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Next generation risk assessment and new approach methodologies for safe and sustainable by design chemicals and materials: Perspectives and challenges for occupational health

Veruscka Leso [a,*], Bernd Nowack [b], Achilleas Karakoltzidis [c,d], Fotini Nikiforou [c,d], Spyros Karakitsios [c,d], Denis Sarigiannis [c,d,e,f], Ivo Iavicoli [g,h]

[a] Department of Public Health, Section of Occupational Medicine, University of Naples Federico II, Naples, Italy
[b] Empa - Swiss Federal Laboratories for Materials Science and Technology, Technology & Society Laboratory, St. Gallen, Switzerland
[c] HERACLES Research Center on the Exposome and Health, Center for Interdisciplinary Research and Innovation, Aristotle University of Thessaloniki, Thessaloniki, Greece
[d] Environmental Engineering Laboratory, Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece
[e] National Hellenic Research Foundation, Athens, Greece
[f] University School of Advanced Study IUSS, Pavia, Italy
[g] Dipartimento di Sicurezza e Bioetica, Catholic University of Sacred Heart, Rome, Italy
[h] Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy

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ABSTRACT

Europe is facing increasingly challenging threats to health and well-being, including chemical pollution, climate change, and biodiversity loss. To counter such threats, the European Union has developed a series of policy strategies, including the Chemicals Strategy for Sustainability and the Zero Pollution Action Plan that pointed out the need for safe-and-sustainable-by-design (SSbD) chemicals/materials. The SSbD and the "zero pollution" ambition will inevitably lead to a transformation of the conditions of exposure to chemicals both in general living environments and workplaces with the consequent need to adequately anticipate and manage the chemical risk, starting from the assessment of the hazard and risk characterization. Among those, next generation risk assessment (NGRA) is defined as a human-relevant, exposure-led, hypothesis driven risk assessment approach, designed to prevent harm. To date, application of NGRA has been restricted to assessing the use of cosmetics, and it has not been implemented in occupational risk assessment. Occupational safety assessment represents an area that would benefit from increasing application of NGRA to safety decision making. Additionally, the application of new approach methodologies (NAMs) can support the generation of data useful to implement the operationalization of the SSbD framework, favorably impacting the adoption of suitable management strategies. In turn, the historical occupational preventive and protective approach to the health and safety of workers may provide support to adequately implement NGRA in the occupational context. Therefore, this work aims to provide an overview on the principal available NAMs and their possible implications for occupational chemical risk assessment and management.

1. Introduction

Europe is facing increasingly challenging threats to health and well-being, including chemical pollution, climate change, and biodiversity loss (EEA, 2025a). Unsustainable patterns of production and consumption, together with the challenging issues of waste management, can be included among possible causes of such conditions. Transdisciplinary and multi-sectoral collaboration in science, policy and society is necessary to face these issues according to a holistic and system-based One Health approach that recognizes the interconnection between human, animal, plants, and environmental health (EEA, 2025b; WHO, 2022).

To these aims, the European Union (EU) has developed a series of policy strategies regarding climate, environment, energy, transport, industry, agriculture and sustainable finance (EU 2021a). Among those,

* Correspondence to: Department of Public Health, Section of Occupational Medicine, University of Naples Federico II, Via S. Pansini 5, Naples 80131, Italy
E-mail address: veruscka.leso@unina.it (V. Leso).

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the Chemicals Strategy for Sustainability (CSS) (European Commission, 2020) published by the European Commission (EC) on 14 October 2020, and the Zero Pollution Action Plan (European Commission, 2021a, b), adopted on 12 May 2021, pointed out the need for safer and more sustainable chemicals and materials according to the safe-and-sustainable-by-design (SSbD) framework. SSbD is a voluntary and (pre-market) systems approach aimed at proactively addressing safety and sustainability beginning at the early stages of innovation and throughout the lifetime of the chemicals/materials (European Commission, 2020). This approach is in line with the goals of the European Green Deal (European Commission, 2019), the roadmap leading the EU to climate neutrality by 2050, finalized to encourage technological progress, while maximizing health and environmental protection as part of the ambitious goal to tackle pollution from all sources and move towards a toxic-free environment (European Commission, 2019, 2020).

The EC proposed a framework that can assist in the definition of safety and sustainability criteria, to guarantee coherence between actors, sectors and value chains (EC, 2022; Leso et al., 2024; Soeteman-Hernandez et al., 2019; Caldeira et al., 2022). The framework encompasses both a (re)design phase and an assessment phase of safety and sustainability (EC, 2022). The stepwise approach for addressing these two former elements is characterized by the assessment of the hazard of the chemical/material; human health and safety aspects in the chemical/material production and processing phase; human health and environmental aspects in the final application phase; the environmental sustainability. Additionally, the analysis of the social and economic impacts of the chemical's/material's life cycle can be implemented.

SSbD and the "zero pollution" ambition is inevitably leading to a transformation of the conditions of exposure to chemicals in both general living environments and workplaces, possibly decreasing the levels of exposure while enhancing the likelihood of contact with innovative chemicals of concern or mixtures of them, thus requiring to update the way we assess and manage chemical risks. In the general risk assessment process, traditional methodologies, primarily based on animal testing, are too time-consuming and expensive to keep up with the large number of chemicals available or expected to enter the market in the next future. SSbD moves the shift towards more rapid, cost-effective and versatile tools, including in silico tools, applicable in early innovation stages with a limited amount of information. In addition, most of the current traditional risk assessment frameworks struggle to assess risks in emerging conditions of exposure, such as those related to chemical mixtures, in the disposal or recycling phases of the life-cycle of chemicals/materials, focusing more on production and use phase related risks. The SSbD framework, as a holistic approach, takes into account the entire life-cycle of a chemical or material, including the end-of-life or reuse stages. Up to now, a chemical or material could be nontoxic and safe to use, without necessarily being environmentally sustainable or vice versa. This separation underlines some of the traditional assessment limitations, where safety and sustainability have been treated separately. From this perspective, SSbD integrates both safety and sustainability aspects in a proactive way, early in the design phase.

