Concept Article

The Virtual Human Platform for Safety Assessment (VHP4Safety) Project: Next Generation Chemical Safety Assessment Based on Human Data

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Abstract

The Virtual Human Platform for Safety Assessment (VHP4Safety) project aims to build a virtual human platform (VHP) to protect human health and revolutionize the safety assessment of chemicals and pharmaceuticals by transitioning from animal-based to human-based approaches. The goal of this article is to introduce the project and its interdisciplinary approach to co-creation with multiple academic, regulatory, industrial and societal partners covering the entire safety assessment knowledge chain. Three research lines drive the project: 1) building the VHP; 2) feeding the VHP with human data; and 3) implementing the VHP. The project focusses on three case studies that incorporate human-relevant scenarios not included in current animal-based safety assessment strategies. The VHP is built on tools and services, including pharmacokinetic and computational models, and integrates several data sources within each case study, including data on human physiology, epidemiology, toxicokinetic and -dynamic parameters, as well as data on chemical characteristics and exposures. In addition, the VHP integrates new data generated within the project using new approach methodologies representing key events within adverse outcome pathways. Implementation of the VHP is investigated using an innovation systems approach, engaging stakeholders, and organizing training and education. Central to the VHP4Safety project is our co-creative approach, which is facilitated by biannual designathons and hackathons that foster active involvement of all project participants from over 30 partner organizations. By integrating technological innovations with transparency and stakeholder collaboration, the VHP4Safety project will help shape the transition to next generation safety assessment in which animal testing becomes redundant.

Plain language summary

The Virtual Human Platform for Safety Assessment (VHP4Safety) project will build a virtual human platform (VHP) to determine the safety of chemicals and pharmaceuticals for human health based solely on human biology. By integrating innovations in data science, new approach methodologies, and transition science, the VHP4Safety project will help shape the transition to safety assessment in which animal testing becomes redundant. The goal of this article is to introduce the project and its interdisciplinary approach to co-creation with multiple academic, regulatory, industrial and societal partners covering the entire safety assessment knowledge chain. We invite stakeholders who support our vision to collaborate and provide input in order to enhance transparency and acceptance of the VHP in next generation safety assessment based on human data.

# contributed equally
§ http://www.vhp4safety.nl (under 'About')

1 Introduction

Imagine a world in which we perform precision safety testing of chemicals and pharmaceuticals without using laboratory animals. Imagine that the safety of chemicals and pharmaceuticals can be assessed for vulnerable groups such as infants, the elderly or the diseased. Imagine that we know how these substances interact with human biology and physiology and how they can be used safely at home, at school or at work over the course of our lives. This is the vision that underlies the Virtual Human Platform for Safety Assessment[1] (VHP4Safety) project, an interdisciplinary and multi-stakeholder collaboration that aims to help shape the transition from animal-based testing to innovative animal-free safety assessment.

Current legal and regulatory frameworks for the assessment of the risk of chemicals and safety of pharmaceuticals (further referred to in this article as "safety assessment") for human health rely predominantly on data from *in vivo* animal studies. However, the accuracy of animal studies to predict toxicity in humans is limited due to reasons that include the inability of animal studies to reflect human-relevant scenarios, such as differences in susceptibility due to age and sex, timing of exposure or disease state (Piersma et al., 2018; Westmoreland et al., 2022). In an international arena urgently calling for the reduction of animal testing, including the European Commission's launch of a new roadmap with the aim to ultimately move to an animal-free regulatory system (EC et al., 2023), there is an increasing need for a human-relevant and animal-free approach towards human safety assessment. Therefore, the opportunities offered by state-of-the-art technologies in human health and data science need to be explored in the realm of safety assessment. The mission of the VHP4Safety project is to improve the prediction of the potential harmful effects of chemicals and pharmaceuticals based on a holistic definition of human health, using a human-relevant and animal-free approach.

The goal of this paper is to summarize the structure, objectives, and approach of the VHP4Safety project, as well as the expected outcomes. We refer to the project as "VHP4Safety" and to the platform as the "VHP". The VHP, which is being developed in VHP4Safety, aims to integrate data on human physiology, chemical characteristics, and perturbations of biological pathways and will incorporate: 1) human-relevant scenarios to discriminate vulnerable groups; 2) substances from different sectors (pharma, consumer products, and chemical industry); and 3) different regulatory and stakeholder needs. This project addresses the emerging societal challenges of the transition to animal-free safety assessment by integrating various scientific disciplines and working with multiple stakeholders towards implementation and societal acceptance of an approach to chemical and pharmaceutical safety assessment that is based on human rather than animal data.

