

Symposium on NAMs @ FSC
10:00 ~ 13:00 5th December 2024

International Activities Related To NAMs

NAMs: New approach methodologies / New approach methods

Chemicals Evaluation and Research Institute, Japan (CERI)

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NAMsに関するシンポジウム @ 食品安全委員会
2024年12月5日 10:00～13:00

NAMsに関する国際動向

NAMs: New approach methodologies / New approach methods

一般財団法人化学物質評価研究機構（CERI）

赤堀有美

Outline

[1] Introduction

- **Background of NAMs**
- **Definitions of NAMs**
- **Example technologies as NAMs**

[2] International activities to implement NAMs

[3] Example applications of NAMs from known case studies

Outline

[1] はじめに

- **NAMsの背景**
- **NAMsの定義**
- **NAMsに含まれる技術の例**

[2] NAMs導入に向けた国際動向

[3] 既存のケーススタディによるNAMsの適用事例

Background of NAMs

2007	NRC: Published "Toxicity testing in the 21st century: a vision and a strategy" US-EPA: Launched ToxCast Program
2008	US-EPA, NIH/NTP&NCGC: Initiated Joint Program Tox21 (US-FDA joined in 2010)
2010	US-EPA: Launched Next Generation Risk Assessment (NexGen) program EU: "Directive 2010/63/EU on the protection of animals used for scientific purposes"
2013	EU: Ban on the use of animal testing for cosmetics
2014	US-EPA: Published "Next Generation Risk Assessment: Incorporation Of Recent Advances In Molecular, Computational, And Systems Biology (Final Report)"
2016	ECHA: Held "Topical Scientific Workshop - New Approach Methodologies in Regulatory Science"
2017	US-FDA: Published "FDA Predictive Toxicology Roadmap"
2018	US-EPA: Published " Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program" ICCVAM: Published " A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" OECD: Published "Guidance Document on Good In Vitro Method Practices (GIVIMP) "
2020	US-EPA: Launched the plan for TSCA to phase-out the use of animal testing in the future
2021	EFSA: Published " EFSA strategy 2027 science safe food sustainability" US-EPA: EPA New Approach Methods Work Plan
2022	EFSA (external scientific report): Published "Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment"
2023	ECHA: Held "New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals".
2024	ICCVAM: Published "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies"

The US and Europe have legal background for the use of NAMs.

NAMsの背景

2007	NRC: "Toxicity testing in the 21st century: a vision and a strategy" US-EPA: ToxCast Programを開始
2008	US-EPA, NIH/NTP&NCGC: Joint Program Tox21を開始 (US-FDAは2010年に参加)
2010	US-EPA: Next Generation Risk Assessment (NexGen) programを開始 EU: "Directive 2010/63/EU on the protection of animals used for scientific purposes"
2013	EU: 化粧品における動物試験使用の禁止
2014	US-EPA "Next Generation Risk Assessment: Incorporation Of Recent Advances In Molecular, Computational, And Systems Biology (Final Report)"の公開
2016	ECHA: "Topical Scientific Workshop - New Approach Methodologies in Regulatory Science"の開催
2017	US-FDA: "FDA Predictive Toxicology Roadmap"の公開
2018	US-EPA: "Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program" の公開 ICCVAM: "A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States"の公開 OECD: "Guidance Document on Good In Vitro Method Practices (GIVIMP) "の公開
2020	US-EPA: Launched the plan for TSCA to phase-out the use of animal testing in the future将来的にTSCAにおいて動物試験の利用を無くす計画を発表
2021	EFSA: "EFSA strategy 2027 science safe food sustainability"を公開 US-EPA: "EPA New Approach Methods Work Plan" を公開
2022	EFSA (external scientific report): "Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment"を公開
2023	ECHA: "New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals"を開催
2024	ICCVAM: "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies"を公開

米国や欧州ではNAMs利用に関する法的背景がある

Definitions of NAMs

... “there is no formal and legally agreed definition of a NAM” (ECHA, 2023).

ECHA (2023) Report on the European Chemicals Agency’s “New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals”

<p>ECHA 2016, Topical Scientific Workshop</p>	<p>NAM as an overarching term for all methods including toxicological methods that serve as (replacement, reduction or refinement) alternatives to animal testing, as well other sources of information such as exposure data. Thus, NAMs may include <i>in silico</i> approaches, in chemico and <i>in vitro</i> assays.</p>
<p>US EPA 2018, TSCA Strategic Plan</p>	<p>NAM has been adopted as a broadly descriptive reference to any technology, methodology, approach (including computational/<i>in silico</i> models (i.e., QSARs)), or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals. In the context of TSCA, NAM encompasses any alternative test methods and strategies to reduce, refine or replace vertebrate animal testing.</p>
<p>ICCVAM 2018, Strategic roadmap</p>	<p>NAM has been adopted as a broadly descriptive reference to any alternative test method or methodology that can be used to provide information on chemical hazard and risk assessment. These new approaches include IATAs, defined approaches for data interpretation, and performance-based evaluation of test methods. In this context, alternative test methods include non-animal test systems and phylogenetically lower species, methods that reduce the number of animals required for a specific test or refine animal use to lessen or avoid pain and distress.</p>
<p>US EPA 2021, New approach methods workplan</p>	<p>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.</p>
<p>EFSA 2022, Development of a Roadmap for Action on NAMs in Risk Assessment</p>	<ul style="list-style-type: none"> ● the term new approach methodologies (NAM) refers to any new approach which can be used to provide toxicological information in the context of human hazard and risk assessments. These new approaches include integrated approaches to testing and assessment (IATAs), defined approaches for data interpretation, and performance-based evaluation of test methods. ● From glossary: New approach methodologies are any non-animal-based approaches (<i>in silico</i> or <i>in vitro</i>) to predict components of hazard or risk.
<p>ECHA 2023, New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals</p>	<p>NAMs denote alternatives to traditional toxicity methods that typically involve animal testing. These alternatives are useful for predicting and assessing chemical risks and hazards, by providing mechanistic information for biologically complex endpoints. They include, e.g. <i>in vitro</i>, in chemico methods and <i>in silico</i> computational models, which may be used alone or in combination with other methods and have the potential to be quicker, cheaper and use less animals.</p>
<p>ICCVAM 2024, Validation, Qualification, and Regulatory Acceptance of NAMs</p>	<p>A broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment and that supports replacement, reduction, or refinement of animal use (3Rs).</p>

IATA: Integrated Approach to Testing and Assessment. 3R: Replacement, Reduction, Refinement. **Blue:** Information provided by NAMs. **Purple:** 3R or avoiding animal testing.

