National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Towards the future of toxicity testing

Landscape New Approach Methodologies (NAMs) safety assessment pharmaceutical products

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Introduction

To ensure that pharmaceutical products optimally benefit human health, they need to be tested for quality, efficacy and safety. In the European Union (EU) and European Economic Area (EEA), legislative frameworks exist that require non-clinical testing, primarily using animal studies, to assess the quality, efficacy and safety of these pharmaceutical products. They include small molecules, biotechnological products (e.g. recombinant proteins, antibodies), advanced therapy medicinal products (ATMPs) and oligonucleotides. These legislative frameworks are established for pharmaceutical products consisting of a new active substance, novel pharmaceutical products or a new indication applied for marketing, which are defined under article 8(3) of directive 2001/83/EC. Generally, article 8(3) procedures are applied centralised through the European Medicines Agency (EMA), who is responsible for the evaluation and supervision of medicines in the EU. The directive prescribes the use of animal tests to support the non-clinical safety, efficacy and kinetics package, where the most animal studies are typically performed to test safety. As a general rule, short term repeat-dose toxicity tests, safety pharmacology tests and genotoxicity tests are required in the pre-clinical phase, while more lengthy and animal-intensive tests, such as long-term repeat-dose toxicity, reproductive toxicity and carcinogenicity tests are required to support later clinical phases 2 and 3.

Over the last decades, increasing attention is given to replace, reduce and refine animal studies, according to the 3R (replacement, reduction, refinement of animal use) principles, with tests that use no or less animals, or cause less harm to animals, mandated by the Directive 2010/63/EU and seen in the recent EU resolution and actions (see also Trends). A term that is increasingly being used for these methods is New Approach Methodologies (NAMs). A recent definition of NAMs encompasses simple *in vitro* tests, organotypic or microphysiological systems and *ex vivo* tissues, in silico tools for interpretation of the *in vivo* relevance of the data from these methods, (i.e. physiologically based pharmacokinetic (PBPK) models), and human low-dose testing and microdosing studies, among others. (Turner et al., 2023) (see also: Trends).

NAMs can potentially replace or reduce animal testing in the non-clinical domain.

NAMs cannot be readily implemented for use in regulatory frameworks to replace pivotal animal safety studies. Only qualified models are allowed to be used. To stimulate the uptake of NAMs as qualified tests for non-clinical safety testing of pharmaceutical products, an overview is needed of all phases and (international) organisations and regulatory bodies involved in the process. For example, guidance of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and regional guidance provide considerations for test approaches that can be used to support the development of a pharmaceutical product. These test approaches are currently mainly animal-based studies. An overview of the phases and stakeholders involved can potentially stimulate adoption of qualified NAMs.

These phases and stakeholders involved are depicted in the implementation curve of NAMs, as shown in Figure 1 and explained in Table 1, which illustrates the process towards adoption and uptake of NAMs in the pharmaceutical regulatory framework for safety assessment (RSNN, 2023). Stakeholders from (international) organisations can influence and stimulate the qualification, adoption and uptake of NAMs. Cooperation between the stakeholders in and between the phases is essential for efficient implementation of NAMs (Avila et al. 2020; Turner et al. 2023).

