NAMs

Use of NAMs in Food Regulations

Louise Fortunato and Falko Partosch

RAMBOLL

Bright ideas.
Sustainable change.

Ramboll in brief

Independent architecture, engineering, and consultancy company

Creating sustainable solutions across energy, real estate, transport, water, waste, industry, finance, technology, healthcare and public sectors.

Founded 1945 in Denmark

Owned by Rambøll Fonden – The Ramboll Foundation providing long term stability

SBTi approved reduction targets consistent with the most ambitious goals of the Paris Agreement to keep warming below 1.5°C

>35

Countries covered by global office network

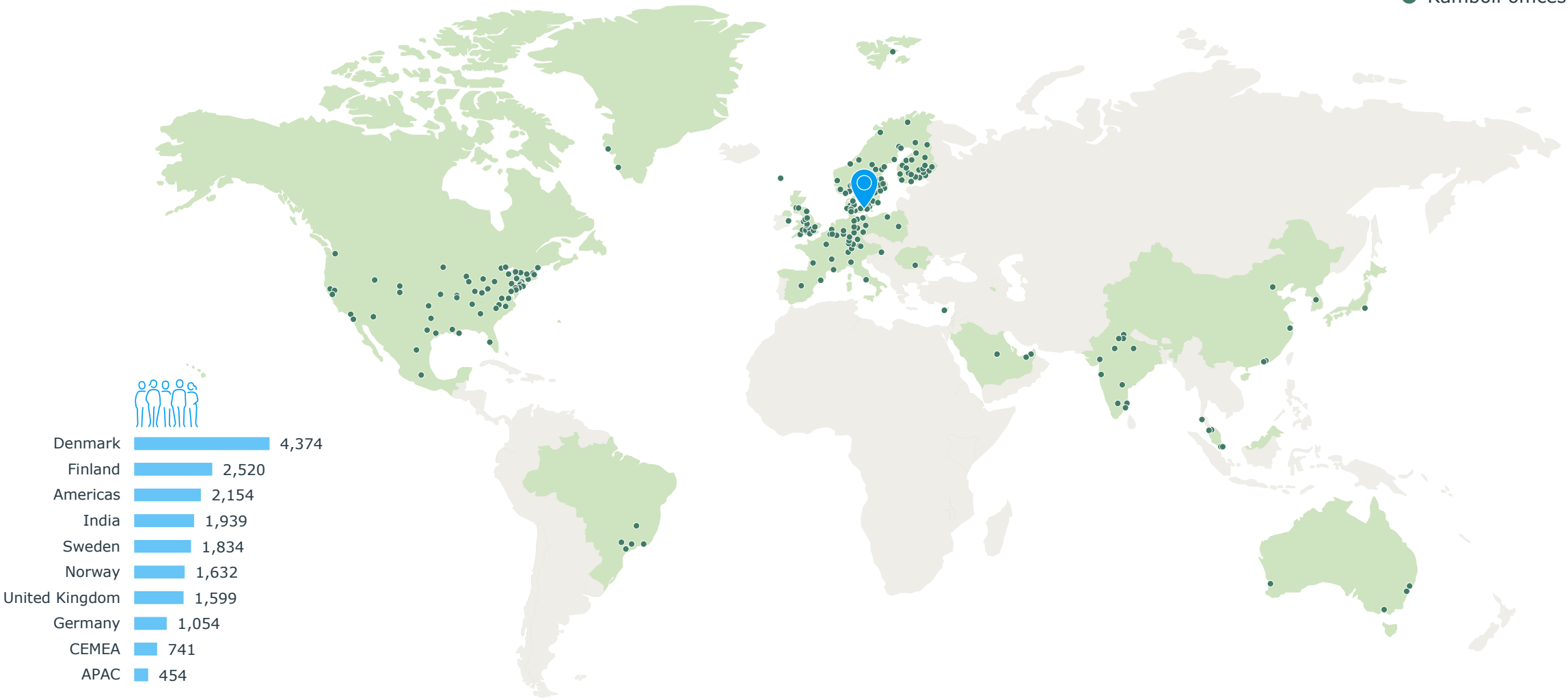
>18,000

Experts

2.3 Bn

Global revenue, in 2023 across all markets

Geographical footprint



Environment & Health

Pedigree

- Leading environmental & **health sciences consulting expertise**
- Solutions for the most challenging environmental, health & social issues
- Private & public clients worldwide
- Deep scientific expertise and technical acumen

Key figures

4,254

Revenue 2023
(mDKK)

