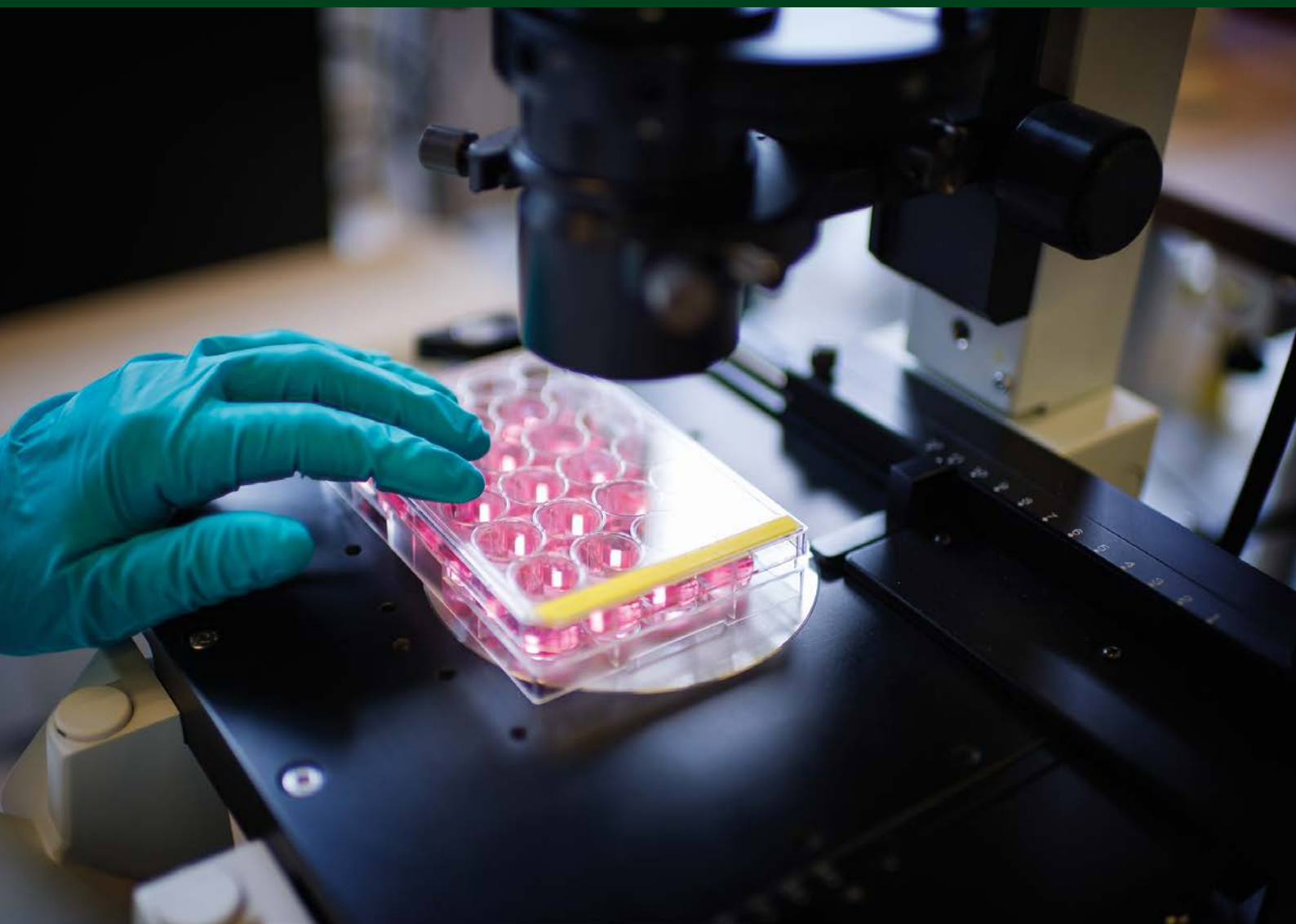
National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Landscape New Approach Methodologies (NAMs) *safety assessment chemical substances*



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Introduction

Chemical substances need to be safe for human health (for example consumers and workers) and the environment. Chemical substances are used in industrial processes. They can also be found in food as food additives (dyes, preservatives) and as contaminants (pesticide residues, chemical substances released from food contact materials). Chemical substances can also be used for the production of consumer products such as cleaning products, paint, cosmetics and many types of articles (clothes, furniture and electronics). Various EU-regulations and/or Dutch regulations are in place to ensure the safety of these chemical substances. More information about these regulations can be found on the "Risico's van Stoffen"-website for chemicals substances and products and food (both in Dutch).

The regulatory frameworks require toxicological information to assess the safety of chemical substances. The amount and type of information needed is different in each framework, because of the wide variety of chemical substances, products and applications. Within a regulatory framework the general rule is that the more intrinsically dangerous a chemical substance and/or the higher the market volume, the more information is required (RIVM, 2020 (in Dutch)).

The information needed for a safety assessment is obtained from a number of different tests. The information from these tests can show whether a chemical substance is an irritant, has carcinogenic properties, can cause harmful effects in specific organs, or does no harm at all. Some of these tests that need to be performed are animal studies. In the last few years, increasing attention is given to replace these animal studies with tests that use no or less animals, or cause less harm in the animal (e.g. as stated in the recent EU resolution and the Lab animal policies of the Dutch government (in Dutch). This is also called 3R: replacement, reduction and refinement of laboratory animals. In the context of safety assessment, the term New Approach Methodologies (NAMs) is used (see also: Trends).

The implementation curve of NAMs, as shown in Figure 1 and explained in Table 1, illustrates the phases that a NAM has to go through in order to be implemented in the regulatory frameworks (OECD Series on testing and assessment no. 34; Hartung et al., 2004; RIVM, 2020 (in Dutch)). In every phase of this curve there are (international) organisations and advisory bodies involved. They can influence and stimulate the validation, acceptation and implementation of NAMs. Corporation between the stakeholders in and between the phases is essential for efficient implementation of NAMs. (Bos et al., 2020; Knight et al., 2021; Pistollato et al., 2021; Patterson et al., 2021).

