Conference Report

Alternatives to Laboratory Animals
2024, Vol. 52(1) 60–68

DOI: 10.1177/02611929231218378
journals.sagepub.com/home/atl
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Brazilian National Network of Alternative Methods (RENAMA) 10th Anniversary: Meeting of the Associated Laboratories, May 2022

Renato Ivan de Ávila[1], Julia Fentem[1], Izabel Villela[2], Debora Somlo[3], Ana Marisa Fusco Almeida[4], Maria José S. Mendes-Giannini[4], Andrezza Di Pietro Micali Canavez[5], Bruna Bosquetti[5], Carolina Motter Catarino[5], Desiree Cigaran Schuck[5], Bruna Nardy Valadares[6], Gustavo Facchini[7], Bianca Marigliani[8], Ana Carolina Migliorini Figueira[9], Rosângela Hickson[10], Daniela Morais Leme[11], Carlos Tagliati[12], Larissa C.R. de Souza[12], Silvya Stuchi Maria Engler[13], Lorena Rigo Gaspar Cordeiro[14], Janice Koepp[15], Jose Mauro Granjeiro[16], Humberto de Mello Brandao[17], Michele Munk[18], Katherine Antunes de Mattos[19], Bruna Pedralli[20], Marcella Miranda Siqueira Furtuoso Rodrigues[20], Ana Clara Stival[20], Jordana Andrade[20], Lara Barroso Brito[20], Thais Rosa Marques dos Santos[20], Jacqueline Leite[20,21], Artur Christian Garcia da Silva[20] and Marize Campos Valadares[20]

[1]Unilever's Safety and Environmental Assurance Centre (SEAC), Colworth Science Park, Bedfordshire, UK
[2]InnVitro Support and Management in Toxicology, Porto Alegre, Brazil
[3]Unilever Brazil Industrial Ltda, WTorre Morumbi, São Paulo, Brazil
[4]Laboratory of Proteomics and Clinical Mycology, Department of Clinical Analysis, Faculty of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil
[5]Safety Assessment Management, Grupo Boticário, Curitiba, Brazil
[6]T&E Analytics, Analytical and Scientific Center, Campinas, Brazil
[7]Kosmoscience Group, Campinas, Brazil
[8]Research and Toxicology Department, Humane Society International (HSI), Rio de Janeiro, Brazil
[9]National Biosciences Laboratory, CNPEM, Campinas, Brazil
[10]Promove Faculty, Belo Horizonte, Brazil
[11]Department of Genetics, Federal University of Paraná, Curitiba, Brazil
[12]Lab Tox, Federal University of Minas Gerais, Belo Horizonte, Brazil
[13]Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil
[14]Faculty of Pharmaceutical Sciences of Ribeirão Preto, Ribeirão Preto, Brazil
[15]Biocelltis Biotechnology SA, Florianópolis, Brazil
[16]National Institute of Metrology, Quality and Technology, Fluminense Federal University, Rio de Janeiro, Brazil
[17]Innovation Laboratory in Nanobiotechnology and Advanced Materials for Livestock Embrapa Gado de Leite, Juiz de Fora, Brazil
[18]Federal University of Juiz de Fora, Juiz de Fora, Brazil
[19]Microbiological Control Laboratory, Quality Control Department, Bio-Manguinhos, Fiocruz, Rio de Janeiro, Brazil
[20]Laboratory of Education and Research in *In vitro* Toxicology, Faculty of Pharmacy, Federal University of Goiás, Goiânia, Brazil
[21]Institute of Biological Sciences, Federal University of Goiás, Goiânia, Brazil

Corresponding author:
Marize Campos Valadares, Laboratory of Education and Research in *In Vitro* Toxicology–Tox In. Alameda Flamboyant, Quadra K, Edifício LIFE, Parque Tecnológico Samanbaia, Rodovia R2, n. 3.061, Campus Samambaia — Universidade Federal de Goiás, Goiânia 74.690-631, Brazil.
Email: marizecv@ufg.br