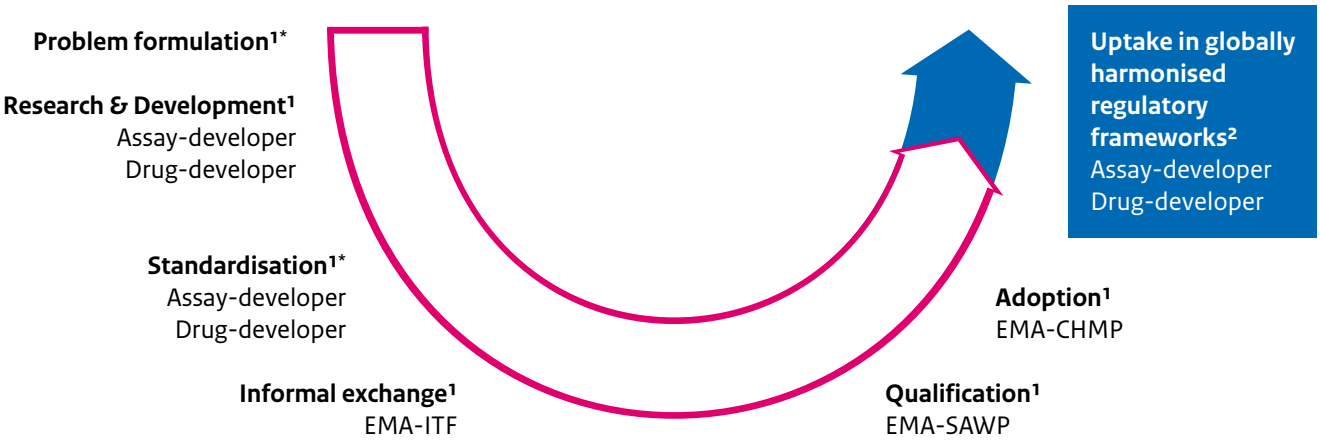
Gaining insight in the application of NAMs for pharmaceutical product development and the roles of the various stakeholders involved is an essential first step to accelerate the implementation of NAMs. This report is a follow-up of a previous report, where we explored the implementation curve for NAMs in chemical safety assessment (RIVM, 2022). In this report, we focus on NAMs for the safety assessment of pharmaceutical products. Efficacy of pharmaceutical products, quality control of pharmaceutical products during manufacturing (e.g. batch testing of vaccines), and the effect of pharmaceutical products on the environment are outside the scope of this landscape.

Also factors regarding risk management and the decision-making process around pharmaceutical products with or without the use of NAMs are not considered here.

The phases from qualification to uptake in global guidance mainly take place in an international setting where various (international) organisations and regulatory bodies are involved in consultation and decision-making about test methods. Experts from the Netherlands are active in these committees and can in that way stimulate the qualification, adoption and uptake of NAMs.

This landscape document is a reiteration of the Landscape New Approach Methodologies (NAMs) safety assessment chemical substances, which was published in 2022. Information to set up the landscape for pharmaceutical products was drawn from two workshops (RSNN, 2023) and in collaboration with the Dutch Medicine Evaluation Board (MEB).

Figure 1. The NAM Implementation curve



*This curve describes the phases a NAM could pass through, from Research & Development towards the uptake in globally harmonised guidance. Every block includes the organisations who are primarily responsible for that specific phase in the implementation curve. The white arrow represents phases for a specific context of use, the blue arrow applies to a broad context of use. [1] Specific context of use, [2] Broad context of use, * Relatively novel phases, which are further explained under "Trends". Adapted from the "Landscape New Approach Methodologies (NAMs) safety assessment chemical substances" (RIVM, 2022) and the RSNN expert workshop on NAMs (RSNN, 2023).*

Table 1. Explanation of phases in the implementation process.

Phase	Description
Problem definition*	The problem definition outlines the Context of Use for a NAM that will be developed. According to the European Medicine Agency (EMA), the Context of Use is the critical reference point for the regulatory assessment of any qualification application (EMA/750178/2017). Currently, a specific owner of this phase seems to be lacking.
Research & Development	This phase encompasses the development of a NAM, including development of *in silico*, *in vitro* and *in chemico* test (strategies) for establishing toxicity and kinetics (ADME/TK) of drugs, and models and statistical tools that are needed for the interpretation of the results.
Standardisation*	Standardisation is the process making a NAM (strategy) robust and reproducible. Currently, the definition and requirements for standardisation (criteria) is diffuse, but various initiatives are ongoing to provide clarity (see Trends).
Informal exchange	Early in the development or as an exploratory conversation on the status of an application, a developer of a pharmaceutical product or developer of a NAM (strategy) can consult the EMA Innovation Task Force (EMA-ITF), who provide informal non-legally binding feedback.
Qualification	The qualification procedure (EMA/CHMP/SAWP/72894/2008) describes the process of how a sponsor should present the a NAM and the underlying data which demonstrates that this NAM is fit for purpose within its Context of Use in drug development. The qualification procedure results in a submission of the qualification dossier to EMA. Via the Scientific Advice Working Party (EMA-SAWP), a formal qualification advice or opinion is provided by the Committee for Medicinal Products for Human Use (EMA-CHMP). Qualification opinions and letters of support are published on the EMA website.
Qualification advice	When in the early phase of qualification a NAM is not (yet) deemed acceptable for the qualification procedure, a qualification advice is given. It contains recommendations for future studies in order to generate the data required to support the proposed Context of Use of the method in drug development. This advice is not made public.
Qualification opinion	When the NAM has passed through the whole qualification procedure, a qualification opinion is given. This is a formal scientific advice in which EMA accept that a NAM can be used in a defined context-of-use. The opinion is made publicly available.
Adoption	This concerns the adoption of a qualification opinion in which a test method is accepted by the EMA-CHMP for a specific Context of Use.
Uptake in globally harmonised regulatory frameworks	This phase comprises the uptake of qualification criteria or examples of NAMs in guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The European Commission approves the (updated) guideline and implements it for the European Economic Area (EEA).