2 Structure and objectives

VHP4Safety[1] started in 2021, following a successful grant application to the Netherlands Research Council/Netherlands Research Agenda: Research on Routes by Consortia (NWA-ORC). The project has a duration of 5 years and brings partners from 32 organizations together (Tab. 1), representing stakeholders (scientists,

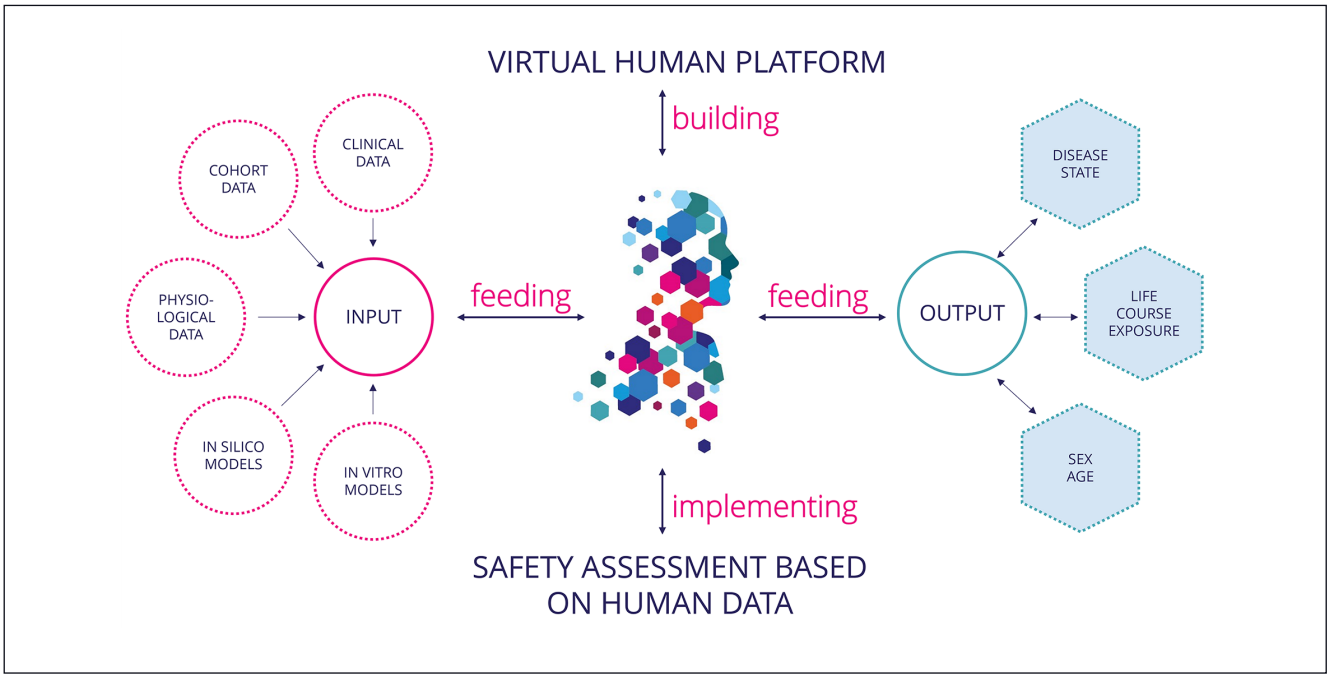

Fig. 1: Conceptual overview of the Virtual Human Platform for Safety Assessment (VHP4Safety) project

[1] www.vhp4safety.nl

Tab. 1: The VHP4Safety Consortium

Research organizations	
1.	Utrecht University (UU)
2.	University of Applied Sciences Utrecht (HU)
3.	National Institute for Public Health and the Environment (RIVM)
4.	Maastricht University (UM)
5.	Leiden University (UL)
6.	Wageningen University & Research (WUR)
7.	Amsterdam University Medical Centers (AUMC)
8.	Vrije Universiteit Amsterdam (VU)
9.	Netherlands Organization for Applied Scientific Research (TNO)
10.	Erasmus University Medical Center Rotterdam (ErasmusMC)
11.	University Medical Center Utrecht (UMCU)
Co-funding organizations	
12.	ORTEC Logiqcare
13.	Certara UK
14.	Charles River Laboratories
15.	Unilever
16.	Shell
17.	Bayer
18.	Galapagos NV
19.	International Collaboration on Cosmetics Safety
20.	Dutch Association Innovative Medicines
21.	KWR Water Research Institute
22.	Dutch Ministry of Agriculture, Nature and Food Quality
23.	Proefdiervrij
24.	Dutch Burns Foundation
25.	Dutch Kidney Foundation
Cooperation partners	
26.	Medicines Evaluation Board (CBG)
27.	Institute for Human Organ and Disease Model Technologies (hDMT)
28.	Samenwerkende Universitaire RekenFaciliteiten (SURF)
29.	Dutch Techcentre for Life Sciences (DTL)
30.	United States Environmental Protection Agency
32.	Uppsala University

industry, regulators, policy makers, clinicians, non-governmental and patient organizations) working in co-creation towards implementation and societal acceptance of an approach to safety assessment that is based on human data, *in vitro* data, and predictive computer models rather than animal data.

