DELIVERABLE REPORT 2.1

Report on NAMs' requirements and validation strategy

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1 R= Document, report (excluding the periodic and final reports); DEM = Demonstrator, pilot, prototype, plan designs; DEC = Websites, patents filing, press & media actions, videos, etc.; DATA =Data sets, microdata, etc.; DMP = Data management plan; ETHICS = Deliverables related to ethics issues; SECURITY = Deliverables related to security issues; OTHER = Software, technical diagram, algorithms, models, etc.

2 PU = Public, fully open, e.g. web (Deliverables flagged as public will be automatically published in CORDIS project's page); SEN = Sensitive, limited under the conditions of the Grant Agreement; Classified R-UE/EU-R = EU RESTRICTED under the Commission Decision No2015/444; Classified C-UE/EU-C = EU CONFIDENTIAL under the Commission Decision No2015/444; Classified S-UE/EU-S = EU SECRET under the Commission Decision No2015/444

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Acronyms Listed in this Document	
EC	European Commission
WP	Work Package
3R	Reduction, Replacement, and Refinement
AOP	Adverse Outcome Pathway
ECVAM	European Centre for the Validation of Alternative Methods
ECHA	European Chemicals Agency
EU	European Union
FAIR	Findable, Accessible, Interoperable, and Reusable
FDA	Food and Drug Administration
GD	Guidance Document
GIVIMP	Good *In vitro* Method Practices
GLP	Good Laboratory Practice
IATA	Integrated Approaches to Testing and Assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JACVAM	Japanese Center for the Validation of Alternative Methods
MAD	Mutual Acceptance of Data
MIE	Molecular Initiating Event
MOA	Mode of Action
NAM	New Approach Method
OECD	Organisation for Economic Co-operation and Development
PBK	Physiologically Based Kinetic
SOP	Standard Operating Procedure
TRL	Technology Readiness Level
WP	Work Package

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Executive Summary

The CHIASMA Project, funded by the European Union's Horizon Europe Research and Innovation Programme, aims to develop, and validate innovative New Approach Methods (NAMs) for the safety and sustainability assessment of chemicals and materials, minimising the reliance on animal testing. This deliverable, focused on Work Package 2 (WP2), outlines the development and validation strategy for both *in vitro* and computational NAMs, focussing on compliance with OECD guidelines to ensure regulatory readiness. The Project integrates biological and computational approaches, aiming for a combinatorial framework that enhances mechanistic understanding and safety assessment efficiency. The structured validation process, including intra-laboratory and inter-laboratory evaluations, aims to produce reliable, reproducible, and ethically sound NAMs, setting new standards in toxicological testing and safety evaluation.

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1 Introduction

The foremost goal of CHIASMA is to develop novel and innovative methods for the safety and sustainability assessment of chemicals and materials. Work package 2 (WP2) focuses on the development of experimental New Approach Methods (NAMs) for the safety assessment of chemicals and materials, ensuring their relevance for regulatory practice. The primary objective of WP2 is to advance the CHIASMA NAMs, generate Good Laboratory Practice (GLP) ready methods, and conduct intra-laboratory and inter-laboratory validation of the developed NAMs. The tasks involved in WP2 include defining the requirements and validation strategy for NAMs, developing, and optimising experimental NAMs, and predicting long-term health effects using NAMs.

The focus of this deliverable is Task 2.1 of WP2, where established guidelines to develop a comprehensive validation strategy for NAMs are being brought into the CHIASMA NAMs development programme. This deliverable demonstrates the necessary procedures to prepare the NAMs under development in CHIASMA for regulatory submission.

2 New Approach Methods Overview

The 3R principles of reduction, replacement, and refinement, emphasise minimising animal use in experiments by adopting alternative *in vitro* models (1). Modern toxicology is increasingly focused on employing models that elucidate the mechanisms through which chemicals, drugs, materials and substances affect measurable readouts and phenotypes in biological systems. To achieve accurate mechanistic material safety assessments, it is imperative to develop and utilise relevant biological test systems. New Approach Methods (NAMs), which include *in silico* (computer-based), *in chemico* (chemical-based), *in vitro* (cell culture-based), and ex vivo (tissue-based) techniques, are promoted by regulatory bodies globally (OECD, ECHA, FDA and more), as effective alternatives to animal testing. These methods provide essential risk assessment data for chemicals and materials while significantly reducing the reliance on animal experimentation (2,3).

