EU-FORA SERIES 5

APPROVED: 31 August 2022

doi: 10.2903/j.efsa.2022.e200908

The use of NAMs and omics data in risk assessment

German Federal Institute for Risk Assessment (BfR), Berlin, Germany,
Andrea Miccoli[1,2,3], Philip Marx-Stoelting[1] and Albert Braeuning[2]

[1]Department Pesticides Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[2]Department Food Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[3]Department for the Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy

Abstract

The animal-centric approach so far predominantly employed in risk assessment has been questioned in recent years due to a number of shortcomings regarding performance, consistency, transferability of results, sustainability, costs and ethical reasons. Alternatives to animal testing, collectively termed NAMs, may have the potential to deliver sound, cost-effective, prompt and reliable information, but their regulatory acceptance has not been established yet. The main reasons behind this are mostly related to actual methodological obstacles, with particular reference to addressing complex endpoints such as repeated-dose toxicity, the issue of translating the concept of adversity to NAMs, and doubts of stakeholders about the level of chemical safety ensured by NAMs. With the aim of providing an updated view on major conceptual and methodological developments in the field of toxicology, a symposium and a workshop were organised by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR) and Helmholtz Centre for Environmental Research on 15–17 November 2021 in Berlin. The conference, entitled 'Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology' brought together eminent scientists with representatives from industry and regulatory authorities. The organisation, day-to-day operations and the reporting of the event main outcomes in a position paper were the main focus of the present EFSA EU-FORA work programme. Tasks pertaining to 'The use of NAMs and omics data in risk assessment' were implemented under the shared supervision of units 'Testing and Assessment Strategies Pesticides' and 'Effect-based Analytics and Toxicogenomics' of the German Federal Institute for Risk Assessment.

Keywords: New Approach Methods, Animal experiment, Omics, Risk assessment, 3Rs principle, Workshop

Correspondence: eu-fora@efsa.europa.eu

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: This report is funded by EFSA as part of the 2021-2022 EU-FORA Fellowship Programme.

Suggested citation: Miccoli A, Marx-Stoelting P and Braeuning A, 2022. The use of NAMs and omics data in risk assessment. EFSA Journal 2022;20(S2):e200908, 9 pp. https://doi.org/10.2903/j.efsa.2022.e200908

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

New approach methodologies (NAMs) are defined as any non-animal technology, methodology, approach or combination thereof that can be used to provide information on chemical hazard and human risk assessment (Dent et al., 2018). NAMs can be used as alternative or complementary methods to traditional animal testing, and comprise mostly *in silico* (e.g. QSAR, PBK models, machine learning models and artificial intelligence) (Hartung and Tsatsakis, 2021; Thompson et al., 2021), *in vitro* (cell cultures, organoids and other microphysiological systems) (Marx et al., 2020; Pamies et al., 2022), *ex vivo* (e.g. the large field of omics applications, which are anyway also applicable to entirely *in vitro* investigations) (Buesen et al., 2017; Mav et al., 2018; Gwinn et al., 2020; Malinowska et al., 2022), and *in chemico* (i.e. a term for generally referring to the use of abiotic methods aimed at identifying chemical reactivity) (Gerberick et al., 2008) approaches. When information related to human adverse effects and exposure are used, NAMs represent an enhancement of traditional animal testing with regard to risk assessment aimed at ensuring chemical safety and human health protection.

Alternative approaches are commonly utilised by industry and regulatory agencies, at least internally, for preliminary prioritisation and read-across purposes (Ball et al., 2020; van der Stel et al., 2021).

Over the last 15 years, several European projects have explored the regulatory capacity and acceptance of NAMs, and the just-started 7-year long PARC project (Horizon Europe 2021–2027 framework, 200 partners, 28 countries, 400 million € budget) aims at designing a next generation chemical risk assessment (NGRA) by leveraging on one of the largest scale and innovative partnerships of its kind.