As shown in Figure 1, the VHP is built by integrating input data on human physiology, clinical and epidemiological data, data from *in vitro* and *in silico* models, chemical characteristics, and perturbations of biological pathways. The input data is incorporated in human-relevant scenarios to provide outputs that predict chemical safety, initially based on disease state, life course exposure, and sex and age. The platform incorporates substances from different sectors such as pharmaceuticals, food ingredients, and industrial chemicals, and with this different regulatory and stakeholder needs.

In VHP4Safety, we have started with output focusing on three scenarios for which biological, toxicological and exposure data from humans and human models are collected and/or generated and integrated with existing human physiological information to form the input that will feed into the VHP (Fig. 1). These scenarios, described in more detail below, will provide a first proof-of-concept for the VHP and will demonstrate its technical feasibility and help identify challenges. At the same time, we investigate what is needed to fulfil the requirements of stakeholders for the implementation of the VHP so as to understand the uncertainties and ensure trust in the outcomes of the VHP. As such, we have engaged many relevant stakeholders (including representatives from academia, industry, government, and society) in a consortium to help ensure that the outcomes of the VHP are reliable, sustainable, acceptable, and have translational value to human health. In a collaborative effort of co-creation each stakeholder will share knowledge and expertise. In addition, this project sets the framework for future versions of the platform, with more data, models and features also based on feedback.

The project is structured into three research lines (RLs), which address the three main project objectives:

i. To build the VHP (RL1), we are developing a platform to identify, collect, integrate, and analyze relevant human data, using state-of-the-art technology for FAIR[2] (findable, accessible, interoperable and reusable) data integration to ensure the transparency and security of the process combined with advanced computer models and well-structured knowledge. To this end, we integrate and analyze high-quality data about human biology and chemical and pharmaceutical perturbation for the purpose of risk assessment. Importantly, we aim to ensure a high predictive ability of components used to build the VHP that will aid in making safety predictions for the case studies in RL2.
ii. To feed the VHP (RL2), we perform case studies based on each human-relevant scenario. In these case studies, we collect existing human-relevant data and generate new human data for defined sets of chemicals and pharmaceuticals. We use adverse outcome pathways (AOPs) to define and model the mechanisms underlying perturbations to human biological pathways

2 https://www.go-fair.org/fair-principles/

and identify model systems that represent the key events. Substances are case study-specific and include pharmaceuticals and other chemical substances such as pesticides. This will include data on exposure, kinetics, and toxicological effects, generated with advanced *in vitro* human models.

iii. To implement the VHP (RL3), we investigate what is needed to gain confidence and trust in the VHP. As such, we aim to ensure a sustainable transition towards animal-free safety testing while striving for improved reproducibility and predictability in science. To this end, we engage relevant stakeholders from the start to determine the needs and requirements for the VHP to be applied in safety assessment and to identify incentives to change. Ample attention has been paid to co-creation, training, and educating (prospective) professionals. Ultimately, our goal is to embed the governance, implementation, and sustainability of the VHP within the safety assessment frameworks for chemicals and pharmaceuticals.

3 Research lines

3.1 Research line 1: Building the Virtual Human Platform

The platform is being built by developing a research infrastructure that hosts a number of human and computer accessible services with which a user or computer workflow can dynamically integrate data and software tools that allow for safety assessment. We use large language models and agent-based data retrieval systems to integrate data and software tools to build these workflows. A user-interface and application programming interface (API) layer allows workflows to be executed that combine data from the human-relevant scenarios described in more detail below, from human studies (epidemiological, clinical and pharmacovigilance data), from patient registries, and from various knowledge resources, using the platform as the gateway to relevant databases and computational models. Databases that will be linked to the VHP include the recommended international data collections connected through ELIXIR[3], the European infrastructure for biological data (Harrow et al., 2021; Martens et al., 2021).

The VHP is set up as a Docker-based platform, where data and tools are made available in a FAIR and flexible format. To ensure scientific (re)usability, we set objective quality criteria, and datasets undergo a process in which the quality is verified and established before being added to the platform. Development of individual Dockerized services happens on GitHub, where possible in open repositories[4]. Data collections are published in ELIXIR deposition databases or on Zenodo[5], and all tools will be described on bio.tools[6]. Docker containers themselves are shared on DockerHub[7], workflows on Zenodo, and described on WorkflowHub[8]. Documentation is available on a cloud-integrated documentation platform[9], and all training material is shared on ELIXIR's training and support platform TESS[10] (Bacall et al., 2023). Together this not only ensures availability, quality control, and interoperability, it also effectively makes efforts to build the platform more sustainable and facilitates collaboration with other cloud- and container-based scientific platforms, including those in other toxicology projects and in the European Open Science Cloud (EOSC).