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NAMsの定義

公式に合意されたNAMの定義はない (ECHA, 2023).

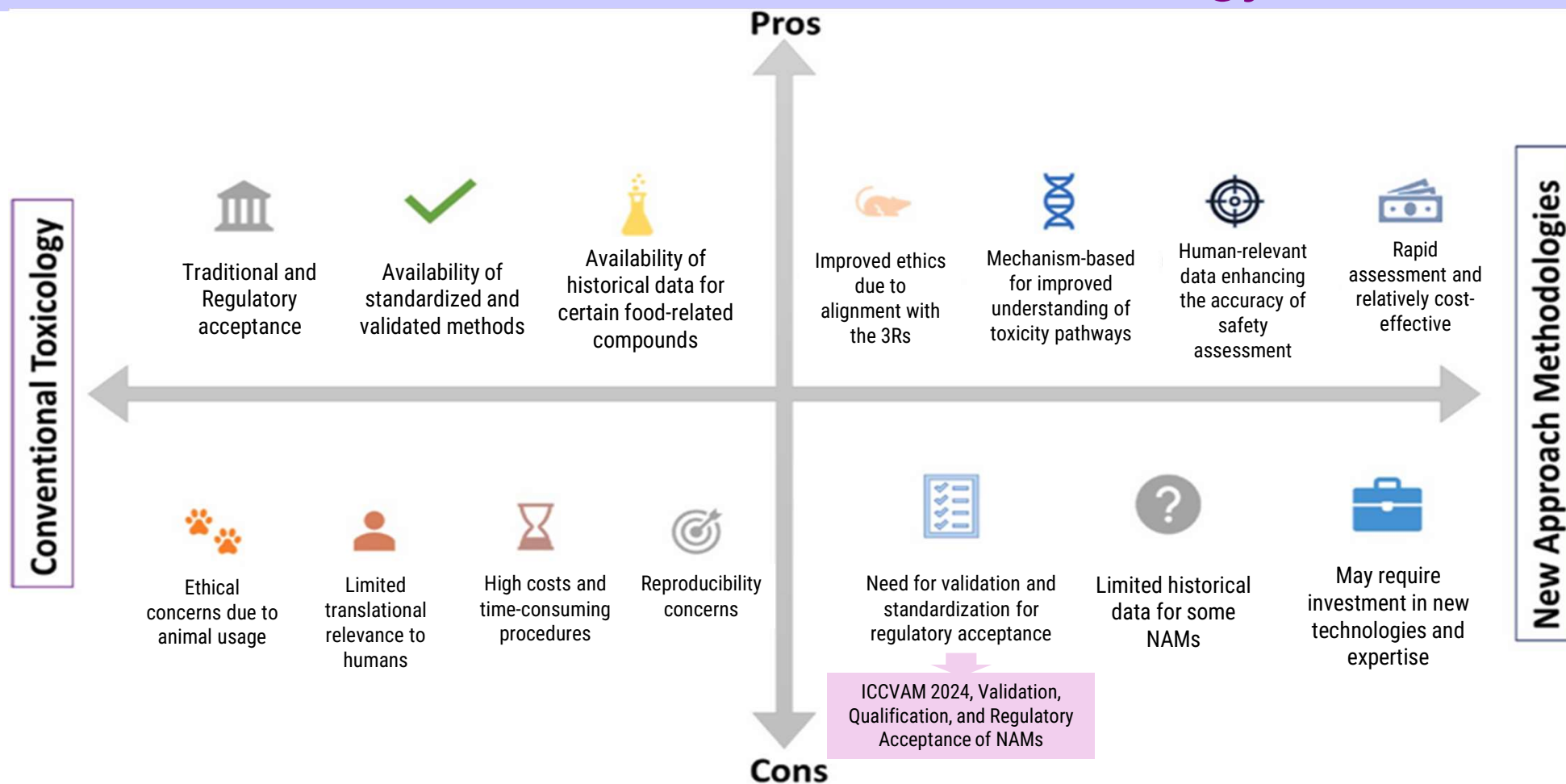
ECHA (2023) Report on the European Chemicals Agency's "New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals"

<p>ECHA 2016, Topical Scientific Workshop</p>	<p>NAMは、動物実験の代替(代替、削減、(苦痛の)軽減)となる毒性学的手法や、ばく露データなどの他の情報源を含むすべての手法を包括する用語であり、<i>in silico</i>アプローチ、<i>in chemico</i>、<i>in vitro</i>アッセイが含まれる。</p>
<p>US EPA 2018, TSCA Strategic Plan</p>	<p>NAMは、試験動物の使用を回避し、化学物質の有害性及びリスクの評価に関する情報提供のために使用できる、あらゆる技術、方法論、アプローチ(計算/<i>in silico</i>モデル(すなわち、QSAR)を含む)、またはそれらの組み合わせに対する広範な記述的参照として受け入れられている。TSCAの文脈では、NAMは、脊椎動物試験を代替、削減し、(苦痛の)軽減するための代替試験方法及び戦略を包含する。</p>
<p>ICCVAM 2018, Strategic roadmap</p>	<p>NAMは、化学物質の有害性及びリスクの評価に関する情報提供のために使用できる代替試験法又は手法の広範な記述的参照として受け入れられている。このような新しい手法には、IATA、データ解釈のための定義された手法、試験法の性能に基づく評価などが含まれる。この文脈では、代替試験法には、動物を用いない試験系や系統的に下位の生物種、特定の試験に必要な動物数削減方法、あるいは痛みや苦痛を軽減または回避するために動物使用を改良する方法などが含まれる。</p>
<p>US EPA 2021, New approach methods workplan</p>	<p>NAMsとは、動物実験の使用を回避し、化学物質の有害性とリスク評価に関する情報を提供できる技術、方法論、アプローチ、またはその組み合わせと定義される。</p>
<p>EFSA 2022, Development of a Roadmap for Action on NAMs in Risk Assessment</p>	<ul style="list-style-type: none"> ● 新しいアプローチ方法論(NAM)とは、ヒトの有害性評価及びリスク評価において、毒性学的情報を提供するために使用できるあらゆる新しいアプローチを指す。これらの新しいアプローチには、試験と評価の統合的アプローチ(IATA)、データ解釈のための定義されたアプローチ、試験法の性能に基づく評価などが含まれる。 ● 用語集での説明: 新たなアプローチ方法論とは、ハザードやリスクの構成要素を予測するための、動物を用いないアプローチ(<i>in silico</i>または<i>in vitro</i>)である。
<p>ECHA 2023, New Approach Methodologies Workshop: Towards an Animal Free Regulatory System for Industrial Chemicals</p>	<p>NAMは、一般的に動物実験を伴う従来の毒性試験法の代替法である。これらの代替法は、生物学的に複雑なエンドポイントに対する機序情報を提供することにより、化学物質のリスクや有害性の予測や評価に有用である。これには、<i>in vitro</i>、<i>in chemico</i>法、<i>in silico</i>計算モデルなどが含まれ、これらは単独で、あるいは他の方法と組み合わせて用いることができ、より迅速で、より安価で、より少ない動物数で実施できる可能性がある。</p>
<p>ICCVAM 2024, Validation, Qualification, and Regulatory Acceptance of NAMs</p>	<p>化学物質の有害性及びリスク評価に関する情報を提供するために使用できる、あらゆる技術、方法、アプローチ、またはそれらの組み合わせについて、広く記述した参考資料。また、動物使用の代替、削減、または(苦痛の)軽減(3R)を支援する。</p>