3,000

FTEE 2023

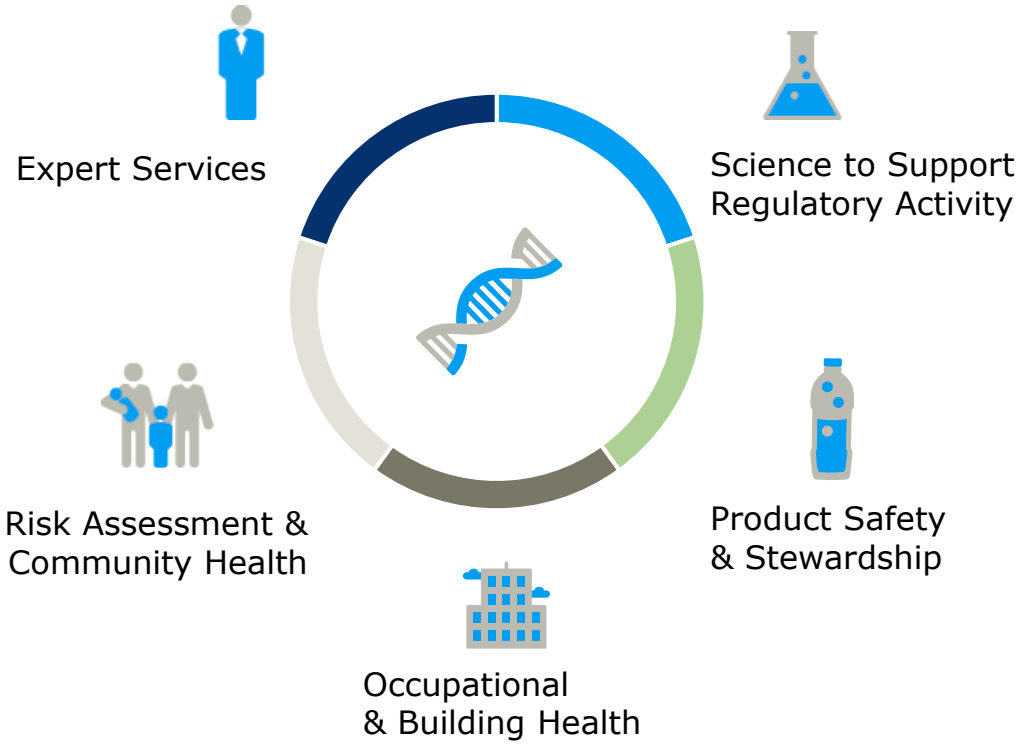
Fields of expertise

- Air & Climate
- Biodiversity & Ecosystems
- Circular Economy & Resource Management
- Contaminated Site & Facility Solutions
- Decarbonisation
- EHS Compliance Assurance & Performance
- Environmental Due Diligence
- ESG
- **Health Sciences**
- Impact Assessment

Applying science for a healthy society

Our 170 health sciences specialists work in seamless interdisciplinary global teams to create sustainable solutions for a healthy, flourishing society.

Our Health Sciences Services



Our experience – regulated food products

- Deep expertise in toxicology, including toxicokinetics, microbiology and chemistry;
- Leading insights to exposure assessment, including consumption, intake and uptake models for general and sensitive or vulnerable populations;
- Significant experience in conducting safety assessments and obtaining regulatory approvals for food and food ingredients around the world (including EU, US, Canada, and Australia);
- Substantial experience assessing safety of contaminants and impurities in foods;
- An established team based in the US and EU that work closely together to ensure submissions in multiple jurisdictions that are aligned, consistent and optimised for success; and
- Focus on delivering technically robust dossiers based on all available data and integrated strategies that minimise need for further testing.

40 years

expertise in the chemical characterisation of food ingredients

Safety assessments and obtaining regulatory approvals for food and food ingredients around the world

Support Beyond Food regulations

Other issues affecting food manufacturers

- emerging markets such as medical foods
- focus on endocrine disruption
- issues management such as chlorate and bromide
- contaminants
- related regulations such as pesticides, biocides and FCM
- policy development such as chlorate and bromide
- crisis management
- sustainability

Louise Fortunato

Senior Consultant

UK Health Sciences

London, UK

Professional Registrations

Associate Member of the UK Royal Society of Chemistry

Member of the UK Royal Society of Biology

Education

2014 – 2017
Bournemouth University
BSc (Hons) Forensic Science

2017 – 2018
Bournemouth University
MSc Forensic Toxicology (by Research)

Relevant experience

2016
Bournemouth University
Research assistant - Summer placement

2017
Bournemouth University
Research assistant - Summer placement

2018 – 2020
Delphic HSE Limited
Junior Toxicologist

2020 – 2021
Delphic HSE Limited
Toxicologist

2021 – present
Ramboll UK Limited
Senior Consultant

Dr Falko Partosch

Senior Consultant

DE Health Sciences

Essen, DE

Professional Registrations

Member of the German Society of Experimental and Clinical Pharmacology and Toxicology (DGPT)

Member of the German Society for Toxicology (GT)