We collect and improve *in silico* tools for toxicokinetics to enable the translation of *in vitro* data obtained in human-relevant bioassays to the *in vivo* situation. RL1 develops generic physiologically based kinetic (PBK) computer models, enabling quantitative *in-vitro*-to-*in-vivo* extrapolation (QIVIVE). Models will be shared and organized in project-level resources like FAIRDOM[11] to allow model versioning and published in the BioModels[12] database where possible. We aim to make these models less language-dependent, e.g., using systems biology markup language (SBML) and more explainable, for instance through automated conversion to LaTeX, which will allow discussion of model content independent of the modelling language used. By making the models more modular it will be easier to evaluate various physiological conditions, for instance by explicitly adding specific organs and exposure routes.

We also employ and integrate key *in silico* tools to describe computational toxicodynamics, including machine learning, structure-based modelling, and bioinformatics. Biological activity spectra are predicted using quantitative structure-activity relationships (QSAR) and proteochemometric modelling (PCM) based on literature data available in the public domain.

AOPs play a key role in the toxicodynamic part of the project and will serve as the basis to describe and understand results from human and *in vitro* studies from RL2. AOPs are developed or extended by expert groups and published on AOP-Wiki[13]. Semantic web versions of these AOPs allow quick searches, quality control, extensive evaluation and comparison, and aid in the development of more interoperable versions. This is then used to create molecular versions of these AOPs on a WikiPathways community page[14], which can be used for omics analysis on key event level and can

[3] https://elixir-europe.org/
[4] https://github.com/vhp4safety/
[5] https://zenodo.org/communities/vhp4safety
[6] https://bio.tools/
[7] https://hub.docker.com/
[8] https://workflowhub.eu/
[9] https://docs.vhp4safety.nl/en/latest/index.html
[10] https://tess.elixir-europe.org/
[11] https://fair-dom.org/
[12] https://www.ebi.ac.uk/biomodels/
[13] https://aopwiki.org/
[14] https://www.wikipathways.org/communities/aop.html

be converted into molecular networks to allow extension with other datatypes and study with a large variety of network biology approaches (Martens et al., 2022). Alternatively, this process can be started in CellDesigner, and the created SBML is converted to WikiPathways format. Another approach to get quantitative information about functionally related genes in key events that we employ uses the TXF-MAPr[15] tool to evaluate effects relative to known co-expression changes during tissue toxicity development (Callegaro et al., 2021). Quantitated changes in key events obtained in these two ways, or directly from dedicated *in vitro* models, can then be used for calculation of quantitative key event relationships described in differential equations.

3.2 Research line 2: Feeding the Virtual Human Platform

In order to feed the VHP built in RL1, data on human exposure, kinetics including adsorption, distribution, metabolism and excretion (ADME), and toxicological effects are collected, interpolated, and/or generated in RL2. To establish exposure-effect relations, clinical and epidemiological data are collected and assessed. Toxicokinetic and toxicodynamic data are generated by performing experiments with advanced human-relevant new approach methodologies (NAMs) based on stem cell technology, including induced pluripotent stem cell (iPSC) and organoid models for, e.g., skin, liver, lung, thyroid, gut and kidney cells. Furthermore, we set criteria and guidelines for the human relevance of the models and the quality of the data used. In addition, the possibility to reliably interpolate missing data with artificial intelligence and machine learning will be assessed. RL2 is the "home" for experimental research in the three scenarios and associated case studies, which are described in more detail below. In addition, safety assessment of the chemicals and pharmaceuticals within the case studies is performed in RL2, using outcomes from modelling in RL1 and criteria established in RL3.

The three human-relevant scenarios on disease state, life course exposure, and sex and age were selected because they are insufficiently addressed in current safety assessment based on animal testing. These scenarios also demonstrate the potential variation in human physiology and sensitivity that can play a significant role in the safety of substances. From the perspective of disease state, data is needed not only on how chemicals and pharmaceuticals may induce chronic diseases but also how chronic disease status (i.e., impaired organ function) affects the toxicity of substances. From the perspective of life course exposure, mechanisms of aging from childhood to adulthood need to be considered with respect to the toxicity of substances. Alterations in metabolic state, nutritional deficiencies, and organ deterioration in the elderly influence the adverse effects of substances. From the perspective of sex, input data is required not only on differences in reproductive function and hormone cycle, but also on kinetics, genetic-molecular differences, body size and composition, and behavior. From the perspective of age, data on chronic exposure during a substantial portion of life or even before birth, decrease in metabolic activity, and changes in body mass index (BMI) need to be considered. To address these complex scenarios, we selected three specific case studies corresponding to each scenario to research in the project, namely kidney disease and pharmacovigilance, life course exposure and neurodegenerative disease, and thyroid hormone-mediated neurodevelopmental toxicity.

