EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

3Rs Working Party Biennial report 2023/2024

An agency of the European Union

Table of contents

Foreword by Sonja Beken, EMA 3Rs Working Party Chair

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It is a pleasure to introduce this biennial report of the EMA 3Rs Working Party (3RsWP), the first since the 2016/2017 report of the Joint CVMP/CHMP 3Rs Working Group. This report clearly reflects EMA's continued commitment to the 3Rs principles—Replacement, Reduction and Refinement—in regulatory testing of medicines for human and veterinary use.

Since its establishment in 2022, the 3RsWP has embraced its transversal mandate, revising key guidance documents, updating its reflection papers providing an inventory on animal testing requirements and current and future opportunities for 3Rs implementation, initiating the drafting of context-of-use based qualification criteria for complex *in vitro* models, and promoting 3Rs implementation in batch release testing. We have fostered stakeholder dialogue, launched the International Medicines Regulators' Working Group on 3Rs, and contributed to EU and global initiatives including the EC Roadmap on phasing out animal testing.

As Chair, I am proud of the profound dedication shown by our members, experts and drafting groups, and EMA staff. Together, we are shaping a future where regulatory science, technological innovation, and ethical responsibility converge, ensuring the 3Rs actively drive transformation in medicines testing.

1. Executive summary

This report provides an overview of the EMA's key activities and achievements in 2023 and 2024 related to the 3Rs principles of replacement, reduction and refinement of animal use for medicines development. These two years represent a hugely significant period in terms of the Agency's commitment to the 3Rs, covering some of the first plenary meetings of the standing 3Rs Working Party (3RsWP), established in 2022. It describes the advances made in relation to the provision of guidance, engagement with stakeholders, and the development of training on 3Rs. It also describes the strategic focus of the Agency and the working party to support integration and acceptance of 3Rs-compliant, new approach methodologies (NAMs) that have the potential to replace or reduce animals used for regulatory testing.

Highlights over the reporting period include: the publication of the first 3RsWP work plan (as part of the EMA Non-clinical Domain), the conduct of the working party's first two annual stakeholder meetings and the establishment of a European Specialised Expert Community (ESEC) on NAMs and an Operational Expert Group (OEG) on batch release testing. Additionally, three key guidance revisions were initiated, a new reflection paper was developed, and ongoing procedural work to support EMA's Innovation Task Force (ITF) and Qualification of novel methodologies procedures, where 3Rs-relevant, was provided.

The work carried out over this period is a continuation of EMA's long standing commitment to the 3Rs. However, the Agency last reported on 3Rs activities for the period of 2016/2017. This is due, in part, to the challenges posed by Brexit (resulting in the relocation of EMA), and subsequently by the COVID-19 pandemic. These difficult circumstances resulted in the introduction of business continuity plans (BCP), which restricted some of the Agency's activities to ensure continuation of core, procedure-related work. These BCP measures were lifted in May 2023.

Given the challenges faced, we are pleased to reintroduce this reporting activity as an opportunity to showcase EMA's ongoing work on 3Rs. NAMs research and development has grown exponentially over the last number of years, and at present, there is significant momentum towards integration of these methods in a regulatory context. As such, this is an exciting period for the 3Rs field, with EMA playing an active role.

2. Introduction

As described in the 2016/2017 report, EMA has been active in 3Rs since 2010 when, aligned with the publication of Directive 2010/63/EU, the Joint CHMP/CVMP Expert Group on 3Rs (JEG 3Rs) was established. This was succeeded in 2016 by the Joint CVMP/CHMP Working Group on the Application of the 3Rs in Regulatory Testing of Medicinal Products (J3RsWG). Both groups were mandated to provide advice and recommendations to the Agency's human and veterinary committees on all matters relating to the use of animals in regulatory testing of medicinal products.

In 2020, EMA published its Regulatory Science Strategy (RSS) to 2025, with the aim of building a more adaptive regulatory system and encouraging innovation in human and veterinary medicine. This contained specific recommendations in relation the 3Rs and NAMs, signalling the topic as a strategic priority for EMA. It also reinforced the importance of having a central group for the coordination of 3Rs activities, with links to the relevant working parties of EMA's scientific committees. As a result, in 2022, as part of the reorganisation of its working parties, EMA created the 3RsWP, the Agency's first permanent, standing group on 3Rs. As with its predecessor groups, the 3RsWP is a joint working party of CHMP and CVMP and comprises experts from human and veterinary fields from member state national competent authorities. The membership is based on the best available expertise across the network and a small core working party is supported by ancillary expert communities, operational expert groups and drafting groups for the provision of guidance.