One of EFSA's vision is to routinely make use of NAMs (e.g. omics and associated bioinformatic approaches) in relevant RAs by 2030 (EFSA, 2021) to ultimately support the transition into a NGRA that is actually exposure-led, hypothesis-driven, truly data-centric and AOP-based.

At last, the traditional animal testing approach currently in place notoriously suffers from many shortcomings, the most severe of which are related to reproducibility issues, interspecies concordance, poor sustainability of toxicological data generation resource- and time-wise, and ethical concerns (Luechtefeld et al., 2018; Smirnova et al., 2018; Karmaus et al., 2022).

Despite the potential of several NAMs was demonstrated, their regulatory acceptance has mostly not been established yet, mainly due to the difficulty of addressing complex end-points (e.g. repeated-dose toxicity), the issue of translating the concept of adversity to NAMs, doubts of stakeholders about the level of chemical safety ensured by NAMs, and lack of internationally harmonised guidance on the interpretation of NAM-derived data.

The present EFSA EU-FORA work programme was entitled 'The use of NAMs and omics data in risk assessment' and was implemented at the German Federal Institute for Risk Assessment under the shared supervision of Prof. Dr. Braeuning (Unit 51, 'Effect-based Analytics and Toxicogenomics') and Dr. Marx-Stoelting (Unit 66, 'Testing and Assessment Strategies Pesticides'). Its overarching aims were (i) to provide the most up-to-date picture on the potential of NAMs in risk assessment, (ii) to clarify the reasons of the slow progress in regulatory uptake as well as (iii) to suggest possible ways forward in terms of testing strategies, standardisation, harmonisation and validation procedures, knowledge transfer and confidence building, and the required changes in regulation for facilitating the integration of NAM into risk assessment.

2. Description of work programme

2.1. Aims

The implementation of the work programme was centred on the international conference and workshop entitled 'Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology', organised by the hosting site in partnership with UFZ on 15–17 November 2021. Knowledge in the current state of integration of NAM and omics techniques in toxicological risk assessment of food contaminants, pesticides and other areas, as well as into technical limitations and steps needed for further implementation was generated.

2.2. Activities/Methods

The work programme activities of the fellow can be classified into three main categories, i.e. pre-, during and post-conference.

2.2.1. Pre-conference

The pre-conference activities revolved around (i) the acquaintance of the fellow to the subject of NAMs and omics in risk assessment, (ii) the refinement of the conference schedule, (iii) the screening of the responses to a preliminary questionnaire sent to invited workshop participants up to 4 weeks prior to the conference start and (iv) the preparation of the material to be presented at the workshop for moderation purposes. In particular, activity (i) was implemented via relevant literature searches of latest scientific (2009–2022 time frame), institutional (e.g. JRC's Reference Laboratory for alternatives to animal testing, Scientific Committee on Consumer Safety, US EPA, US FDA) and intergovernmental (OECD) publications/reports, as well as with an in-depth analysis of 17 breakthrough projects funded by the EU both within the Horizon 2020 and Horizon Europe frameworks (i.e. EURION cluster, ASPIS cluster, PARC) and US federal funds (e.g. Tox21). Such information later served in the preparation of one of the position paper tables, providing a comparative outlook with regards to project aims, methods, *in vivo* and *in vitro* test models, *in silico* and high-throughput approaches, toxicological targets, investigated toxicity and interaction with regulatory agencies.

2.2.2. Conference

The activities performed during the conference were mainly related to the day-to-day operations and the moderation of the workshop, during which a synthesis of all questionnaire responses received was presented, interpreted and used for stimulating discussion.

2.2.3. Post-conference

The activities performed following the conference pertained to the compilation of the input from all symposium sections and the subsequent drafting of a position paper on the use of NAM and omics in regulatory toxicology based on the conference contributions, panel discussion and invite-only workshop discussion and visions.