Abstract

The Brazilian National Network of Alternative Methods (RENAMA), which is linked to the Ministry of Science, Technology and Innovation, is currently comprised of 51 laboratories from CROs, academia, industry and government. RENAMA's aim is to develop and validate new approach methodologies (NAMs), as well as train researchers and disseminate information on their use — thus reducing Brazilian, and consequently Latin American, dependence on external technology. Moreover, it promotes the adoption of NAMs by educators and trained researchers, as well as the implementation of good laboratory practice (GLP) and the use of certified products. The RENAMA network started its activities in 2012, and was originally comprised of three central laboratories — the National Institute of Metrology, Quality and Technology (INMETRO); the National Institute of Quality Control in Health (INCQS); and the National Brazilian Biosciences Laboratory (LNBio) — and ten associated laboratories. In 2022, RENAMA celebrated its 10th anniversary, a milestone commemorated by the organisation of a meeting attended by different stakeholders, including the RENAMA-associated laboratories, academia, non-governmental organisations and industry. Ninety-six participants attended the meeting, held on 26 May 2022 in Balneário Camboriú, SC, Brazil, as part of the programme of the XXIII Brazilian Congress of Toxicology 2022. Significant moments of the RENAMA were remembered, and new goals and discussion themes were established. The lectures highlighted recent innovations in the toxicological sciences that have translated into the assessment of consumer product safety through the use of human-relevant NAMs instead of the use of existing animal-based approaches. The challenges and opportunities in accepting such practices for regulatory purposes were also presented and discussed.

Keywords

Brazil, Brazilian National Network of Alternative Methods, conference, NAMs, Network to Alternative Methods, new approach methodologies, next-generation risk assessment

Introduction

The Brazilian National Network of Alternative Methods (RENAMA), which is linked to the Ministry of Science, Technology and Innovation (MCTI), is currently comprised of 51 laboratories from contract research organisations (CROs), academia, industry and government (https://www.renama.tec.br). RENAMA's aim is to develop and validate new approach methodologies (NAMs), as well as train researchers and disseminate information on their use — thus reducing Brazilian, and consequently Latin American, dependence on external technology. Moreover, it promotes the adoption of NAMs by educators and trained researchers, as well as the implementation of good laboratory practice (GLP) and the use of standardised products.

The approval of *Law No. 11,794/2008*,[1] known as the Arouca Law, was a milestone for the regulation of activities related to the use of animals in scientific experimentation and education in Brazil and Latin America.[2] Its implementation also supported the creation of the National Council for the Control of Animal Experimentation (CONCEA), whose responsibilities include monitoring and evaluating the uptake of alternatives to animal testing.

The idea for the creation of RENAMA came about at the end of 2010; however, the network started its activities in 2012 through an MCTI authorisation, *Ordinance No. 491/2012*,[3] and a public call executed by the National Council for Scientific and Technological Development (CNPq)/MCTI, providing funding for its establishment. This MCTI/CNPq public call No. 25/2012, entitled 'Support to projects for the structuring of the National Network of Alternative Methods (RENAMA)', officially started RENAMA's activities, and it initially encompassed three central laboratories (the National Institute of Metrology, Quality and Technology (INMETRO); the National Institute of Quality Control in Health (INCQS); and the National Brazilian Biosciences Laboratory (LNBio)) and ten associated laboratories.

In 2014, CONCEA mandated that institutions interested in validating alternative methods to replace the use of laboratory animals must be associated with RENAMA,[4] highlighting the importance of the network. In 2016, with the consolidation of efforts to increase the number of professionals using NAMs in Brazil, the Regional Platform of Alternative Methods to the Use of Animals (PReMASUR — see https://www.gov.br/mcti/pt-br/acompanhe-o-mcti/premasul) was created. PReMASUR relies on the assistance of RENAMA to run education and training activities, giving professionals from the Southern Common Market (MERCOSUR) countries (Argentina, Brazil, Paraguay and Uruguay) training opportunities to reproduce non-animal methods and also become disseminators of the learnt methodologies.[5]

Considering the positive impacts of implementing NAMs and the need to expand the training of professionals, Ordinance No. 3,586[6] was issued in 2017 with the main objective of maintaining RENAMA and its structure for another three years — a process that was repeated 2021.[7] Also in 2021, a training workshop was promoted by the associated members of RENAMA, on the theme of 'Authenticity of test systems'. This workshop attracted national

participants from the associated laboratories, as well as international participants.