Figure 1: Schematic timeline of EMA 3Rs activities

The 3RsWP and its Work Plan

The EMA working parties are organised in five topical domains (i.e. quality, non-clinical, clinical, methodology and veterinary); 3RsWP is under the governance of the non-clinical domain, alongside the Non-clinical Working Party (NcWP), recognising that there are shared interests across these groups. The 3RsWP initially comprised six members; however, this was increased to eight members in 2024 with the support of EMA management and the Domain Governance, as a recognition of the increasing importance of the 3Rs from a global political perspective, placing additional expectations and demands on the working Party.

With the 3RsWP now in place as the focal hub for 3Rs activities at EMA and across the European Medicines Regulatory Network (EMRN), one of the first actions was the development and publication of a comprehensive and ambitious work plan. The first Non-clinical Domain Consolidated Work plan covered the period from May 2022 to December 2024; it identified topics where NcWP and 3RsWP were in the lead and, in relation to 3Rs and NAMs, incorporated the recommendations from the Regulatory Science Strategy. The work plan is organised by strategic, tactical and operational goals and the initial publication was supplemented by the publication of priorities for 2023 and 2024 respectively. The work plan has been the main driver for work conducted in 2023/2024 as outlined below.

3. Guidance Activities

A primary tactical goal of the 3RsWP as outlined in the work plan is to continuously review existing guidance and evaluate emerging regulatory science in order to identify opportunities for new or improved guidance. This is to encourage and support increased use of 3Rs-compliant methodologies by medicines developers

3.1. Revisions of existing guidance

3.1.1. Reflection papers on the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs

A flagship activity of the EMA's previous 3Rs working groups, continued by 3RsWP, has been to provide an inventory of regulatory provisions that require(d) animal testing, and opportunities for application of the 3Rs in the form of reflection papers; these opportunities are categorised as 'implemented' (e.g. already possible under existing guidance) or 'newly identified' (i.e. likely to become available in the future). The 3RsWP identified a need to update the two reflection papers relating to human and veterinary medicinal products, originally published in 2018, to ensure the most up-to-date and state-of-the-art guidance and 3Rs opportunities are included. Subgroups of the 3RsWP began the significant undertaking of revising these reflection papers in 2023, consulting relevant EMA working parties/committees to ensure completeness of the overview. The drafts were finalised and adopted in 2024, with release for public consultation in early 2025 (EMA/CHMP/CVMP/3Rs/742466/2015 Rev. 1, EMA/CHMP/CVMP/3Rs/164002/2016 Rev. 1).

3.1.2. Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches

Since this guideline's initial implementation in 2012, scientific, technological and regulatory knowledge on 3Rs-compliant methods has significantly evolved. Therefore, there was a need for more up-to-date, specific guidance to define the regulatory acceptance criteria for specific models. A concept paper (EMA/CHMP/CVMP/452614/2023) outlining the planned revision of the "Guideline on the principles of regulatory acceptance of 3Rs testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012)" was released for public consultation in 2023. It summarises the proposed stepwise revision of the guideline. As well as a revision of the core text of the current guideline, this proposed revision will include a terminology section to provide clear and aligned definitions of critical 3Rs-related terminology. In addition, two annexes are being prepared that will provide regulatory acceptance criteria for complex *in vitro* models (CIVMs) for specific contexts of use (cardiac CIVMs for safety pharmacology testing and CIVMs, such as liver-on-chip, for detecting drug induced liver injury (DILI)). These were chosen based on the state-of-the-art of CIVMs, including micro physiological systems (MPS) such as organ-on-chip technologies, the current large-scale initiatives related to performance assessment of CIVMs (e.g. IQ MPS Affiliate; European Organ on Chip Society (EUROoCs)), the main causes of safety-related drug attrition, and the maturity of current regulatory guidance (e.g. Q&A ICH S7B/E14). The revision aims to provide detailed information for applicants in order to further foster the regulatory acceptance of NAMs. During the public consultation, more than 180 comments were received from 20 interested parties, which are now being incorporated in the ongoing revision. The drafting is being overseen by a 'steering group' that coordinates the work of sub-groups responsible for drafting specific sections of the revised guideline (e.g. annexes, terminology section). These drafting groups are composed of relevant experts from the 3RsWP and the NC NAMs ESEC (see section 5.1).