There are many players and stakeholders involved in the implementation of NAMs. The goal of the Landscape NAMs is to provide a current overview of who is involved, which initiatives are undertaken and which trends can be observed in the field of NAM implementation for the safety assessment of chemical substances. Gaining insight in this arena and in the roles of organisations is an essential first step to accelerate the implementation on NAMs. The domain of safety assessment of drugs and the effect of chemical substances on the environment are outside the scope of this landscape. Also factors regarding risk management and the decision-making process around chemical substances with or without the use of NAMs are not considered here.

The phases from validation to uptake in legislation and regulation mainly take place in an international setting where various (international) organisations and advisory 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 validation, acceptation and implementation of NAMs.

The basis for this landscape is the "Landschap 3V-methoden risicobeoordeling chemische stoffen" (in Dutch) published in 2017. In 2021, four rounds of interviews were held with a total of 27 experts working in institutions and organisations that are involved in the implementation of NAMs. Using this input the previous version of the landscape was updated and expanded to include projects, funding sources, trends and (informal) networks and committees for NAMs.

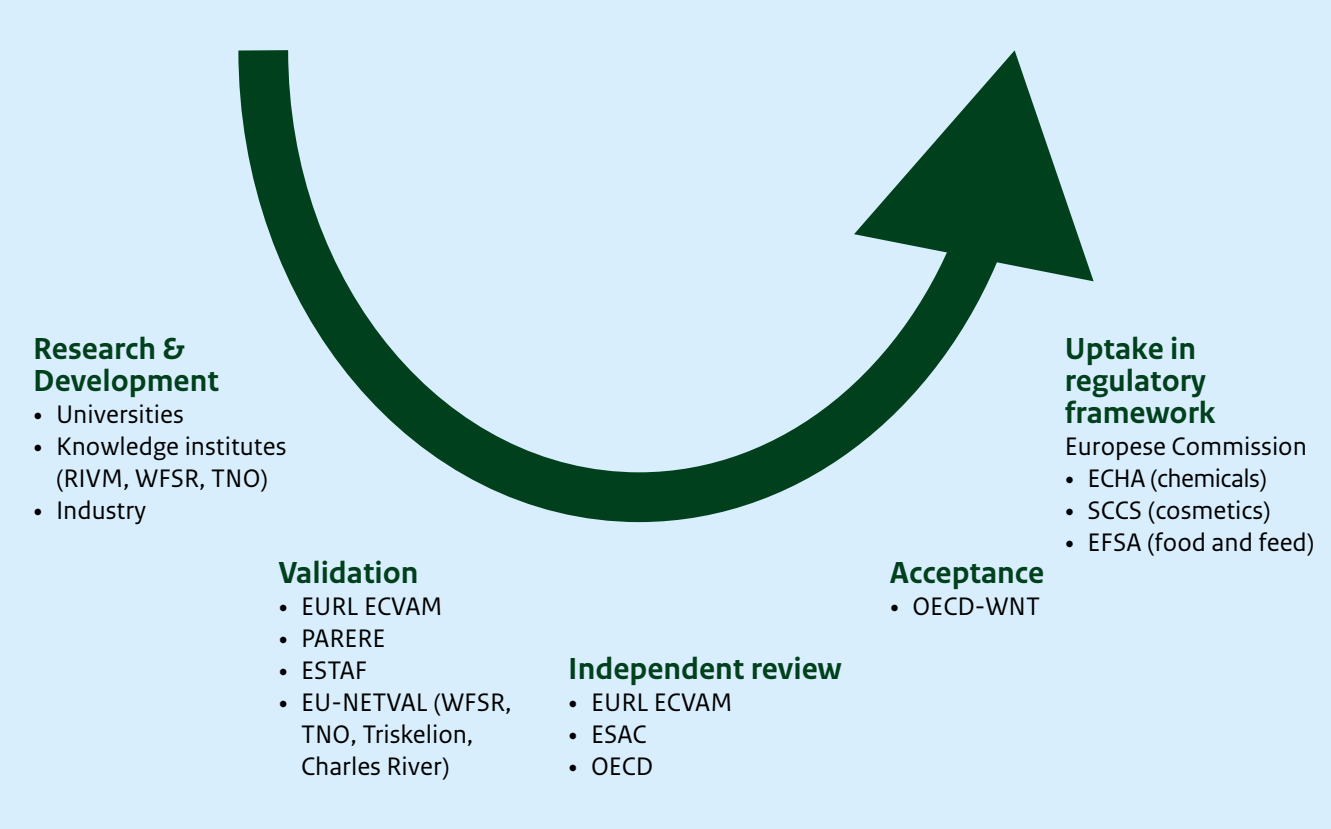

Figure 1. Implementation curve of NAMs. This curve describes the phases that a new NAM has to move through from research to uptake in regulatory frameworks (based on RIVM, 2017 and RIVM, 2013, both in Dutch).

Table 1. Description of phases in the implementation route.

Phase	Description
Research & Development	This phase encompasses the development of NAMs, including development of *in silico* and *in vitro* tests for establishing toxicity of chemical substances and kinetics (ADME/TK), and models and statistical tools that are needed for the interpretation of the results. Scientific research that is focussed on unravelling mechanisms can also contribute to the development of Adverse Outcome Pathways (AOPs). This knowledge can be applied to develop test methods and strategies that are based on these mechanisms.
Validation	Validation of NAMs is a flexible process in which the reliability and relevance of a test method for a specific goal needs to be proven. Validation can be performed in a prospective as well as in a retrospective manner. The chances of successful regulatory acceptance are higher when these studies are conducted according to the principles formulated by the OECD (Series on testing and assessment no. 34, no. 211, no. 286 and the modular approach of EURL ECVAM). The Tracking System for Alternative methods towards Regulatory acceptance (TSAR) database contains all tests that are currently in the process of validation in one of the validation centres. The EURL ECVAM DataBase service on advanced and Alternative Methods (DB-ALM) contains all test methods that are validated by EURL ECVAM. In silico models such as read-across, (Q)SAR and PBK models are not validated according to these principles. OECD has defined criteria for the latter two models with which the reliability and relevance can be assessed (QSAR: Series on testing and assessment no. 49, no. 69; PBK: no. 331).
Prospective validation	Prospective validation studies are conducted for newly developed test methods or strategies. These are technically validated for relevance and reliability. The reliability is based on the reproducibility within and between laboratories, the relevance of the toxic effect that is measured, the regulatory goal for which the test can be used, insights in the limitations and advantages, applicability domain (which classes of chemical substances can be tested) and the predictive value (accuracy, sensitivity and specificity).
Retrospective validation	Retrospective validation of test methods or strategies is conducted based on existing data. These data are evaluated to determine the relevance and reliability of the test method or strategy.
Independent review	After completion of the formal validation study (externally or via a validation centre), the results are evaluated by an independent advisory body according to set criteria, for example those of the EURL ECVAM Scientific Advisory Committee (ESAC). Aspects of a test method such as accuracy, reproducibility and applicability domain are assessed. ESAC also evaluates whether the test method generates data that are useful for the safety and risk assessment of chemical substances for humans and the environment. EURL ECVAM can optionally provide a recommendation along with the ESAC opinion about a test method, which are both open for public consultation. ECVAM, with permission of JRC, publishes the ESAC opinion and optional recommendation in the JRC repository. An independent review can also be performed by a peer-review panel at the OECD or by another (international) validation centre.