Therefore, to ensure the safety and health of exposed workers and consumers, there is a growing need for advanced risk assessment strategies that can efficiently and effectively identify hazards and characterize risks during the early stages of innovation. Alternative approaches, such as next generation risk assessment (NGRA), may be useful for their cost-effectiveness, but also for their ability to integrate mechanistic data, prioritize early hazard identification, and support proactive risk management (Hristozov et al., 2024).

NGRA is defined as a human-relevant, exposure-led, hypothesis driven risk assessment approach, designed to prevent harm (Farmahin et al., 2017; Alexander-White et al., 2022; Schmeisser et al., 2023; Moné et al., 2020). The process begins with hypotheses about the association between exposure to a particular chemical and biological effects based on available information, and the hypotheses are used as a departure point to guide the next step in the assessment, which is done in an iterative, step-by-step manner, until a suitable conclusion is achieved (Dent et al., 2018; Hristozov et al., 2024). This assessment is specifically focused on the biological effects caused by the chemical exposure and should have an independent and transparent assessment logic that ideally do not depend on the experience of the assessor, with specification and quantification of uncertainty factors (Farmahin et al., 2017; Alexander-White et al., 2022). In this scenario, recent technological advancements in toxicology, such as "new approach methodologies" (NAMs) can offer the opportunity for more rigorous hazard characterization, more effective dose-response and accurate exposure assessment (Schmeisser et al., 2023; Wang et al., 2018).

To date, the application of NGRA has been restricted to the assessment of cosmetics, and it has not been implemented in occupational risk evaluation (Wood et al., 2024). However, the occupational hazard evaluation and risk assessment represent areas that would benefit from increasing application of NGRA. Moreover, the application of NAMs can support the generation of data useful to implement the operationalization of the SSbD framework, favorably impacting decision-making processes and the adoption of suitable risk management strategies. In turn, the historical occupational preventive and protective approach to the health and safety of workers may provide support to adequately implement NGRA in the occupational context. Therefore, the aim of this paper is to provide an overview on the principal available NAMs and their possible contribution to the SSbD framework application and to highlight the implications for occupational chemical risk assessment and management.

2. New approach methodologies (NAMs)

NAMs intended as "new approaches methodologies" (ECHA, 2014, 2020) or "novel alternative methods" (NIH, 2022), depending on the different definitions proposed, can include combinations of in silico tools, complex in vitro systems, organ models and omics approaches in conjunction with physiologically based kinetic (PBK) modelling and complex exposure models (Marx-Stoelting et al., 2023). In this paper we refer to NAMs as "any technology, methodology, approach, or combination of approaches that can provide information on risk assessment without the use of vertebrate animal studies" (ECHA, 2020). The use of NAMs can help improve hazard identification by combining more robust and relevant resources from biological mechanisms and effect studies (Dent et al., 2018), which can predict exposure levels as well as the adverse outcome pathways (AOPs) more accurately than traditional hazard identification methods (Fay et al., 2017). NGRA, combining data from different NAMs, may allow, in principle, for an accurate, efficient and ethical prediction of AOPs, exposure pathways, and exposure levels of hazards improving the accuracy and reliability of safety and risk assessments. For instance, NAMs can predict the exposure pathways by integrating physicochemical properties data such as e.g., solubility, volatility with biological activity data (e.g., receptor binding assays) that estimate how the chemical can migrate within or interact with environmental or biological systems. The models, for instance, can be employed for calculating dermal absorption from general molecular properties or could be supported by some "organ-on-a-chip" kind of model to assess inhalation exposure. In combination with a well-established PBK model, this approach can be employed to approximate internal doses in occupational settings or moving a step forward to be integrated with advanced dose-response models that will have been calibrated with in vitro data.

Additionally, NAMs can support the transformation of the current regulatory landscape based on human-relevant methodologies and provide valuable mechanistic insight (Schmeisser et al., 2023; Karakoltzidis et al., 2024). Generally, NAMs can be broken down into *in vitro*, *in chemico* and *in silico* methods. *In vitro* data may be used to directly inform cellular targets and chemical-induced molecular mechanisms potentially leading to adverse effects (Ankley et al., 2010; Chauhan et al., 2021; Leist et al., 2017; OECD, 2021; Hartung et al.,

2012, 2017; Beronius et al., 2020; Rotter et al., 2018). *In chemico* is a general term referring to the use of abiotic chemical reactivity methods (Gerberick et al., 2008). *In silico* methods refer to computational tools aiming at modelling endpoints such as toxicokinetics or metabolism, or at predicting effects based on chemical structural features (Thompson et al., 2021; Leonard, 2019).

2.1. In vitro models

High throughput and high content testing with potential real-time transmission of results can be achieved through advanced *in vitro* physiologically realistic models, such as 3D- and co-cultures, organoids, spheroids, and organotypic tissue models (Kastlmeier et al., 2022; Kokot et al., 2020; Pyrgiotakis et al., 2018; Toprani et al., 2021; Watson et al., 2014; Yang et al., 2020). Among those, e.g., a lung-on-a-chip model is a sophisticated 3D presentation of a living breathing human lung on a microchip (Jaber and Billet, 2024). This new model enables researchers to reproduce the structure and functions of the human lung, making it easier to assess the pulmonary toxicity of contaminants, particularly in the case of airborne absorption as the primary route of exposure for occupational xenobiotics (Rothbauer et al., 2021). In the attempt to extrapolate data that may inform occupational risk assessment, it is important to expose these biological models to compounds, either alone or in combination, in a way that may effectively represent workplace exposure. In this sense, toxicological tests should be planned to ensure determination, production and maintenance of concentrations of xenobiotics resembling those occurring during job activities. To this aim, combined dosimetry models have been developed as an *in vitro* to *in vivo* extrapolation (IVIVE) strategy to link cell responses to the whole organism responses (Romeo et al., 2020). Such models estimate the air concentration for humans to *in vitro* doses. These may be useful to verify whether doses used *in vitro* are in a realistic range. This may allow us to estimate and compare human Benchmark Doses and Benchmark Doses-derived human exposure from *in vitro* data and testing the possibility to estimate Benchmark Doses- derived human exposure level ranges from the particle characteristics and the conditions of the experimental settings. Combined dosimetry models can be employed retrospectively to assess the doses used in *in vitro* studies, but also prospectively to select realistic doses during the design of an experiment.