* *Phases marked with an asterisk are relatively novel phases and are further explained under "Trends".*

The implementation curve step by step

Problem definition

The problem definition outlines the Context of Use for a NAM that will be developed. In a definition by EMA this means "a full, clear and concise description of the way a novel methodology is to be used and the medicine development related purpose of the use". The Context of Use is the critical reference point for the regulatory assessment of any qualification application, thereby defining the need for and extent of qualification (EMA/750178/2017). Currently, a specific owner of this phase seems to be lacking. For more information, see Trends.

Research & Development

Research & Development (R&D) of NAMs happens at universities, universities of applied science, knowledge institutes and small and large companies (which can be pharmaceutical companies, contract research organisations, method developers, etc.), either in combination with or without development of pharmaceutical products. The pharmaceutical industry uses NAMs primarily in-house for early drug development, which support both efficacy testing and serve as screens for safety testing before moving into animal studies (Avila et al., 2020, Morgan et al., 2018, Turner et al., 2023). As opposed to regulatory safety testing, tests used to establish the efficacy of a pharmaceutical product do not need to be officially qualified. It is currently explored how NAMs could be accepted into regulatory use to support non-clinical safety testing as well (Avila et al., 2020, Turner et al., 2023). Many toxicology research projects are mainly focused on application for chemical substances, rather than pharmaceutical application (although pharmaceuticals are often used as testing compound). NAMs developed for the chemical domain could also be useful for pharmaceutical products, even though they were not primarily made for this purpose.

Funding for NAM-related projects is available from various sources, e.g. government ministries, national funding programmes, industry partners and foundations. In context of EU framework programmes research is financed through the EU Horizon Europe and Innovative Health Initiative programme (Table 2).

The development of NAMs is often not initiated with qualification criteria in mind to make them regulatory applicable. However, there are a number of projects and initiatives that do explicitly take a regulatory application perspective in mind (see examples in Table 3). Trending technologies in pharmaceutical industry are microphysiological systems, including organs-on-chips, with the promise to enhance efficacy and safety assessment of pharmaceuticals (Schneider et al. 2021). There are ongoing efforts to standardise these platforms and to make them more suitable for regulatory purposes (see Trends). Each year, the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) publishes an overview of the progress of research projects in the EU relating to NAM development (EURL ECVAM Status Report 2023).

Dutch organisations contributing to research and development of NAMs and innovative technologies:

Universities, universities of applied sciences, (biotech) industry, TNO, RIVM, hDMT, ministries (e.g. Ministry of Health, Welfare and Sport (VWS), Ministry of Agriculture, Nature and Food Quality (LNV), the MEB, national funding programmes and foundations.

Table 2. Examples of Dutch and European funding programmes for research and development of NAMs for the purpose of safety testing of pharmaceutical products.

