance of the NAMs in food safety risk assessment

Important to collaborate and form stakeholder partnerships



Collaboration on Product Safety in China

Unilever China CPSC Centre
联合利华中国消费者产品安全合作中心

中国消费者产品安全合作中心

中国毒理学会 Chinese Society Of Toxicology
NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research
EURL ECVAM
HUMAN TOXICOLOGY PROJECT CONSORTIUM
OECD
上海市食品药品监督管理局 SHANGHAI MUNICIPAL FOOD AND DRUG ADMINISTRATION
CFDA
FDA
CFSA China National Center for Food Safety Risk Assessment
EPA
SOT 2017
Simulations Plus, Inc.
ScitoVation
charles river
cyprotex
BioSpyder
中国医科大学 CHINA MEDICAL UNIVERSITY
BROWN
PEKING UNIVERSITY 1898
HB NIES
生态环境部 南京环境科学研究所 Nanjing Institute of Environmental Sciences,MEE
中国香料香精化妆品工业协会
cpaa The European Partnership
CCAI

Thank you

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