Education

2004 – 2008
Bielefeld University
BSc Bioinformatics and Genome Research

2008 – 2010
Charité – Berlin University Medicine
MSc Toxicology

2011 – 2015
University of Potsdam
PhD Toxicology

Relevant experience

2010 – 2015
Charité – Berlin University Medicine
Research assistant

2015 – 2022
Scientific Adviser of a RAC Member
ECHA

2016 – 2020
University Medical Center Göttingen
Research assistant - German MAK Commission

2020 – 2022
Fraunhofer ITEM Hannover
Research assistant

2022 – present
Ramboll
Senior Consultant

Agenda

01 What is NAMs?

02 Regulatory use of NAMs (non-food)

03 Use of NAMs in US Food Regulations

04 Use of NAMs in UK Food Regulations

05 Use of NAMs in EU Food Regulations

06 Case study

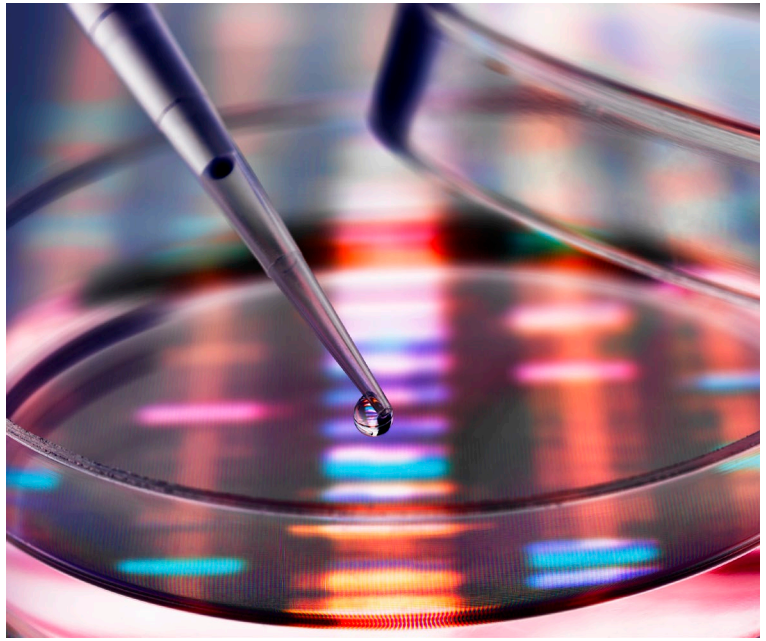
01 What is NAMs?

What are NAMs?

- New Approach Methodologies (NAMs) include any *in vitro, ex vivo, in chemico, omics* or computational (*in silico*) methods.

- NAMs are not limited to the above methodologies, NAMs include any methodology that intends to replace, refine, or reduce animal testing (3Rs).
  - This can include animal studies which have been refined to use less animals, for example.

- In other regulations, NAMs may be strictly Non-Animal Methodologies, such as the EU and UK Cosmetics Regulation, where all animal testing is banned.



UK FSA (2024). New Approach Methodologies (NAMs) to Support Regulatory Decisions for Chemical Safety.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

Product Safety and Metrology etc. (Amendment) (EU Exit) Regulations 2021.

Nothing new about NAMs…

The emergence of molecular biology, computational modelling, and high-throughput screening in the late 20th and early 21st centuries laid the foundation for NAMs.

Researchers began leveraging read-across from structurally similar substances, *in vitro* (cell-based) assays, *ex vivo* assays (e.g., precision cut tissue slice system) organ-on-a-chip technologies, and in silico (computer-based) models to predict human responses more accurately without relying on animal testing.

The advent of omics technologies, such as genomics, proteomics, and metabolomics, has further refined NAMs by allowing deeper insights into biological mechanisms.

Regulatory bodies have also played a key role in the evolution of NAMs. Agencies such as the U.S. Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) have increasingly embraced NAMs in risk assessment frameworks. Collaborative initiatives, like the Tox21 program and the EU's REACH regulation, have promoted the development and validation of alternative testing methods.

Definition of NAMs by Regulators

- ECHA

"NAMs denote alternatives to traditional toxicity methods that **typically involve animal testing** NAMs WS Report (europa.eu)

- EFSA

„**any non-animal-based** approach that can be used to provide toxicological information in the context of hazard assessments." Theme (Concept) Paper - New Approach Methodologies - - 2022 - EFSA Supporting Publications - Wiley Online Library

"**Alternatives to animal testing**, collectively termed NAMs" The use of NAMs and omics data in risk assessment | EFSA (europa.eu)

- EPA

"NAMs are defined as any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment to **avoid the use of animal testing**" New Approach Methods Work Plan (epa.gov)

- FDA

„…**reduce the number** of animals used in testing; refine the **methods still requiring animals** so they are less stressful to the animals; and to replace animal testing whenever possible." New Approach Methods (NAMs) | FDA