Phase	Description
Acceptation	This concerns the formal acceptation of a test method by the OECD or European Union in their test guidelines programme. OECD test guidelines are harmonised and internationally recognised test methods and strategies that can be used for testing, evaluating and managing chemical substances. The OECD test guidelines fall under the Mutual Acceptance of Data (MAD) system. This means that when a study is performed in an OECD member state under Good Laboratory Practices (GLP) and according to the test guideline, it will be accepted in all other member states. This reduces duplicate testing of chemical substances. The EU regulation for test methods (EC 440/2008) describes harmonised and accepted methods that are used in EU legislation (e.g. REACH, Cosmetics Regulation). There is close interaction with the OECD Test Guidelines programme to ensure global harmonisation. Specifically, it means that when a test guideline is accepted by the OECD it will in most cases be taken up in the EU regulation for test methods.
Uptake in regulatory framework	The uptake of test guidelines in regulatory frameworks takes place at the European Commission (EC) and European Parliament. Advice about the uptake of test guidelines in regulation on the EU level is given by the European CHemicals Agency (ECHA), the European Food Safety Authority (EFSA) and the Scientific Committee for Consumer Safety (SCCS).

The implementation curve step by step

Research & Development

Much international effort is taking place concerning NAM research and development for safety assessment of chemical substances. In the Netherlands, multiple institutions invest in this endeavour, including knowledge institutes, universities, universities of applied sciences and industry partners. Funding for NAM-related projects is available from various sources, e.g. government ministries, national funding programmes, industry partners and foundations. Moreover, in context of EU framework programmes research is financed through the EU Horizon 2020 programme (Table 2).

Dutch institutions currently participate in or coordinate numerous national and international projects focused on research and development of NAMs for safety assessment of chemical substances. This research concerns the development of NAMs to reduce or replace animal test methods for certain toxicological endpoints. Other research focuses on developing NAMs for toxicological endpoints or substances for which no (good) test method exist, e.g. endocrine disruption and nanomaterials, respectively. Test methods developed for these purposes include, but are not limited to, NAMs. In addition, innovative technologies are being developed to better resemble human biology and physiology, such as organ-on-chip models, which are not necessarily focused on the refinement or reduction of animal models. These models could, however, be used for other purposes as well, such as in safety assessment of chemical substances. Both research and development of NAMs and innovative technologies may contribute to less animal testing in the future. 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 (EURL ECVAM Status Report 2021).

Dutch institutions and organisations contributing to research and development of NAMs and innovative technologies:
RIVM, TNO, Wageningen Food Safety Research (WFSR), universities, universities of applied sciences, (biotech) industry, ministries (e.g. Ministry of Health, Welfare and Sport (VWS) and Ministry of Agriculture, Nature and Food Quality (LNV) and regulatory authorities (Netherlands Food and Consumer Product Safety Authority (NVWA)), national funding programmes and foundations.

Table 2. Overview of funding programmes for research and development of NAMs.

Funding programme	Institution/organisation	Overview of ongoing projects
National		
Meer Kennis met Minder Proefdieren (MKMD)	Netherlands Organisation for Health Research and Development (ZonMw)	https://www.zonmw.nl/nl/onderzoek-resultaten/fundamenteel-onderzoek/programmas/programma-detail/meer-kennis-met-minder-dieren/t/modules/
Nationale wetenschapsagenda (NWA)	Dutch Research Council (NWO)	https://www.nwo.nl/en/projects
Humane meetmodellen	Samenwerkende Gezondheidsfondsen (SGF)	https://www.nwo.nl/en/researchprogrammes/partnership/partnership-programmas/human-measurement-models-20-health-research
-	Dutch Society for the Replacement of Animal Testing (Stichting Proefdiervrij)	https://proefdiervrij.nl/en/for-science
International		
Horizon 2020 / Horizon Europe	European Union	https://ec.europa.eu/programmes/horizon2020/en/h2020-sections-projects https://ec.europa.eu/info/research-and-innovation/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-europe_en
Long-Range Research Initiative (LRI)	CEFIC	http://cefic-lri.org/projects/
Long Range Science Strategy (LRSS)	Cosmetics Europe	https://www.lrsscosmeticseurope.eu/lrss-projects-and-research/
Crack-IT	NC3Rs	https://nc3rs.org.uk/crackit/view-challenges
-	EPAA	https://ec.europa.eu/growth/sectors/chemicals/european-partnership-alternative-approaches-animal-testing/project-platform_en

Table 3. Overview of national and international projects focused on the development of new approach methodologies (NAMs) in which Dutch organisations participate.