Additionally, real contact between the pollutant and the cells enabling the bioavailability of the compound should be followed to support occupational risk assessment. Several techniques have been developed for these latter aims: submerged cell exposure, intermittent procedures, and air-liquid interface (ALI) methods. This latter method requires the generation of test atmospheres enabling the cell exposure at the ALI (Upadhyay and Palmberg, 2018). However, the use of ALI exposure devices remains costly and requires extensive optimization due to the lack of standardized calibration guidelines, recommended flow rates, exposure durations, and specifications for temperature and relative humidity to be applied in the test atmosphere.

Materials, either substances or mixtures which may or may not yet fulfil the definition of an article under REACH and may be of natural or synthetic origin (Patinha Caldeira et al., 2022; EC, 2007), and in particular nanomaterials, pose challenges due to their complex properties that are affected by both the material itself (intrinsic properties), but also the surrounding medium (extrinsic properties) (Lynch et al., 2014). Several approaches have been developed for materials that also enable the use of *in vitro* data for human hazard assessment (Wu et al., 2024; Romeo et al., 2020). The one suggested by Romeo et al. (2020) is based on the characteristics of nanomaterials and utilizes *in vitro* human data. After selecting the appropriate *in vitro* models and conducting tests with relevant dose units, data from submerged *in vitro* cultures can be adjusted to reflect the actual dose reaching the cells, using an *in vitro* dosimetry model like the one-dimensional Distorted Grid model (DeLoid et al., 2017). To enhance the alignment with *in vivo* dose-response relationships, the *in vitro* results can be integrated with kinetic models, such as the PBK model and the Multiple-Path Particle Dosimetry (MPPD) model, which link *in vitro* responses to whole-organism exposure outcomes (Anjilvel and Asgharian, 1995). In cases where kinetic models are unavailable, a Relative Potency Factor (RPF) approach can be used, although it comes with certain limitations (Salieri et al., 2020). This represents an attempt to link a cellular response to an *in vivo* one. A RFP approach allows the estimate of *in vivo* chemical potency (i.e. the dose that yields a given level of response) from the comparison with the potency of a better characterized reference substance using subhuman data (e.g., subcellular, cellular, animal) (Calle and Zaighemi, 2000). However, the correlation between *in vitro* and *in vivo* data, without explicitly describing or modelling any process occurring between the cellular and whole organism level (e.g. the kinetics of the substances) is an assumption that needs to be verified before this approach could be extensively implemented. As a final result, the necessary values for risk assessment are provided to evaluate human health impacts.

The final aim of the advanced *in vitro* toxicological evaluation of chemicals and materials is understanding the cytotoxicity of xenobiotics and the underlying mode of action, e.g., oxidative stress, inflammation, DNA damage, epigenetic changes, that appears important to inform occupational health actions (Despréaux et al., 2023; Kastner et al., 2013; Sanchez-Guzman et al., 2021). Omic techniques, including proteomics, metabolomics, transcriptomics and genomics, in this scenario, are of high importance in the overall risk assessment process, as they can provide insights into the chemical interaction with the biological system and its mode of action, while enabling the prediction of toxicological effects (German Federal Institute for Risk Assessment , 2022; OECD, 2023; EC, 2025).

Overall, the *in vitro* derived mechanistic information may be useful to anticipate AOPs and possible effects in exposed workers and provide guidance to the identification of possible biomarkers of early effect for human biological monitoring (Fig. 1). This is even more important to inform risk assessment strategies in case of exposure to substances' mixtures and to establish possible biological exposure limits.

2.2. In chemico

In chemico NAMs are, by definition, abiotic methodologies, focused on risk screening by measuring properties that are directly associated with exposure or hazard potential (Hristozov et al., 2023). Well-known examples include the measurement of size, shape and surface properties (texture, defects or interfaces which may result in different surface reactivity (e.g., the number of reactive sites), catalytic activity, generation of radicals, as well as the measurement of the dissolution rates and halftimes under simulated physiological conditions (Bañares et al., 2025). OECD has developed a guideline for an *in chemico* method aimed at identifying skin sensitization based on peptide reactivity (OECD, 2024a). This test guideline is key event based and used to identify the molecular initiating event in the skin sensitization pathway. The method assesses the reactivity of test chemicals with synthetic peptides or amino acid derivatives, including lysine or cysteine. As an additional example, because reactivity is an extrinsic property considered a key parameter to describe the interaction of nanomaterials with biological systems (Kraegeloh et al., 2018), the development of abiotic *in chemico* assays to evaluate surface reactivity and link it with key events in reactive-based nanotoxicity would help to fundamentally understand the modes of action and better group nanomaterials while minimizing *in vivo* testing. Alcolea-Rodriguez et al. (2024a) through a methanol probe chemisorption assay quantified the number of reactive sites at the surface of different engineered nanomaterials, enabling normalization per reactive site in reactivity and toxicity tests, rather than per mass or physical surface area. The reactive nature of surface sites (acidic, basic, redox or combination thereof) was then addressed through temperature-programmed surface reaction of chemisorbed methanol and a dithiothreitol probe oxidation reaction was used to evaluate the oxidation capacity.