- OECD

„According to some definitions of NAMs, **animal tests are also included**, if they serve to reduce or refine another animal test Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA) (oecd.org)

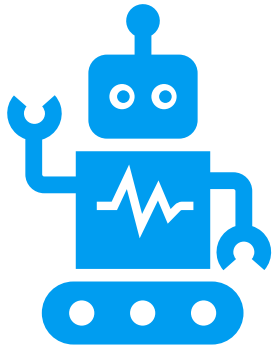
UK Definitions

- HSE
  - NAMs include in chemico, in vitro and in silico (computational) approaches, amongst others
  - In some cases, they can be used as **stand-alone replacements for animal tests**.

- FSA
  - include the **best available science** and are
  - consistent with the reduction, refinement, and replacement (3Rs) of animal testing approaches

- Definition of NAMs according to the NC3Rs:
  - New approach methodologies are replacement technologies (full and partial) for use in assessing chemical or drug toxicity.
  - Includes *in vitro, ex vivo, in silico* **and *in vivo* test methods**.

NAMs Today



Innovations in artificial intelligence, machine learning, and advanced 3D tissue models, paving the way for more predictive, human-relevant, and ethical safety assessments.

As regulatory acceptance grows and scientific capabilities expand, NAMs are set to become the standard for assessing chemicals, drugs, and environmental hazards in a more sustainable and humane manner.

02 Regulatory use of NAMs (non-food)

Use of NAMs: Cosmetic Regulations

- **Cosmetic Products Regulation (EC) No 1223/2009**
- Article 18 **bans animal testing** for finished cosmetic products and ingredients, but it also encourages the use of alternative testing methods
- Significant driver of NAMs in the cosmetics industry
- The SCCS includes various techniques in NAMs for:
  - In silico models, read across, in vitro assays, other mechanistic techniques such as 'omics'

"No validated alternative method is available yet for determining the repeated dose toxicity of a substance, which poses a problem for new compounds…"

SCCS (2018). The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 10th Revision

Use of NAMs: REACH

- **Annex XI of REACH Regulation (EC) No 1907/2006** provides provisions for the **use of alternative methods**, including *in vitro* and computational methods, to gather safety data for chemicals: "promotion of alternative test methods on an international and national level including computer supported methodologies, *in vitro* methodologies, such as appropriate, those based on toxicogenomics, and other relevant methodologies"

- **CLP Regulation (EC) No 1272/2008**

  "Implementation of Regulation (EC) No 1272/2008 should be based on the promotion and use of New Approach Methodologies (NAM), suitable for the assessment of health and environmental classification of chemicals, **wherever possible**."

Use of NAMs: Pesticides

Pesticides Regulation (EC) No 1107/2009, Article 8: flexibility for use of alternative methods for the evaluation of pesticide active substances ("...*for each test or study involving vertebrate animals, a justification of the steps taken to avoid animal testing and duplication of tests and studies on vertebrate animals"*)

EFSA: support of NAMs through their guidance on pesticide risk assessments, including a focus on validating and integrating alternative methods.

03 Use of NAMs in US Food Regulations

Use of NAMs in US Food Regulations: Current state

- **Animal Testing Requirements**:
  - Existing FDA and global guidelines often mandate **animal testing for market approval**, but use of NAMs is encouraged
  - approaches that **reduce the number of animals** used accepted by FDA

- **Role of NAMs**:
  - NAMs are permitted and encouraged as **complementary or alternative approaches**.
  - Contribution to the "**weight of evidence**" in regulatory decisions.

"Though it is not yet possible for NAMs to cover the full array of all human target organ systems, they can still be deployed in certain regulatory decision frameworks for both the pre-and-post-market product assessments"

Potential Approaches to Drive Future Integration of New Alternative Methods for Regulatory Decision-Making

Use of NAMs in US Food Regulations: NAM Tools

Current and Emerging NAMs Tools at FDA

- **Systems Biology**: Evaluating multi-cellular and tissue responses for a holistic view of organismal impact.
- **Engineered Tissues**: Using scaffolded cell structures to simulate biologically active tissues.
- **Artificial Intelligence (AI)**: Leveraging in silico methods for predictions and modeling.
- **Alternative Organisms**: Zebrafish and *C. elegans* as alternative model organisms.
- **Microphysiological Systems (MPS)**: Organs-on-chip technologies to replicate human organ systems.

- Collaboration with international agencies and sectors like the U.S. EPA.

- In the US, regulators such as the EPA and FDA both fund and perform a significant proportion of the research into NAMs meaning that they can be expected to have a deep understanding of the potential and shortcomings in the research.