3.1.3. Guideline on non-clinical local tolerance testing of medicinal products

This guideline (EMA/CHMP/SWP/2145/2000-Rev.1) was last updated in 2016. The guideline for human medicinal products was developed by EMA's Safety Working Party (currently NcWP), and in 2023 the 3RsWP proposed a second revision to bring the guidance in line with best practices in relation to 3Rs. The proposal was accepted by NcWP and incorporated into the work plan as a joint activity of both working parties. The proposed revision will include an increased emphasis on *in vitro* testing, especially with regards to skin and eye irritation. In addition, the guideline will integrate the use of NAMs for assessing skin sensitisation potential of topically applied pharmaceuticals in accordance with OECD test guidelines 442C, 442D, 442E, and 497. OECD Guideline 497 on defined approaches on skin sensitisation uses *in silico*, *in chemico* and *in vitro* test data (TG 442C-E) to reach a conclusion on potential dermal sensitisation hazard. These defined approaches have been demonstrated to provide the same, or increased, level of information compared to the murine Local Lymph Node Assay for hazard identification. A joint expert drafting group has been established with work on this revision beginning in 2025.

3.1.4. Veterinary user safety guidelines

In accordance with updated veterinary medicines legislation, revisions of both the Guideline on user safety for pharmaceutical veterinary medicinal products (EMA/CVMP/543/03-Rev.1) and the Guideline on user safety of topically administered veterinary medicinal products (EMA/CVMP/SWP/721059/2014)) were initiated, led by CVMP Safety Working Party (SWP-V). This was identified as an additional opportunity to strengthen the 3Rs provisions in these guidelines. As a result, the 3RsWP is contributing to both drafting groups. Similar to the revision of the guideline on non-clinical local tolerance testing, the proposed revisions will include reference to the OECD *in vitro* methods for local tolerance testing as well as cross referencing the veterinary reflection paper on 3Rs opportunities (see section 3.1.1). Furthermore, the overarching user safety guideline will include additional considerations on "significant user exposure" to avoid unnecessary *in vivo* developmental toxicity testing where exposure to the veterinary medicinal product is expected to be negligible. In addition, the topical product guideline will include a tiered approach for the derivation of toxicological reference values to potentially avoid *in vivo* testing for dermal toxicity. The two guidelines are expected to be released for public consultation at the end of 2025.

3.2. New guidance

3.2.1. Reflection paper on non-human primates (NHPs) in safety testing of human medicinal products and opportunities for 3Rs implementation

Directive 2010/63/EU recognises specific ethical and practical concerns regarding studies using NHPs. This directive limits their use to biomedical areas that are essential for human benefit and where no other alternative replacement methods are yet available. During the COVID-19 pandemic, the preexisting shortage of NHP specimens was further exacerbated, prompting a critical assessment of regulatory opportunities to minimise NHP use for medicine safety testing. As a joint activity of the NcWP and 3RsWP, drafting of a Reflection Paper on the topic commenced in 2023. It was acknowledged that significant work was ongoing in this area by the pharmaceutical industry and other stakeholders, and thus to avoid duplication of efforts, an ad hoc Interested Parties meeting was held by EMA, bringing together experts from the European Federation of Pharmaceutical Industry Associations (EFPIA), the Health and Environmental Sciences Institute (HESI), the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) and the UK National Centre for 3Rs (NC3Rs). This meeting served as an initial brainstorm for the development of the reflection paper, which was progressed throughout 2024. Its release for public consultation is expected in Q4 of 2025.

3.3. Input to external guidance

In addition to the development and revision of EMA guidance documents, the 3RsWP has also provided comments on a number of key guidances developed by the World Health Organisation (WHO) as part of their consultation processes. These included the draft "Guidelines on the phasing out of animal tests for the quality control of biological products" and the "Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of COVID-19", a proposed addendum to Annex 2 of WHO Technical Report Series, No.1048.