Funding programme	Institution / organisation	Overview of ongoing projects
More Knowledge with Fewer Animals (Meer Kennis met Minder Proefdieren (MKMD))	Netherlands Organisation for Health Research and Development (ZonMw)	www.zonmw.nl/en/program/more-knowledge-fewer-animals
Nationale wetenschapsagenda (NWA)	Dutch Research Council (NWO)	www.nwo.nl/projecten
Humane meetmodellen	Samenwerkende Gezondheidsfondsen	www.gezondheidsfondsen.nl/humanemeetmodellen
-	Dutch Society for the Replacement of Animal Testing (Stichting Proefdiervrij)	https://proefdiervrij.nl/en/for-science
Horizon Europe	European Union	https://commission.europa.eu/funding-tenders/find-funding/eu-funding-programmes/horizon-europe_en
Innovative Health Initiative	European Union and pharmaceutical products industry	www.ihi.europa.eu/apply-funding
	European Partnership for Alternative Approaches to Animal Testing	https://single-market-economy.ec.europa.eu/sectors/chemicals/european-partnership-alternative-approaches-animal-testing/project-platform_en
Crack-IT	NC-3R	https://nc3rs.org.uk/crackit/view-challenges

Table 3. Examples of national and international projects focused on the development of new approach methodologies (NAMs) in which Dutch organisations have a coordinating or contributing role.

Name of project	Duration	Brief description
Centre for Animal-Free Biomedical Translation (CPBT)	2025-2035	Establishment of a centre for the development and dissemination of animal-free biomedical innovations and expertise. The centre will implement the developed methods, tools and expertise together with researchers and companies, and will offer education, training, advice, and support to enhance the acceptance and use of animal-free biomedical innovations.
NXTGEN HIGHTECH Biomedical	2023-2029	Within the applicability domain Biomedical, the projects Organ-on-a-chip and the overarching project One-Stop-Shop work on an integral infrastructure needed for manufacturing and scaling up of organ-on-a-chip models, the development of a qualification framework, needed for regulatory acceptance and the necessary standards to ensure a broad application of these innovative models, including for the safety assessment of pharmaceuticals.
VHP4Safety	2021-2026	The aim of this project is to develop a virtual platform of humans in order to better predict harmful effects of chemicals and medicines. This is done on the basis of a holistic and interdisciplinary approach that will accelerate the transition from animal testing to innovative methods.
Inno4Vac	2021-2027	The aim of this project is to harness advances in the fields of immunology, big data and artificial intelligence, 3D tissue models and human infection models, and incorporate them into the vaccine industry to accelerate the development of new vaccines.
EUROoC	2018-2023	The goals of the project are the implementation of innovative research projects aiming for industrial translation, the training of early career researchers who will carry this forward in the future, and the foundation of a network of OoC researchers.
eTRANSAFE	2017-2023	The goal of this project is to develop an integrative data infrastructure and computational methods and tools that aim to improve the feasibility and reliability of translational safety assessment during the drug development process.

Standardisation

Standardisation is the process making a NAM (strategy) robust and reproducible. Currently, the definition and requirements for standardisation (and its criteria) is diffuse, but various initiatives are ongoing to provide clarity. For more information, see Trends.

Informal exchange

Applying NAMs in non-clinical testing can be done in two ways: one could consider a formally validated NAM (see Landscape NAMs chemical substances for the process steps) that is considered qualified for pharmaceutical products as well. But more often, in pharmaceutical product safety evaluation, a NAM will be assessed on a case-by-case basis in the context of a specific pharmaceutical product or product portfolio.

When the developer of a NAM and/or pharmaceutical product wants to use a NAM to establish safety of a pharmaceutical product in the regulatory dossier, this can be discussed with the EMA Innovation Task Force (EMA-ITF). The EMA-ITF provides a multidisciplinary discussion forum which can include scientific, regulatory and legal experts from the EMA committees and/or working parties, and from the EU regulatory network, and is responsible for the **informal advice meetings**. They provide a free-of-charge forum for early dialogue with the method developer and any other stakeholder on innovative aspects in medicines development, which includes the use of NAMs in non-clinal testing. A developer or stakeholder can apply for an *ITF briefing meeting* where information and guidance can be provided for the regulatory acceptance of NAMs for a specific context-of-use. The advice that the ITF provides is not legally binding. Developers are welcome at the ITF from the moment they have a proof of concept of a NAM.