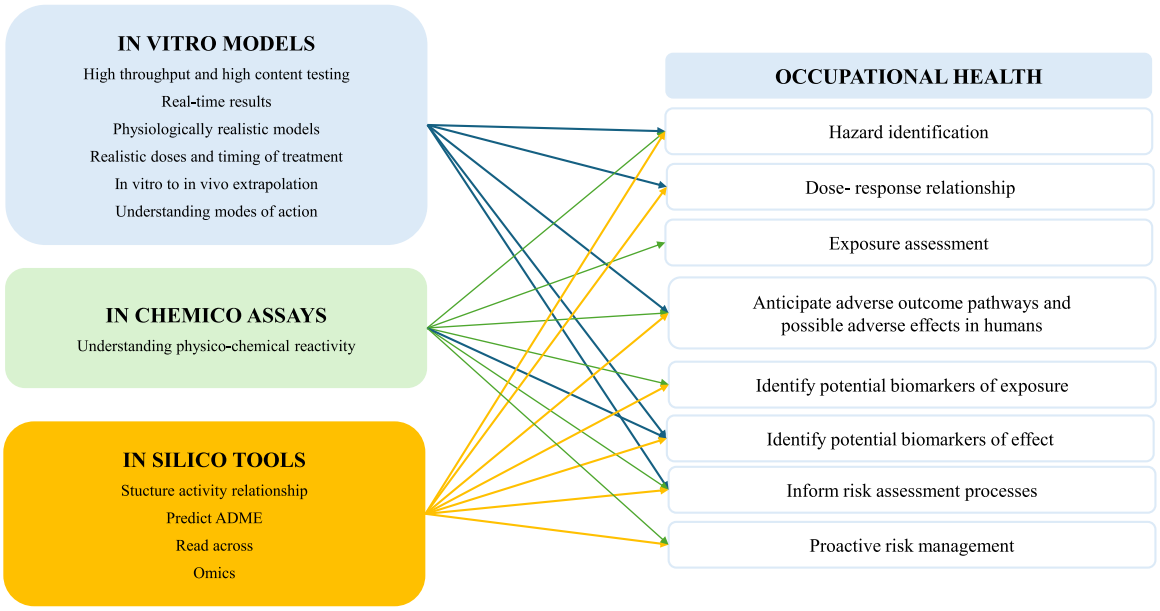

Fig. 1. New approach Methodologies' contribution to occupational health.

Additionally, in case of multicomponent chemicals, the potential transformation of chemical and/or physical structure under physiological conditions can be assessed by suitable *in chemico* NAMs (Petersen et al., 2023). The idea is to develop chemical probe descriptors that correlate with reactivity-based toxicity trends. This may fill information gaps, expecially in case of innovative materials, concerning exposure occurrence, physico-chemical characterization, toxicity measurements, correlating toxicity with specific parameters, regulatory enablement. Additionally, *in chemico* models should be able to predict molecular initiating events, required to understand both the adverse outcome pathways and the properties at the biological-chemical interface (Alcolea-Rodriguez et al., 2024b).

To support the exposure assessment, the *in chemico* NAM has to simulate the stress that a chemical or a chemical enabled product will experience during its life cycle (Kleinekorte et al., 2020). One of the *in chemico* NAM employed for exposure screening addresses dustiness, which provides, in its standardized form, the respirable dustiness index as a measure of the propensity of a powder to generate dust when agitated, as well as the qualitative composition of a multicomponent substance (Ribalta et al., 2024). The application of the dustiness index may be useful for the characterization of emission sources and the prediction of occupational exposure during powder handling. Multiple chemical and biological assays probe the diverse reactivity of chemicals/materials so that these may be grouped with related adverse effects, that should consider both the source material, and the transformation products. This approach may simplify research by clustering specific environmental fate, exposure and adverse effects preferentially connected with certain physicochemical reactivity profiles (Alcolea-Rodriguez et al., 2024b). Overall, this may contribute to an early warning detection of chemicals of emerging concern, thus favouring the adoption of preventive and protective measures for the health and safety of firstly exposed workers (Fig. 1).

2.3. *In silico*

The *in silico* approach uses computer simulations to predict the behavior of a substance in the body, its internal dose and potential adverse effects based on chemical structural features and association with legacy data (Clippinger et al., 2018; Thompson et al., 2021; Leonard, 2019). The Structure - Activity Relationships (SARs) models include approaches addressing the relationship between a molecular feature (chemical structure) and its toxicity, requiring expert input for assessment (Allen et al., 2018; Wedlake et al., 2020; Veith, 2004). The Quantitative Structure - Activity Relationships (QSARs) are methods able to predict the quantitative relationship between a chemical's molecular structure and its toxicity (OECD, 2021; Cherkasov et al., 2014). QSARs may be categorized as either machine learning algorithms or requiring expert input. QSAR are primarily applied for their capability to identify the toxicity of chemicals as a function of structural attributes and are today recognized as a cost and time efficient alternative to traditional testing methods for specific regulatory applications, such as the toxicity screening of pharmaceutical impurities and the metabolism simulation as addressed by the OECD QSAR Toolbox (Allen et al., 2020a, b; Cherkasov et al., 2014; Yordanova et al., 2019). QSARs also play a primary role in planning targeted follow-up *in vitro* testing, for example prioritization of substances toward a certain test method.

The PBK modelling is a mathematical modelling technique to predict the behavior of a chemical in the body, or 'ADME' (Absorption, Distribution, Metabolism, Excretion) processes, by incorporating key factors such as blood flow, plasma protein binding and tissue composition, organ volumes as well as metabolism rate. PBK can combine *in silico* and *in vitro* models to predict human bioavailability and target organ concentrations (Cai et al., 2006). It represents a physiology-based approach, uses body compartmentalization, simulates kinetic processes, and can be adapted to different animal species. However, PBK models are detailed and mechanistic, considering various physiological factors and requiring extensive data for parameterization, they are considered low-throughput models due to their time- and labor-intensive nature (Pletz et al., 2020). On the other side, high-throughput kinetic models are simplified and aim to be rapid and scalable, often relying on *in silico* data and generic model structures, designed for rapid processing and able to be applied to screen large chemical libraries (Geci et al., 2024). This may provide greater efficiency when data on compounds are still limited as well as for a prompted SSbD operationalization.