Furthermore, the 3RsWP provided comments on guidances developed by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). These included the VICH guideline 22 "Studies to evaluate the safety of residues of veterinary drugs in human food: reproduction testing" and the VICH guideline 23 "Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing".

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>
> **Sonja Beken**,
> EMA 3Rs Working Party Chair

4. Stakeholder interaction

A central tenet of the 3RsWP work plan is communication and engagement with stakeholders on an EU and international level. The 3RsWP has, as permanent observers, European Commission (EC) Services (DG SANTE, DG RTD, DG ENV, DG JRC (EURL ECVAM)) and the European Directorate for the Quality of Medicines and Healthcare (EDQM). DG GROW was added as an observer to the 3RsWP in 2024, given their leading role in the development of the "Roadmap towards phasing out of animal testing for chemical safety assessment" (see also section 4.2). The contribution of a wide variety of stakeholders in the 3Rs field is recognised, and a core group of relevant interested parties was established, in collaboration with EMA's stakeholder and industrial liaison offices. These include pharmaceutical industry trade associations, animal welfare and non-governmental organisations (NGOs), research consortia, EU agencies for food and chemical safety ECHA and EFSA and the Organisation for Economic Cooperation and Development (OECD). These stakeholders receive targeted communications from EMA relevant to 3Rs and are invited to participate in annual stakeholder meetings. The stakeholder group is dynamic, and the 3RsWP welcomes the possibility of including additional relevant stakeholders

4.1. Annual stakeholder meeting

Since its inception, the 3RsWP has hosted annual stakeholder meetings. In addition to sessions with invited stakeholders, these meetings have included a public session with an open broadcast presenting the 3RsWP work plan and annual priorities. In addition, these meetings provide an opportunity to stakeholders and the public to comment and provide their views on the working party's activities (see meeting reports from 2023 and 2024). These meetings have been instrumental in shaping the working party's activities, as well as giving a perspective of the ongoing work across sectors. Some key elements emerging from

these meetings as being of high priority for stakeholders include the validation/qualification of NAMs, harmonisation of regulatory acceptance criteria, and international cooperation in relation to 3Rs.

4.2. Collaboration with European Commission and other EU agencies

As outlined above, the 3RsWP engages with a number of key European stakeholders as observers or interested parties. In 2023/2024, this engagement intensified, partially due to the development of a "Roadmap towards phasing out of animal testing for chemical safety assessment" by the EC. This action was in response to a European Citizen's Initiative (ECI), "Save Cruelty Free Cosmetics", which received over 1 million signatories across 22 EU member states. In July 2023, the Commission published its response to the ECI, including a commitment to the development of a roadmap outlining milestones and specific actions, to be implemented in the short to longer term, to reduce animal testing across relevant pieces of chemical legislation. Human and veterinary medicines legislations are included in the scope, and the commission communication states that the development of the roadmap will be supported by the expertise of EMA. The roadmap is restricted to safety assessment of chemicals, which excludes other types of medicinal products (biotechnology-derived products such as antibodies, vaccines, gene and cell therapy, blood products, proteins and peptides) regulated by EMA. It also excludes animal testing that is required to demonstrate efficacy of a medicinal product, the mechanism of action, or as proof-of-concept for the effect in a particular indication (e.g. disease models), as well as testing required to demonstrate the product meets certain quality requirements. However, the roadmap activity is considered a valuable opportunity to showcase EMA's dedication to 3Rs advancements. EMA has participated in the EC and Agencies Interservice Group, supporting the roadmap development, as well as a number of working groups established to contribute to the drafting. EMA has also worked extensively with ECHA and EFSA to identify areas of common interest (while acknowledging sector-specific differences), and to ensure sharing of knowledge and learnings where relevant.

4.3. International Medicines Regulatory Working Group 3Rs (IMRWG3Rs)

Internal discussions within the 3RsWP and feedback from stakeholders identified the need for increased global regulatory alignment on 3Rs. Given the global context in which medicines are developed, a harmonised approach is required to achieve successful integration of NAMs and meaningful reductions in animal use. With this in mind, EMA led in the establishment of an "International Medicines Regulators' Working Group on 3Rs (IMRWG3Rs)". The group consists of international medicines regulators from the USA (FDA), Canada (Health Canada), Switzerland (Swissmedic), Australia (TGA), and Japan (PMDA). The first meeting of the group was held in January 2024 and the terms of reference, outlining the objectives of the group, were adopted in October 2024. The overarching aim of the IMRWG3Rs is "To foster a consistent global approach across regulatory jurisdictions to achieve internationally harmonised 3Rs recommendations and assist in the implementation of new alternative approaches for testing of human and veterinary medicinal products, wherever possible". Other specific goals agreed by the group include facilitating agreement on acceptance criteria for NAMs, encouraging broader application of the 3Rs in batch release testing and developing a regulatory position paper on 3Rs to be shared with other medicines regulatory authorities.