IATA: Integrated Approach to Testing and Assessment. 3R: Reduction, Refinement. **Blue:** NAMsが提供する情報. **Purple:** 3R あるいは動物試験回避

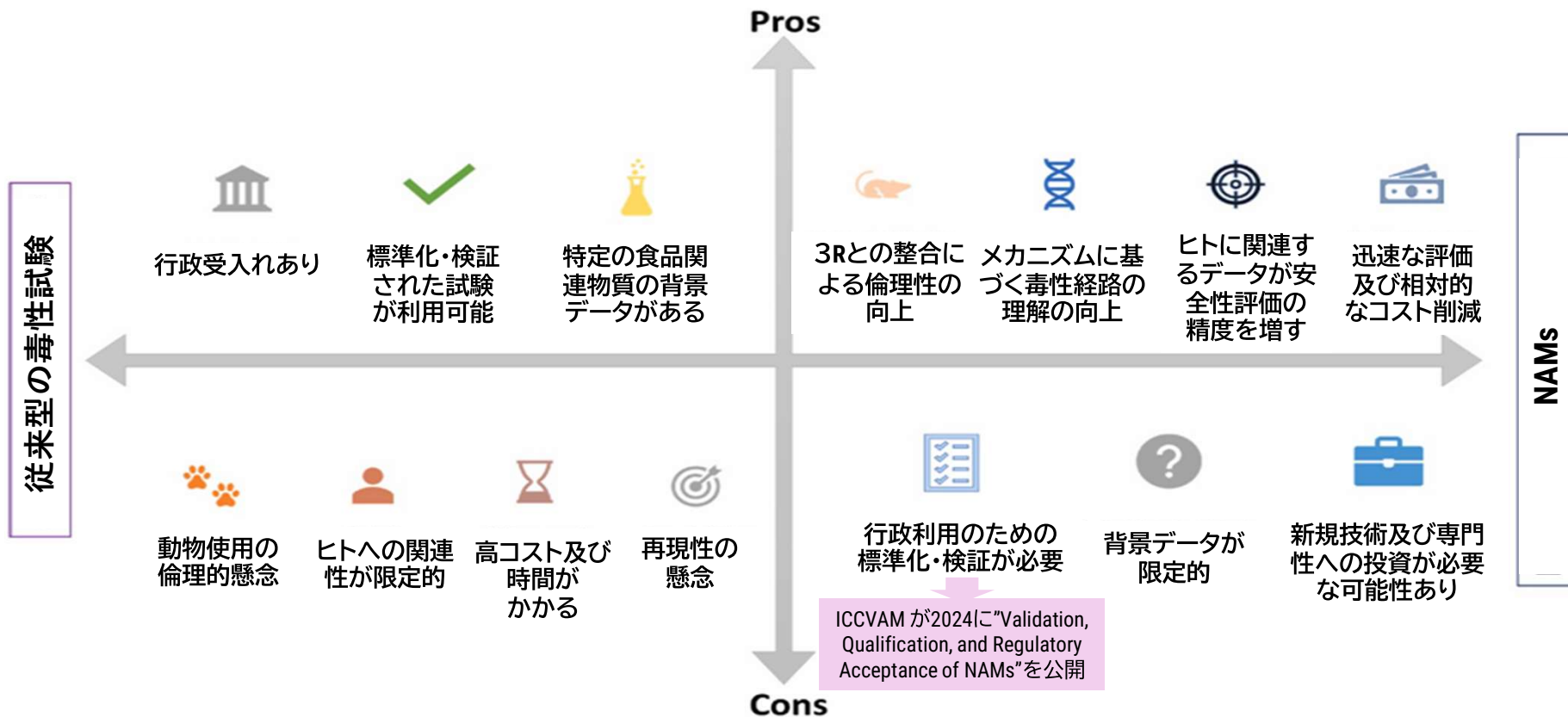
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Pros and Cons of the Conventional Toxicology vs. NAMs



from Manful *et al.* (2023) New Approach Methodologies (NAMs) for safety testing of complex food matrices: A review of status, considerations, and regulatory adoption. *Trends in Food Science & Technology*, 142: 104191

従来型評価とNAMs利用評価のPros/Cons



Manful et al (2023) のFig. 3. Pros and cons of the traditional toxicology and New Approach Methodologiesより抜粋・改変

Manful et al. (2023) New Approach Methodologies (NAMs) for safety testing of complex food matrices: A review of status, considerations, and regulatory adoption. Trends in Food Science & Technology, 142: 104191

Example Technologies as NAMs



Toxicodynamics

- *in vitro* guideline tests
- Omics, including genomics, transcriptomics, proteomics, metabolomics...
- TTC
- Quantitative AOP (model)

- Mode of action
- Mechanism of action
- AOP

- QSAR/SAR
- Machine learning and AI
- Read across
- Cell-based assay
- Reporter gene assays
- High throughput Screening (HTS)
- High content Imaging/Screening (HCI/HCS)
- 3D-Organoids
- Micro-physiological systems (MPS)
- Organ-on-a-chip / Human-on-a-Chip
- Testing using alternative organisms

Toxicokinetics

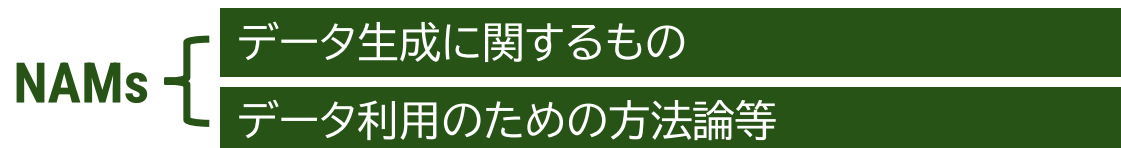
- High Throughput Toxicokinetics (HTTK)
- ADME model
- Physiologically-based kinetic (PBK) model
- Reverse Toxicokinetics (RTK)
- (Quantitative) *In vitro* to *in vivo* extrapolation ((Q)IVIVE)

Weight of Evidence (WoE) / Uncertainty analysis /
 Integrated Testing Strategy (ITS) / Integrated Approach to Testing and Assessment (IATA) / Defined approach (DA)

Hazard assessment / Risk assessment

Magurany *et al.* (2023) A pragmatic framework for the application of new approach methodologies in one health toxicological risk assessment. *Toxicological Sciences*, 192(2), 155–177.
 Schmeisser *et al.* (2023) New approach methodologies in human regulatory toxicology – Not if, but how and when! *Environment International*, 178, 108082.
 OECD(2020) GD329. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA).