3. Conclusions

3.1. New Approach Methodologies in human regulatory toxicology

Invited workshop participants were asked to fill a questionnaire within 4 weeks prior to their attendance to the workshop. The questionnaire was composed of six sections, namely (i) Current uses of NAMs, (ii) Regulatory toxicology areas currently covered by NAMs, (iii) Regulatory toxicology areas currently not covered by NAMs, (iv) Challenges for the implementation of NAMs, (v) Possible ways forward and (vi) Approach to exploit the full potential of NAMs in the regulatory field. Based on a synthesis of the stakeholders' responses, the fellow prepared the material to be presented for moderating the workshop and stimulating the discussion.

The conference 'Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology' took place at the Langenbeck-Virchow-Haus venue in Berlin on 15–17 November 2021, and encompassed 4 sessions, namely 'Recent advances in the application of NAM' (6 presentations), 'Use of omics techniques in experimental toxicology' (7 presentations), 'Use of omics techniques in regulatory toxicology' (5 presentations), 'Current applications – a regulatory authority perspective' (6 presentations, 1 panel discussion, 3 position talks). An invite-only workshop entitled 'Future concepts and strategies', to which representatives from relevant stakeholders participated, was conducted at the end of the 3-day programme. Contributions from academia, industry and regulatory authorities were presented and stimulated the discussion both in presence and remotely. The full programme of the event, together with select presentation and streaming recording, is available at https://www.bfr-akademie.de/english/archive/2021/omics.html.

A total of 485 people (54 in presence, 431 remotely) attended the event. The event was advertised through the following networks: EUROTOX (Federation of European Toxicologists & European Societies of Toxicology), German Toxicology Society, European Partnership for Alternative Approaches to Animal Testing (EPAA), EU-ToxRisk project, UFZ, hosting site's LinkedIn announcement and webpage. A high level of satisfaction with the conference was perceived from the follow-up questionnaire sent to all participants, which was compiled by 113 people (23.3% of the total participants, 17 in presence and 96 remotely). The most valued features of the event were the level of expertise of contributors (score 4.7/5), the support provided during the event (4.9/5), and future practical applicability of the knowledge obtained (4.2/5).

A position paper was produced based on the main outcomes of the event. The manuscript explores the potential of NAMs, which alone promise faster and more efficient hazard and exposure assessments, to transform today's regulatory work, ultimately allowing for a more human-relevant decision-making, as well as the obstacles still hampering the expansion of regulatory applications of NAMs. A conceptual perspective was also included as to how NAMs can be gradually integrated into chemical risk assessment, until an animal-free NGRA is achieved (Figure 1).

The first draft of the position paper was circulated internally already by December 2021. The second draft was later circulated among all co-authors in May 2022 after a second internal revision and is currently (July 2022) at a consolidated version. The tentative title of the manuscript is 'New Approach Methodologies in human regulatory toxicology – not an if question, but how and when!'. The manuscript will be expectedly submitted to a high-rank scientific journal within August 2022. Shared first authorship of the manuscript was assigned to the fellow and to Dr. Sebastian Schmeisser (BfR), whose guidance and support throughout the pre- and post-conference activities proved precious.

The list of co-authors and corresponding affiliations is included in Table 1.

Table 1: Names and affiliations of tentative authors of the position paper on NAMs in regulatory toxicology

Name of co-author	Affiliation
Von Bergen M	UFZ
Berggren E	European Commission's Joint Research Centre
Braeuning A	BfR
Busch W	UFZ
Desaintes C	European Commission's Directorate General for Research and Innovation
Gourmelon A	OECD, Environment Directorate
Grafstoem R	Karolinska Institute
Harrill J	Center for Computational Toxicology and Exposure, U.S. EPA
Hartung T	Johns Hopkins Center for Alternatives to Animal Testing
Herzler M	BfR
Kass G	EFSA
Kleinstreuer N	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
Leist M	Johns Hopkins Center for Alternatives to Animal Testing - Europe
Marx-Stoelting P	BfR
Poetz O	Natural and Medical Science Institute, University of Tübingen; SIGNATOPE GmbH
van Ravenzwaay B	Wageningen University and Research
Roggeband R	European Partnership for Alternative Approaches to Animal Testing; Procter and Gamble Services Company NV/SA
Rogiers V	Scientific Committee on Consumer Safety; Vrije Universiteit Brussel
Roth A	F. Hoffmann-La Roche Ltd
Sanders P	French Agency for Food, Environmental and Occupational Health & Safety
Vinggaard AM	National Food Institute, Technical University of Denmark
Vinken M	Vrije Universiteit Brussel
van de Water B	Leiden University
Luch A	BfR
Tewes T	BfR