4.4. Other outreach activities

During 2023 and 2024, EMA and/or members of the 3RsWP have actively promoted the work of the working party through participation in scientific and regulatory conference and events, as well as important collaborative fora in which 3Rs and NAMs are discussed. EMA is represented on the scientific committee of the European Partnership for Alternative Approaches to Animal Testing (EPAA) and participates in the OECD Advisory Group on Emerging Science in Chemicals Assessment (ESCA). Meetings in which EMA and/or the 3RsWP has participated in 2023/2024 include: the MPS World Summit, the Safety Pharmacology Society Annual Meeting, the Drug Information Association (DIA) Global Meeting, EUROoCs Annual Meeting, the Global Summit on Regulatory Science, the EPAA Annual Meeting, as well as meetings/symposia organised by the Federal Agency for Medicines and Health Products under the Belgian Presidency of the Council of Europe, ECHA, EFSA, The Organisation for Professionals in Regulatory Affairs (TOPRA), Humane Society International (HSI), The Danish 3R-Center, the Innovative Health Initiative (IHI) and the National Contact Points (NCP) for implementation of Directive 2010/63/EU.

Furthermore, given the societal interest in 3Rs, the agency frequently responds to requests for information on the topic from members of the public, animal welfare organisations and the press. These include questions posed through the public platform, AskEMA, the EMA Press Office and the 3Rs mailbox. In 2023/2024, EMA staff participated in an TV documentary produced by NDR/ARD (German public broadcaster) and in an interview with the French daily newspaper Le Monde on 3Rs and NAM-related topics.

EMA is committed to continued engagement with stakeholders and outreach as well as improving its communication strategy in relation to 3Rs. It is however acknowledged that this requires significant allocation of time and resources.

5. Establishment of Expert Groups

Per the EMA Working Parties Model, small working parties, such as 3RsWP, are supported in their activities by drafting groups, operational expert groups (OEG) and European specialised expert communities (ESECs).

5.1. Non-clinical and new approach methodologies ESEC

The Non-clinical and New Approach Methodologies ESEC (NC NAMs ESEC) was established with the aim of providing a platform for information sharing and communication to support the delivery of the Non-clinical Domain Work plan. More specifically, given that a lot of research and development of NAMs occurs within an academic setting, the community is intended to include experts from both inside and outside the EMRN with expertise in *in vitro*/*in silico* models, human cell-based systems that facilitate improved translation of non-clinical data, qualification/validation of NAMs, *in vitro*/*in vivo* extrapolation and non-animal alternatives to batch release or quality control testing of medicines.

The mandate, objective and rules of procedure of the NC NAMs ESEC was published in August 2023, and an open call for experts launched. As of the end of 2024, the community comprised 114 members, 48 of whom are part of NcWP, 3RsWP and the veterinary Safety Working Party (SWP-V) and automatically included. The additional participants are predominately assessors from the EMRN, though academia, EU agencies and NGOs are also represented.

The NC NAMs ESEC, overseen by the 3RsWP has held a series of webinars on topics including regulatory requirements and opportunities for implementation of the 3Rs (in line with the above-mentioned Reflection Papers), qualification frameworks from the perspective of EMA and EURL ECVAM and NAMs for cardiotoxicity from an academic and regulatory perspective. Webinars planned for 2025 include the topics of NAMs for skin sensitisation and read-across approaches for non-clinical risk assessment.

ESEC members are eligible, subject to an evaluation of their declared interests, to participate in guidance drafting groups and members of the NC NAMs ESEC are contributing to the revisions of the guidelines on regulatory acceptance of 3Rs testing approaches and non-clinical local tolerance testing.