Name of project	Duration	Brief description	Dutch coordinating organisation[a]
VHP4Safety	2021-2026	The mission of the Virtual Human Platform is to improve the prediction of the potential harmful effects of chemicals and pharmaceuticals based on a holistic, interdisciplinary definition of human health by developing the Virtual Human Platform and accelerating the transition from animal-based testing to innovative safety assessment.	RIVM, UU-IRAS, HU
EU-ToxRisk	2016-2022	The vision of EU-ToxRisk is to drive the required paradigm shift in toxicological testing away from 'black box' animal testing towards a toxicological assessment based on human cell responses and a comprehensive mechanistic understanding of cause-consequence relationships of chemical adverse effects. EU-ToxRisk will integrate advancements in cell biology, omics technologies, systems biology and computational modelling to define the complex chains of events that link chemical exposure to toxic outcome.	UL-LACDR
ASPIS cluster	2021-2026	This cluster is led by the European Commission and aims to synergise efforts and increase visibility of three EU-supported projects focused on improving safety assessment without animal testing (RISK-HUNT3R ONTOX PRECISIONTOX).	UL-LACDR, UU-IRAS, HU
PANORAMIX	2021-2026	PANORAMIX will use a mixture modelling, case studies and experimental data to deliver a web-based interface for calculating risks to chemical mixtures and to define effect-based trigger values for in vitro effects that can be directly measured in water, food, and blood to identify when mixture exposure poses a health threat.	VU

[a] UU-IRAS: Utrecht University – Institute for Risk Assessment Sciences, LU-LACDR: Leiden University – Leiden Academic Centre for Drug Research, HU: HU University of Applied Sciences, VU: VU Amsterdam, MU: Maastricht University, LU: Leiden University, UU: Utrecht University, WUR: Wageningen University & Research, Radboudumc: Radboud University Medical Centre, KWR: KWR Water Research.

Name of project	Duration	Brief description	Dutch coordinating organisation[a]
Eurion cluster	2019-2024	EURION is a cluster group of eight research projects (e.g. FREIA, GOLIATH, ENDpoiNTS) from the Call SC1-BHC-27-2018 – New testing and screening methods to identify endocrine disrupting chemicals (EDCs).	VU, UU-IRAS, UM
PARC	2021-2026	PARC is an EU-wide research and innovation programme to support EU and national chemical risk assessment and risk management bodies with new data, knowledge, methods, networks and skills to address current, emerging and novel chemical safety challenges. It will facilitate the transition to next generation risk assessment to letter protect human health and the environment, in line with the Green Deal's zero-pollution ambition for a toxic free environment and will be an enabler for the EU Chemicals Strategy for sustainability.	RIVM, WFSR, TNO, UL, UU, WUR, Radboudumc, VU, KWR

[a] UU-IRAS: Utrecht University – Institute for Risk Assessment Sciences, LU-LACDR: Leiden University – Leiden Academic Centre for Drug Research, HU: HU University of Applied Sciences, VU: VU Amsterdam, MU: Maastricht University, LU: Leiden University, UU: Utrecht University, WUR: Wageningen University & Research, Radboudumc: Radboud University Medical Centre, KWR: KWR Water Research.

Validation

Validation is a flexible process in which the reliability and relevance of new approach methodologies (NAMs) are evaluated for a defined purpose. Guidance documents about the principles of validation have been published by the OECD and EURL ECVAM. There are, however, no specific instructions or rules for validation studies. But experiences on validations of NAMs have been extensively described. Developers and end-users of NAMs can coordinate and perform validation studies within one or multiple laboratories depending on the purpose. Within Europe, validation studies can be coordinated by the EU Reference Laboratory for alternatives to animal testing, as defined in EU directive 2010/63/EU. EURL ECVAM publishes a yearly report on the progress made in the development, validation and regulatory application of alternative methods (EURL ECVAM Status Report 2021).

EURL ECVAM has multiple advisory bodies (PARERE, ESTAF) that help to collect the opinions of regulators and stakeholders on aspects of regulatory relevance and user relevance, respectively, of a new test method or approach. In the US, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) also has a mandate to coordinate and conduct validation studies. In addition, national validation centres in Canada, Japan, China, Brazil and Korea have a role in validation. Together, they are united in ICATM (International Cooperation on Alternative Test Methods). ECVAM coordinates most validation studies in the EU. If international harmonisation is desired, test methods and related validation studies are submitted to the OECD for inclusion of the test method into OECD test guidelines.

Dutch institutions and organisations involved in committees and discussion groups for validation:

Ministry of LNV, RIVM, TNO, Triskelion BV, Charles River Laboratories Den Bosch BV (CRL) and WFSR.

Table 4. Overview of advisory and consultation committees of EURL ECVAM.

Name	Brief description	Dutch institutes/organisations
EURL ECVAM's Preliminary Assessment of Regulatory Relevance (PARERE)	The PARERE network has been established in context of the EU Directive (2010/63/EU) on the protection of animals used for scientific purposes. It consists of representatives of member states, EU agencies (EFSA, ECHA, EMA) and committees (SCCS, SCHEER). Every member state has appointed a 'focal point' to represent it in this network (for the Netherlands, this is an expert of RIVM). The PARERE network advises EURL ECVAM on the regulatory relevance and applicability of a test method prior to validation. In addition, EURL ECVAM consults this network when writing a recommendation for a validated test method.	RIVM
ECVAM Stakeholder Forum (ESTAF)	ESTAF consists of European representatives of industry, scientific federations and international animal welfare organisations. This forum represents multiple stakeholders in test methods and advises EURL ECVAM on the relevance and applicability of test methods for end-users prior to validation. EURL ECVAM consults this forum when writing a recommendation for a validated test method.	*Not applicable as EU stakeholders are involved*
European Union Network of Reference Laboratories for the Validation of Alternative Methods (EU NETVAL)	EU NETVAL is a network of reference laboratories which supports the European Commission in carrying out validation studies. EURL ECVAM coordinates this network and selects reference laboratories. In the Netherlands, the Ministry of LNV is responsible for the implementation of EU Directive (2010/63/EU) on the protection of animals used for scientific purposes, and together with EURL ECVAM selects the reference labs in the Netherlands.	WFSR, TNO en Triskelion BV, CRL BV, LNV
EURL ECVAM Scientific Advisory Committee (ESAC)	ESAC is an independent committee which evaluates the scientific quality of a validation study. ESAC advises EURL ECVAM, which forms the basis for the recommendation for a validated test method provided by EURL ECVAM in case EURL ECVAM provided one besides an opinion of ESAC.	**-***

** Experts are involved in a personal capacity and not as a representative of their organisation.*

Independent review

Upon completion of a validation study, results are evaluated by a scientific independent peer-review panel. This independent review of a validated test method is performed by ESAC (ECVAM Scientific Advisory Committee) within EURL ECVAM or via another national or international validation centre. ESAC issues an opinion on the scientific validity of a test method or approach, which can be found in the JRC Publications Repository. When appropriate, EURL ECVAM can issue a recommendation taking into account ECVAM's own evaluation, the ESAC opinion and input from stakeholders and regulators (ESTAF/PARERE), international organisations (ICATM), the public and also the test submitter. For the development of an OECD test guideline, both the opinion and recommendation (if available) are submitted to the OECD and reviewed by the Working Party of National Coordinators of the OECD Test Guidelines Programme (OECD-WNT).