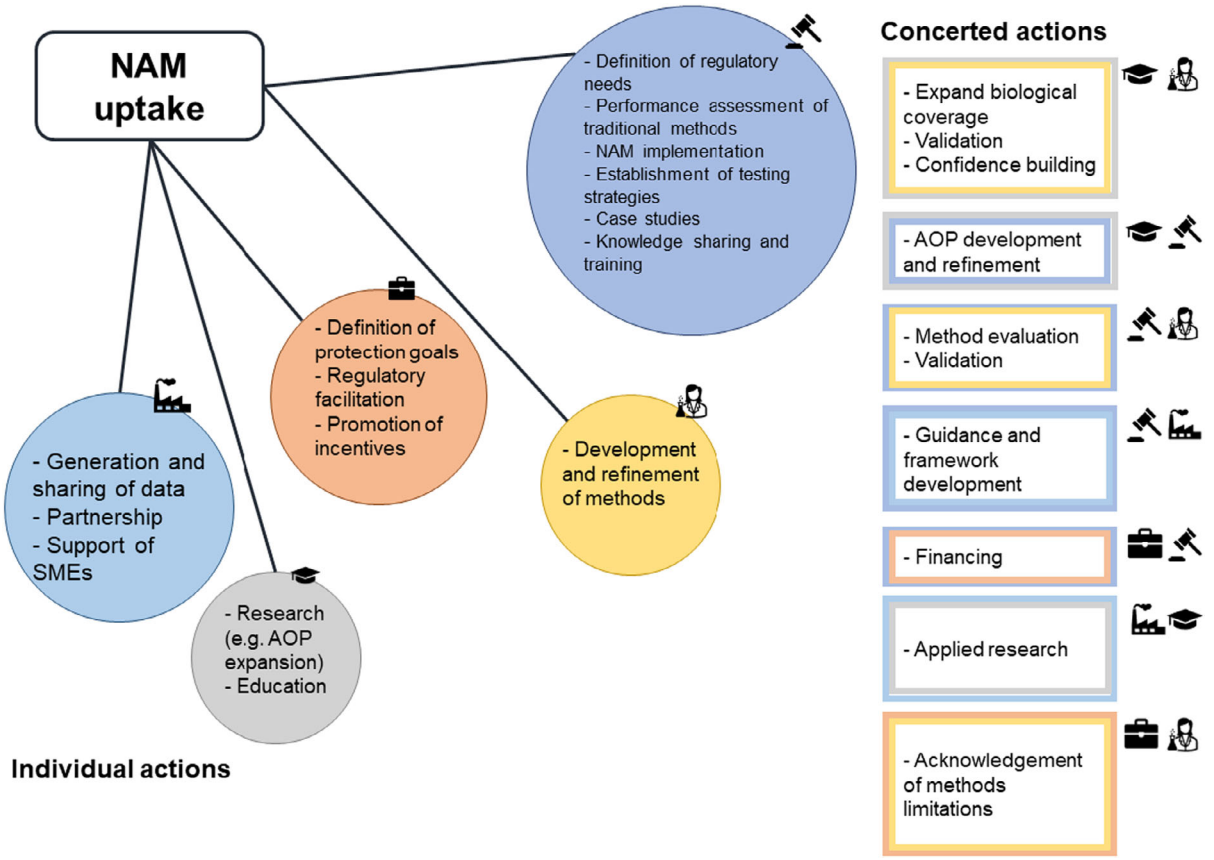

Figure 1: Responsibilities of the different actors involved in a transition towards NAM implementation in regulatory risk assessment. Light blue: Industry; Orange: Politics; Violet: Regulatory community; Yellow: Developers; Grey: Academia.