In 2022, RENAMA celebrated the 10th anniversary of its creation, a milestone commemorated in a meeting attended by different stakeholders, including the RENAMA-associated laboratories, academia, non-governmental organisations (NGOs) and industry. The meeting, held on 26 May 2022 in Balneário Camboriú, SC, Brazil, as part of the programme of the XXIII Brazilian Congress of Toxicology 2022 (CBTox 2022), was attended by 96 participants. Significant moments in the history of RENAMA were celebrated, and new goals and discussion topics were established, in order to promote innovative methods for the replacement of animal experimentation. In addition, a keynote lecture and two presentations were delivered virtually, by industry and biotechnology research representatives. A summary of presentations, as well as the proceedings and conclusions of the meeting, are presented.

Summary of the presentations

Assessing the safety of consumer products by using animal-free methods *(Julia Fentem; Unilever's Safety and Environmental Assurance Centre, Sharnbrook, UK)*

In the keynote lecture, Dr Julia Fentem provided an industry perspective based on the experience and collaborative work of the Unilever's Safety and Environmental Assurance Centre (SEAC; https://seac.unilever.com/) in performing safety assessments for cosmetics and other consumer products without animal testing. In a broad overview, the presentation covered recent innovations in toxicological science that have been applied to the assessment of the safety of consumer products by using advanced human-based approaches instead of animal testing, and the challenges and opportunities in gaining acceptance of such practices for regulatory purposes.

From a consumer perspective, it was shown that different populations worldwide, such as Brazil, Mexico, France and the UK, oppose animal testing for personal care and cosmetic products. In line with this, it was highlighted that it is crucial to use the latest science to understand human chemical exposure and use information from different sources obtained with advanced technologies that do not rely on animal experimentation. For instance, cell culture methods, biological analytical techniques, physiologically based kinetic (PBK) models, and high throughput transcriptomics (HTTr) can provide relevant safety data to support risk assessment decisions for consumer products.

The path is not easy and has demanded multidisciplinary work and global partnerships with different stakeholders, such as governmental institutions, leading research teams from academia, CROs and animal protection NGOs, to support wider acceptance and use of non-animal tools and approaches. This is important not only for educational purposes but also to promote the regulatory acceptance of innovative human-relevant safety science and technology. The cosmetic industry has pioneered the demonstration that it is not necessary to use animal experimentation to protect consumers, workers and the environment. Regarding this, the industry has been building consumer confidence through independent brand certification and consumer-facing no animal testing claims, e.g. the People for the Ethical Treatment of Animals (PETA) certification and the Cruelty Free International Leaping Bunny scheme.

In addition, stakeholders have been involved in education and training activities to support others in building expertise in novel safety assessment practices. An example is the movement created in Latin America through the PReMASUR, set up by the MCTI to establish human resources and infrastructure to promote NAMs in the MERCOSUR countries. The majority of the PReMASUR trainings has been in a 'hands-on' format, and the programme has covered the expenses of the attendees, as well as the costs of the laboratory supplies required for such activities. With this Brazilian initiative, several scientists from academia, industry, regulatory bodies and CROs have been trained in NAMs.

Dr Fentem also mentioned the Animal-Free Safety Assessment (AFSA) Collaboration (https://www.afsacollaboration.org/) that brings together corporate and non-profit worldwide organisations to accelerate the global adoption of NAMs for safety assessment. The AFSA Master Class covers the entire next-generation risk assessment (NGRA) process for cosmetics and cosmetic ingredients, focusing on understanding the information generated by non-animal methods and how to use this information for safety assessment decision making. The freely available online course is divided into nine modules, plus a separate module on the global regulatory landscape. More information about the AFSA Master Class and review webinars can be found at https://www.afsacollaboration.org/masterclass/.