NAMsに含まれ得る技術の例



Toxicodynamics

化学物質により与えられる影響

- *in vitro* ガイドライン試験
- Omics, including genomics, transcriptomics, proteomics, metabolomics...
- TTC
- Quantitative AOP (model)

- Mode of action
- Mechanism of action
- AOP

- QSAR/SAR
- Machine learning and AI
- Read across
- Cell-based assay
- Reporter gene assays
- High throughput Screening (HTS)
- High content Imaging/Screening (HCI/HCS)
- 3D-Organoids
- Micro-physiological systems (MPS)
- Organ-on-a-chip / Human-on-a-Chip
- 代替生物を用いた試験

Toxicokinetics

化学物質が生体から受ける影響

- High Throughput Toxicokinetics (HTTK)
- ADME model
- Physiologically-based kinetic (PBK) model
- Reverse Toxicokinetics (RTK)
- (Quantitative) *In vitro* to *in vivo* extrapolation ((Q)IVIVE)

Weight of Evidence (WoE) / Uncertainty analysis /
 Integrated Testing Strategy (ITS) / Integrated Approach to Testing and Assessment (IATA) / Defined approach (DA)

有害性評価 / リスク評価

Magurany *et al.* (2023) A pragmatic framework for the application of new approach methodologies in one health toxicological risk assessment. *Toxicological Sciences*, 192(2), 155-177.
 Schmeisser *et al.* (2023) New approach methodologies in human regulatory toxicology - Not if, but how and when! *Environment International*, 178, 108082.
 OECD(2020) GD329. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA).

Defined Approach (DA) at OECD

A **defined approach** is a formalised decision-making approach consisting of a fixed data interpretation procedure used to interpret data from a defined set of information elements. (OECD GD286, 2018)

- **Guideline No. 497: Defined Approaches on Skin Sensitisation (2021)**
 - ✓ *Able to predict skin sensitization by combining in silico and in vitro results.*
 - ✓ *Depending on the combination type of the results, it is also possible to classify for sub-categorization 1A/1B in GHS.*
- **Test Guideline No. 467: Defined Approaches for Serious Eye Damage and Eye Irritation (2022)**
- **Guidance Document No. 377: Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) *In-Vitro* Testing Battery (2023)**
 - …DNT *in vitro* testing battery is not yet adapted as an OECD test guideline
 - ✓ Integrate the results from the various *in vitro* assay (and *in silico* tools) using IVIVE and PBK modeling

When it comes to OECD test guidelines, it becomes subject to Mutual Acceptance of Data (MAD)
→ There is a possibility that Food Safety Committee (FSC) need to evaluate results from DA

OECDにおけるDefined approach (DA)

Defined approachとは、定義された情報要素の集合から得られたデータを解釈するために使用される固定されたデータ解釈手順から構成される、公式化された意思決定アプローチである(OECD GD286, 2018)

■ **Guideline No. 497: Defined Approaches on Skin Sensitisation (2021)**

- ✓ *in silico* 及び *in vitro*の結果の組合せにより皮膚感作性を予測する
- ✓ 結果の組合せの種類によっては、GHSにおける細分類1A/1Bの分類もできる

■ **Test Guideline No. 467: Defined Approaches for Serious Eye Damage and Eye Irritation (2022)**

■ **Guidance Document No. 377: Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) *In-Vitro* Testing Battery (2023)**

- …DNT *in vitro* testing battery はまだOECDテストガイドラインにはなっていない
- ✓ IVIVEやPBKモデリングを用いて様々な*in vitro*試験からの結果を統合して評価する

OECDテストガイドラインになると、Mutual Acceptance of Data (MAD:データの相互受入れ) の対象となる
→ 食品安全委員会でもDAによる結果の評価が必要になる可能性がある

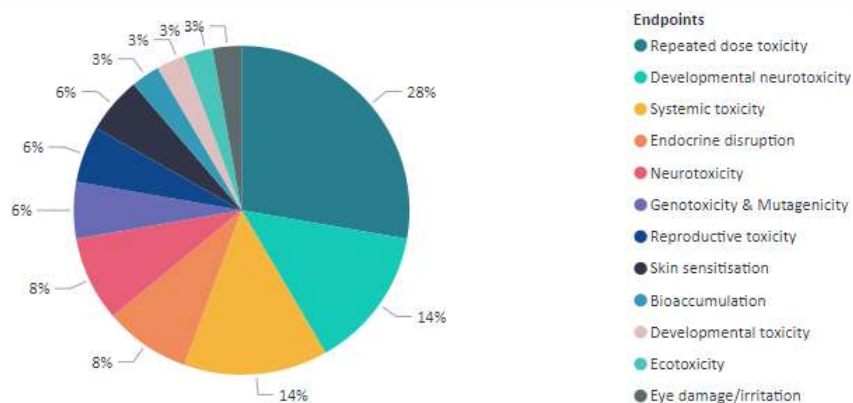
Activities at OECD

OECD IATA Case Studies Project (since 2014)

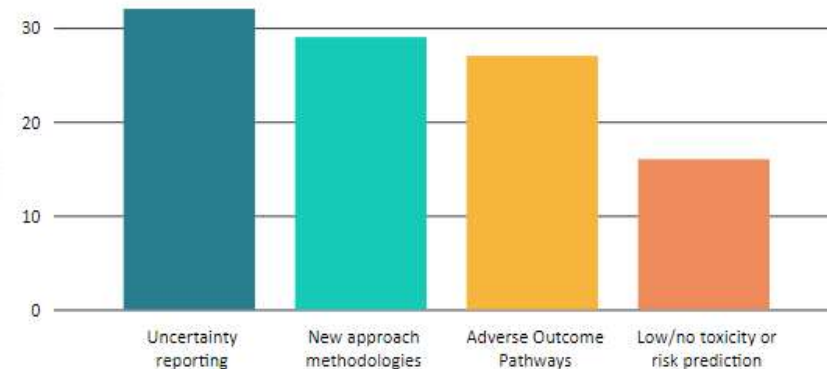
Case studies under the OECD IATA Case Studies Project

<https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html>

Percentage of case studies by endpoints



Number of case studies by IATA topics



“ ... allows countries to share and explore the use of novel methodologies in IATA for evaluating the safety of chemicals within a regulatory context.”

