Department
for Environment
Food & Rural Affairs

Recommendations for the Adoption of New Approach Methodologies (NAMs) in UK Chemical Regulation

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* Items with an asterisk are defined in Appendix 1

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Foreword

Ten years after the 2012 opinion on New Approach Methodologies (NAMs) prepared by DEFRA's Hazardous Substances Advisory Committee (HSAC), which indicated that the scientific basis was not yet sufficiently advanced for NAMs to replace traditional animal tests, DEFRA invited the HSAC to provide an updated opinion and recommendations on the adoption of NAMs for assessment of chemical safety in the UK under a post-Brexit regime. The HSAC accepted the commission and Terms of Reference, and agreed that we would follow a 2-step approach, in which we would:

- Evaluate the use of NAMs broadly and produce a high-level Brief focused on key recommendations for DEFRA regarding the adoption of NAMs for chemical risk assessment (the current document), and
- Follow-up with a more detailed analysis of the evidence for post-Brexit opportunities for the UK, including some case studies demonstrating how NAMs can be, and are already being, applied.

For the Brief we have intentionally avoided making explicit reference to the literature, as this would detract from the core message, and could, at best, give a snapshot of the complexities and the breadth of literature on the topic. The Brief has been extensively peer-reviewed by experts from within DEFRA and its arms-length bodies, before formal publication. Comments and suggestions received were taken on board where they addressed aspects of clarification or precision at the high level of the Brief. Some of the comments received will be addressed in the follow-up evidence-based report, where specific examples and the broader landscape will be presented.

Acknowledgements

Contributions from HSAC: John Colbourne, Luigi Margiotta-Casaluci, Susan Chilton, Kevin Jones, Iseult Lynch, Stewart Owen and reviewed by HSAC in 2023

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Executive summary

HSAC defines New Approach Methodologies (NAMs*) as every method that can enhance the assessment and regulation of hazardous chemicals by improving the relevance, performance and reliability of toxicological testing, based upon an understanding of the modes of action of substances (MoAs*) and support a transition away from mammalian testing, apical endpoints and improve throughput. To take advantage of existing scientific capabilities, foster technological improvements and leverage existing know-how regarding the mechanistic evaluation of chemicals, HSAC proposes that Defra set criteria for NAMs within a framework for progressively integrating NAMs into chemical safety assessment as the science advances, through the following key recommendations.

Key Recommendations for UK Chemicals Regulation include:

- Adoption of a technology agnostic definition of NAMs based on an understanding of chemical modes of action.
- Setting of criteria for NAMs to be considered within a progressive regulatory framework, beginning with criteria for their use in grouping and prioritisation.
- Application of a Progressive NAMs Regulatory Framework that utilizes NAMs for a wide range of regulatory applications as the certainty of the findings for hazard assessment increases. NAMs may already be used to support grouping of chemicals to prioritise higher-tier testing.
- Establishment of UK centres of excellence and a UK national reference laboratory for the development and validation of NAMs to ensure the uptake of technological improvements within the government and private sectors.
- Incentivisation of chemical registrants under UK REACH to provide NAMs data indicative of the modes of action of their substances to support the implementation of a “group first” approach to chemical safety assessment.

Some Key Takeaways:

- The UK is well positioned to emerge as the global leader, and to achieve substantial economic benefits and high standards of human and environmental protection, in using NAMs to obtain findings that are useful for assessing and, when appropriate, regulating chemical exposures.
- NAMs are advancing, leading to better understanding of chemical MoAs and adverse outcome pathways (AOPs*). NAMs are presently viable for regulatory applications by improving the confidence that chemicals belong to defined groups because they induce similar biological responses as other members, as a basis for prioritisation.
- Although UK REACH currently provides a legal basis for applying NAMs, strategic implementation of a NAMs approach within UK REACH and GB-CLP can significantly reduce, refine, and potentially replace the use of mammalian animals (the 3Rs) for chemical safety testing while improving hazard assessment robustness and efficiency.

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Background: HSAC Remit

The Hazardous Substances Advisory Committee (HSAC) was requested to provide a view and recommendations on the adoption of NAMs for the assessment of chemical safety in the UK under a post-Brexit regime.