Conducting safety assessments without animals is not only a scientific challenge and societal demand but a paradigm change. In the short history of alternatives to animal testing, *in vitro* and *in silico* tests were initially used in the 1980s for the hazard identification of mainly local effects (e.g. eye irritation, skin corrosion); more recently, the tools and approaches have evolved toward being more mechanistic — for instance, with the understanding of toxicity pathways/adverse outcome pathways (AOPs) and the establishment of integrated approaches to testing and assessment (IATAs) for risk assessment.[8–16] Such frameworks started to gain pace in the 2000s, especially after the publication of the National Research Council report, 'Toxicity testing in the 21st century: A vision and strategy', at the request of the US Environmental

Protection Agency (US EPA). This report proposed a shift from the traditional animal tests to more human-relevant *in vitro* models that allow better understanding of the potential hazard and risks to human health induced by exposure to chemicals.[17] This approach has paved the way for the implementation of NAMs and NGRA in regulatory practices worldwide. For instance, this was reflected in guidance from the Scientific Committee on Consumer Safety[18] and the recent report of the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM).[19] Dr Fentem pointed out that Brazil has been a pioneer in the Latin American region by updating its legal framework and regulatory practices, mainly driven by the publication of *Law No. 11,794* in 2008 (also known as the 'Arouca Law'),[1] a milestone in the implementation of alternative methods in Brazil.[2] In addition, the outstanding efforts of RENAMA in creating a taskforce to disseminate NAMs expertise and knowledge among the associate laboratories was highlighted.

The maximisation of the use of existing information and animal-free approaches (e.g. *in silico* predictions, read-across, exposure-based waiving and history of safe use) assure consumer safety without animal testing as established by the NGRA. Having a fundamental principle of 'Protection not Prediction', it represents a migration from traditional prescriptive and processual methods to investigative and integrative strategies using existing information and NAMs aiming at human protection. In contrast to the traditional risk assessment based on animal experimentation, NGRA aims to be exposure-led, hypothesis-driven and bring the best science behind NAMs to ensure transparency and robustness to regulatory decisions, thus protecting human health.[20,21]

Collaborative research programmes have tried to fill the current gaps in the safety science to improve the NGRA approaches. For example, the research initiative 'Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)' developed an *ab initio* chemical safety assessment workflow. Based on exposure considerations, it is also divided into tiers which guide the use of NAMs to derive points of departure (PoDs) to estimate a safe external dose in a repeated use scenario.[22] This progress has enabled safety assessments of various ingredients, including those used in cosmetics and home care products, without the need for animal models. In this way, it is important to take advantage of the latest science behind NAMs and exposure tools to develop NGRA frameworks to make scientific weight-of-evidence decisions on, for example, systemic safety,[23] skin sensitisation potential[24] and developmental/reproductive[25] safety.

Moving from the science to the regulatory space, there are challenges to face in different geographies, such as hygiene products and disinfectants testing in China, as well as ingredients testing challenges in Europe due to the EU Chemical Strategy for Sustainability (https://environment.ec.europa.eu/strategy/chemicals-strategy_en), under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), which has requested new animal testing for widely used chemicals with existent safety data.[11,26] On the other hand, there has been progress toward the uptake of non-animal approaches — such as the publication of the Scientific Committee on Consumer Safety (SCCS) guidance notes for cosmetic safety assessment,[18] as well as the Organisation for Economic Co-operation and Development (OECD) case studies showing how NAMs can be integrated and used in practice for weight-of-evidence safety decisions (https://www.oecd.org/chemicalsafety/series-testing-assessment-publications-number.htm).

In Brazil, there has been the recent recognition of validated alternative methods by the National Council for the Control of Animal Experimentation (CONCEA — see https://www.gov.br/mcti/pt-br/composicao/conselhos/concea/paginas/publicacoes-legislacao-e-guia/metodos-alternativos-reconhecidos-pelo-concea) through normative resolutions,[27–30] and acceptance of these methods by the National Health Surveillance Agency (ANVISA).[31] In addition, the use of NAMs for assessing food safety is currently in the loop of regulatory bodies as the example of the European Food Safety Authority (EFSA) Strategy 2027 (https://op.europa.eu/webpub/efsa/strategy-2027/en/). Also worthy of mention is the pioneering work plan for adopting NAMs for assessing chemical safety that is being led by the US EPA (https://www.epa.gov/system/files/documents/2021-11/nams-work-plan_11_15_21_508-tagged.pdf).