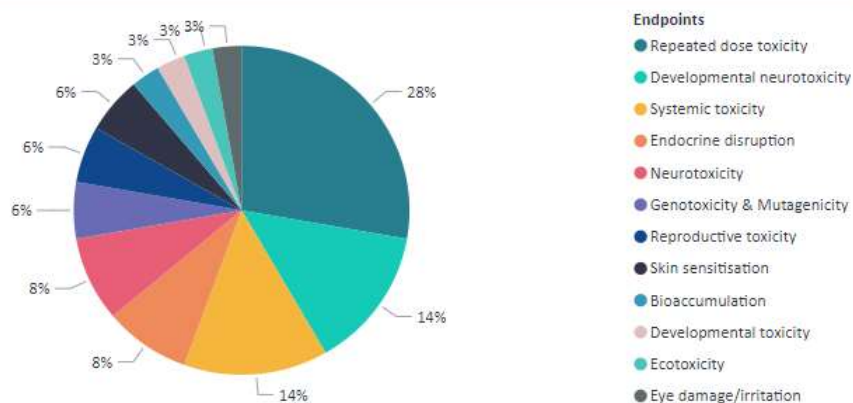
Reports on considerations from case studies and its review outcomes have been published almost every year, including identified items requiring guidance and reporting templates.

OECDでの取組み

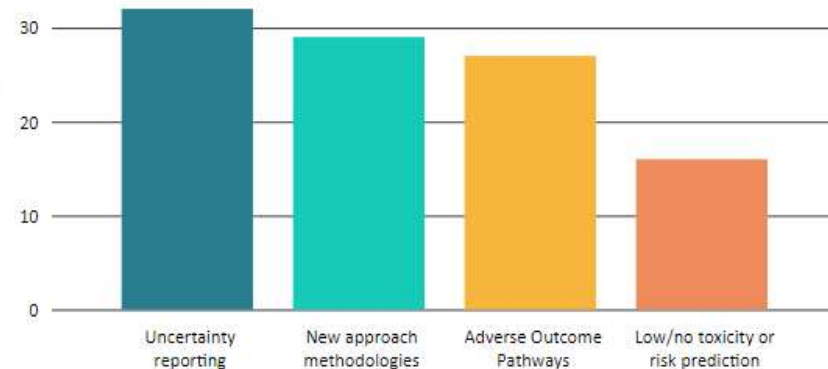
OECD IATA Case Studies Project (since 2014)

OECD IATA Case Studies Projectにおけるケーススタディ <https://www.oecd.org/en/topics/sub-issues/assessment-of-chemicals/integrated-approaches-to-testing-and-assessment.html>

Percentage of case studies by endpoints



Number of case studies by IATA topics



「...規制の枠組みにおいて、IATAにおける化学物質の安全性評価のための新たな方法論の利用を各国が共有し、検討することを可能にする。」

ケーススタディからの考察及びレビュー結果に関する報告書は、ほぼ毎年発行されており、ガイダンスが必要な項目や報告書のテンプレートなどが特定されている。

Activities at APCRA*

*Accelerating the Pace of Chemical Risk Assessment

<https://apcra.net/#/>

- **Started from 2016**
- **Goals of APCRA:**
 - **Common understanding of current state of the science applications of NAMs, including the regulatory context.**
 - **Increased understanding of realistic benchmarks for performance of NAMs in different regulatory contexts.**
 - **Determine mechanisms to enhance data sharing capabilities.**
 - **Increase engagement and commitment to development and sharing of case studies of mutual interest.**
 - **Increased cross-Agency collaboration to strategically address barriers and limitations of use of NAMs in a regulatory context.**
- **Partnership:** US-EPA, ECHA/EFSA, Health Canada
- **Working or worked in total 13 case studies**

APCRA*での取組み

*Accelerating the Pace of Chemical Risk Assessment

<https://apcra.net/#/>

- 2016年から開始
- APCRAが目指していること:
 - 法的背景を含め、NAMsの科学応用の現状に関する共通理解
 - 異なる法的背景におけるNAMs性能の現実的なベンチマークに対する理解の向上
 - データ共有能力を高めるためのメカニズムの決定
 - 相互に関心のあるケーススタディの開発と共有に対する関与とコミットメントの向上
 - 法的背景におけるNAMs使用の障壁と限界を戦略的に解決するための省庁間での協力の拡大
- パートナーシップ: US-EPA, ECHA/EFSA, Health Canada
- 13のケーススタディを実施

Activities at PARC

<https://www.eu-parc.eu/>

<https://www.anses.fr/en/content/european-partnership-assessment-risks-chemicals-parc>

- European Union's framework programme for the 2021-2027 period
- “**Partnership for the Assessment of Risks from Chemicals (PARC) aims to develop next-generation chemical risk assessment (NGRA) to protect human health and the environment.**”
- 200 partners including national agencies and research organizations, ECHA, EFSA, EEA (budget: 400 million euros).
- Wide range of activities are included (example keywords: NGRA, Human health/Environment, Monitoring methods, Mixtures, Biodiversity protection, shift away from animal testing etc.).