When developed according to the rigorous validation guidelines established by the OECD, NAMs offer the advantage of consistent and reproducible endpoints, which contrasts with the variable outcomes often associated with animal experiments (4). Furthermore, NAMs enhance resource efficiency, allowing for high-throughput experimentation that generates ample data for robust analysis within shorter timeframes, and algorithmic methods (*in silico*, *in chemico*) are only limited by computational requirements and the availability of curated FAIR (Findable, Accessible, Interoperable and Reusable) data. Effective mechanistic assessments from biological systems necessitate testing multiple concentrations and exposure times, increasing sample numbers and the throughput required. NAMs are well-suited for such demanding experimental designs due to their resource efficiency, and coupled with

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computational methods that can bring information from the wealth of publicly available data, NAMs are highly applicable for safety assessment. As a result, NAMs are becoming the foundation of contemporary toxicology and safety assessments, despite ongoing challenges to their universal acceptance and implementation (3,5). The adoption of NAMs marks a significant shift towards more ethical and efficient testing methodologies, paving the way for advancements in chemical safety evaluation without the extensive use of animals.

3 New Approach Methods in CHIASMA

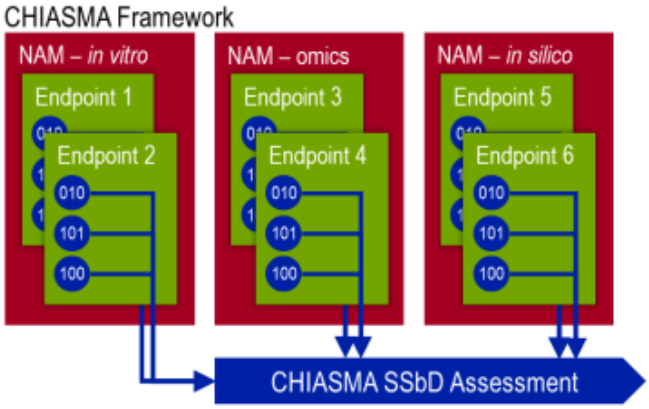

Figure 1: The CHIASMA framework for the development of NAMs for safety assessment

The final goal of CHIASMA is to develop combinatorial NAMs, consisting of *in vitro*, omics and *in silico* components, which have each been developed in an iterative fashion (Table 1). From the outset, *in silico* and omics methods will use publicly available data based on the exposure substances selected in WP1. The *in vitro* biological NAMs will be exposed to the chemicals defined in WP1, and data from these experiments, including omics data under WP3, will be used to fill and data gaps in the development of the *in silico* and omics NAMs approaches. In turn, the combined data and developed methods will be brought together to refine these combinatorial NAMs (Figure 1). Each of these stages requires an understanding of, and compliance with, the necessary regulatory guidelines for each of the methodologies employed, with an eventual goal to have regulatory ready NAMs over the course of the CHIASMA Project that can be submitted to the OECD.

Table 1: A full list of the NAMs being developed in CHIASMA.

NAM name	Lead partner	Target endpoint
Biological *in vitro* NAMs		
ALIsens	LIST	Respiratory Sensitisation Genotox (COMET) Genotox (micronuclei)

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		Irritation Acute Toxicity
Liver spheroids	SU	Chromosomal damage DNA strand breaks Point mutations Gene expression changes Inflammatory response
Placenta model	EMPA	Barrier integrity Endocrine Function Cell Viabilty Barrier translocation
Skin model	EMPA	Irritation Sensitisation
Intestinal model	EMPA	Barrier integrity Cell viability Inflammation Assay Lipid Uptake
Neuroendocrine model	NMBU	Neuroreproductive toxicity BPG axis: GnRH, LH and FSH production and receptors
Gonadal model	NMBU	Reproductive toxicity: egg/sperm maturation, fertilisation
Developmental neurotoxicity model (Neurosphere assay)	IUF	hNPC proliferation (NPC1) radial glia migration (NPC2a) neuronal migration (NPC2b) oligodendrocyte migration (NPC2c) neuronal differentiation (NPC3) neurite outgrowth (NPC4) oligodendrocyte differentiation (NPC5)
Developmental neurotoxicity model (human neural network formation assay, hNNF)	IUF	Spike-, burst- and network-related parameters based on mixed neuron/glia neural networks, exposure during network formation
Neurotoxicity model (hMNR Assay)	IUF	Spike-, burst- and network-related parameters based on mixed neuron/glia neural networks, acute exposure of matured networks (effects on individual neuronal subtypes detectable)
Placenta-embryo model	UniTOV	Barrier Integrity, barrier translocation (when possible), fetal effects
Blood-brain barrier spheroid *in vitro* model	AIT	Barrier integrity, cell viability, cell toxicity