The OECD usually establishes ad-hoc expert groups which evaluate the new test method according to OECD criteria.

There is frequent interaction and discussion on harmonisation within the aforementioned organisations. The 'Environmental Health and Safety' (EHS) program works on international harmonisation of test methods and strategies that can be applied for testing, evaluating and managing of chemical substances.

Dutch institutions and organisations:
The National Coordinator of the OECD Test Guidelines Programme (OECD-WNT) for the Netherlands is an expert of RIVM. The coordinator consults and informs experts within the Netherlands (e.g. TNO, WFSR, Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), RIVM, universities and contract research laboratories) when new or modified methods will be submitted within the OECD Test Guidelines Programme. Furthermore, this coordinator represents the Netherlands within the PARERE network of EURL ECVAM.

Acceptance

Acceptance means the approval of a test method as a test guideline by the OECD or the EU. An OECD test guideline has to meet certain requirements. For example, a detailed method protocol should be readily available in the public domain (OECD Series on testing and assessment no. 34). In the OECD, all member state national coordinators are represented in the OECD-WNT, which is the responsible body for establishing test guidelines. The OECD test guidelines (link to Risk assessment network in the Netherlands – chemicals domain) comprise internationally accepted test methods applied in regulatory safety tests required for notification and registration of chemical substances. A test method does not have to be approved by the OECD prior to adoption into legislation and regulations in the EU, but usually it is. Similarly, in most cases a test method is validated before submission to the OECD, but it is not required to follow each validation step via a validation centre such as EURL ECVAM and/or ESAC. The OECD examines the test method according to the principles set out for validation.

Upon acceptance by the OECD-WNT, a test method is approved at the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology of the EHS programme. An extensive overview of various EHS working groups can be found on the OECD's website.

Various OECD working groups have an interest in supporting NAMs, such as the development and evaluation of *in silico* methods (e.g. (Q)SARs), *in vitro* assays and integrated test strategies and to facilitate the development of adverse outcome pathways (AOPs). This is done in the Extended Advisory Group for Molecular Screening and Toxicogenomics (EAGMST) and OECD IATA working groups, for example.

Dutch institutes and organisations:
Ministry of Infrastructure and Water Management (IenW), with other ministries (VWS and LNV) and competent authorities, such as Bureau REACH and Ctgb, RIVM/Bureau REACH (national coordinator).

Uptake in regulatory framework

The uptake of test methods concerns formal adoption of test methods in EU regulations under the Council Regulation (EC) for test methods (EC 440/2008). In certain cases regulation is less specific and only specifies which toxicological endpoints need to be assessed. ECHA and EFSA, which are EU agencies, are responsible for the implementation of regulations regarding chemical substances and food, respectively. ECHA and EFSA write guidance documents on how to conduct hazard and risk assessments, e.g. for the use of new test methods, within EU guidelines and regulations. These EU agencies have multiple working groups, panels and committees whose members include scientific experts, who represent organisations or serve a in a personal capacity.

Advisory committees within ECHA, EFSA or SCCS play an important advisory role in the implementation of NAMs but do not have a mandate for the acceptance of a new test method. These advisory committees often work on scientific opinions and recommendations for the European Commission, e.g. on NAMs, which can result in changes in legislation and regulations. Based on these opinions and recommendations, coordination and decision-making take place within decision-making committees, which consist of representatives of all member states. Table 5 provides an overview of advisory and decision-making committees for each regulatory context.

EU Agencies can use data of new (new approach) test methods for which no OECD test guideline have been established yet, although this option is used to a limited extent. Hence, a NAM does not have to follow each step of the implementation curve to be used in hazard and risk assessment. However, the use of not (fully) validated test methods in risk assessment has to be assessed on a case-to-case basis in the regulatory context when making decisions on this.

Dutch institutes and organisations:

- in decision-making committees: Ministries of IenW, LNV and VWS (supported by Ctgb, Bureau REACH and RIVM);
- in advisory committees: Ministries of IenW and VWS, NVWA-BuRO, RIVM, TNO, University Medical Centre Groningen (UMCG).

Table 5. Overview of European advisory and decision-making committees involved in hazard and risk assessment and/or legislation and regulations for chemical substances relevant to implementation of NAMs.

Regulatory context	Committee	Brief description	Dutch organisation
Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) & Classification, Labelling and Packaging (CLP)	*Competent Authorities for REACH and CLP (CARACAL)*[a]	CARACAL consists of representatives of the member state competent authorities for REACH and CLP regulations. Preparations for changes to regulations take place here, for example. CARACAL provides recommendations to the European Commission (DG GROW and DG ENVI).	IenW, VWS, Bureau REACH[+]
	ECHA Member State Committee (MSC)[b]	This committee is involved in several REACH evaluation processes, such as dossier evaluations, test proposals, substance evaluations and proposals for the identification and prioritising of a substance of very high concern (SVHC). Decisions are adopted unanimously and coordination between member states and ECHA takes place here. Together with ECHA, MSC decides which information and test methods are required.	IenW, Bureau REACH[+]
	Risk Assessment Committee (RAC)[a]	RAC is a scientific committee that advises the European Commission on the human health and environmental hazards and risks of chemical substances. RAC issues opinions on proposals for harmonised classification and labelling under CLP, Annex XV Restriction dossiers, and applications for authorisation of a SVHC under Annex XIV of REACH. In addition, RAC sets indicative (occupational) exposure limits for certain chemical substances on behalf of the European Commission and provides ad-hoc advice at the request of ECHA.	RIVM[*]
	REACH Committee[b]	This committee consists of representatives of member state competent authorities. This committee works according to a comitology decision-making process regarding REACH and CLP (changes to the Annexes of REACH and CLP and granting an authorisations).	IenW, VWS, Bureau REACH[+]
Biociden	*ECHA Committee for Biocides (BPC)*[a]	The BPC issues opinions that involves multiple procedures according to the Biocidal Products Regulation (BRP; EU 528/2012). The European Commission is responsible for the final decision. The BPC is involved in discussions regarding information requirements and evaluates new test guidelines.	Ctgb