■ NAMs related activities under PARC:

Addressing unknown mixtures in risk assessment through monitoring data and NAMs

NAMs based on artificial intelligence and machine learning approaches

Development of NAMs

- For metabolism disrupting chemicals
- For non-genotoxic carcinogens
- For immunotoxicity
- For neurotoxicity

Mapping of regulatory implementation of NAMs

PARCでの取り組み

<https://www.eu-parc.eu/>

<https://www.anses.fr/en/content/european-partnership-assessment-risks-chemicals-parc>

- 2021-2027に実施されるEUの枠組みにおけるプログラム
- “ヒト健康と環境を保護するために、Partnership for the Assessment of Risks from Chemicals (PARC) は、次世代リスク評価(NGRA)の開発を目的とする”
- 各国省庁、研究機関、ECHA、EFSA、EEAを含む200のパートナー (予算規模: 400 million € (約650億円)).
- 幅広い取り組みが含まれている (キーワード例: NGRA, ヒト健康/環境, モニタリング方法, 混合物, 生物多様性保護, 動物実験からの移行 等).
- PARCにおけるNAMs関連の取り組み:

モニタリングデータ及びNAMsによるリスク評価における未知の混合物の特定

人工知能及び機械学習アプローチに基づくNAMs

NAMsの開発

- 内分泌かく乱物質の代謝関連
- 非変異発がん物質関連
- 免疫毒性関連
- 神経毒性関連

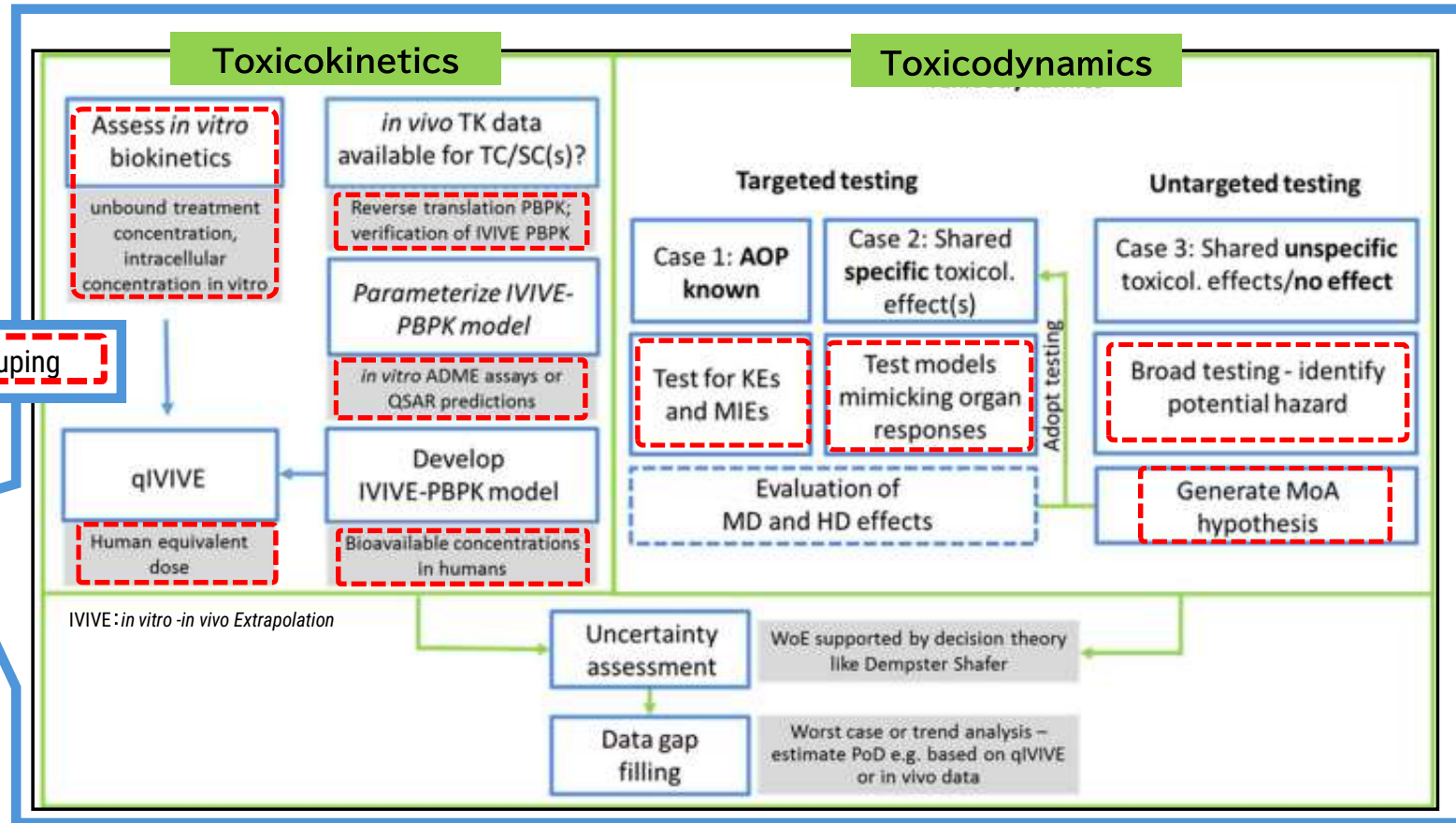
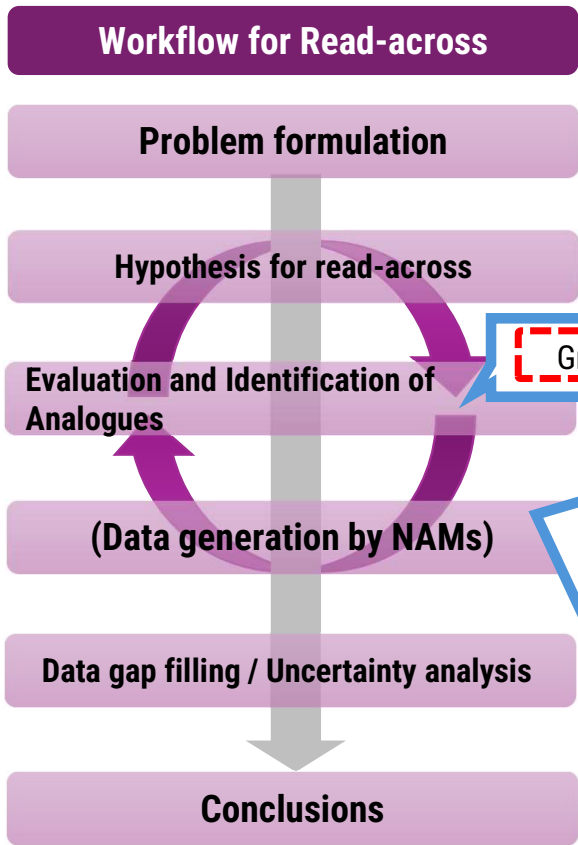
行政適用できるNAMsのマッピング

[3] Example applications of NAMs from known case studies

Example area of NAMs application in IATA Case Studies

Use of NAMs in read-across (also applicable for other purpose other than read-across)

Example of possible NAM applications



Escher *et al.* (2019) Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project. Archives of Toxicology (2019) 93:3643–3667.