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Blood-brain barrier Transwell *in vitro* model as alternative	AIT	Barrier integrity, cell viability, cell toxicity, toxin permeability (dep. on availability of analytics)
Kidney model	VU	Barrier integrity, cell viability, Lactate/Glycolysis OCR/Mitotox/ Gene changes
Computational *in silico* NAMs		
PODs from OMICS	TAU	Reconstruction of molecular MOA
AOP fingerprint	TAU	AOP reconstruction from OMICs data; prediction of MIE/AO
PBK models	NTUA	Biodistribution prediction under different exposure scenarios, risk assessment employing AOPs information

Table 1: A full list of the NAMs being developed in CHIASMA. Light Blue = biological in vitro NAMs, Light Red = Computational NAMs.

3.1 CHIASMA *in vitro* NAMs

In vitro new approach methods (NAMs) represent a significant shift in the field of toxicology and safety assessment, moving away from traditional animal testing towards more humane, efficient, and often more human relevant alternatives. *In vitro* NAMs use cell culture systems to evaluate the safety and efficacy of materials, chemicals, drugs, and other substances by studying their effects on cultured cells, tissues, or organs outside of a living organism, in highly controlled experimental setups. One of the primary drivers behind the development and adoption of *in vitro* NAMs is the ethical concern regarding animal testing. Traditional chemical safety assessment methods often involve significant animal suffering and are increasingly seen as morally unacceptable. Additionally, these traditional methods can be time-consuming, costly, and sometimes fail to accurately predict human responses due to interspecies differences. *In vitro* NAMs address these issues by providing more ethically sound and potentially more relevant human-based data (6).

A total of 10 biological systems are represented by *in vitro* experimental NAMs being developed within the CHIASMA consortium (Table 2). The selection of these biological NAMs was taken to cover the primary organs responsible for response to exposure, being either exposure sites themselves (lung, skin, intestine), involved in the metabolic response to exposure (liver, kidneys) or then critical sites of exposure susceptibility (developing neurons, blood-brain-barrier, blood-placental-barrier, placenta-embryo model etc.). On top of this, a broader definition of NAMs has been developed to include *in silico* and *in chemico* approaches within CHIASMA.

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Table 2: The biological system modelled by in vitro NAMs with the method developer (Develop.) and the partner who will validate the method (Valid.) in an inter-laboratory validation.

NAMs/Models	Developer	Validation by
Lung	LIST	SU/RIVM
Skin	EMPA	LIST
Intestine	EMPA	LIST
Liver	SU	VU
Reprotox	NMBU	UniTOV
Reprotox	UJniTOV	NMBU
Kidneys	VU	AIT
Brain and ED-DNT	IUF	AIT
Blood-Brain-Barrier	AIT	IUF
Blood-Placenta-Barrier	EMPA	UniTOV
Placenta-Embryo-Model	UniTOV	EMPA

3.1.1 OECD GD 211

The rapid development of next generation *in vitro* assays, means that the historical guidelines developed for validation and regulatory acceptance (such as OECD GD 34) are often not easily useable for the validation of these new and complex technologies and as they are slow to update, regulators increasingly are met with non-guideline *in vitro* assay data that, whilst not validated, is still useful information for their evaluation of the safety of the test chemical (5,7). The OECD has recognised this and provided the Guidance Document for Describing Non-Guideline *In vitro* Test Methods OECD GD 211, in an attempt to provide a framework for *in vitro* method developers to describe the applicability of their methods in safety assessment despite the fact they haven't passed through the full battery of validation processes required to become a test guideline (7).