Disease state scenario: kidney disease and pharmacovigilance

For the disease state scenario, the selected case study addresses kidney disease and pharmacovigilance by modelling kidney disease. Nephrotoxicity as a consequence of pharmacotherapy is a frequently reported problem in clinical medicine, and the incidence of drug-related acute kidney injury (AKI) can be as high as 26% in the adult, hospitalized population (Yousif et al., 2023). If insufficiently treated or in case of prolonged exposure and/or insufficient repair, AKI may result in chronic kidney disease. These chronic drug-related effects are currently not included in safety testing. In this case study, we expose human adult stem cell-derived organoids that closely mimic healthy human kidney proximal tubules (named tubuloids), adult human proximal tubule cell lines, as well as patient-derived organoids to nephrotoxic drugs (Schutgens et al., 2019; Jansen et al., 2022). With these models we study kinetics and nephrotoxic pathology and predict AKI upon pharmaceutical exposure for specific patient groups, which will be translated to *in silico* models and validated with available human clinical data. Molecular AOPs are designed aiming at unravelling the molecular mechanisms underpinning how drug-induced nephrotoxicity results in kidney failure and to identify relevant biomarkers with high *in-vitro*-to-*in-vivo* translational value to aid in risk assessment.

Life course exposure scenario: life course pesticide exposure and neurodegenerative disease

Neurodegeneration (e.g., Parkinson's disease) is a major cause of death in humans and has substantial impact on society, including health care costs and quality of life. Neurodegeneration cannot be adequately tested in animal studies, since cognitive decline, a common trait in neurodegeneration, is difficult to measure in animals. Little is known about the effect of exposure to chemicals and the onset of neurodegenerative disease in humans. For some compounds, there are indications of a causal relationship between exposure and neurodegenerative disease (Meerman et al., 2023b). This case study focusses on the putative relationship between pesticide exposure and Parkinson's disease by mapping the critical pathways and mechanisms underlying the disease as well as collecting data on life course exposure to pesticides in human biomonitoring studies. The first AOP network for Parkinson's disease has been developed (Meerman et al., 2023a), and dose-response relationships for pesticides are determined with relevant *in vitro* models (e.g., human neuronal iPSC models, SH-SY5Y and LUHMES cells) (Scholz et al., 2011; Ducray et al., 2020; Kumar et al., 2014).

Age and sex scenario: thyroid hormone mediated neurodevelopmental toxicity

Mammalian central nervous system development is critically dependent on maternal and fetal thyroid hormone homeostasis.

[15] https://txg-mapr.eu/

Perturbations of the thyroid hormone system during brain development might result in maldevelopment of the brain, with morphological and functional consequences that affect quality of life. Thyroid-related disease prevalence is age dependent and differs between sexes. Quantitative models describing the AOP network governing thyroid homeostasis-mediated brain development are needed to understand the quantitative role of thyroid hormones in human brain development and disease. This case study takes advantage of the wealth of human clinical data and experimental data available to support physiological and disease mapping in this area (Dierichs et al., in prep.). Molecular AOPs are designed with the aim to unravel the molecular mechanisms underpinning how thyroid hormone imbalances impact neuronal cells and to enhance our understanding of the specific effects of neurodevelopmental conditions caused by such disturbances (Martens et al., 2023). Quantitative kinetic models of human thyroid homeostasis have been developed and provide the core of physiological mapping and qAOP derivation. We investigate age and sex specificity in thyroid-mediated adverse effects on brain development using existing and newly developed human-relevant *in vitro* (thyroid organoid) and *in silico* models to evaluate the effects of compounds, verified with human clinical and epidemiological data.

3.3 Research line 3: Implementing the Virtual Human Platform

In RL3, we focus on the implementation of the VHP and provide insight into how a transition towards animal-free safety assessment can be realized. The approach relies on insights from management of socio-technical transitions and innovation systems (Hekkert et al., 2007) as well as the monitoring and evaluation of the societal impact of science and technology (Schot and Rip, 1997; Smit and Hessels, 2021). RL3 starts from the observation that transitions are not only dependent on scientific and technological advances but require equal attention to the needs, responsibilities, practices, and interests of relevant stakeholders. VHP4Safety takes an innovative approach that aims to contribute to paradigmatic change in the system of animal-based safety assessment as it proposes human biology and physiology as the basis and new reference for human safety assessment. The pathways to widespread acceptance of this innovative revolutionary approach, however, are uncertain. This requires participatory approaches to VHP development, active monitoring, and evaluation of how the project contributes to the transition towards animal-free safety assessment and educating stakeholders, amongst others.