Potential Approaches to Drive Future Integration of New Alternative Methods for Regulatory Decision-Making

Use of NAMs in US Food Regulations: Limitations

Limitations of NAMs

No assays fully capture the critical hazard endpoints for assessing all currently existing human or animal organ systems

No elimination of the use of integrated physiological systems such as in animal and human trials

Currently best used **in conjunction with traditional methods**, not as stand-alone solutions for hazard identification / risk assessment

03 Use of NAMs in UK Food Regulations

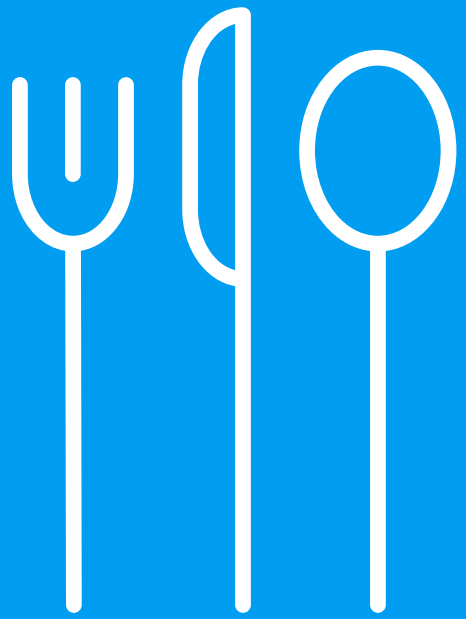
Use of NAMs in UK Food Regulations: NC3Rs

- National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs)
- UK Government funded research centre with a primary mission is to accelerate the replacement, reduction and refinement of the use of animals in research.
- Focuses on the 3Rs

https://nc3rs.org.uk/

Use of NAMs in UK Food Regulations

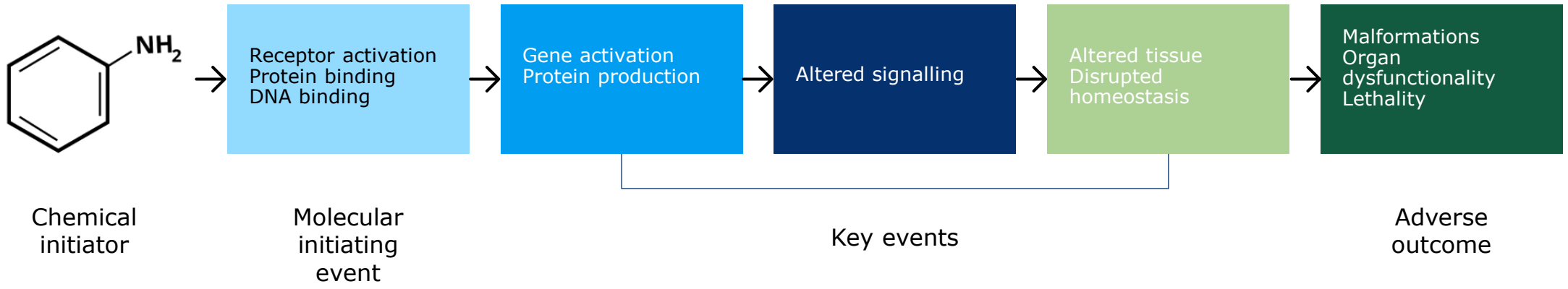
- UK FSA paper on the use of NAMs to support regulatory decisions in chemical safety
  - Scientific community opinion and literature-based evidence on the use of NAMs.
- Animal test methods are still regarded as the “gold standard”
- Observational (animal studies) vs predictive (NAMs) approach.
- Multiple NAMs would be required to address a given endpoint.
- Validated against human data rather than animal data.



UK FSA (2024). New Approach Methodologies (NAMs) to Support Regulatory Decisions for Chemical Safety. FSA Research and Evidence. https://doi.org/10.46756/001c.122591.