The information that GD 211 suggests should be minimally provided for an *in vitro* method can be summarised as follows. General Information: assay names, summary, dates, contact details, developers, and laboratories, as well as references to main scientific papers using the method and information on any proprietary elements. Test Method Definition; with a detailed description of the assay's purpose and scientific principle; information on the tissue, cells, or extracts used, their species source; description of the experimental system, exposure regime, response measurement, and quality/acceptance criteria; and identification of any technical limitations and related assays. Data Interpretation and Prediction Model; with information on how the assay's data is used in prediction models; a description of the prediction model, software used, and any relevant data analysis processes. Test Method Performance; with information on the robustness of the method, including within-laboratory and between-laboratory reproducibility; details on reference chemicals, performance measures, predictive capacity, and scope/limitations of the assay. Potential Regulatory Applications;

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describing context of use, including how the assay supports regulatory decisions such as priority setting, screening level assessments, and integrated approaches to testing and assessment (IATA).

The OECD GD 211 aims to ensure that non-guideline *in vitro* methods are described comprehensively and transparently, facilitating their use in regulatory contexts, and streamlining their potential eventual submission for consideration as guideline methods. By providing a standardised framework, GD 211 helps in assessing the scientific validity and regulatory applicability of these methods, promoting their acceptance and integration into safety assessment frameworks. With that said, one criticism aimed at GD 211 however, is that it is primarily targeted at regulators and lacks the necessary detail to fully equip test method developers (often bench scientists) with knowledge of the exact scope of information they should include when creating a method description conforming to GD 211. Therefore, an annotated toxicity test method template (ToxTemp) was developed for filling in by *in vitro* test method developers, which meets all requirements of GD211, has information explaining to ToxTemp users the detail of information required, includes sections to define the acceptance criteria for tests, and sections that define the cells used in the *in vitro* method with sufficient detail and transparency (8).

In CHIASMA, partners developing *in vitro* methods have been directed to provide the complete set of information suggested in GD 211 in the form of a ToxTemp. Over the course of the CHIASMA Project, if partners fail to develop their *in vitro* NAMs to full regulatory readiness as per GIVIMP and OECD GD 34 (see following), then the *in vitro* NAM description outlined in the ToxTemp and following GD 211 has been set as the minimum requirement at the cessation of CHIASMA.

3.1.2 OECD GD 286

The OECD's Guidance Document on Good *In vitro* Method Practices (GIVIMP) 286 aims to improve the reliability, robustness, and regulatory acceptance of *in vitro* methods for human safety assessment. GIVIMP was developed by the OECD Working Group on Good Laboratory Practice (WG GLP) and the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) agreed and coordinated by the validation body European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM). GIVIMP provides detailed guidance on scientific, technical, and quality practices throughout the lifecycle of *in vitro* methods (9).

GIVIMP focuses on human safety assessment using mammalian cell and tissue cultures. There are strictly defined roles and responsibilities defined by GIVIMP, where of particular importance to CHIASMA NAM development, *in vitro* method developers are responsible for developing, documenting, and ensuring the reproducibility and regulatory readiness of *in vitro* methods. If the *in vitro* method is to become a test system then those responsible for developing the method must ensure the quality, authenticity, and contamination-free status of biological systems provided. Quality assurance (QA) and quality control (QC) are critical to the success of *in vitro* methods. Under GIVIMP this

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entails risk assessments, quality control charts, and the integrity of electronic data with necessary audit trails, all ensured by an appropriate auditing body. Ensuring the quality of test systems, consumables, reagents, and staff training is essential.

The performance of *in vitro* methods under GIVIMP involves establishing stringent acceptance criteria, robust experimental design, reproducibility via in-house validation, and the use of necessary control chemicals to guarantee reliability and reproducibility. Data analysis of the detailed endpoints must be robust and transparent to support the credibility of results. GIVIMP also mandates rigorous storage and retention protocols, including data integrity measures, archiving, and backup procedures, to ensure the traceability and long-term availability of records and materials. Data management, storage, handling and FAIRification is being developed in WP4 of CHIASMA.

GIVIMP provides guidelines for Good Cell Culture Practices (GCCP) for the sourcing, transportation, handling, and maintenance of test systems. This includes cryopreservation, thawing, contamination screening, and verification of cell line identity. GIVIMP also covers test and reference/Control Items so that model developers know how to guarantee proper preparation, concentration range, solubility, and stability of test items, avoiding interference with the test system and ensuring accurate exposure.