Dutch organisations involved in informal exchange:

MEB

Qualification

When a NAM is considered sufficiently mature to provide evidence for safety assessment in a regulatory submission dossier, the drug developer or the method developer can apply for a qualification of the NAM. The Scientific Advice Working Party (EMA-SAWP) is responsible for assessing a NAM in a **formal qualification procedure**. The EMA-SAWP will invite experts from the 3Rs Working Party of the EMA (EMA-3RsWP) and the Non-clinical Working Party (NcWP), or when appropriate from the EU regulatory network.

The guidance on Qualification of Novel Methodologies for Drug Development (EMA/CHMP/SAWP/72894/2008) outlines how the procedure works. The guideline on the principles of regulatory acceptance of 3Rs testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012), provides principles of regulatory acceptance of 3R methods, which is currently being updated (EMA/CHMP/CVMP/452614/2023). Considerations for successful qualification of novel methods are described in EMA/750178/2017. Based on recommendation from the SAWP, the Committee for Medicinal Products for Human Use (EMA-CHMP) either gives a *qualification advice or a qualification opinion*. If a NAM is not (yet) accepted for qualification the procedure results into the *qualification advice*, in which future protocols and methods for further method development towards qualification are recommended. This advice is not made publicly available.

The *qualification advice* may contain recommendations for future studies in order to generate the data required to support the proposed Context of Use of the method in drug development. When the new data is generated, the applicant may request a qualification opinion. Based on the review of the preliminary qualification data submitted in a qualification advice procedure, the CHMP may propose a letter of support for the NAM to encourage further studies and data sharing, which can lead to a positive qualification opinion.

When a NAM is considered suitable for further evaluation, the CHMP proceeds with the qualification procedure. If the NAM is accepted for qualification a *draft qualification opinion* will be given by the CHMP, based on recommendations from the SAWP. In this opinion, the CHMP describes the acceptability of the NAM for a specific Context of Use regarding research and development of a pharmaceutical product.

The CHMP publishes its evaluation for public consultation by the scientific community, which has six weeks to respond. Depending on the outcome, EMA may organise a workshop for clarification. All letters of support and qualification opinions are published on the EMA website.

Qualification meetings can be attended by (non-) EU regulatory agencies who have a confidentiality agreement with the EMA, e.g. the EDQM and non-EU countries.

Dutch organisations involved in qualification:

In SAWP: MEB, individual experts
In CHMP: MEB

Adoption

When the qualification procedure has been successfully completed, the EMA-CHMP may adopt the final qualification opinion. This means that they accept that the NAM can be used for a defined context-of-use in a research and development context, based on the assessment of submitted data. All EMA qualification opinions can be found on the EMA website.

Dutch organisations involved in adoption:

MEB

Uptake in globally harmonised regulatory frameworks

When a NAM has a positive qualification opinion in at least one regulatory region (e.g. EEA, United States of America, Japan) and the data is shared with the other regions, or has positive opinions in multiple regions, consensus may be reached to take up the NAMs in guidelines of the ICH. Alternatively, NAMs may not be explicitly mentioned in an ICH guidance. Rather, qualification criteria could allow the use of data generated from NAMs for product-related decision making, stand alone or in a weight of evidence approach. In summary, NAMs may be explicitly (see ICH S7B) or implicitly (see ICH S5(R3)) mentioned in ICH guidance.

The ICH brings together regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of pharmaceuticals and develop ICH guidelines. The Netherlands and other EU and EEA-countries are represented in the ICH by members of the European Commission (EC). EMA, in particularly the EMA-CHMP, supports the EC on a technical and scientific level. In the EMA-CHMP, each EEA Member State is represented by one member.

The Management Committee of the ICH can provide recommendations for harmonisation of new topics, based on topic proposals by member states. New topics as well as updates of existing guidelines go through a formal ICH procedure with well described steps. During the step of public consultation, each member country can provide input on a draft guidance.

NAMs can also be taken up in region-specific guidance. As a first step, this may stimulate further global acceptance and uptake in ICH guidance.

Dutch organisations involved in uptake:

Not applicable.