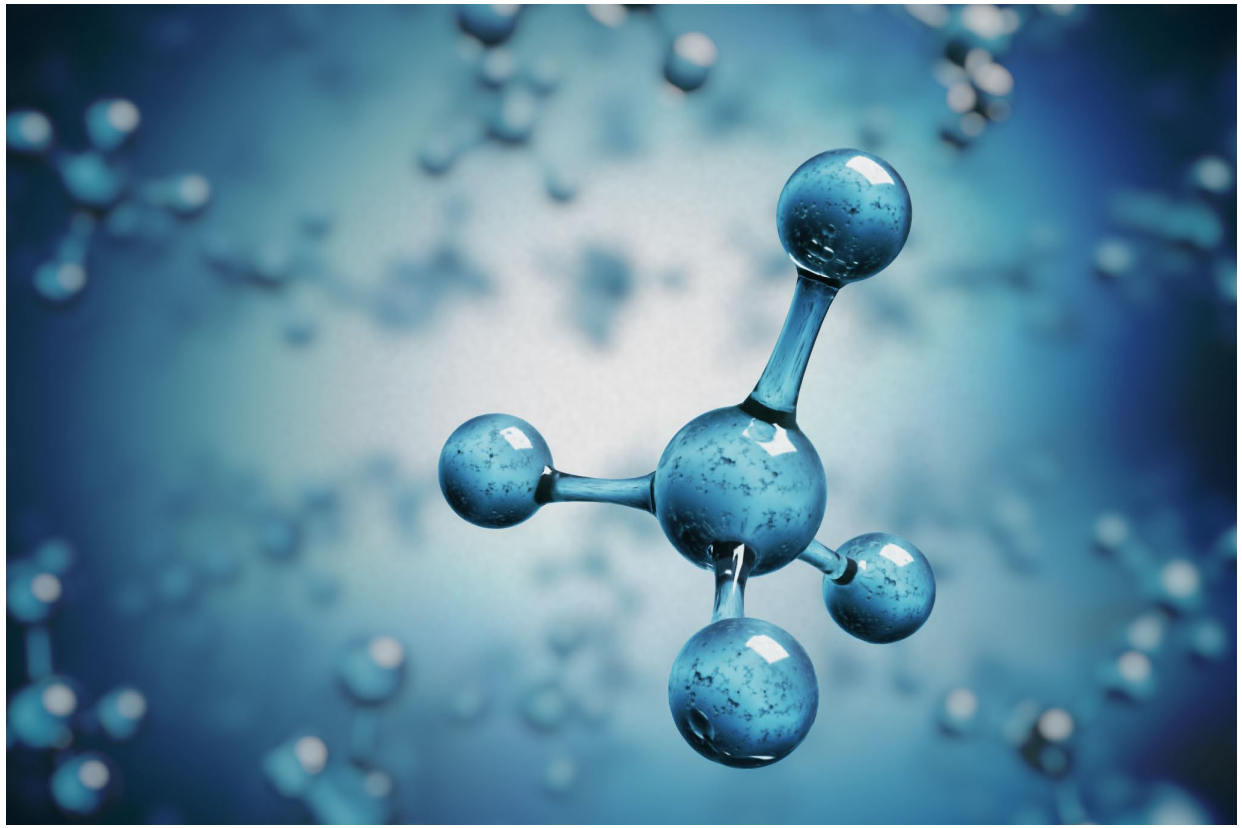
Use of NAMs in UK Food Regulations: Approaches to developing NAMs

- Most NAMs use a battery of tests based on the known Adverse Outcome Pathway (AOP) for a given adverse effect.
  - Substance agnostic
  - AOP approach is a simplistic view of the complex biology
  - Potential for incomplete AOPs or missing biological effects.
  - Still lacking an understanding at an organ level.



Use of NAMs in UK Food Regulations: Approaches to developing NAMs

- NAMs based on Mode of Action (MoA) approach:
  - Comprehensive molecular description of every biological event in the pathway from an initial interaction with a specific chemical
  - e.g., IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (Boobis et al., 2006).

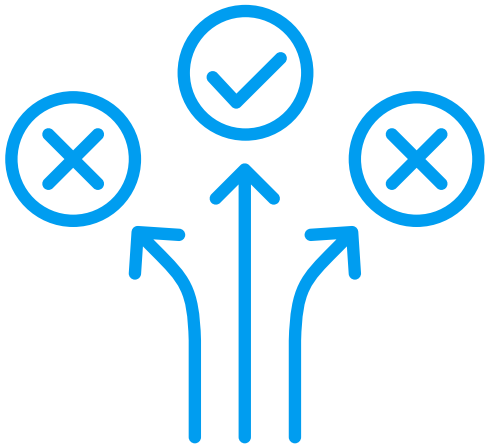


Use of NAMs in UK Food Regulations: challenges for the uptake of NAMs

- Rigorously validated models for systemic toxicity still do not exist.
- Need for determining health-based guidance values.
- No legally binding definition exists yet
- CROs do not usually offer NAMs, unless already validated
- The cost of a battery of NAM studies is usually higher than the singe animal study.
- The UK FSA recommends the use of NAMs to justify **Read-Across or Weight-of-Evidence** to reduce the amount of animal data produced.