From an occupational health perspective, PBK and high throughput kinetic models can inform risk assessment processes through the estimation of a chemical internal exposure and the prediction of possible effects in a dose-related manner (Fig. 1). This modelling offers the opportunity to assess risks derived from occupational and general living exposure, helping to establish safe exposure limits. Overall, the kinetic modelling methodology serves as a powerful and flexible tool for simulating and analyzing the kinetic behavior of chemical substances in

biological systems, making significant contributions to toxicology (Reale et al., 2024). This may allow us to overcome the challenging issues related to the definition of occupational exposure limits for less data-rich substances for which less human data are available. In these cases, in fact, animal data are primarily used as the starting point with a standard modification of the dose descriptor to extrapolate to workers, considering, however, the complexity in extrapolating data from the animal experimental settings (interspecies differences and in routes of exposure) to the real human contexts.

Read across is a method to infer toxicological endpoint information from the known endpoints for other chemicals (or analogues) that are 'similar' to the target from a structural and/or biological point of view (Escher et al., 2022; Gadaleta et al., 2020; Koch et al., 2014; Schultz and Cronin, 2017; Read Across Assessment Framework, 2017). Read across is usually used as a standalone method and in some scenarios, may be used to predict the toxicological properties of a chemical in addition to QSARs, as part of a NGRA strategy (Alexander-White et al., 2022). Although read across is less amenable to automation because expert opinion remains a key requirement, research is underway to employ computational approaches to automate some aspects of the methodology, namely analogues selection, properties prediction and data gap filling (Gadaleta et al., 2020; Tate et al., 2021; Luechtefeld et al., 2018; Alves et al., 2018). Overall, computational methods are the ideal preliminary step in a multi-tier screening approach for large-scale chemical hazard assessment, being relatively fast and cheap to perform before further confirmatory *in vitro* tests (Helma, 2005).

3. Discussion

The transformation of the conditions of chemical exposure both in general living and occupational environments in Europe has inevitably raised the need to adequately anticipate chemical risks in conditions of generally low-dose, multiple-chemical exposure and develop prevention based-governance strategies. The NGRA and NAMs, in this context, offer great opportunities, although requiring significant technical, practical, but also cultural and societal mindset shifts. Occupational medicine and toxicology, in their long tradition of preventive disciplines are in line, from a cultural and educational point of view, with such innovative and dynamic approaches, considering the need experienced by these disciplines to adapt to the continuously changing conditions of work. Therefore, the relationship between the NGRA and occupational health can be viewed from a bidirectional perspective.

3.1. The role of NAMs in occupational health

NAMs are promising techniques to support the risk assessment process in occupational settings (Fig. 2). A combined approach of multiple NAMs may be useful in this sense (Jin et al., 2021). An accurate IVIVE, for example, requires synergistic utilization of *in vitro*, *in vivo* and *in silico* information, that may be useful to read across existing information and/or to generate new data. PBK and *in vitro* dosimetry models can link *in vitro* concentrations to *in vivo* responses (Romeo et al., 2022). The accuracy and reliability of toxicity predictions obtained combining *in vitro* and *in silico* approaches can be finally verified in real occupational settings.

Specifically, the focus should be on how NAM-derived data can be effectively interpreted and integrated into comprehensive risk evaluation frameworks. *In vitro* experiments, for example, can be used to provide data on a chemical's toxicity, such as its effects on cell viability, gene expression, or protein function. *In silico* QSAR or PBK models, through the employment of specific input data, such as those referring to the ADME of the substances, can be used for predicting the toxicity of related substances (Ruiz et al., 2020). Furthermore, *in vitro* testing can help overcome some of the limitations of *in silico* technologies, such as a lack of knowledge of complex biological processes and intercellular connections, and to define the complex datasets necessary for building *in silico* models that may characterize an additional bias for their operationalization. *In vitro* experiments can provide more precise and cost-effective information on the molecular and cellular causes of toxicity, as well as help identify critical events and biomarkers to be used