Pharmaceutical product legislation in Europe

The evaluation of the safety of pharmaceutical products is mainly regulated at the level of the EEA through EC-directives and regulations.
Various regulatory contexts are available for pharmaceutical products in the EEA related to their application (Table 4). In strict legal sense, pharmaceutical product regulations do not contain barriers for the use of alternatives to animal testing (RIVM, 2015). When alternative tests including NAMs are used for the purpose of decision making on the safety of a medicinal product, they should be qualified. In a report in 2015 it was stated that pharmaceutical product legislation does not offer many incentives for the use of alternative tests including NAMs. This is reflected in, for example, the cautious wording when talking about NAMs and that "EU-law, the European Pharmacopoeia and the guidelines issued by the ICH, the EMA and in the context of OCABR (Official Control Authority Batch Release) still refer to, require and/or set the standards for animal testing in pharmaceutical research and development or the batch release of biologicals. In this sense, there may be no formal legal barrier, but the informal barrier of soft law guidelines is almost as strong as formal law." (RIVM, 2015). More recently, a reflection paper was published that provide an overview of testing requirements, and current and newly identified opportunities for 3Rs implementation (EMA/CHMP/CVMP/3Rs/742466/2015). This reflection paper is currently being updated.

Table 4. Overview of European legal assessment frameworks for pharmaceutical products.

Regulatory context	Regulation	Brief description
Pharmaceutical products for human use	Directive 2001/83/EC	Community code relating to pharmaceutical products for human use.
Pharmaceutical products for human use	Regulation (EC) No 726/2004	Community procedures for the authorisation and supervision of pharmaceutical products for human and veterinary use and establishing a European Medicines Agency.
Pharmaceutical products for rare diseases ('Orphan medicines')	Regulation (EC) No 141/2000	Community procedure for the designation of medicinal products as orphan medicinal products and to provide incentives for the research, development and placing on the market of designated orphan medicinal products.
Pharmaceutical products for paediatric use	Regulation (EC) No 1901/2006	Rules for the development of medicinal products for human use to meet the specific therapeutic needs of the paediatric population.
Advanced therapy pharmaceutical product (ATMP)	Directive 2009/120/EC	Community code relating to pharmaceutical products for human use as regards advanced therapy pharmaceutical products. Address scientific and technical requirements needed to demonstrate the quality, safety, and efficacy of ATMPs during the development stage.
ATMP	Regulation (EC) No 1394/2007	Rules for the authorisation, supervision, and pharmacovigilance of advanced therapy medicinal products.

Besides the European directive and regulations, there are guidelines established by the EMA-CHMP. EMA mostly refers to ICH guidelines and has several additional EMA-specific guidelines (Table 5). The EMA and ICH guidelines are non-binding and do not specify the type of study required, but adhering to these guidelines gives the highest guarantee that the test results are accepted. Deviations from the guidelines need to be justified by the drug developer. Most of the guidelines provide space for NAMs to be used to replace part of the *in vivo* test, except for establishing neurotoxicity and toxicokinetics, pharmacokinetics and biodistribution.

Table 5. Overview of animal testing requirements for non-clinical studies for human pharmaceuticals, the ICH and/or EMA test guidelines and whether there is explicit mentioning of NAMs.

ICH and EMA safety guidance	Toxicological endpoint(s)	Explicit mention of possibilities to use NAMs
M3	Toxicokinetics and pharmacokinetics, and biodistribution, Chronic toxicity	Yes
S1A, B, C CPMP/SWP/2877/00 CHMP/SWP/2592/02 CPMP/SWP/372/0	Carcinogenicity	Yes
S2	Genotoxicity, chronic toxicity	Yes
S3	Toxicokinetic and pharmacokinetics, and biodistribution	No
S4 CPMP/SWP/1042/99	Chronic toxicity	No
S5 EMEA/CHMP/SWP/169215/2005 CPMP/SWP/2600/01 Final	Reproduction toxicity (fertility and developmental toxicity)	Yes
EMEA/273974/2005	Reproduction toxicity (fertility and developmental toxicity)	No
S6	Chronic toxicity	Yes
S7A	Cardiac toxicity, neurotoxicity, respiratory toxicity	Cardiac, respiratory: Yes, neuro: No
S7B	Cardiac toxicity	Yes
EMEA/CHMP/SWP/94227/2004	Neurotoxicity	No
S8	Immunotoxicity	No
S9	Chronic toxicity	Yes
S10	Phototoxicity	Yes
EMA/CHMP/SWP/2145/2000	Local tolerance	Yes
EMEA/CHMP/SWP/150115/2006 (reflection paper)	Hepatotoxicity	Yes
S11	Multiple	Yes
EMEA/CHMP/GTWP/125459/2006	Multiple	Yes
S12	Biodistribution	No