3.2. Additional activities

In addition to the work programme-specific goals, the fellow engaged in extracurricular laboratory- and desktop-based activities, as follows:

i) Within the October 2021–August 2022 time frame, the fellow participated remotely to the following symposia and workshops, listed in chronological order:

   a) April 2022 – EURION cluster – 'Cross-omics discovery of adverse outcome pathways linked to exposure to endocrine disrupting compounds'.
   b) February 2022 – American Society for Cellular and Computational Toxicology (ASCCT) – 'Serum-Free Cultures: Why and How?', presented by Dr. Barbara Jozef (Swiss Federal Institute of Aquatic Science and Technology) and Dr. Aline Chary (Luxembourg Institute of Science and Technology).
   c) November 2021 – EU-ToxRisk – 'The final outcomes and legacy of EU-ToxRisk, including case studies and a comprehensive collection of learnings and gaps in the application of NAM-enhanced read-across'.
   d) November 2021 – ASCCT – 'How to Control and to Maintain the Quality of Cell Cultures', presented by Dr. Oliver Wehmeier, acCELLerate GmbH.
   e) October 2021 – 'Next Generation Approaches for identifying Endocrine Disruptors'.
   f) October 2021 – EU-ToxRisk – 'Application of a science-driven approach to solve the needs of regulatory and industry communities in the implementation of NAM-supported read-across in regulatory dossiers'.

ii) October 2021 – ASCCT – 'Practical applications of new tools in toxicology'.
iii) The fellow participated in presence to bi-weekly hosting site's Unit 51 seminars and presented an oral contribution on 1 March 2022. The presentation reported on the main

outcomes of the conference 'Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology' as well as introduced further involvement of the fellow in hosting site activities.

iv) On 9 November 2021 the fellow attended a whole-day course held by the hosting site entitled 'Risk assessment and risk management of Genetically Modified Organisms'.

v) The fellow engaged actively in laboratory work since 30 January 2022, within the framework of the project 'Analysis of key functional events in cell culture models', run jointly by the hosting site, the Natural and Medical Science Institute of the University of Tübingen, and SIGNATOPE GmbH (http://www.signatope.com/). The aim of the project is the investigation of cytotoxicity and transcriptomic/proteomic signatures of two human hepatic and renal cells lines caused by the single exposure to six pesticidal active compounds and five food contaminants in a multifactorial design concentration- and time-wise. In some cases, available cytotoxicity information was used as starting point for dose selection in the current project. Of note, the substances have been characterised with regards to toxicological effects by means of traditional *in vivo* experiments (Table 2). The significance of the project, therefore, lies in the verification or identification of the MoA by means of NAMs, and will ultimately contribute to progressing in the slow but steady establishment of NAMs in regulatory risk assessment. Results will be expectedly published in relevant peer-reviewed scientific journals.

Table 2: Known *in vivo* toxicological effects of *in vitro* test substances

Substance	Usage	Known *in vivo* toxicity
Cyproconazole	Azole fungicide	Liver: Hypertrophy, Steatosis
Fluxapyroxad	Pyrazole anilide fungicide	Liver: Hepatocellular necrosis, Steatosis
Azoxystrobin	Strobilurin fungicide	Liver: Hepatocellular cell death, Hypertrophy
Chlorotoluron	Urea herbicide	Kidney: Tumours, Tubular hyperplasia
Thiabendazole	Benzimidazole fungicide	Liver: Lesions of biliary epithelium Kidney: Pelvis and tubular hyperplasia
2-Phenylphenol	Pesticides, etc.	Kidney: Cell death due to oxidative stress
PFOS	PFAS	Liver: Steatosis, Cholestasis
PFOA	PFAS	Liver: Steatosis, Cholestasis
Aflatoxin B1	Mycotoxin	Liver: Tumours
Cadmium	Heavy metal	Kidney: Tubular toxicity
Lasiocarpine	Pyrrolizidine alkaloid	Liver: Hepatic veno-occlusive disease, Tumours