The partners developing *in vitro* NAMs in CHIASMA have been instructed to follow GIVIMP guidelines, and during the crystallisation and optimisation of the biological NAMs in CHIASMA, the procedures described in GIVIMP are being developed into Standard Operating Procedures (SOPs) as outlined in the OECD Principles on Good Laboratory Practice (10). SOPs must be well-designed, robust, unambiguous, and clear, to reduce variability and ensure consistent application of *in vitro* methods. By adhering to GIVIMP and developing the necessary SOPs, partners in CHIASMA developing *in vitro* methods are safeguarding the scientific validity, regulatory readiness, and ethical standards of their work, and will increase the likelihood that the developed NAMs will be accepted for follow up validation and eventual acceptance into OECD test guidelines.

3.1.3 OECD GD 34

The ultimate aim is to bring NAMs within CHIASMA to a state of readiness so that they can be submitted either to EURL-ECVAM for validation, or to the OECD via a Standard Project Submission Form (SPSF) for review to potentially become a test guideline. The OECD GD 34 is a comprehensive guidance document designed to provide a detailed framework for the validation of new or updated test methods. The document is divided into several key sections, each focusing on different aspects of test method validation and regulatory acceptance. This guideline document stresses the need for internationally recognised principles and criteria to standardise the validation process of test methods.

GD 34 defines what constitutes a test method, including its chemical, biochemical, or biological basis. It covers the rationale for test relevance and the endpoints to be measured. Definition of the test method is crucial, and it must have relevance to an internationally recognised issue, with relevant endpoints developed. The first phase of

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validation is crucial for establishing the scientific foundation and intended use of the test method.

GD 34 gives guidelines on prospective and retrospective validation studies. Prospective studies involve generating new experimental data, while retrospective assessments evaluate existing data. A modular approach to validation is proposed, organising information into distinct modules like test definition, repeatability, reproducibility, and predictive capacity. Within CHIASMA, NAMs are being generated with the intention to be submitted for retrospective validation, as the data developed in CHIASMA should be sufficient to cover the retrospective validation requirements. This may or may not come with an intermediary step of submission of one or more of the CHIASMA NAMs to a validation centre such as EURL-ECVAM, ICCVAM, JACVAM for a full validation prior to submission to the OECD via an OECD SPSF (Standard Project Submission Form), which is a formal document used to propose a new or revised test method for consideration as an OECD Test Guideline.

GD 34 provides guidance on the purpose of validation, the necessity of prevalidation to refine and optimise test protocols, and the formal inter-laboratory validation process. It emphasises the importance of standardisation, reproducibility, and reliability in validation studies. The section on independent evaluation of validation studies (peer review) describes the mechanisms for conducting peer reviews, including the selection and roles of peer reviewers. It outlines the process for ensuring that the validation criteria have been met and provides a transparent assessment of the test method's performance. Within GD 34, the international regulatory acceptance of validated tests is discussed. The criteria for regulatory acceptance, the process of moving from test protocols to official test guidelines, and the importance of early regulatory involvement in the validation process are emphasised. This section highlights the need for harmonisation of test methods to facilitate international acceptance and mutual recognition of data.

The Mutual Acceptance of Data (MAD) agreement is a foundational principle first adopted by the OECD in 1981, which has since been amended multiple times to include amongst other things the necessity of compliance with GLP principles. Under the standards of MAD, member countries facilitate the harmonisation of chemical safety assessments. The agreement ensures that data generated from testing chemicals in one member country, according to OECD Test Guidelines and Principles of Good Laboratory Practice (GLP), are accepted in other member countries for regulatory purposes. The primary goal of the MAD system is to avoid duplicative testing, reduce the use of test animals, and streamline the regulatory process by providing a mutually recognised framework for chemical safety data. GD 34 and the validation of *in vitro* NAMs necessitates adherence to the principles of MAD. In the final section of GD 34, the necessary components of a submission package to the OECD are described, including scientific and regulatory rationale, test method protocols, performance data, and other supporting materials. Over the duration of CHIASMA, NAMs will be brought to regulatory readiness by developing the necessary components of the submission package after

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proving that the NAMs are functional and relevant for the safety assessment they undertake.

3.2 CHIASMA computational NAMs

Computational modelling and *in silico* approaches are beginning to become a crucial component in modern toxicology and safety assessment NAMs. Such computational NAMs involve using computer algorithms and simulations to predict the toxicity and safety of substances based on their chemical structure and known biological data. *In silico* models can rapidly analyse large libraries of data, to predict the potential risks of test substances, thereby reducing the need for extensive *in vitro* or *in vivo* experiments (11).