Trends

The NAMs field is in transition. A typical feature of a transition is that new terms, concepts, and frameworks are emerging. A few of these trends are explained below. Besides the numerous initiatives that are being undertaken, various (informal) networks involved in the implementation of NAMs are founded. Examples of these are listed in Table 6.

New phase: Problem definition, and Context of Use
There is an increasing realisation that for a NAM to be fit-for-purpose, the problem definition should be clear, i.e. defining the problem that needs to be solved to which a NAM can contribute. As such, it is important to define the Context of Use for the NAM that will be developed. The term Context of Use has been defined as "a full, clear and concise description of the way a novel methodology is to be used and the medicine development related purpose of the use", and is the critical reference point for the regulatory assessment of any qualification application (EMA/750178/2017). Currently, it is not yet defined who would be the owner responsible for the definition of the problem and the Context of Use. According to experts that were consulted during the RSNN workshop (RSNN, 2023), potential owners include preferably a combination of stakeholders that are either developers and end users of the NAMs, e.g. working in industry, academia, hospitals, regulatory agencies, and members of society, mainly represented by patient and animal welfare organisations. Initiation of funding rounds can direct research towards developing NAMs for specific contexts of use. These funding rounds could include requirements for multi-stakeholder collaboration and could first focus on low hanging fruits.

New phase: Standardisation
There are increasing efforts ongoing for the standardisation of NAMs. The definition of standardisation is currently diffuse. A suggestion mentioned by experts in the field is that standardisation should involve activities such as standardisation of starting materials (e.g. cell banking, dimensions of microphysiological or 3D systems), data generation, upscaling and the assessment of method for amongst others reproducibility and accuracy (RSNN, 2023).

A recent example a standardisation effort is the CEN-CENELEC Focus Group on Organ on chip for standardisation of organ-on-chip (OoC) systems, which the Netherlands chairs (TU Twente) and holds the secretariat (Nederlandse Norm). Another example are the Guidelines for Stem Cell Research and Clinical Translation developed by the International Society for Stem Cell Research that, amongst other, sets quality standard for the use of stem cells depending on its application. As such, standardisation is a prerequisite for a NAM to become qualified within a certain Context of Use. In this process, consensus-building is needed between stakeholders such as industry, governmental organisations, interest groups and standards organisations.

Establishing qualification criteria for NAMs
The EMA 3Rs Working Party (EMA-3RsWP) is currently developing guidance and position papers to define criteria towards regulatory acceptance of NAMs (EMA/CHMP/CVMP/452614/2023). For example, input for qualification criteria are now ongoing for microphysiological systems (MPS, OoCs). The availability of guidance for NAM qualification criteria for a specific Context of Use should result in well-defined, general criteria that can be applied to a wide range of NAMs for that specific Context of Use. This guidance should not restrict innovation by recommending only one or several NAMs, while still offering flexibility or update of qualification criteria when these are needed based on accumulated experience. However, too flexible, and general guidance could lack clarity. Therefore, there should be a balance between flexibility and clarity.

Advanced therapy medicinal products
The rise of novel types of pharmaceutical products comes with the need for new guidelines and an increasing realisation that the information needs to assess the safety of the product cannot always be obtained with the currently available animal experiments. This necessitates tiered risk-based strategies that define the need for and type of studies (EMA/CAT/CPWP/686637/2011). An example of such an approach are advised for ATMP development, which reveal the limited relevance

of using (immunocompromised) animals in toxicity testing and drive the need for use of NAMs (van Meer et al., 2020).