Regulatory context	Committee	Brief description	Dutch organisation
EFSA remit	*EFSA Advisory Foruma*	The Advisory Forum consists of representatives of member state food authorities. This is an advisory forum of EFSA regarding science-related topics, priorities and upcoming risks. The director of NVWA-BuRO is the Dutch representative in this forum.	NVWA-BuRO
EFSA remit	*EFSA Scientific Committee (SC)a*	The EFSA SC develops harmonised guidelines, e.g. NAMs, for risk assessment for scientific panels in areas where no EU-wide accepted methods are available (e.g. read-across, TTC, nanomaterials). In case a substance involves the domain of multiple scientific panels, this committee will do the risk assessment. This horizontal committee provides scientific support for EFSA's mandate. With a mandate from the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF), EFSA advises the European Commission (DG SANTE), the European Parliament (ENVI committee) and member states.	
Plant Protection Products	*EFSA Panel on Plant Protection Products and their Residues (PPR)a*	The PPR panel provides scientific advice on risk assessment of plant protection products (PPPs), including the development of new methods for risk assessment. Market approval of PPP is granted by the member state competent authority. The Ctgb in the competent authority in the Netherlands.	RIVM*, WUR*
Novel foods and nutrition and health claims	*EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA)a*	The NDA panel deals with risk assessments of novel foods and assesses nutrition and health claims.	NVWA-BuRO*
Food contact materials, aids, additives and flavourings	*EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP)a*	The CEP panel evaluates the safety of food contact materials and chemical substances added to food, and related processes.	RIVM*
	EFSA Panel on Food Additives and Flavourings (FAF)a	The FAF panel focuses on the safety of chemical substances used as food additives and flavourings. This mainly concerns chemical substances evaluated by EFSA before their use can be authorised in the EU.	TNO*, RIVM*
Additives for animal feed	*EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)a*	The FEEDAP panel evaluates the safety and/or efficacy of additives, products, and chemical substances in animal feed for the target species, user, consumer and the environment.	^
Contaminants	*EFSA Panel on Contaminants in the Food Chain (CONTAMa*	The CONTAM panel provides scientific advice on contaminants in the food chain and undesirable substances, such as natural toxicants, mycotoxins and residues of unauthorised substances.	WFSR*

Regulatory context	Committee	Brief description	Dutch organisation
Committee for plants, animals, food and animal feed	*EC Standing Committee on Plants, Animals, Food and Feed (SCoPAFF)*[a]	This committee plays a key role in ensuring that EU measures on food and feed safety, animal health and welfare, and plant health are practical and effective. It consists of several sub committees. The mandate of these committees focuses on the entire food chain. SCoPAFF grants EFSA mandates for risk assessment. Ctgb and RIVM provide advice to the Ministries of LVN and VWS. The Ministry of Social Affairs and Employment (SZW) provides input to the Ministries of LNV and VWS.	LNV, VWS, Ctgb[+], RIVM[+]
Cosmetics	*Scientific Committee for Consumer Safety (SCCS)*[a]	The SCCS evaluates the safety of non-food consumer products (e.g. cosmetic ingredients) and provides guidance on which test methods could be used for risk assessment through the Notes of Guidance. The SCCS is part of DG SANTE and receives mandates from DG GROW.	RIVM[*], UMCG[*]
	SCCS Working group on cosmetic ingredients[a]	This working group evaluates the safety of cosmetic ingredients.	RIVM[*], UMCG[*]
	SCCS Working group on nanomaterials in cosmetic products[a]	This working group evaluates the safety of nanomaterials in cosmetic products.	RIVM[*], UMCG[*]
	SCCS Working group on methodology[a]	This working group examines new, mainly animal-free, test methods. External experts from science or industry present these new test methods. The working group annually drafts an update of the Notes of Guidance, in which new test methods can be included.	RIVM[*], UMCG[*]
	Standing Committee for Cosmetic Products (COSCOM)[b]	COSCOM consists of representatives from member state competent authorities and is chaired by a European Commission representative. It makes decisions regarding changes to the annexes related to approved/ banned cosmetic ingredients upon consultation of SCCS.	VWS, RIVM[+]

[a] *Advisory committee*
[b] *Decision-making committee*
[*] *Experts are involved in a personal capacity and not as a representative of their organisation.*
[+] *Experts are involved in an advisory role.*
[^] *Retired employees of Dutch organisations.*

Chemical substances regulations in the EU

The assessment of chemical substances is mainly regulated at EU level. Various regulatory contexts are available for chemical substance in the EU related to the application of a chemical substance (Table 6). Within all regulatory contexts, a NAM or other new test method can only be applied when it is implemented in regulation. These regulatory frame works offer room for the application of NAMs in safety assessment of chemical substances. However, the applicability of a NAM must be substantiated and/or validated. The room for application of NAMs within a regulatory context varies per context and toxicological endpoint (RIVM, 2020; RIVM, 2014).

Table 6. Overview of EU regulatory contexts for chemical substances. For a complete overview of New Approach Methodologies (NAMs) in safety assessment of consumer products and food see RIVM, 2020.

Regulatory context	Regulation	Brief description
REACH	*EG/1907/2006*	The purpose of REACH is to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances (REACH Article 1). REACH Article 13 states that whenever possible alternative methods, such as *in vitro* methods, qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across), should be used instead of vertebrate animal tests and in accordance with the use of alternative (Annex XI).
CLP	*EG/1272/2008*	The CLP (Classification, Labelling & Packaging) regulation ensures that employees and consumers in the EU are informed regarding the hazards of chemicals or chemicals used in mixtures. CLP uses the hazard criteria and labelling guidelines as set out by the United Nations and documented in the "Globally Harmonised System of Classification and Labelling of Chemicals (GHS)".
Biocides	*EU/528/2012*	This regulation aims to improve the functioning of the biocidal products market in the EU, while ensuring a high level of protection for humans and the environment. Biocidal products consist of active chemical substances and are used to protect humans, animals, materials or products against harmful organisms like pests or bacteria.