Use of NAMs in UK Food Regulations: Roadmap

- Moving from a 3R approach to a 6R approach: includes reproducibility, relevance, and regulatory acceptance
- Clearly define the goal of an assessment and thus a NAM
- The uncertainty of NAM method should be defined
  - A clear link between *in vitro* and *in vivo* dosimetry must be established (e.g. PBK modelling, IVIVE dose extrapolation)
- Challenge the current validation procedures



Use of NAMs in UK Food Regulations: COC opinion on alternatives to the 2-year Bioassay

The 2-year rodent assays (usually in 2 species) was the gold standard for carcinogenicity assessment:

- High animal use, high costs and low relevant to human risk assessment (e.g. different MoAs)

Emerging alternatives:

- Short-Term In Vivo Assays
- In Vitro Cell Transformation Assays
- Omics Technologies and High-Throughput Screening
- Integrated Approaches to Testing and Assessment (IATA)

COC (2019). Alternatives to the 2-year bioassay. Available from: https://assets.publishing.service.gov.uk/media/5ce3bdf8ed915d247d0ba73e/G07_Alternatives_to_the_2-year_Bioassay_v1.1.pdf

Jacobs et al (2020). Chemical carcinogen safety testing: OECD expert group international consensus on the development of an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens. https://link.springer.com/article/10.1007/s00204-020-02784-5

05 Use of NAMs in EU Food Regulations

Use of NAMs in EU Food Regulations: New Guidances

- No EU regulatory definition of NAMs (no legally binding definition)
- The use of NAMs is allowed in certain circumstances in EU Food related Guidances
- Regulators encourage the submission of NAMs once validated by an official body (e.g. OECD, JRC)
  - If not validated NAMs, can be submitted together with standard animal data requirements
- Both new Draft EFSA Guidances released on Novel Foods and Food Additives submission include a tiered approach to testing in order to minimise the use of animals.

EFSA (2024). Draft guidance on the scientific requirements for an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283.

EFSA (2024). Draft guidance on the preparation of an application for authorisation of a food additive submitted under Regulation (EC) No 1331/2008.

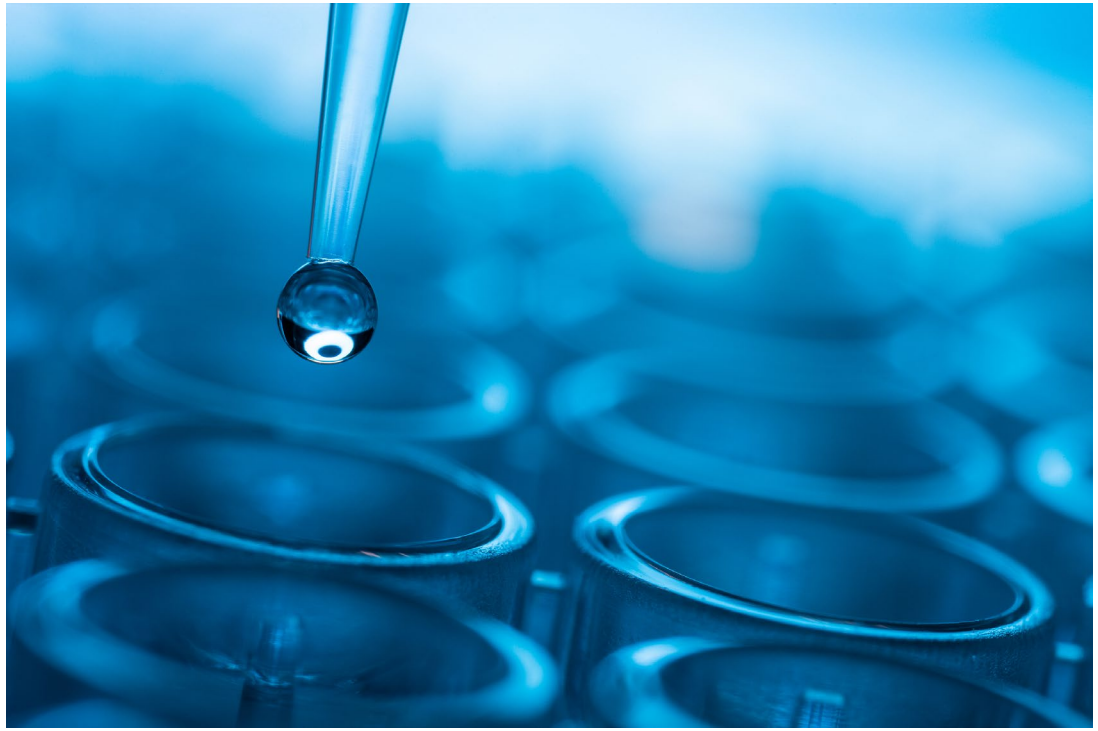
Use of NAMs in EU Food Regulations: Tiered approach

- **New Food Additive Guidance**:
  - Literature research
  - *Tier 0:* in vitro genotoxicity studies
  - *Tier I:*
    - ***in vitro* TK / ADME**;
  - *Tier II:*
    - ***in vitro* comparative ADME studies**

- **New Novel Food Guidance**:
  - Literature research
  - *Tier I:*
    - ***in vitro* genotoxicity (OECD TG 471 and OECD TG 487)**
    - ***in vitro* TK / ADME** including comparative studies