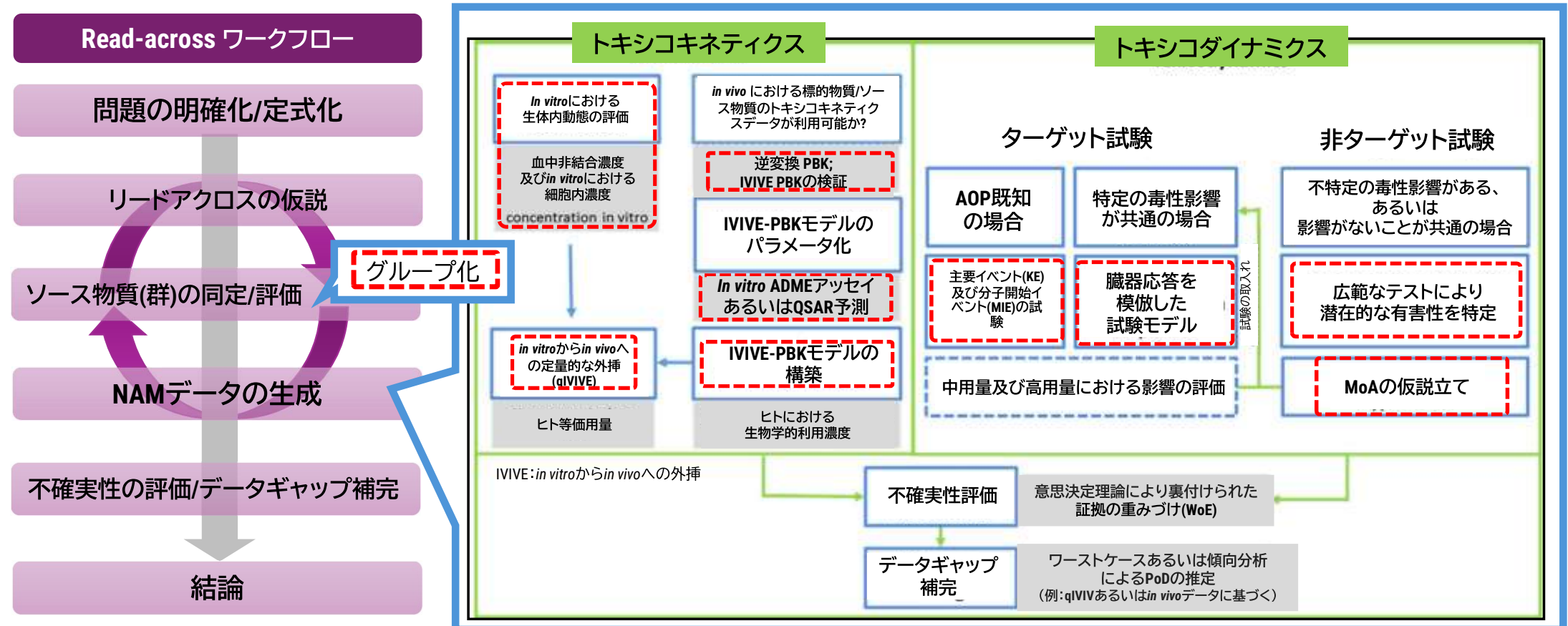
[3] 既存のケーススタディによるNAMsの適用事例

IATA Case StudiesにおけるNAMsの活用例

Read-acrossにおけるNAMsの活用

(Read-acrossに含まれるNAMsの活用は他の目的にも利用できる)

NAMsの活用場面の例

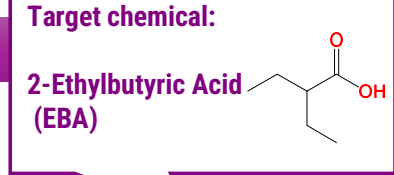


Escher et al. (2019) Towards grouping concepts based on new approach methodologies in chemical hazard assessment: the read-across approach of the EU-ToxRisk project. Archives of Toxicology (2019) 93:3643–3667.

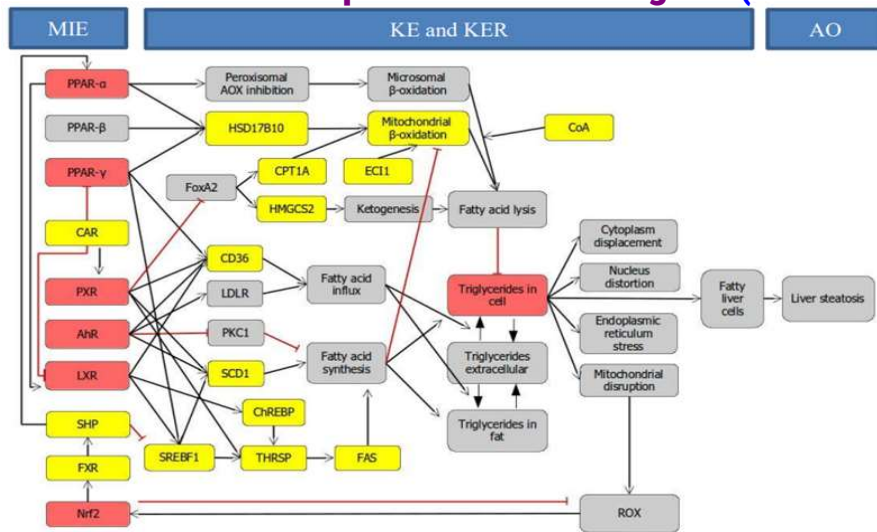
[3] Example applications of NAMs from known case studies

Case study on 90-D repeated dose toxicity assessment using read-across

Hazard Characterization by NAMs



● Steatosis was suspected from analogues (Source-4 & -5)