Dr Fentem concluded the discussion on the science regulatory use gap by emphasising the need to re-think and modernise the approach to conducting animal tests. Animal testing is currently perceived as a regulatory requirement, but its use might not always be scientifically justified. Additionally, specific laws and regulations, rather than scientific evidence, are hindering the paradigm shift toward modern animal-free safety science. To improve the protection of workers, consumers and the environment, a change in the regulatory approach to chemical safety evaluation is necessary. This change should not be anchored in predicting the apical toxicity effects seen in high-dose animal studies, but instead should focus on modern, more exposure-relevant safety science methodologies. Her thoughts on priorities are that the regulatory change can be achieved through developing a modern, science-based, chemicals regulatory framework, which facilitates the use of 21st century science and technology to protect people and the environment better. Dr Fentem also emphasised that is crucial to establish open dialogue on, and transparent scientific evaluation of, NAMs strategies for specific chemicals/chemical groups through the assessment of case studies. There is a need to accelerate knowledge transfer and training in advanced safety science and NAM-based chemical assessments through initiatives like PRe-MASUR and the AFSA Collaboration, and to stimulate capacity building in NAMs to increase the number of service providers of the new 'NAMs Toolbox'.

Case studies on the application of non-animal approaches for local and systemic safety assessments of cosmetic ingredients *(Renato Ivan de Ávila; Unilever's Safety and Environmental Assurance Centre, Sharnbrook, UK)*

To illustrate some aspects discussed by Dr Fentem, Dr de Ávila presented Unilever SEAC's experience of the use of NAMs to successfully perform transparent human safety assessments of ingredients and products, without generating new animal data. The presentation focused on a common question in the real-life risk assessment process: Can we safely use x% of ingredient y in product z?

Dr de Ávila showed that assuring consumer safety is possible by maximising the use of existing information of suitable quality and animal-free approaches, including exposure-based waiving approaches (e.g. toxicological threshold of concern),[32] history of safe use,[33] *in silico* predictions, read-across,[34] data obtained from established methods (e.g. OECD Test Guidelines), other human-relevant *in vitro* tools (e.g. High-Throughput Transcriptomics; HTTr), and computational modelling.[20,35] Therefore, the whole existing and newly generated data have to be integrated with weight-of-evidence within an NGRA strategy for decision-making that protects human health.[20,21,36,37]

To illustrate the practical application of this, Dr de Ávila discussed two published hypothetical safety assessment case studies: one focused on assessment of the systemic safety,[23] and one on assessment of the skin sensitisation potential,[24] of 0.1% coumarin, to be used in different consumer products (such as a face cream). Coumarin was considered as if it were a novel fragrance, and there were no *in vivo* data — such as historical information or *in silico* predictions based on *in vivo* assays — used in these particular case studies. Dr de Ávila showed that, in general, the workflows for skin allergy and systemic safety NGRA frameworks used the following components, which are anchored in the principles underpinning the use of NAMs in the safety risk assessment of cosmetics:[21,22,38,39]

- the collation of existing information (e.g. literature review), consumer exposure estimation and problem formulation;
- the generation of NAMs data for *in vitro* biological activity characterisation;
- the determination of PoDs, i.e. the concentration at which coumarin induced bioactivity in *in vitro* assays through a concentration–response analysis;
- the determination of a risk metric, by calculating the bioactivity exposure ratio (BER), also referred to as the 'margin of safety' or 'margin of exposure'; and
- the formulation of a risk assessment conclusion, based on a weight-of-evidence approach, after considering all the available information.

Some details of the strategies involving NAMs data are summarised below:

Systemic safety assessment. Considering that one of the critical principles of NGRA is that of exposure-led assessment, exposure information was defined by analysing consumer habits and practices. Associated with this, a PBK model was used to estimate the level of internal exposure to coumarin, i.e. plasma C_{max}. For biological activity characterisation, three high-throughput broad biological coverage NAMs were then used to derive PoDs: HTTr and an *in vitro* cellular stress panel[40] using 2-D and 3-D cell models, and *in vitro* pharmacological profiling[35] involving several targets associated with human adverse drug effects. To evaluate specific targets of concern identified by computational predictions, two *in vitro* laboratory tools were used to assess the potential mechanism-based genotoxicity and immunomodulatory effects of coumarin — namely, ToxTracker assay[41] and BioMap Diversity 8 panel,[42] respectively. The ratios of the obtained PoDs and the relevant internal exposure C_{max} estimate were calculated, in order to determine the margin of safety.[23] Further analyses have been performed to understand how effective this NAMs-based strategy is for protecting human health, through benchmarking against historical systemic safety decisions for a chemical data set. Preliminary results from these analyses have indicated that up to 69% (9/13) of the low-risk scenarios were identified, and a high protection rate (5/5) was demonstrated for the high-risk scenarios.[35]