5.2. Batch Release Testing OEG

During the mandates of JEG 3Rs and the J3RsWG, these working groups reviewed protocols related to the batch release testing of a significant number of human and veterinary medicinal products authorised via the centralised procedure between 1996 and 2013, with a view to promote and implement 3Rs-compliant testing methods within these batch release processes as far as possible.

In 2024, the Batch Release Testing OEG (BRT OEG) was established under the oversight of the 3RsWP. The group was mandated to review the batch release and quality control testing in centrally authorised veterinary and human medicinal products between 2013-2023. The OEG consists of EU network and 3RsWP experts who are tasked with reviewing the *in vivo* batch release or quality control testing protocols in quality dossiers of marketed products to identify potential 3Rs opportunities. Based on these assessments, letters are sent to the marketing authorisation holders (MAHs) providing 3Rs recommendations where appropriate. During the reporting period, this process was completed for veterinary biological products (67), resulting in letters being sent on behalf of CVMP to MAHs of 13 products. The equivalent process is ongoing for human biological products (297). Recommendations for VMPs included use of available non-animal alternative methods for batch release/quality control or encouraged method development/refinement. They also provided information on opportunities for interactive dialogue with EMA to support the development of novel 3Rs-compliant methods e.g. the Innovation Task Force (see Section 7).

During the review of the veterinary biologicals, the OEG identified opportunities for the revision of several European Pharmacopoeia (Ph. Eur) monographs where relevant *in vitro* methods are now available. These were communicated to the relevant EDQM expert group.

6. Training

The Non-clinical domain work plan included, as a tactical goal, the development of training activities on 3Rs methods/best practices and their application, and facilitation of information exchange through the NC NAMs ESEC.

The EU Network training centre (EU NTC) is a platform offering training opportunities to the staff of medicines regulatory authorities in the EMRN and is overseen by the EMA and Heads of Medicines Agencies (HMA). Training resources can be accessed via a single digital platform and are organised into curriculum frameworks to deliver comprehensive training in specific knowledge areas. A number of 3Rs trainings were already available via the EU NTC, and included mainly within non-clinical or veterinary curricula, or in some cases unassigned to a specific curriculum.

Notable 3Rs trainings added to the EU NTC catalogue in 2023/2024 included courses on the revision of ICH S5 (NAMs in the frame of DART testing programs) and a comprehensive course on CIVM and MPS, provided by the IQ Consortium MPS affiliate. In addition, recordings of all webinars organised in the frame of the NC NAMs ESEC have also been added to the EU-NTC platform. By increasing 3Rs training offerings within the EU NTC, the overall objective is to increase knowledge and awareness of 3Rs and NAMs topics amongst assessors, thereby increasing confidence in NAM data submitted for regulatory acceptance.

The 3RsWP has engaged in mapping exercise of training resources within the EU NTC to identify those directly addressing the 3Rs and those which mention 3Rs opportunities indirectly. Based on this analysis there may be scope for the development of a specific 3Rs curriculum, which will be explored in the future.

7. Operational activities/ interaction mechanisms

EMA fosters regulatory acceptance of NAMs by offering various mechanisms by which 3Rs method developers can interact with EMA at different stages of development. These include ITF briefing meetings, qualification of novel methodologies (QoNM) procedures, scientific advice, portfolio and technology meetings (PTMs) and voluntary data submission (VDS) (“safe harbour”). The 3RsWP provides regular scientific and regulatory input to these procedural activities. In particular, the ITF and QoNM identify the 3Rs as areas of special interest, and developers of new 3Rs-compliant methodologies are encouraged make use of these opportunities to interact with regulators from the EMRN.

The ITF provides developers with a forum for early dialogue with EMA on innovative medicines and novel methodologies. Analysis has shown a steady increase in the number of requests for ITF briefing meetings in relation to 3Rs and NAMs in 2023/2024 compared with previous years. This information was compiled by the EMA’s Regulatory Science and

Innovation Task Force and included in a Horizon Scanning report on NAMs published in 2025. The report also includes data on the number of PTMs and scientific advices pertaining to the 3Rs and NAMs. The overall goal of the horizon scanning report is to enable the EMRN to proactively respond to forthcoming challenges and opportunities related to the development and integration of NAMs in medicines development.