Regulatory context	Regulation	Brief description
Plant protection products	*EG/1107/2009*	This regulation lays down rules for the authorisation of plant protection products, protecting crops and plants against harmful organisms, in commercial form and for their placing on the market, use and control. The assessment is focussed on the active substance to ensure the protection of human and animal health and the environment.
	EG/283/2013 *EU/284/2013*	These regulations describe which information is required for the risk assessment of active substances (EC/283/2013) and plant protection products (EC/284/2013).
	EG/396/2005	This regulation establishes maximum levels of pesticide residues in or on food and feed of plant and animal origin.
Novel foods	*EU/2015/2283*	This regulation lays down rules for the marketing of novel foods and novel ingredients. Novel foods means any food not consumed to a significant degree by humans in the EU before 15 May 1997.
Food contact materials	*EG/1935/2004*	This regulation applies to materials which are intended to be brought into contact with food and is focused on the safe use of such materials and components thereof that are released to food.
Agents to improve food: • **Enzymes** • **Additives** • **Flavourings**	*EG/1331/2008* *EG/1332/2008* *EG/1333/2008* *EG/1334/2008*	These regulations concern agents added to improve food. This includes enzymes, additives and flavourings, and may only be used in food when safe for human consumption.
Additives for animal feed	*EG/1831/2003* *EG/767/2009*	These regulations concern additives used in animal feed to improve its quality or health. Marketing authorisation is granted after a scientific evaluation demonstrating the absence of harmful effects to human and animal health and to the environment.
Cosmetics	*EG/1223/2009*	This regulation sets out rules that any cosmetic product put on the market must comply with, in order to ensure a high level of protection of human health. This regulation lists UV filters, preservatives and (hair) colouring products approved for use in cosmetics. It also describes which substances are prohibited or restricted in cosmetic products. Pursuant to Article 18 of this regulation, animal testing is prohibited for cosmetic products and ingredients.
Contaminants in foodstuffs	*EG/1881/2006*	This regulation sets maximum levels for contaminants in foodstuffs that are toxicologically acceptable, so as to protect public health.

EU regulations often refer to specific guidelines established by EU agencies (ECHA, EFSA). These guidelines mainly consist of OECD test guidelines (Table 7). Remarkably, numerous validated in vitro test guidelines are currently available for toxicological endpoints such as genotoxicity and eye and skin irritation, but far fewer in vitro test guidelines are available for more complex toxicological endpoints like repeated dose toxicity and carcinogenicity.

Table 7. Toxicological endpoints and the OECD test guidelines available for in vivo and invitro methods accepted as OECD test guideline (adapted from Heringa et al., 2020 / RIVM, 2020).

Toxicological endpoint	OECD test guideline for *in vivo* methods	OECD test guideline for *in vitro* methods
Acute toxicity (3 routes: oral, inhalation, dermal)	401, 402, 403, 436, 425, 423, 420, 433	
Irritation/corrosion (eye and skin)	404, 405	460, 437, 438, 491, 492, 430, 431,435, 439
Phototoxicity		432, 489
Skin sensibilisation	406, 429, 442A, 442B	442C, 442D, 442E, 497
Repeated dose toxicity	407, 408, 409, 410, 411, 412, 413, 452	
Genotoxicity	488, 489, 483, 478, 475, 474, 473, 485, 484	471, 490, 487, 476
Carcinogenicity	451, 453	
Reproductive toxicity (fertility, sexual function and development)	443, 414, 415, 416 (421 and 422 for screening only)	
Neurotoxicity	424, 419, 418, (426)	
Endocrine disruption*		493, 455, 458, 456
Dermal absorption and toxicokinetics	440, 441	428
	417, 427	

* OECD guidance document GD150 and ECHA and EFSA gives an overview of OECD test guidelines for other endpoints in which information on endocrine disruption can be obtained (repeated dose toxicity, carcinogenicity, reproductive toxicity and TG426). This has been adopted in some test guidelines.

Trends

In recent years the term '3R method' is making way for 'NAM' within, amongst others, the field of chemical safety assessment. This term was coined at ECHA in 2016 as abbreviation of 'New approach methodologies'. ECHA's definition of NAMs is: "*NAMs include in silico approaches, in chemico and in vitro assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment. They also include a variety of new testing tools, such as "high-throughput screening" and "high-content methods" e.g. genomics, proteomics, metabolomics; as well as some "conventional" methods that aim to improve understanding of toxic effects, either through improving toxicokinetic or toxicodynamic knowledge for substances."* The US Environmental Protection Agency (EPA) defines NAMs as follows: *"it is broadly descriptive reference to any non Vertebrate Animals technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment."*

European strategies for the reduction of animal studies
In the European Chemicals Strategy for Sustainability the EC has committed itself to stimulate the development of advanced materials, methods and models for the assessment of chemical substances, and to move away from animal studies. Also on the international level, e.g. at the OECD, the EC wants to advocate this commitment in order to become less dependent on the use of animal studies (for example: EU resolution about the reduction of animal studies and the European Partnership for Chemicals Risk Assessment).

Another development in the EU is the effort to coordinate risk assessment for different regulatory domains (one substance - one assessment), which is described in the **European Green Deal**. This can also lead to a reduction in animal testing. Another interesting development in the field of plant protection products is the shift from chemical substances towards the use of micro-organisms as plant protection product, see the website of the Ctgb (in Dutch) for more information. This category of plant protection products already requires less animal studies and after modification of the test guidelines even less animal studies are needed. This also leads to a reduction in animal testing.

Evolution / revolution approach

Two potential ways towards animal-free safety assessment are an evolutionary or revolutionary approach. The evolutionary approach is focussed on the implementation of NAMs in the current framework for safety assessment. These are mainly NAMs that can fulfil the current information requirements, which are still predominantly tailored to information that can be obtained from animal studies. The revolutionary approach is focused on a completely animal-free safe assessment system. Human biology and physiology are at the centre of this approach (and does not regard the animal experiment as the golden standard) and research is focused on how information can be gathered with a combination of NAMs that can provide insights about the mechanism of human toxicity. The revolutionary approach reasons from the information needs and not from the information requirements. More information on evolution and revolution can be found on the webpage on this topic (in Dutch).