Skin sensitisation safety assessment. Taking into consideration all the existing information associated with *in vitro* NAMs data generation, transparent risk assessment decisions within the skin sensitisation NGRA framework are made by using the Skin Allergy Risk Assessment (SARA) model in a weight-of-evidence approach, as presented as part of the coumarin case study.[24] The SARA model is a defined approach, used to infer the concentration of a substance that would induce sensitisation in 1% of a human repeated insult patch test (HRIPT) population (ED01 value), based on the integration of any combination of historical *in vivo* data (HRIPT and local lymph node assay) and *in vitro* skin sensitisation OECD Test Guideline assay data, namely the Direct Peptide Reactivity Assay (DPRA),[43] KeratinoSens™,[44] human cell line activation test (h-CLAT) and U937 cell line activation test (U-SENS™).[45] The defined approach also makes use of benchmark exposures related to the use of consumer products with clinical data, to derive a second output that is significant within a risk assessment — i.e. the probability that market exposure to an ingredient in a finished product is low risk.[46,47] Therefore, it was demonstrated that the low-risk conclusion of the assessment was acceptable and consistent under

the investigated exposure scenario of 0.1% coumarin as an ingredient in a face cream.[24]

Safe products depend on toxicological data of quality *(Izabel Villela; InnVitro Suporte e Gestão em Toxicologia, Porto Alegre, Brazil)*

Dr Villela discussed the importance of better-quality data to ensure the safety of consumer products. An evaluation strategy that is based on NAMs, integrates *in silico*, *in chemico* and *in vitro* approaches, in order to understand the initial mechanistic endpoints that lead to the adverse effects observed *in vivo*. Dr Villela emphasised that these approaches are not designed to become direct substitutes for *in vivo* methods, but aim to provide improved evidence about adverse effects on different target species.[48] However, all of the data generated during a toxicological assessment must be fully reliable, in order to permit regulatory acceptance. To achieve this reliability, all experiments should be accurately planned, executed and reported.

As recently reviewed,[48] some of the main points regarding the regulatory acceptance of NAMs in Brazil are that:

- validated methods are used within the scope for which they were validated;
- various criteria are met, in order that high-quality experiments are carried out by high-quality laboratories, e.g. facilities with GLP accreditation; and
- full test reports are presented, not least including information on the reference items used, historical laboratory data generated, numbers of replicates, and the responsible researcher.[48]

Each test method has its own specificities, to be addressed before it can be fully adopted. Before the method can be used appropriately, three aspects must be carefully considered, namely: the method; the test system; and the test item.

The **test method** must be selected and applied within the scope for which it was initially validated, considering the toxicological endpoint and type of test item (sample) to be tested. Before applying a method for hazard assessment, the laboratory must be able to perform the method proficiently, according to the specificities of each test method.

The **test system** can be a molecule, a cell, a tissue, or organ. It must be appropriately characterised, independently of the test method, to include confirmation of, for example, its identity, passage number and sterility. The constant use of reference items to control the test system response, and the generation of historical laboratory data, are fundamental to maintaining laboratory quality.

The **test item** doses should be selected with regard to solubility, cytotoxicity, or the maximal dose recommended in the test method. The number of doses should also be subjected to a dose–response relationship assessment.

Dr Villela highlighted that these points should be addressed, in order to generate high-quality and reliable data. A collective effort, from regulators, industry, CROs and the academic environment, is needed to ensure the generation of this high-quality data and thus to build regulatory confidence in the use of NAMs.

Discussion

During CBTox 2022, the 10th anniversary of the creation of RENAMA was celebrated through a meeting with different stakeholders, revisiting the accomplishments, sharing knowledge, promoting synergies and increasing discussion between national and international research groups.