NAM developers can apply for a QoNM procedure if they have generated sufficient and robust data to demonstrate the utility and regulatory relevance of a NAM for a specific context of use. A context of use describes the circumstances under which the NAM is applied in the assessment of human or veterinary medicines. A qualification team composed of experts from EMA and the European medicines regulatory network then assesses the data submitted to support the use of the NAM.

In both 2023 and 2024, 3RsWP members participated in 10 ITF briefing meetings related to 3Rs or more general non-clinical topics and 3RsWP members have also been part of the qualification teams for ongoing QoNM procedures.

8. Collaborative success stories

8.1. Overall reduction in animal testing (ALURES data)

Since the implementation of Directive 2010/63/EU, member states of the EU are legally obliged to submit information on the statistics of the animals they used for scientific purposes. The EC makes this information available through a public open access database, ALURES. Within this database it is possible to filter animal use by high-level purpose (e.g. regulatory use) and specific use categories (e.g. batch potency, specify toxicity testing categories such as skin sensitisation). Moreover, uses can be associated with specific legislations including those on medicinal products for human use and on medicinal products for veterinary use and their residues. Currently available reporting years in ALURES are from 2015 to 2022. A comparison of the total numbers of animal uses per year for regulatory purposes related to medicines between 2015 and 2022 revealed a reduction of over 1 million (1,886,694 in 2015 vs 820,874 in 2022). Further comparisons in specific categories of use during the same period showed that batch potency testing was reduced by 57%, pyrogenicity testing fell by 53% and skin sensitisation testing by 76%. While these numbers do not relate to the period covered by this report, they show that significant strides have been made in relation to reduction of animal use in medicines regulatory testing since the introduction of the Directive and the initiation of EMA's activity on 3Rs. Once available, the ALURES data for 2023 and 2024 will be reviewed to determine whether continued 3Rs improvements can be identified.

8.2. Botulinum potency testing

In April 2023, the European Coalition to End Animal Experimentation (ECEAE) presented a petition to EMA calling for the abolition of the mouse potency (LD_{50}) test for botulinum neurotoxin-containing products, comprising over 164,770 signatures. Based on the current

wording of the European Pharmacopoeia (Ph. Eur) monographs for botulinum neurotoxin, a complete abolition of the *in vivo* potency test is not possible at present. However, EMA's 3RsWP is committed to the full application of the 3Rs principles with respect to botulinum neurotoxin testing, and the submission of this petition was recognised as a significant call for action.

The 3RsWP sought supplementary expertise from the EMRN in relation to quality control and batch release, specifically in relation to botulinum neurotoxin products, and determined that, while alternative cell-based methods are now routinely used for the determination of botulinum neurotoxin potency for final lot release, *in vivo* LD_{50} testing is still being used in other stages of the botulinum neurotoxin production process (e.g. drug substance (DS) release and stability testing, DS characterisation, reference standard qualification and stability testing, validation of alternative assays, and process validation). The 3RsWP agreed to draft a letter to MAHs of botulinum neurotoxin products querying their use of animals at different stages within the manufacturing process, any hurdles faced in implementing *in vitro* assays at these stages, and their efforts to refine *in vivo* potency testing where it is still required.

For botulinum neurotoxin products authorised within the EU, thirty-seven individual MAHs were identified. However, all of these were found to belong to seven parent companies. With the support of EMA's committees, letters were sent by the chairs of the 3RsWP to the QPPV (qualified person responsible for pharmacovigilance) of each of the parent MAHs. It was determined that two of these seven companies hold a marketing authorisation for the purposes of parallel import and distribution only and are not engaged in any animal testing. The remaining five MAHs provided a response to the request for information, indicating their willingness to engage with EMA and the 3RsWP. Where animal numbers were provided, they were generally for 2022.

Some of the key results of the survey are listed below:

- One MAH did not carry out any *in vivo* testing within the EU
- Only one MAH used *in vivo* testing drug product (DP) release, with a relatively small number of animals used
- As anticipated, most of the animal use was in other production steps. Those listed in the responses included:
  - Reference standard qualification
  - Analytical method validation and in-process control testing
  - Characterisation of drug substance (DS)
  - DS release and stability testing
  - Equivalence testing
  - Operator training
- For four of the MAHs, cell-based potency assay (CBPA) alternatives for DS stability and release testing are either in development or at regulatory review stage
- Of the four MAHs conducting *in vivo* potency in the EU, three noted the inclusion of humane endpoints as a refinement measure

- The total number of animals used in 2022, as declared by the surveyed MAHs was 13,340. However, one MAH carried out reference standard testing, but did not declare the number of animals used.