In vivo data available

Examples of <i>in vitro</i> assays	Source-1 2-Propylheptanoic acid PHP	Source-2 2-Ethylheptanoic Acid EHP	Source-3 2-Propylhexanoic acid PHA	Source-4 2-Ethylhexanoic Acid EHA	Source-5 Valproic Acid VPA	Source-6 2-Ethylpentanoic Acid EPA	Source-7 2-Methylhexanoic Acid MHA	Source-8 2-Methylpentanoic Acid MPA	target 2-Ethylbutyric Acid EBA	Source-9 2-Methylbutyric Acid MBA	negative Pivalic Acid PVA
Resazurin reduction POD	4000	4000	8000	8000	>8000	4000	8000	>8000	>8000	8000	>8000
supernatant lactate POD	8000	8000	>8000	>8000	>8000	>8000	>8000	>8000	8000	>8000	>8000
anti-PR CALUX	-3.6	-3.5	-3.9	-3	-3.1	>-3	>-3	>-3	>-3	>-3	>-3
PXR CALUX	-4.3	-4	-4	-3.6	-4.1	-3.5	>-3	>-3	>-3	>-3	>-3
PPARα CALUX	-4.5	-4.5	-4	-4	-4	-3.9	-4.5	-3.5	-3.5	>-3	>-3
PPARδ CALUX	-3	-3.1	>-3	>-3	>-3	>-3	>-3	>-3	>-3	>-3	>-3
Nrf2 CALUX	-4	-3.7	-3	-3	>-3	-3.4	>-3	>-3	>-3	-3.1	>-3
Steatosis 3D HepaRG	834	2330	2350	1270	1570	5290	3820	>8000	>8000	>8000	>8000(665(NS))
ATP 3D HepaRG	109	601	320	409	209	558	568	1600	579	3000	4720
GSH MEC/IC20 HepG2	7383	1311	>8000	>8000	218	6235	>8000	>8000	>8000	>8000	>8000
MMP IC50 HepG2	150	803	>8000	>8000	no data available	>8000	>8000	>8000	>8000	>8000	>8000
Lipid accumulation MEC/EC20 HepG2	83	1598	810	1203	69	>8000	>8000	>8000	>8000	>8000	>8000

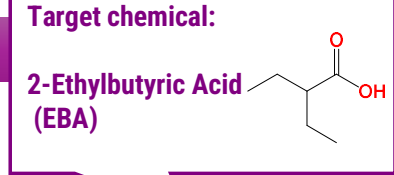
● The AOP for steatosis had been developed and *in vitro* assays for MIEs and KEs in AOP were available.

● Source-4 & -5 showed both MIE activations and lipid accumulation in *in vitro* assays. On the other hand, target (EBA) only showed PPARα activation but no lipid accumulation.

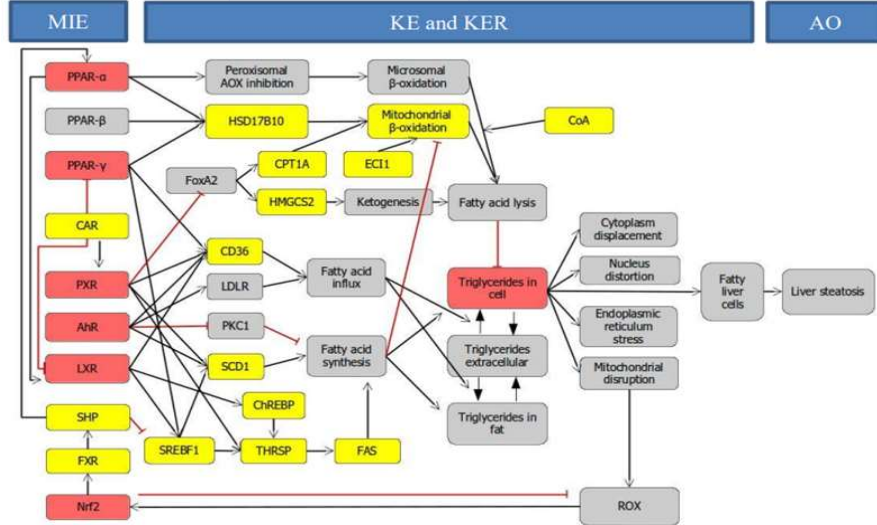
→ Steatosis by EBA is unlikely. Human oral equivalent dose was calculated based on the concentration of cytotoxicity.

Read-acrossを用いた90日間反復投与毒性評価事例

NAMsによる有害性の特性化



●類似物質 (Source-4 & -5)から脂肪肝が疑われた



In vivo データ有

Examples of <i>in vitro</i> assays	Source-1 2-Propylheptanoic acid PHP	Source-2 2-Ethylheptanoic Acid EHP	Source-3 2-Propylhexanoic acid PHA	Source-4 2-Ethylhexanoic Acid EHA	Source-5 Valproic Acid VPA	Source-6 2-Ethylpentanoic Acid EPA	Source-7 2-Methylhexanoic Acid MHA	Source-8 2-Methylpentanoic Acid MPA	target 2-Ethylbutyric acid EBA	Source-9 2-Methylbutyric Acid MBA	negative Pivalic Acid PVA
Resazurin reduction POD	4000	4000	8000	8000	>8000	4000	8000	>8000	>8000	8000	>8000
supernatant lactate POD	8000	8000	>8000	>8000	>8000	>8000	>8000	>8000	8000	>8000	>8000
anti-PR CALUX	-3.6	-3.5	-3.9	-3	-3.1	>-3	>-3	>-3	>-3	>-3	>-3
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PPARδ CALUX	-3	-3.1	>-3	>-3	>-3	>-3	>-3	>-3	>-3	>-3	>-3
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Lipid accumulation MEC/EC20 HepG2	83	1598	810	1203	69	>8000	>8000	>8000	>8000	>8000	>8000

●脂肪肝に対するAOPは開発済みであり、AOPにおけるMIE及びKEの*in vitro*アッセイを使用

●Source-4及び-5は、*in vitro*アッセイにおいてMIE活性化と脂質蓄積の両方を示した。一方、標的物質(EBA)はPPARα活性化のみを示したが、脂質蓄積はなかった。

→EBAによる脂肪肝は考えにくい。ヒト経口等価用量は細胞毒性濃度に基づいて計算された。

Take home messages

At present, in Europe and the US, case studies are being conducted to accumulate experiences in the use of NAMs for regulatory application. Also, the development of NAMs are accelerated.

- **There are legal background for the use of NAMs in Europe and the US
→ It should be recognized that such background to be dealt with is different from that in Japan.**
 - **The human resources and its scale (capacity) allocated for safety assessment differ between Japan and Europe or the US.**
 - **On the other hand, NAMs are being incorporated into OECD test guidelines and this trend will continue (e.g., DNT, non-mutagenic carcinogens).**
 - **NAMs data may be submitted to FSC for assessment (e.g., for contaminants).**
- ⇒ The safety assessment using NAMs may be inevitable.**

メッセージ

現在、欧米では、規制への応用に向けたNAMsの利用に関する経験を蓄積するためのケーススタディが行われている。また、NAMsの開発も加速している。

- 欧米ではNAMsの利用に法的背景がある
→ 対応すべき背景が日本とは異なることを認識すべきである
- 日本と欧米では安全性評価に割く人的資源や規模(キャパシティ)が異なる
- 一方、NAMsはOECDテストガイドラインに取り入れられつつあり、この傾向は今後も続く(例: DNT、非変異原性発がん物質)
- NAMsによるデータは評価のために食品安全委員会に提出される可能性がある(例: 汚染物質)

⇒ NAMsを活用した評価は不可避である