The introduction of *in silico* methods into test guidelines under the OECD has been slow to progress. *In silico* methods, such as computational models and simulations, require extensive validation to ensure they are as reliable and accurate as traditional *in vivo* or *in vitro* methods. This involves demonstrating their predictive accuracy across a wide range of chemicals and conditions, which can be a time-consuming and computationally costly process. On top of this, *in silico* methods rely heavily on large datasets for training and validation. The availability of high-quality, comprehensive datasets is crucial for developing accurate models. However, obtaining such datasets can be difficult, and there may be issues related to data sharing and confidentiality. Furthermore, regulatory bodies have notoriously and understandably been cautious about adopting new methodologies, especially those that significantly deviate from established practices. The regulatory acceptance of *in silico* methods ultimately depends on their ability to meet stringent safety and efficacy standards, which requires robust evidence and consensus among international stakeholders (11).

For *in silico* methods to be widely accepted and integrated into OECD guidelines, there must be standardised protocols and guidelines. Developing and validating *in silico* models that can accurately predict complex biological interactions and toxicological outcomes is technically challenging. These models need to account for various biological variables and mechanisms over thousands of data points, which adds to the complexity and development time and requires best practices in the fields of data transformation and statistics. Despite all these challenges, there are precedents for *in silico* methods within the current battery of OECD test guidelines including GARDSkin. GARDSkin, OECD Test Guideline (TG) 442E, is an *in silico* method designed to predict skin irritation potential of chemicals using a combination of machine learning algorithms and large datasets of known skin irritants to develop predictive models (12). The model measures the gene expression response of a panel of genes, which are transformed and analysed to predict the likelihood of skin irritation. SkinGard exemplifies how *in silico* methods can be successfully integrated into OECD guidelines, providing a reliable and efficient alternative to traditional testing methods while adhering to rigorous validation standards (13). Its success highlights the potential for broader acceptance and use of computational approaches in regulatory toxicology.

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In silico methods in CHIASMA consist of several computational approaches. These include the identification of Points of Departure (PODs) from OMICS data, and the reconstruction of molecular Modes of Action (MOA). The utilisation of transcriptomics data for the generation of Adverse Outcome Pathway (AOP) fingerprints, for the prediction of Molecular Initiating Events (MIE) and Adverse Outcomes (AO) for exposure substances. Also, Physiologically Based Kinetic (PBK) models are employed for predicting biodistribution under different exposure scenarios for risk assessment. Whilst there is an OECD guidance document 331 for PBK modelling (14,15), the guidance for the validation of toxicogenomics is lacking in part due to the challenges of standardising analysis but efforts are still underway to bring toxicogenomics data into greater regulatory use (16). With that said, there is some regulatory guidance on Integrated Approaches to Testing and Assessment (IATA) within GIVIMP GD 211, and a guidance document on the the use of AOPs in developing IATA has been developed (17), which both inform how to manage data and use AOPs as a platform for organising molecular data such as transcriptomics, an effort towards which is already underway amongst CHIASMA partners (18). That omics data can be useful for chemical safety assessment is not in doubt, but the strategy to bring it to regulatory readiness within the omics computational NAMs developed in CHIASMA requires extensive expertise and application of general regulatory principles of traceable and FAIR data management, robustness and reproducibility. Expertise which is already developed amongst the collaborations between CHIASMA partners.

4 Validation strategy for NAMs in CHIASMA

Regulatory acceptance and integration of NAMs into safety assessment frameworks are essential for their widespread adoption. As seen in the previous sections, international efforts, such as those led by the Organization for Economic Co-operation and Development (OECD), aim to standardise and validate NAMs to ensure their reliability and reproducibility. These efforts are crucial for gaining regulatory approval and fostering confidence in the use of NAMs for safety evaluations.