In situ validation and case studies

In situ validation is a concept that entails the extensive characterisation of a NAM or strategy of multiple NAMs within a defined setting, using some of the principles of validation, but not yet in the form of a full validation study. One way to do an *in situ* validation is by doing an extensive characterisation within one laboratory. Another way that is now increasingly used is through the development of case studies. This type of *in situ* validation is focussed on further developing new test methods or strategies towards robust and standardised tests for regulatory purposes. Such an integrated testing strategy is characterised in a larger consortium, often trying to perform a safety assessment on a specific compound without the use of animal studies. These consortia are composed of for example universities, companies and regulators.

An example of a case study initiative is the IATA case studies project. These case studies all aim to perform part of a safety assessment of a chemical substance without animal studies. Case studies are firstly evaluated by the OECD working party on hazard assessment (WPHA) and subsequently approved by the OECD-WNT before they are published as an approved OECD case study. Furthermore, there is another initiative from amongst others ECHA, EFSA and US EPA (Accelerating Pace of chemical risk assessment; APCRA) to work interactively with various organisations on case studies in which the use of new technologies for regulatory safety assessment is explored. Here too the case studies form the basis for the use of new technologies applied to regulatory safety assessment. Cosmetics Europe also works on various case studies to support the concept of Next Genration Risk Assessment (NGRA). Lastly, several of the EU-funded projects are built around case studies such as EU-ToxRisk and RISK-HUNT3R. Development in these and comparable projects are reported annually by EURL ECVAM.

Table 8. Overview of national and international networks concerning NAMs.

Network	Stakeholders	Goal (as defined by network)	Activities	NL organisations
National				
https://www.animal-freeinnovationtpi.nl/ **(TPI)**	Government, society, industry and academia.	Create space for the development of models and tests without the use of animals, and increase trusts in these innovations.	Initiatives in different areas (content, process and/or system) and work on experimental ideas that are happening in the many domains of animal-free innovations.	VWS, IenW,, OCW, Defensie, EZK, LNV, NFU, Proefdiervrij, RIVM, Samenwerkende GezondheidsFond-sen, Life Sciences & Health, VSNU, ZonMw, KNAW, NCad
International				
ILMERAC NAM working group	Food authorities and research organisations	(informal) sharing of information about NAMs in food safety	Working group meetings	RIVM, NVWA-BuRO
Unece Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) - Informal working group Use of non-animal testing methods for classification of health hazards	Informal working group, set up in 2015 by the Netherlands and United Kingdam. Interested countries, organisations (e.g. JRC) and industry can participate in this working group. A group can submit a proposal and ask for a mandate. These often take the lead in the process.	The task of the working group is to revisit the current GHS text to enable classification based on non-animal methods and approaches (*in silico, in vitro, in chemico*)	The working group works with webinars and twice a year they gather during the meeting of the Subcommittee GHS. The working group advises about implementation of NAMs in the GHS for classification and labelling of chemical substances. In the current situation a stepwise approach is taken, starting with skin corrosion/ irritation (chapter 3.2), eye corrosion/ irritation (chapter 3.3) and skin sensitisation (chapter 3.4)	VWS, RIVM, TNO
Accelerating the Pace of Chemical Risk Assessment **(APCRA)**	Regulatory bodies, global	To promote collaboration and dialogue on the scientific and regulatory needs for the application and acceptance of NAMs in regulatory decision making	Workshops, case studies	RIVM

Network	Stakeholders	Goal (as defined by network)	Activities	NL organisations
Animal-free Safety assessment of chemicals: Project cluster for Implementation of novel Strategies (**ASPIS**)	Researchers of H2020 projects ONTOX, PrecisionTOX, RISKHUNT3R	To organise joint collaboration of the H2020 projects ONTOX, PrecisionTOX, RISKHUNT3R, towards sustainable, animal-free and reliable risk assessment of tomorrow	ASPIS cluster meetings (kick-off meeting, 04.11.2021 Bruxelles), case studies in ONTOX, PrecisionTOX, RISKHUNT3R	RIVM, UU-IRAS, HU, LU- LADCR
WHO chemical risk assessment network	Government and public health institutions, intergovernmental organisations, professional societies, WHO collaborating centres, nongovernmental organisations in official relations with WHO, other non-profit entities with relevant expertise	To improve chemical risk assessment globally through facilitating sustainable interaction between institutions on chemical risk assessment issues and activities	Webinars, community of trainers for human health risk assessment, task groups, guidances, frameworks, toolboxes	RIVM
HESI animal alternatives in environmental risk assessment committee	Scientists from academia, government, industry, NGO's and other strategic partners	To collaboratively identify and help resolve global health and environmental challenges through public and private partnerships	Collaborative environment, meaningful studies, implementation	RIVM
ECETOC	Scientists from academia, governments and industry	To develop and promote trusted and practical scientific solutions which ensure a safe, sustainable and healthy world.	Workshops, Expert Meetings, Task Forces, research projects (Cefic LRI)	KWR (Scientific Committee)
European Partnership for Alternative Approaches to Animal Testing (**EPAA**)	EC, universities, industry federations	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 or are not sufficient to ensure the safety of substances (the '3R principle').	Annual conferences, partners forum, collaboration for training in non-animal methods, alternative methods video tutorials, project teams on development, validation, acceptance and implementation of 3R methods	-

Network	Stakeholders	Goal (as defined by network)	Activities	NL organisations
Cosmetics Europe	Multi-disciplinary partnerships between Cosmetics Europe's member companies and other groups with deep interest in non-animal approaches, including regulators, validating bodies, academia, research institutes, industry partners	To enable animal-free safety assessment of chemicals and cosmetic ingredients after repeated exposure, thereby entirely replacing repeat dose toxicity animal tests.	Support case studies to develop NAMs, implement NAMs in risk assessment, support regulatory acceptance	Nederlandse Cosmetica Vereniging (NCV)
International Cooperation on Cosmetics Regulation (**ICCR**)	Voluntary international group of cosmetics regulatory authorities: Brazil, Canada, Chinese Taipei, the European Union, Japan, Republic of Korea, and the United States	Multilateral framework to maintain and enable the highest level of global consumer protection by working towards and promoting regulatory convergence, while minimizing barriers to international trade.	Yearly discuss common issues on cosmetics safety and regulation, as well as enter into a constructive dialogue with relevant cosmetics industry trade associations.	*Not applicable as it concerns a European position*

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