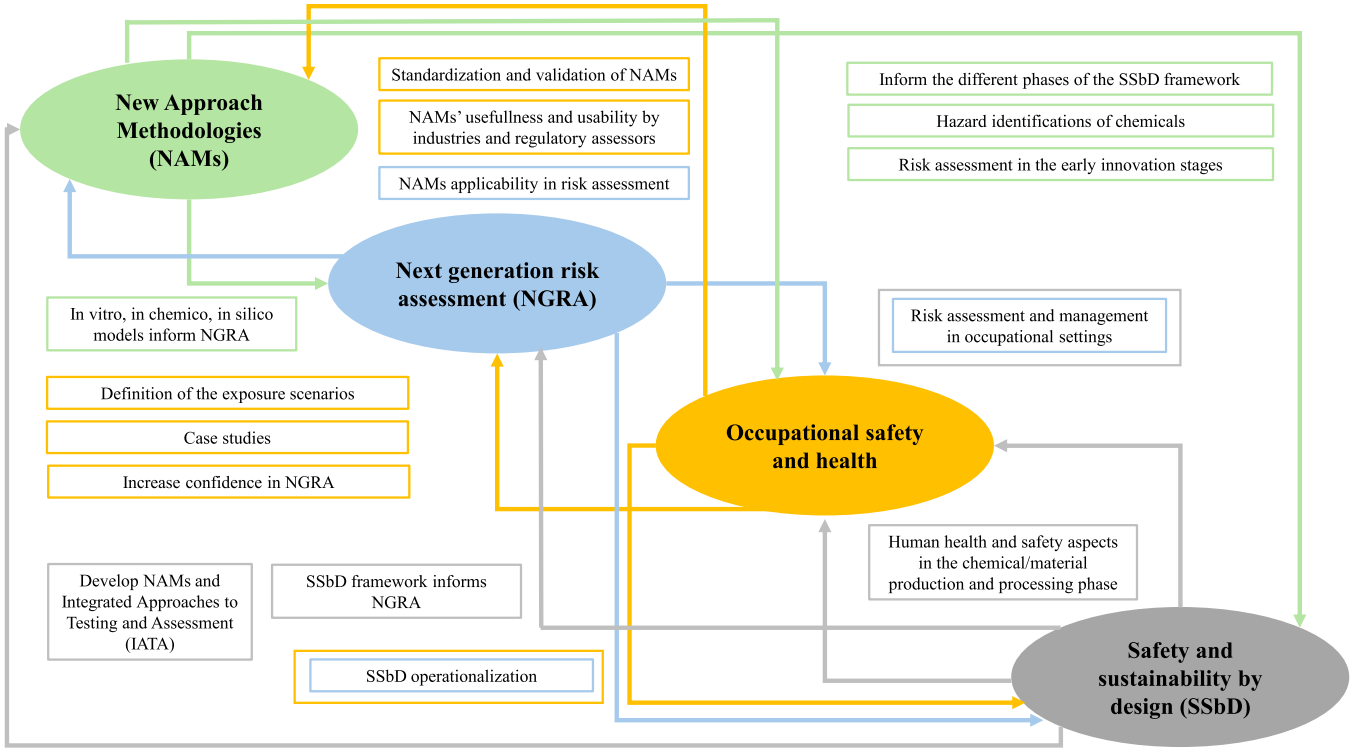

Fig. 2. The relationship between new approach methodologies, next generation risk assessment, occupational safety and health and the safety and sustainability by design.

in developing accurate and predictive *in silico* models. Such a combined approach may benefit from an occupational health contribution providing human data and human biological monitoring (HBM) information that can further confirm the validity of such integrated operational models in anticipating hazards and assessing risks in real exposure contexts.

In turn, the application of NAMs may be useful to overcome some limitations experienced in HBM and risk assessment in real occupational settings. Particularly, as regards the measurement of the internal doses of chemicals with shorter half-lives due to their rapid elimination, limited accumulation, and frequent fluctuations in body concentrations leading to underestimation of their actual exposure levels (Reale et al., 2024). NAMs may support future work needed to develop improved sampling strategies and biomarkers for nonpersistent chemicals. Another limitation is the translation of concentrations from accessible matrices like plasma or urine to specific target tissues. Current advancements in PBK modelling can improve our ability to predict the time course of a chemical in the body, estimate internal exposure levels as well as tissue concentrations, although the reliability of such methods need further research. Additionally, PBK modelling may help in reconstructing external exposure levels from biomarkers of exposure. However, some challenging issues need to be overcome, particularly regarding those cases, like pesticides, where the same biomarker of exposure might originate from different parent compounds, complicating the accurate estimation of external exposure levels and the total margins of exposure for the associated parent compounds. This uncertainty is exacerbated by the HBM strategy of relying on single spot urine samples, which may not provide a reliable reconstruction of external exposure levels. Exposure reconstruction involves the integration of HBM data with other sources of information, such as toxicokinetic data and exposure scenarios, and provides a better understanding of the relationship between external exposure and internal concentrations. Moreover, establishing relationships between exposure to a single chemical and a resulting adverse health outcome is the common practice in risk assessment nowadays. However, evaluation of risks for human health from the simultaneous exposure to multiple chemicals and disease outcomes is increasing (Beronius et al., 2020).

NAMs may provide support to link the aggregate exposure pathway and the AOP frameworks using PBK models, thus improving the confidence in chemical and mixture risk assessment. Understanding the toxicokinetics of a compound is essential, from an occupational health perspective, for developing the HBM strategy, in terms of time and the appropriate number of samples to collect (Koch et al., 2014). This can reduce the number of samples required to have a representative dataset to evaluate exposures, costs and the burden of the study for participants (Connolly et al., 2018; Kohsuwan et al., 2022). Additionally, input data for PBK models can include physiological data of the exposed species (e.g., organ volumes, cardiac outputs to the different organs, urine excretion rate), as well as chemical-specific parameters, such as physicochemical (e.g., blood-tissue partition coefficients) and toxicokinetic properties (e.g., absorption rate, fraction unbound in plasma and tissues, renal clearance, metabolic clearance). Overall, this may support a "real-time" and "personalized" assessment of risks that may take into account the variability of external conditions of exposure, both in ordinary or accidental conditions of work, as well as that of specific groups of exposed subjects to achieve a targeted assessment of risks and prevention. The above-mentioned integrated NAMs should ensure that generated data are managed and shared using the Findable, Accessible, Interoperable and Reusable (FAIR) data principles (Karakoltzidis et al., 2024). This will make it easier for researchers to use computational tools to search, process and analyze large datasets. Standardization with FAIR principles is also crucial for repurposing datasets for secondary research aims. In this view, such an approach should assure possible extrapolation of computational information into real occupational conditions of exposure.

To achieve a suitable risk assessment, NGRA should include Integrated Approaches to Testing and Assessment (IATA) and NAMs to enable use of existing information and targeted generation of new data as far as possible without the use of experimental animals. IATA combine multiple sources of information to conclude on the toxicity of chemicals. These approaches are developed to address a specific regulatory scenario or decision context (OECD, 2022). They follow an iterative approach to answer a defined question in a specific regulatory context, taking into account the acceptable level of uncertainty associated with the decision context. On the other side, NGRA is an exposure-led, hypothesis-driven approach that emphasizes using NAMs, especially non-animal methods, for risk assessment. The data acquisition under NGRA may be supported by IATA with tiers of increasing specificity and complexity of the adopted NAMs.