As such, the VHP4Safety co-funders and cooperation partners are actively involved in RL3 to understand what is needed to develop, validate, and implement the technological approaches that integrate data sciences and toxicology into current practices of safety assessment in industry, government, and academia. We take into account stakeholder's motivations, routines, incentives, and legal responsibilities for using animal-free testing methods as well as scientific and policy uncertainties and complexities associated with development and use of these methods including NAMs. While motivations can be understood on an individual level, we consider how they contribute to resistance to change and compromise our collective ability to ensure a transition towards animal-free safety assessment (Kooijman, 2013). The focus in RL3 on active involvement of relevant stakeholders to address their needs, interests, and values in VHP design and performance revolves around the five activities outlined below.

First, by taking an innovation systems approach (Hoogstraaten et al., submitted), we identify a wide range of key processes that require collaboration between stakeholders in the system to accelerate the transition to animal-free safety assessment. The basis of the innovation systems approach is the implementation curve developed by the Dutch National Institute for Public Health and the Environment (RIVM), which describes the phases for NAMs from research and development to (pre-)validation, acceptance, and ultimately uptake into regulatory frameworks[16] (RIVM, 2022). Beyond the implementation curve that maps phases and actors involved in a more technical role in accelerating the acceptance of NAMs, the innovation systems approach shows the importance of key processes such as legitimacy creation and providing directionality that involve more societal actors such as NGO's, citizens, and policy makers that have a role in driving animal-free safety assessment as well. To develop the innovation systems framework for the purpose of animal-free safety assessment, we learn from prior work on socio-technical transitions in, e.g., the energy, transport, food, and agricultural sector (Hekkert et al., 2007; Hekkert and Negro, 2009; Tziva et al., 2021). As such, in VHP4Safety we integrate knowledge on how transitions can be managed through participatory approaches of the relevant stakeholders in the NAMs innovation ecosystem.

Second, we use technology assessment and design approaches to ensure that the VHP is developed in such a way that it aligns with current and future risk assessment practices in academic, industry and governmental contexts. In close connection with RL1, RL3 defines the performance criteria for users of the VHP4Safety platform such as quality, security, and transparency. This also includes investigating what is needed to gain confidence and trust in the new way in which risk assessment is performed through the platform to ensure a sustainable transition towards safety assessment based on human data while striving for improved reproducibility in science. To this end, we engage stakeholders from the start to determine the needs and requirements for the platform and to identify incentives to change.

Third, throughout the project duration we actively monitor and evaluate the societal impact practices of researchers in the project as well as societal impact practices used in other European-funded large-scale research projects that integrate data sciences and toxicological approaches for risk assessment. Societal impact practices are defined as productive interactions between researchers and external stakeholders and the impact that is achieved through these interactions (de Jong and Balaban, 2022). Collective reflection on these practices in the projects contributes to processes of integration between various forms of expertise and setting directions for stakeholder engagement.