References

Ball N, Madden J, Paini A, Mathea M, Palmer AD, Sperber S, Hartung T and van Ravenzwaay B, 2020. Key read across framework components and biology based improvements. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 853, 503172. https://doi.org/10.1016/j.mrgentox.2020.503172

Buesen R, Chorley BN, da Silva Lima B, Daston G, Deferme L, Ebbels T, Gant TW, Goetz A, Greally J, Gribaldo L, Hackermüller J, Hubesch B, Jennen D, Johnson K, Kanno J, Kauffmann HM, Laffont M, McMullen P, Meehan R, Pemberton M, Perdichizzi S, Piersma AH, Sauer UG, Schmidt K, Seitz H, Sumida K, Tollefsen KE, Tong W, Tralau T, van Ravenzwaay B, Weber RJM, Worth A, Yauk C and Poole A, 2017. Applying 'omics technologies in chemicals risk assessment: Report of an ECETOC workshop. Applying 'omics technologies in chemicals risk assessment: Report of an ECETOC workshop, in: Regulatory Toxicology and Pharmacology. https://doi.org/10.1016/j.yrtph.2017.09.002

Dent M, Amaral RT, Da Silva PA, Ansell J, Boisleve F, Hatao M, Hirose A, Kasai Y, Kern P, Kreiling R, Milstein S, Montemayor B, Oliveira J, Richarz A, Taalman R, Vaillancourt E, Verma R, Posada NVORC, Weiss C and Kojima H, 2018. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Computational Toxicology, 7, 20–26. https://doi.org/10.1016/j.comtox.2018.06.001

EFSA (European Food Safety Authority), Iacono G, Guerra B, Kass G, Paraskevopoulos K, Kleiner J, Heppner C and Hugas M, 2021. Theme (Concept) paper - Application of OMICS and BIOINFORMATICS Approaches: Towards Next Generation Risk Assessment. EFSA Supporting Publications 2021;19(5):E200506, 10 pp. https://doi.org/10.2903/SP.EFSA.2022.E200506

Gerberick F, Aleksic M, Basketter D, Casati S, Karlberg AT, Kern P, Kimber I, Lepoittevin JP, Natsch A, Ovigne JM, Rovida C, Sakaguchi H and Schultz T, 2008. Chemical reactivity measurement and the predictive identification of skin sensitisers. ATLA Alternatives to Laboratory Animals, 36, 215–242. https://doi.org/10.1177/026119290803600210

Gwinn WM, Auerbach SS, Parham F, Stout MD, Waidyanatha S, Mutlu E, Collins B, Paules RS, Merrick BA, Ferguson S, Ramaiahgari S, Bucher JR, Sparrow B, Toy H, Gorospe J, Machesky N, Shah RR, Balik-Meisner MR, Mav D, Phadke DP, Roberts G and DeVito MJ, 2020. Evaluation of 5-day in vivo rat liver and kidney with high-throughput transcriptomics for estimating benchmark doses of apical outcomes. Toxicology Science, 176, 343–354. https://doi.org/10.1093/toxsci/kfaa081

Hartung T and Tsatsakis AM, 2021. Food for thought . . . the state of the scientific revolution in toxicology. ALTEX, 38, 379–386. https://doi.org/10.14573/altex.2106101

Karmaus AL, Mansouri K, To KT, Blake B, Fitzpatrick J, Strickland J, Patlewicz G, Allen D, Casey W and Kleinstreuer N, 2022. Evaluation of variability across rat acute oral systemic toxicity studies. Toxicology Science, 188, 34–47. https://doi.org/10.1093/TOXSCI/KFAC042

Luechtefeld T, Marsh D, Rowlands C and Hartung T, 2018. Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. Toxicology Science, 165, 198–212. https://doi.org/10.1093/toxsci/kfy152