A pivotal step in ensuring regulatory compliance and positioning NAMs in CHIASMA for eventual regulatory readiness and acceptance is first to develop the biological NAMs as per OECD GD 211 (ToxTemp) and GIVIMP. As seen in Figure 2, the development stage of each of the NAMs within CHIASMA is varied, with some in the preliminary stages of optimisation and validation, and others already being moved to submission to the OECD. Computational NAMs are also under development in CHIASMA and resultant NAMs will be a combination of *in vitro* biological and computational methods by for instance the generation of omics data from the biological NAMs that will be used in the downstream computational NAMs. As a means to ensure development of the NAMs within CHIASMA continues to proceed as per the necessary guidelines, and to ensure that a regulatory strategy towards validation of the NAM is being implemented, upon the conclusion of this deliverable, each partner responsible for a biological NAM will be sent a form (Annex A1), which is provided in a format that is accessible despite the heterogeneous stages of development of the NAMs, and covers the necessary criteria

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from OECD GD211 and GIVIMP, as well as asking the partner to provide their plan to validate the NAM in accordance with OECD GD 34. One form will be filled out for each NAM being developed. The answers given in this form can be used to identified further needs of each biological NAM and any weak points in the continued development of the NAM that need to be overcome for the positioning of the NAM for eventual regulatory submission.

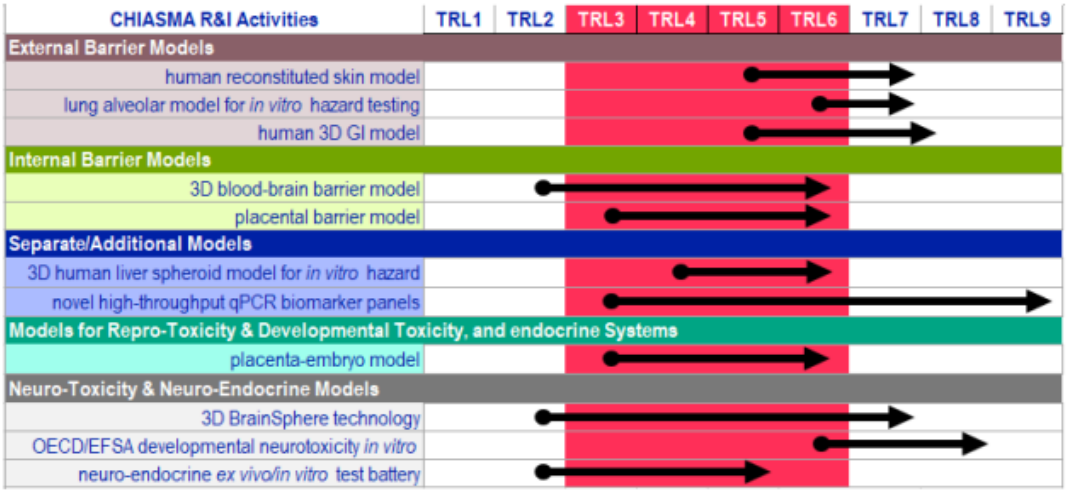

Figure 2 Technology Readiness Level (TRL) on a scale of 1-9 of the NAMs and research and innovation (R&I) activities being undertaken in CHIASMA.

As well as developing robustness with independent intra-laboratory repeats of the NAMs, inter-laboratory validation is also a strict regulatory requirement to ensure the transferability of the NAM to differing institutional environments. Inter-laboratory validation is already underway within CHIASMA as partners are beginning the logistical operations to send their NAMs to their validation partners as seen in Table 2, using GIVIMP as a template for transfer operations of *in vitro* technologies and biological materials. This inter-laboratory validation is an essential component of the validation strategy being undertaken during the Project.

5 Conclusions

This report has detailed the comprehensive requirements and validation strategies employed in the development of New Approach Methodologies (NAMs) within the CHIASMA Project. By adhering to stringent regulatory guidelines and fostering collaboration among partners for inter-laboratory validation, CHIASMA will ensure that NAMs are not only scientifically robust but also regulatory-ready. The implementation of tools such as the 'CHIASMA NAMs Development Form' (ANNEX A1) provides a structured approach to assess the development needs and track progress, ensuring consistent adherence to OECD GD 211 and GIVIMP guidelines throughout the project.

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Moreover, the Project's focus on combining biological and computational NAMs demonstrates an innovative approach to toxicology and safety assessment, bringing in leading edge mechanistic data and computational methods. By integrating biological and computational NAMs and ensuring compliance with regulatory standards, CHIASMA is poised to significantly advance the field of chemical and material safety assessment.