The lectures highlighted recent innovations in the toxicological sciences that have translated into the assessment of consumer product safety through the use of human-relevant NAMs instead of the use of existing animal-based approaches. The challenges and opportunities in accepting such practices for regulatory purposes were also presented and discussed. Also discussed was how these new approaches could be applied to the risk assessment of cosmetic ingredients and their products. Case studies on the application of NAMs for the local and systemic safety assessment of cosmetic ingredients were shown. The importance of better-quality data to ensure the safety of consumer products was also highlighted and discussed.

To reduce Brazil's dependence on external technology and consolidate RENAMA's work on the development and validation of NAMs, as well as training researchers and disseminating information on their use for educational and regulatory purposes, there is a need for continuous collaborative and multidisciplinary action. Attendees acknowledged the extraordinary effort required to accomplish these goals, and the meeting concluded with an emphasis on the need to work together to overcome knowledge and skill gaps.

This collaborative mindset brings crucial motivation to the efforts to develop human-relevant safety science in Brazil, especially with the recently approved CONCEA *Normative Resolution No. 58/2023*,[49] which provides a partial ban on cosmetic testing on animals. Alongside this, some progress has been made on *Bill No. 3062* (previously *Bill No. 70*), which has been under review since 2014. After approval by the Brazilian Senate in December 2022, it is now waiting for approval by the Brazilian Chamber of Deputies (https://www.camara.leg.br/proposicoesWeb/fichadetramitacao?idProposicao=597587). If *Bill No. 3062* is eventually approved and becomes a legal act, it will support *Normative Resolution No. 58/2023*,[49] to ensure a full ban on animal use for cosmetics testing.

Considering this collaborative perspective, it is clear that exploring a wide range of correlated scientific fields is an effective way to further our understanding of how NAMs can be used in safety-related areas. For instance, between 2001 and 2020, Brazil invested over R$ 3 billion in the field of nanotechnology, to investigate nano-safety issues. These efforts led to the creation of and/or participation in four networks: the National System of Laboratories on Nanotechnology (SisNANO 1.0 and SisNANO 2.0); the Brazilian Nanotechnology Initiative (IBN); and participation in the European Regulatory testing of nanomaterials (NANoREG). To support regulatory authorities in providing technical and scientific evidence for establishing guidelines and standards, the MCTI approved and funded the CertificaNano Project in 2017. The aim of this project was to adapt and disseminate knowledge and methodologies from the OECD, ISO, NanoReg and other sources as a reference for developing methods, particularly non-animal methods, that are acceptable to regulators and national authorities. Dr Granjeiro (personal communication) emphasised the need for complementary efforts within RENAMA for the development, or modification, of NAMs for use in the evaluation of nanomaterial safety. Such an approach represents a fantastic opportunity to spread the culture of NAMs use, and expedite the generation of reliable scientific data on the safety of nanomaterials.

During the meeting to celebrate RENAMA's 10th anniversary, the associated laboratories emphasised the need to form working groups that are better equipped, through collaborative efforts, to overcome specific obstacles in Brazil and Latin America. Some of the areas that require attention include: the implementation and application of GLP in laboratory facilities; the interlaboratory validation of manufactured reconstructed tissues (e.g. skin models) that have been developed by Brazilian research groups and companies; and widening the adoption of validated tests (such as the monocyte activation test) by stakeholders. The monocyte activation test is used to evaluate pyrogenic contamination in parenteral products, and is one of the over 40 alternative methodologies already recognised by CONCEA.[29] The ultimate goal of these various initiatives is to ensure the ethical and sustainable protection of both humans and the environment.

Conclusions

The anniversary meeting discussions emphasised the importance of extensive collaboration between laboratories and other organisations and regulatory agencies; this has been facilitated and promoted by RENAMA for the past ten years. Take-home points on the subject of collaboration included the following:

1. There is a need to work together, to overcome knowledge and skill gaps.
2. A collaborative mindset brings crucial motivation to the efforts to develop human-relevant safety science.
3. Exploring a wide range of correlated scientific fields is an effective way to further our understanding of how NAMs can be used in safety-related areas.
4. There is a need to form working groups that are better equipped, through collaborative efforts, overcome specific obstacles.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical statement

Ethical approval was not required for the preparation of this article.

Informed consent

Informed consent was not required for the preparation of this article.

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