[16] https://www.rivm.nl/en/alternatives-to-animal-testing/landscape-nams

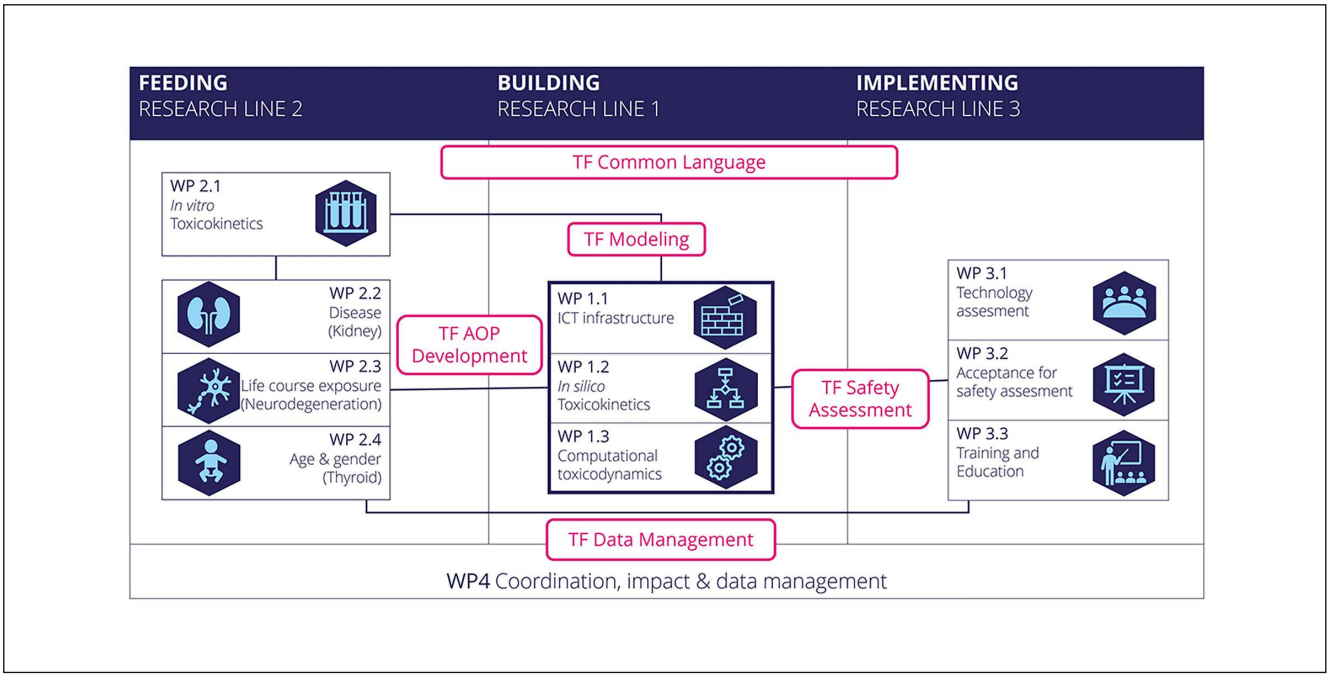

Fig. 2: Approach of the VHP4Safety project, with research lines, work packages (WP) and task forces (TF)

Fourth, the research line includes education and training as a crucial aspect to consolidate transition thinking and innovation capacity of this project. Coordination of capacity building in and outside the partner network and facilitation of the exchange of best practices will be key, e.g., by workshops and training events accessible to (inter)national scientists and regulators.

Fifth, RL3 will also ensure sustainability of the VHP4Safety platform beyond this project, which includes connecting with other initiatives and bodies to gain advice on development, regulatory acceptance, implementation, and the governance of the platform. As such, (inter-)national organizations and projects are represented on the VHP4Safety Scientific Advisory Board, including experts from KWR[17], EURL-ECVAM[18], the H2020 ONTOX[19] project and the Virtual Physiological Human Institute[20] (VPHi), as well as Dr Ellen ter Gast[21], a philosopher specialized in ethics and interdisciplinary collaboration.

4 Approach

4.1 Work packages and task forces

The VHP4Safety project has a dynamic approach to research, ensuring that the work is carried out in co-creation, in which plans are developed and modified in an iterative process as the project progresses. Each research line consists of multiple smaller work packages, of which there are ten in total. As shown in Figure 2, all work packages interrelate. The work packages plan their activities in detail every 6 months based on the evaluation of the previous 6 months. To ensure overarching collaboration between the work packages, interdisciplinary task forces have been introduced with representatives from each research line on the following tasks: common language, AOPs, safety assessment, data management, and modelling.

4.2 Designathons and hackathons

In VHP4Safety, project meetings, which we call "designathons", are organized biannually. The goal of the designathons is for all partners from research organizations, co-funding organizations, and cooperation partners (see Tab. 1) to collaboratively design the first version of the VHP and to decide on connections between elements in technology and scientific content. The designathons are focused on the case studies to leverage the cross-disciplinary nature of the consortium and to design the VHP step by step.

At the first designathon, we designed a safety assessment workflow as the basis for the VHP. We then translated this workflow and systematically categorized all building blocks, including core knowledge resources, international data collections, predictive toxicology methods, AOPs, and *in vitro* assays that build and feed

[17] https://www.kwrwater.nl

[18] https://joint-research-centre.ec.europa.eu/reference-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam_en

[19] https://ontox-project.eu/

[20] https://www.vph-institute.org/

[21] www.ellentergast.com

into the VHP. The first designathon concluded with an innovation systems analysis, where the VHP elements were assessed according to their levels of readiness.

During the first designathon it became clear that working in a manner that encourages people to think outside of their disciplinary silos requires interdisciplinary and work package/research line-transcending task forces. Five task forces were formed (Fig. 2), which meet regularly to discuss specific topics. It also became clear that instead of designing VHP (the platform) on the spot, the designathons appeared to primarily serve as a setting that allowed for lively, interactive discussions with the entire consortium, sharing opportunities and challenges across work packages, research lines, task forces, and disciplines. The designathons are recognized as an important mechanism in VHP4Safety to help co-creation and bring people with different expertise and interests together, building the VHP4Safety community.

In the second designathon, we conducted a brainstorming session to download and assemble our collective knowledge on the state of the science of the AOPs and related questions for each case study. We also worked on integrating the workflows developed in the first designathon and discussed how to operationalize them on the VHP. An important creative component of the designathon from a communication point of view was an exercise to create a common vision for the VHP by considering appropriate metaphors and drawings and sharing these visions. These creations helped us make the application of the VHP more concrete for the different end-users and provide a basis for communication outside the project.