The company using *in vivo* testing for DP release has since switched to an *in vitro* alternative method (via EMA variation procedure in 2023). One company that was performing equivalence testing no longer perform this test routinely, in accordance with EMA scientific advice.

Overall, the MAH responses demonstrated that alternative methods are already routinely used for final lot (DP) release. Replacement of *in vivo* DS stability and release testing with non-animal alternatives will help to further reduce the number of animals used to the absolute minimum feasible. The 3RsWP is committed to support and encourage the regulatory acceptance of alternative methods for the replacement of *in vivo* DS stability and release testing, in collaboration with quality assessment colleagues. The required use of *in vivo* testing to satisfy regulatory requirements in regions other than the EU was noted by MAHs as a hurdle for implementation of *in vitro* alternatives. The IMRWG3Rs represents a useful forum for further discussion on this topic towards a more harmonised global position. The 3RsWP, in collaboration with EMA committees and working parties, will monitor new applications for botulinum neurotoxin products to ensure all possible 3Rs opportunities are implemented with respect to potency testing (including preauthorisation scientific advice procedures).

8.3. Phase out of the rabbit pyrogenicity test

While the ongoing efforts towards phasing out of rabbit pyrogenicity (RPT) testing is not an EMA-led activity, significant milestones were achieved in support of this activity in 2023/2024, led by EDQM. Validated *in vitro* alternatives to this *in vivo* test are available, and EDQM has been at the forefront of efforts to promote their use. In June 2024, the RPT was definitively removed from the Ph. Eur texts. A new general chapter on pyrogenicity will be added outlining a risk assessment approach to determining the appropriate *in vitro* alternative to control the pyrogenicity of a product (e.g. the monocyte-activation test). This will be the responsibility of medicine developers and will have an implementation date of July 1st, 2025. The details of the Ph. Eur revision are included in the revised Reflection Papers on the current regulatory testing requirements for human and veterinary medicinal products and opportunities for implementation of the 3Rs. The EMA Quality Domain have worked to develop guidance for applicants and MAHs on the procedures necessary to remove the RPT from marketing authorisation dossiers, which will be published in 2025.

9. 3Rs objectives going forward

The 3RsWP and its secretariat at EMA are a small but highly dedicated group that managed to achieve significant progress in the first two years following the group's establishment. The coming years will present further challenges and opportunities in the area of 3Rs; to address these, a new work plan has been developed to cover the years 2025-2027 and the 3Rs will continue to be supported by strategic policy of the EMA, as outlined in the European Medicines Agency Network Strategy (EMANS) to 2028.

Some key highlights of the new work plan will be to publish the stepwise revision of the Guideline on principles of regulatory acceptance of 3Rs testing approaches, to continue to foster international harmonisation efforts through the IMRWG3Rs and to support the delivery and implementation of the EC Roadmap towards phasing out of animal testing for chemical safety assessment. In addition, the work plan addresses the use of artificial intelligence (AI) in relation to non-clinical assessment and 3Rs; the domain is committed to tracking developments in this area and to supporting the integration of such methods in guidance and in practice, when appropriate. With respect to modelling and simulation, it is also planned to increase existing collaboration with the EMA Methodology Domain to support the regulatory integration of *in silico* methods supporting the 3Rs.

Another important topic on the horizon for the 3Rs is the implementation of the new pharmaceutical legislation for human medicinal products. In 2023, the EC adopted the EU Pharmaceutical Legislation Package which includes a requirement for applicants to demonstrate that the 3Rs principles have been applied in compliance with Directive 2010/63/EU. The package also indicates flexibility for the incorporation of NAMs, though the practicalities and implications for applicants are still to be clarified.

EMA and 3RsWP, in collaboration with its observers and stakeholders, will continue to support implementation of the 3Rs, while applying scientific rigour to ensure the safety of patients and healthy volunteers.

European Medicines Agency

Domenico Scarlattilaan 6
1083 HS Amsterdam
The Netherlands

+31 (0)88 781 6000

www.ema.europa.eu

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of the European Union