The collective efforts of all partners, through validation processes and continuous assessment of those validation procedures, will contribute to the development of reliable and reproducible NAMs. These methods are expected to meet the high standards required for regulatory acceptance, ultimately reducing reliance on animal testing and promoting more ethical, efficient, and human-relevant safety assessments. As CHIASMA progresses, the Project's outputs will encourage the broader adoption and regulatory integration of NAMs, setting a new standard in the field of toxicological testing and safety evaluation.

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ANNEX A1 - CHIASMA NAMs Development Form

1. Partner Information

Partner Organization Name:
Contact Person:
Email:
Phone Number:
Date of Form Submission:

2. NAM Method Description

Name of the method:
Synonyms for the method:
Brief Description of the NAM (max 300 words):
Relevant Biological Endpoint(s) Addressed:
Assays used:
Associated Adverse Outcome Pathway(s) (AOPs):
How is information from this NAM extrapolated to an *in vivo* context (IVIVE)?:
Are there known related or similar methods to this NAM?:

3. NAM Development

Proprietary elements of the NAM:
Commercialization status of the NAM:
Stage of development (Planning stage/In development/fully developed/other):
Original development documentation available?:
NAM Update documentation available?:
Please list any Standard Operating Procedures (SOPs) available for this NAM:
Please list any references to publications that have used this NAM:
Please list any data repositories linked to this NAM:

4. Procedures According to GIVIMP (Guidance Document on Good *In vitro* Method Practices, OECD GD 286) for Obtaining Biological Material, Maintaining It, and Controlling Its Quality

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4.1 Obtaining Biological Material

Source of Biological Material

Describe the source of the biological material (e.g., cell lines, primary cells, tissues):

Provide information on the origin, including species, strain, and health status:

Ethical Considerations

Compliance with ethical standards and regulatory requirements for the use of human or animal tissues has been ensured (Y/N)?:

Availability of the informed consent forms for human-derived materials, if applicable:

Provide details on the ethical review process and approval documentation:

Material Transfer Agreements

Availability of any material transfer agreements (MTAs) related to the biological material:

Intellectual Property Rights

Are there intellectual property rights (IPR) issues that impact the use of the biological material (Y/N, if yes then explain further):

Pathogen Testing

If pathogen testing has performed, please indicate results and testing methods:

Known Issues

Please list any known issues or limitations with the NAM?:

4.2 Maintenance and Handling of Biological Material

Short-term Storage

Describe the conditions for short-term storage (e.g., temperature, medium):

Describe any specific handling instructions to maintain material integrity:

Long-term Storage

Provide description of procedures for long-term storage, including cryopreservation methods if applicable:

Provide description of procedures for thawing and reusing stored materials?:

Routine Handling

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Outline the standard procedures for handling and maintaining biological material. Include details on cell culture practices, subculturing intervals, and any specific requirements:

Contamination Control

Describe measures in place to prevent contamination, including the use of sterile techniques and contamination monitoring:

Documentation

Are all handling and maintenance procedures are well-documented and traceable?:

Traceability

Describe the traceability system in place to track the biological material from receipt to usage:

4.3 Quality Control

Standard QC

Describe quality control measures, such as viability testing, mycoplasma testing, and authentication of cell lines:

Internal Standards

Specify any internal standards used for quality control (e.g., positive and negative controls, reference chemicals):

Performance Benchmarks

Define performance benchmarks for each biological endpoint:

Provide benchmark values and criteria for acceptance or rejection of data:

Reproducibility Assessments

Describe methods used to assess reproducibility, including inter-laboratory comparisons if applicable:

Briefly describe results of the reproducibility assessments:

Data Analysis

How is experimental data captured, stored, and reported:

Which software are used for analysis (including versions)?:

Which statistical tests are used in analysis?:

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5. Regulatory Applicability

Building Regulatory Readiness

Does this NAM have a method description and the details as outlined in OECD GD 211 (e.g. in the form of a ToxTemp)?:

Describe the strategy toward demonstrating the applicability of the proposed NAM in regulatory applications:

Validation Status

Outline the current validation status of the NAM, including details on any within-laboratory or inter-laboratory validation processes:

Outline your proposed plan to validate the NAM according to OECD GD34 :

6. Additional Information

Any additional comments or relevant information:

7. References

OECD GD 211 **Guidance Document for Describing Non-Guideline *In vitro* Test Methods**

OECD GD 34 **Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment**

OECD GD 286 **Guidance Document on Good *In vitro* Method Practice**

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