NAMs in the 3R context

In the definition of Turner et al. (2023), NAMs are not envisioned as full replacement for animal testing. Rather, there is a vision that NAMs can be effective in a testing strategy, which could result early detection of toxicity and therefore improved lead selection of pharmaceutical products that require testing further down the non-clinical testing line. In non-clinical testing, a drug developer can take a weight-of-evidence approach (which can include data obtained with NAMs, but also previous literature and historical animal data), which can result in having to do an animal test later in the process or even skip it.

European strategy facilitating the use of NAMs

The European Commission is taking action in response to the European Citizen's Initiative *Save Cruelty-free Cosmetics - Commit to a Europe without Animal Testing*'. In the response, a roadmap is proposed to set goals and formulate actions to reduce animal testing, aiming for a transition towards an animal-free testing system for amongst others pharmaceutical product legislation. The roadmap is planned to be made by 2025/2026. Additionally, at the EU-level pharmaceutical legislation is currently being revised that explicitly mentions NAMs to be used in place of animal testing. This may drive change in the uptake of NAMs for non-clinical safety testing.

Table 6. Examples of associations and networks facilitating development and implementation of NAMs in pharmaceutical product safety assessment, many of which the Netherlands is involved.

Network	Stakeholders	Goal (as defined by association /network)	Collaboration activities	Region
Transition Programme for Innovation without the use of animals (TPI)	Government, society, academia and industry	Create space for the development of models and tests without the use of animals, and increase trust in these innovations.	Activities at various levels (science, policy, society), experimenting to facilitate the uptake of animal-free innovation in many areas.	Netherlands
Institute for human organ and Disease Model Technologies (hDMT)	Universities, university medical centers and knowledge institutes	Develop and qualify cell culture models that mimic healthy and diseased human tissues based on Organ-on-Chip technology, and to facilitate their valorisation and implementation.	Share complementary expertise, facilities and ideas within consortium and outside, lobby, workshops, funding matchmaking	Netherlands
HollandBIO	Dutch biotech companies	Represents and creates a network for the Dutch biotech sector.	Network activities, representation, lobby	Netherlands
EMA 3Rs working party (3RsWP)	European experts nominated by CHMP and CVMP members	Joint working party of the EMA-CHMP and the EMA-CVMP. Advises these committees on the use of animals and application of the 3Rs principles for the regulatory testing of medicines.	Liaising with relevant stakeholders, internal training	Europe

Network	Stakeholders	Goal (as defined by association /network)	Collaboration activities	Region
European Organ-on-Chip Society (EURoOCS)	Government, NGOs, universities, knowledge institutes, industry, hospitals, policy makers	Encourage and develop Organ-on-Chip research, and to provide opportunities to share and advance knowledge and expertise in the field towards better health for all.	Annual conference, member platform, webinars	Europe
European Federation of Pharmaceutical Industries and Associations (EFPIA)	Pharmaceutical and biotech industry	Create a collaborative environment that enables our members to innovate, discover, develop and deliver new therapies and vaccines for people across Europe, as well as contribute to the European economy.	Representation, lobby, annual 3R report, forum for partners, coordination of research	Europe
European Partnership for Alternative Approaches to Animal Testing (EPAA)	European Commission, companies, branch organisations	To replace animal testing by innovative, non-animal testing methods, to reduce the number of animals used and to refine procedures where no alternatives exist	Annual conferences, forum for partners, training courses, video protocols, project teams working on development, validation, acceptance and implementation of NAMs, NAM working group	Europe
IQ 3Rs (Replacement, Reduction, Refinement) Translational and Predictive Sciences Leadership Group	Pharmaceutical and biotech industry	Promote sharing and integration of science and technology to advance the 3Rs in the discovery and development of new medicines, vaccines, medical devices and health care products for humans and animals.	Member meetings	Global
IQ Microphysiological systems Affiliate (IQ MPS)	Pharmaceutical and biotech industry	Address challenges and support the implementation of MPS in drug development.	Member meetings	Global
Health and Environmental Sciences Institute (HESI)	Universities, government, industry, NGOs	Collaboratively identify and help to resolve global health and environmental challenges through the engagement of scientists from stakeholders.	Coordinate scientific studies (experimental, frameworks, data integration), workshops, trainings	Global

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