Use of NAMs in EU Food Regulations: NAMs in ADME

- Use of NAMs recommended in both the Novel Foods and Food Additive Guidances.
- Not validated but recommended by EFSA:
  - Absorption from buccal cavity
  - Absorption gastric/intestinal: Caco-2 enterocyte models, Madin-Darby canine kidney (MDCK) cells.
  - Stability in gastric and intestinal fluid, and stability in hepatocytes.
  - Gut microbiota studies:
    - E.g. M-ARCOL, SHIME, Triple coculture
    - Hard to identify labs that offer such tests
- Impact of the Novel food or Food additive on the microbiome itself.
- PBPK modelling



Use of NAMs in EU Food Regulations: Systemic toxicity

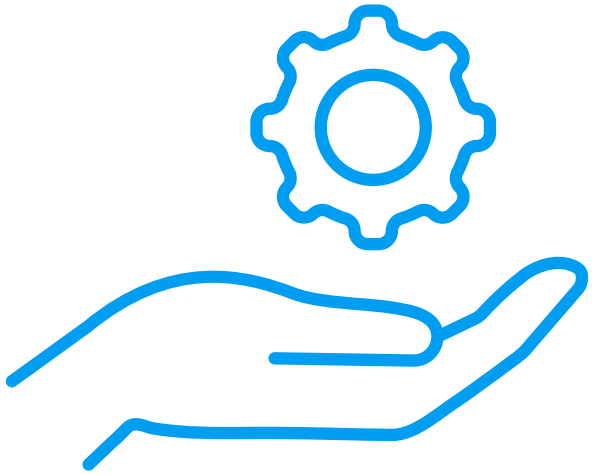
- NAMs only approach is not accepted for the assessment of systemic toxicity

- A thorough literature research is encouraged

- The most efficient way to reduce animal testing is through the use of read-across.
  - NAMs support the use of read-across – e.g. mechanistic or ADME studies to compare the read-across to target.
  - E.g. is mode of action the same?
  - E.g. do the source substances have same or similar metabolites to the target substance.

EFSA (2024). Read-across applications for food and feed ingredients. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2024.EN-8811

Use of NAMs in EU Food Regulations: Read-across (RAX)



- New EFSA Scientific Report on RAX in food and feed
  - Reduce the reliance on new studies
  - Highlight importance of robust data and careful selection of analogues.

- How NAMs can support read-across:
- In silico software tools (e.g. QSAR toolbox)
  - Can only predict phase I metabolites
  - Does not provide quantitative data
- Mode of Action specific assays
- Targeted testing for shared toxicodynamic and kinetic properties

[4] EFSA (2024). Read-across applications for food and feed ingredients. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2024.EN-8811

Use of NAMs in EU Food Regulations: NAMs in allergenicity testing

How to perform an assessment:

- Characterise novel food / food additive:
  - Was it produced with known food allergens?
- Protein not related to known food allergens:
  - Literature research
  - A weight-of-evidence approach
  - Protein identification and characterisation is key.
- Single protein and simple protein mixtures:
  - Bioinformatics
  - Human serum specific IgE binding assay and/ or other immunoassays.
- Complex protein mixtures (in addition to above):
  - In vitro digestibility
  - In vitro / in vivo analysis to identify immunogenicity and sensitising capacity.

06 Case study

Case study for EU Novel Food Additive (FA) application (according to new guidance)

In vitro genotoxicity tests	OECD TG 471 (Ames test) OECD TG 487 (*in vitro* mammalian cell micronucleus test)
In silico and *in vitro* TK studies to determine whether the compound is absorbed systemically.	PBPK modelling Caco-2 cell absorption Absorption from buccal cavity using *ex vivo* buccal models
In vitro stability studies to understand the metabolism of the substance and equivalence to humans.	SGF SIF Hepatocytes (using both rat and human)
Effect of the food additive on the microbiome using *in vitro* studies	In vitro gut microflora studies

Main takeaways

NAMs

- Becoming increasingly important
- Are already being used in various areas
- To be further implemented in the future

Currently mostly used in conjunction with traditional methods, as regulatory acceptance is lacking

The biggest barriers to NAM integration

- Validation
- Lack of confidence and trust
- Preference to working with established approaches
- Education and training of regulators

Main takeaways

A significant amount of research is ongoing which gives a positive outlook for the future implementation of NAMs in regulation.

Some NAMs are already encouraged for use in the food safety space and industry should be making use wherever possible.

Further guidance from regulators on the preferred next steps for the development of NAMs would encourage regulatory acceptance.

- Guidance on reporting of data.
- Guidance on validation of new methodologies.

Bright ideas. Sustainable change.

RAMBØLL

Louise Fortunato
(Senior Consultant)
Louise.fortunato@ramboll.co.uk

Dr Falko Partosch
(Senior Consultant)
Falko.Partosch@ramboll.com