Malinowska JM, Palosaari T, Sund J, Carpi D, Bouhifd M, Weber RJM, Whelan M and Viant MR, 2022. Integrating in vitro metabolomics with a 96-well high-throughput screening platform. Metabolomics, 18, 11. https://doi.org/10.1007/s11306-021-01867-3

Marx U, Akabane T, Andersson TB, Baker E, Beilmann M, Beken S, Brendler-Schwaab S, Cirit M, David R, Dehne EM, Durieux I, Ewart L, Fitzpatrick SC, Frey O, Fuchs F, Griffith LG, Hamilton GA, Hartung T, Hoeng J, Hogberg H, Hughes DJ, Ingber DE, Iskandar A, Kanamori T, Kojima H, Kuehnl J, Leist M, Li B, Loskill P, Mendrick DL, Neumann T, Pallocca G, Rusyn I, Smirnova L, Steger-Hartmann T, Tagle DA, Tonevitsky A, Tsyb S, Trapecar M, Van de Water B, Van den Eijnden-van Raaij J, Vulto P, Watanabe K, Wolf A, Zhou X and Roth A, 2020. Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. ALTEX, 37, 365–394. https://doi.org/10.14573/altex.2001241

Mav D, Shah RR, Howard BE, Auerbach SS, Bushel PR, Collins JB, Gerhold DL, Judson RS, Karmaus AL, Maull EA, Mendrick DL, Merrick BA, Sipes NS, Svoboda D and Paules RS, 2018. A hybrid gene selection approach to create the S1500+ targeted gene sets for use in high-throughput transcriptomics. PLoS One, 13, e0191105. https://doi.org/10.1371/journal.pone.0191105

Pamies D, Leist M, Coecke S, Bowe G, Allen DG, Gstraunthaler G, Bal-Price A, Pistollato F, de Vries RBM, Hogberg HT, Hartung T and Stacey G, 2022. Guidance document on good cell and tissue culture practice 2.0 (GCCP 2.0). ALTEX, 39, 30–70. https://doi.org/10.14573/altex.2111011

Smirnova L, Kleinstreuer N, Corvi R, Levchenko A, Fitzpatrick SC and Hartung T, 2018. 3S - Systematic, systemic, and systems biology and toxicology. ALTEX, 35, 139–162. https://doi.org/10.14573/altex.1804051

Thompson CV, Firman JW, Goldsmith MR, Grulke CM, Tan Y-M, Paini A, Penson PE, Sayre RR, Webb S and Madden JC, 2021. A systematic review of published physiologically-based kinetic models and an assessment of their chemical space coverage. Alternatives to Laboratory Animals, 49, 197–208. https://doi.org/10.1177/02611929211060264

van der Stel W, Carta G, Eakins J, Delp J, Suciu I, Forsby A, Cediel-Ulloa A, Attoff K, Troger F, Kamp H, Gardner I, Zdrazil B, Moné MJ, Ecker GF, Pastor M, Gómez-Tamayo JC, White A, Danen EHJ, Leist M, Walker P, Jennings P, Bennekou SH and van de Water B, 2021. New approach methods (NAMs) supporting read-across: two neurotoxicity AOP-based IATA case studies. ALTEX, 38, 615–635. https://doi.org/10.14573/altex.2103051

Abbreviations

AOP	adverse outcome pathway
BfR	German Federal Institute for Risk Assessment
JRC	European Commission's Joint Research Centre
MoA	mode of action
NAM	new approach method
NGRA	next generation risk assessment
OECD	Organisation for Economic Cooperation and Development
PARC	Partnership for the Assessment of Risks from Chemicals
PBK	physiologically-based kinetic models
QSAR	quantitative structure–activity relationship
RA	risk assessment
SME	small- and medium-sized enterprise
US EPA	Environmental Protection Agency
US FDA	Food and Drug Administration
UFZ	Helmholtz Centre for Environmental Research