In the third designathon, we defined and discussed interdisciplinary collaboration before holding a "systems thinking" workshop to define how to make the output of the VHP more suitable for safety assessment needs., i.e., to integrate *in vitro* data and *in silico* models for safety assessment. We also held an interactive session on addressing stakeholder expectations and reflected on how we could incorporate stakeholder needs in the development of the VHP. From the workshop and stakeholders' session, it became clear that it was time to work on concrete regulatory questions within dedicated work sessions on data integration in the future. The idea of the hackathon was born (see below). We also continued our discussion on the common, shared vision for VHP, reflecting on the metaphors developed in the second designathon as well as defining the VHP end-users and how to explain the VHP clearly and concisely to a target audience.

The fourth designathon focused on connection, starting with connecting with the stakeholders, followed by connecting the platform. Break-out sessions were held to retrieve the input from cooperation partners and co-funders on specific topics such as *in silico* models, building the ICT infrastructure, communication, technology assessment, education and training, and each of the case studies from RL2. In the sessions on connecting the platform, we discussed the sustainability of the VHP, including for whom and what needs to be sustained after the end of the VHP-4Safety project in terms of use, form, and impact (Hoekman et al., in prep).

After the third designathon, the co-creation activities in VHP-4Safety came to a point where the building blocks of the platform needed to be connected towards a first version of the platform. We started the first of a series of custom-designed workshops, which we call "hackathons." During hackathons, VHP4Safety partners work together to come up with solutions for specific questions, requiring everyone to see how research output from the three RLs (i.e., building, feeding and implementing) are connected. In preparation for the first hackathon, the Task Force Safety Assessment was challenged to prioritize two regulatory questions per case study during two preparatory workshops. The first hackathon in VHP4Safety explored how to integrate the six regulatory questions linked to the case studies, the available and newly generated data, and the services, including the predictive toxicology methods. The second hackathon focused on how research output is shared among partners and among RLs. To continue collaborative building of the platform, hackathons are held 2-3 times per year and continue to focus on solving specific questions using the available and newly generated data and services.

With this approach of organizing designathons and hackathons with the complete consortium, VHP4Safety promotes a culture of open collaboration and the use of creativity and innovation to integrate the best solutions into a platform for safety assessment.

5 Expected output of VHP4Safety

We acknowledge that the VHP can only become a driver of the transition towards human-based safety assessment if it is accepted by relevant stakeholders. Only acceptance can create the changes needed for a successful implementation of the VHP in the broader regulatory, economic and societal context. To promote acceptance, VHP4Safety uses a unique approach that combines the development of actionable knowledge on the drivers and barriers of a transition to human-based safety assessment with active co-creation of the VHP by societal stakeholders. Furthermore, the training and education of the consortium partners and the next generation of scientists in a participatory way will accelerate the transition. The knowledge developed within the VHP4Safety project will further contribute to improved preparedness for socio-economic and regulatory changes that are needed. This knowledge will also include insights into impact strategies that address the increased societal demand for reduced animal use and better safety assessment approaches.

The following concrete output is expected at the end of the project:

- Proof of concept of the VHP and user interface, which incorporates both technical aspects and feedback from end-users (scientists, industry, regulators)
- Integration of available human and mechanistic databases and demonstration of how they can be used in a transparent and secure way
- Demonstration of the VHP based on safety estimates generated from the case studies that will give insight into its design and how it can be used

– Roadmap for further development of the VHP, including its future governance, acceptance and implementation

6 Outlook

The ever-increasing scientific body of knowledge on health and disease, together with the ongoing development of innovative *in vitro* and *in silico* NAMs, opens the door to the world's first human biology-based approaches to safety assessment. The financing of projects such as VHP4Safety and the European Horizon2020 projects in the ASPIS cluster[22] marks a milestone that propels innovative animal-free approaches forward. These projects emphasize the need to integrate technological developments with transparency, collaboration, and stakeholder involvement and will have a major impact on shaping a future where animal testing becomes obsolete.

The VHP4Safety project provides a general framework for a virtual platform based on a suite of human *in vitro* and *in silico* tools and lays the groundwork for an integrated *in silico* description of human physiology, using specific scenarios including sex and age, disease state, and life course exposure. During its five-year duration, the project focuses on three specific case studies as proofs of principle. Expansion of the VHP beyond the case studies with new knowledge and additional areas of expertise from other projects and data sources will be promoted to enhance its comprehensiveness and application in the future. Stakeholders who support our vision are invited to collaborate and provide input. Actively sharing our progress and promoting interactive use of the platform among stakeholders will enhance transparency and acceptance of next generation safety assessment based on human data.

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Conflict of interest

We declare that the authors have no conflicts of interest.

Data availability

No datasets were generated or analyzed for this article.

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