3.2. Occupational health contribution in NGRA

Occupational health knowledge can contribute to developing innovative risk assessment strategies given its strong experience in the assessment and management of risks, taking care of the different production cycles, ordinary and non-ordinary working conditions, technological progress in a life-cycle perspective (Fig. 2). As NGRA is exposure-driven, information derived from real occupational settings may provide the correct identification of the exposure scenarios. These may include data on the synthesis, formulation and relevant forms, use and end of life of the chemicals/materials investigated, as well as on exposure routes, combined exposures, exposure-based grouping, worst-case scenarios, that can provide essential inputs for the risk assessment process. Toxicology data, on the other hand, may provide information on the release, human biodistribution, environmental fate, human and environmental hazard for the identified exposure scenarios.

The NGRA approach should ensure proper coverage of a wide variety of molecular/cellular events (to meet the human protection goal) to inform the safety decisions while being pragmatic in terms of implementation. Occupational contexts may provide the scenario to develop, study and verify the operationalization of such advanced tools, or a set of tools, as well as to find the most adequate methods to reconfigure approaches to hazard and risk assessment and to increase confidence in the application of NAMs in animal-free chemical safety assessment. The occupational health may support well-designed future case-studies to achieve these goals across the range of endpoints and available NAMs. The occupational health can provide the know-how to understand and overcome the technical challenges to the implementation of NAMs, to assure their successful application, moving from an early phase of NGRA innovation to a stable and reproducible approach (Fig. 2).

Human data from the 'real world' evidence (e.g., observation studies, adverse events monitoring or epidemiological research) can be combined with the outcomes provided by different NAMs (e.g., *in chemico*, *in vitro*, or *in silico*) to make accurate predictions of the biological activity of chemicals via robust NAM based, exposure-led frameworks for NGRA. Overall, this may contribute to achieve NAMs that are Transparent, Reliable, Accessible, Applicable and Complete (TRAAC) and easily applicable in risk assessment processes and addressing the urgent need for evidence-based guidance on how to use and integrate NAMs effectively (Shandilya et al., 2023). Transparency refers to clear communication about the methods, their strengths, and limitations; reliability measures the quality, correctness, and consistency of output; accessibility refers to the findability and usability of the methods; applicability means that the applicability domain of the methods is clearly communicated; completeness measures how comprehensively the methods align to the relevant regulatory frameworks (e.g. REACH) and cover specific requirements. Additionally, once developed, a NAM needs to be standardized and validated demonstrating biological relevance, reproducibility, transferability and fitness for purpose, and the occupational health settings may support such actions. In this regard, the environmental and biological monitoring of the exposure in workplaces, may be useful to verify the predictions made by NAMs integrating

chemical/material physicochemical properties and kinetic modelling to validate the estimated internal doses and dose-response relationships. Despite the potential advantages of NAMs from a scientific perspective, a change from the status quo can prove uncomfortable: non-technical hurdles include inertia, familiarity, and comfort with established methods, perceptions around what will be expected and accepted by regulatory authorities, uncertainty about how new approaches can be used and applied, as well as concerns around loss of data continuity (i.e. the ability to directly compare new results with previously generated data). There are a few NAMs that have already reached the level of readiness for application in a regulatory context. These include, for example, the evaluation of eye irritation (OECD, 2024b) or skin sensitization (OECD 2024c, 2024d, 2024e; Caloni et al., 2022). The "Defined Approaches for Serious Eye Damage and Eye Irritations" guideline proposes defined approaches combining data generated in vitro methods, with information sources such as physicochemical properties able to achieve a prediction that can be used alone to determine eye hazard potential (OECD, 2024d). For the skin, the determination of a substance's potential to induce sensitisation is an important aspect of regulatory hazard and risk assessment that serves to ensure the safety of workers and consumers. The in chemico Direct Peptide Reactivity Assay (DPRA) addresses the molecular initiating event of the AOP, i.e. the covalent interaction with proteins. In the DPRA, the reactivity of test substances towards synthetic model peptides containing either cysteine or lysine is assessed. The relative depletion of the model peptides is used to support the discrimination between sensitisers and non-sensitisers (Kreiling et al., 2017; OECD 2024c). Additionally, activation of keratinocytes (OECD, 2024d) or that of dendritic cells (OECD, 2024e) have been addressed in key event based test guidelines.

The fact that few NAMs have achieved such limited application may be related to several critical issues that need to be overcome. These include the need for a suitable workflow describing how to develop models, capture their uncertainty, define input data needed and verify the output in the absence of *in vivo* data (Dent et al., 2018). Overall, this requires investment, especially in further case studies that should elaborate scenarios/problem formulations frequently encountered by industry and regulatory safety assessors thus providing confidence that the tools and approaches can reliably discern differing levels of risk.

In this challenging context, the occupational health and toxicology experience may support the cultural change through the demonstration of the potential financial, productivity and/or scientific benefits, including improved protection (Sewell et al., 2024). Moreover, an occupational health perspective may help in verifying NAMs' usefulness and usability by the industries complying with regulations and regulatory requirements (Fig. 2). Therefore, it is crucial to ensure the regulatory readiness of these emerging methods (Bal-Price et al., 2018). The regulatory acceptance of NAMs is particularly important for industries: the application of such alternative approaches can help to reduce R&D and regulatory compliance costs and reduce the time required for new chemicals/materials to reach the market. This can also allow industrial companies to better align to the 3 R principles (Hristozov et al., 2024).

The NGRA approach should ensure proper coverage of a wide variety of molecular/cellular events (to meet the human protection goal) to inform the safety decisions while being pragmatic in terms of implementation. This is particularly important when dealing with substances with no clear mode of action identified. By their nature, many tools and assays used in NGRA are either new or are not yet routinely applied to consumer safety decision-making and need to undergo formal validation. Scientific efforts should be focused at determining which types of data can be trusted to obtain useful bioactivity readouts; reaching a consensus on appropriate data that can be confidently used to implement a new NGRA framework for decision-making; understanding usefulness of the new NGRA framework against traditional approaches (Fig. 2).

3.3. NGRA, SSbD and occupational health

The relevance of an occupational health approach to NGRA is evident also from an SSbD perspective. SSbD is a strategic framework aimed at integrating safety and sustainability considerations into the design and development from the earliest stages. This approach is currently being actively promoted and operationalized by academic researchers, policy makers, and industrial stakeholders across the EU. The SSbD toolbox provides a suite of resources and methodologies designed to support the assessment and mitigation of risks while promoting sustainability (Sarigiannis et al., 2024). Within this framework, NAMs offer significant advantages by enabling the early identification of hazards and the characterization of risks using non-animal, mechanistic, and data-driven approaches (Fig. 3). NAMs align closely with SSbD goals by providing faster, more cost-effective, and human-relevant insights into potential adverse effects, particularly in occupational settings. NGRA fits seamlessly into the SSbD process as it promotes NAMs to anticipate and address risks during the early innovation phase, before materials enter the market or workplace, and are therefore well suited for low tier assessments. Therefore, it is crucial to implement more reliable methods and assessments during these early innovation stages. As a result, the integration of NGRA and NAM methods into the SSbD workflow is significant not only for addressing the hazard of a chemical or material, but also for evaluating the risks derived from the occupational, consumer and environmental exposure, which constitute an integral aspect of the SSbD framework. Within this context, the European Partnership for the Assessment of Risks from Chemicals (PARC) (Marx-Stoelting et al., 2023), is developing an integrative toolbox for the operationalization of the EC SSbD framework (Sarigiannis et al., 2024). The PARC SSbD toolbox aims to render the EC framework operational by providing a user-guided and comprehensive toolbox that will incorporate all the relevant tools, including models, methods, software, and methodologies, for SSbD, while considering the innovation process. Within the conceptual framework of the PARC SSbD toolbox, the integration of NAMs and assimilation of data derived from NAMs are a fundamental of the overall methodological pipeline.

4. Conclusions

This overview provides support to the promising relationship between the NGRA and NAMs' application in the field of occupational health and, in turn, to the possible contribution that occupational health expertise can offer to improve such application. Overall, as the safety and health of occupationally exposed subjects are an essential part of the SSbD assessment approach, the NGRA and Occupational Safety intersection may contribute to its effective operationalization.

CRediT authorship contribution statement

Fotini Nikiforou: Writing – review & editing, Writing – original draft. **Achilleas Karakoltzidis:** Writing – review & editing, Writing – original draft. **Denis Sarigiannis:** Writing – review & editing, Writing – original draft, Supervision. **Spyros Karakitsios:** Writing – review & editing, Writing – original draft. **Ivo Iavicoli:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Bernd Nowack:** Writing – review & editing, Writing – original draft. **Veruscka Leso:** Writing – review & editing, Writing – original draft, Conceptualization.

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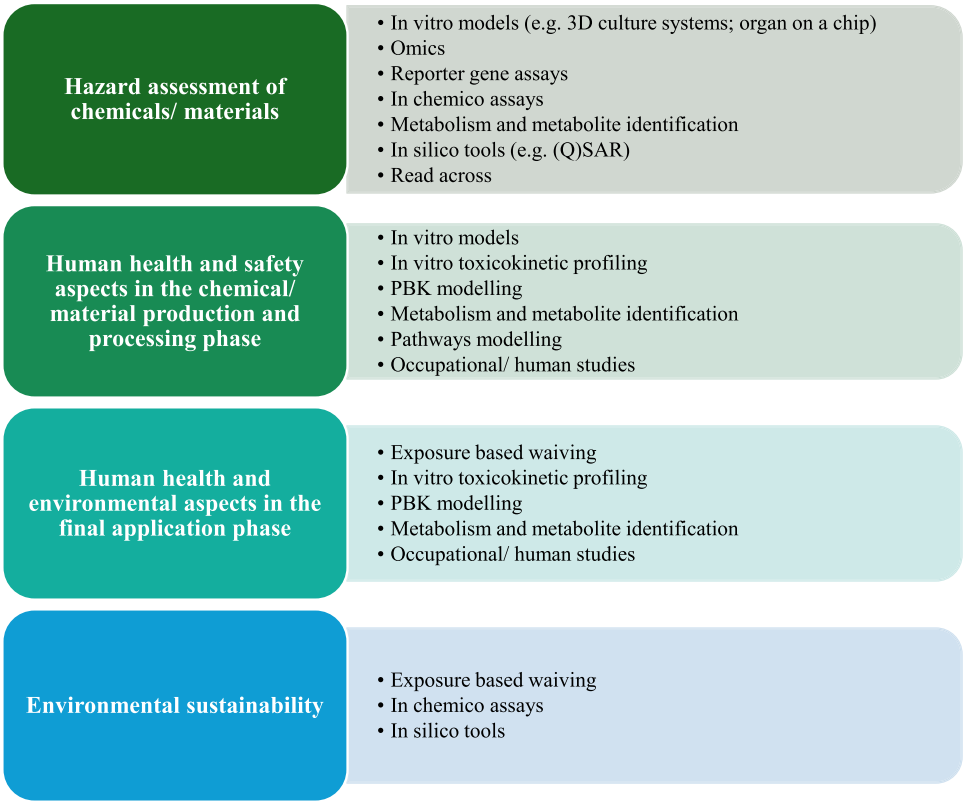

Fig. 3. New approach Methodologies' contribution to the different phases of the "Safe and sustainable by Design" framework. Legend: PBK modelling, physiologically based kinetic (PBK) modelling; (Q)Sar, quantitative structure - activity relationship;.

and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Veruscka Leso reports financial support was provided by European Union. Bernd Nowack reports financial support was provided by European Union. Achilleas Karakoltzidis reports financial support was provided by European Union. Fotini Nikiforou reports financial support was provided by European Union. Spyros Karakitsios reports financial support was provided by European Union. Denis Sarigiannis reports financial support was provided by European Union. Ivo Iavicoli reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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