In our reply, this Committee finds that the UK's technological and scientific capabilities in areas of public health and safety, including the prevention of non-communicable exposure-related diseases, are substantially advanced, thereby offering significant opportunities for the UK to **demonstrate global leadership in chemical safety regulation by integrating NAMs into the regulatory process according to criteria for assessing chemical modes of action**. Explicit and transparent criteria for NAMs are needed to effectively solicit proposals for specific methodologies for using NAMs in chemical safety assessment, including costings. In this document, we offer a framework and a set of criteria for regulatory adoption as an essential preliminary step in facilitating the use of NAMs in chemical regulation.

In 2012, HSAC published a statement on the UK's use of animals in chemical testing that endorsed the government's efforts to fully promote the 3Rs (reduce, refine, and replace animal testing), an aim originating in the UK in the Animals (Scientific Procedures) Act 1986 and continuing through to the current UK REACH regulation. Yet at that time, the Committee could not recommend a change in regulatory practice to NAMs, stating: "Until such alternatives are fully developed and validated so that they are widely acceptable to the scientific and regulatory communities, the HSAC recognises that much toxicological data will be obtained from tests using research animals"[1]. Since then and while a member of the EU, the UK has partaken in (and oftentimes led) numerous cooperative international initiatives for the development of common standards and innovative hazard and risk assessment tools. Notably, research substantially funded by the European Commission's multi-annual research and innovation framework programmes, and within the Organisation for Economic Cooperation and Development (OECD), have substantially advanced the standing of NAMs over the last decade and underwrite the confidence of HSAC in updating its position in **advocating the adoption of NAMs into UK chemical regulation according to their readiness for specific applications.**

This brief outlines why this Committee finds that, after ten years of significant investment and advancement, NAMs are ready to support improved UK chemical assessment under UK REACH. We offer key recommendations for NAMs implementation, proposing a progressive framework for their integration into the regulatory process and emphasizing the immediate need for an explicit set of criteria supported by a UK reference laboratory or

[1] HSAC Statement on the Use of Animals in Chemical Testing

centre for the acceptance of NAMs methodologies and resulting data. Establishing **transparent, technology-agnostic requirements** is an essential first step in facilitating meaningful proposals for NAMs protocols.

Document Structure

We begin this document with a brief recap of the challenges of UK chemical regulation and outline the advancements in NAMs over the past decade, proposing an output-based specification of NAMs. We argue that some NAMs are currently able to provide scientifically relevant answers for the safety assessment of chemicals, outlining a progressive regulatory framework for utilising NAMs according to their increasing certainty. We also argue for the need to establish reporting standards for NAMs based on UK-led advances in data science research so that regulators have confidence in NAMs data submissions. We conclude by proposing general NAMs criteria along with specific criteria for progressive applications within the regulatory process, beginning with chemical grouping and prioritisation for higher-tier testing.

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Problem: Insufficient Chemical Safety Information and 3Rs

Under UK REACH, the burden is on companies to provide data and information on their chemical assessments. Regulatory decisions are made to protect human health and the environment from exposure to toxic substances based on the precautionary principle*. However, **most substances remain untested for their potential hazards, in part because they are below volume thresholds.** Where chemical safety information is provided it is still primarily obtained using animals, despite the UK's commitment to the 3Rs. The UK is ranked 2nd highest among countries in the EU for animal use (1,749,901 animals for experiments in 2018), 25% of which are used for chemicals regulation.

Small rodents have typically been used for chemical safety testing, not because they have proven to provide the best approximations of human response (compared to other animal species) but because they have traditionally been viewed as convenient human surrogates (by virtue of our shared mammalian evolutionary ancestry) and because they have been used for experimental extrapolation to human health for over a century. These **animal-to-human extrapolations have now been shown to be associated with substantial uncertainties** with respect to the conclusions reached. Yet when REACH dossiers are submitted by industries attempting to avoid animal testing in concordance with legal requirements to minimize animal use (UK REACH Articles 1 and 25), these dossiers are often rejected at the earliest stage of compliance checking.

Data requirements for low-tonnage substances (of which there are more than 9,500 substances produced or imported into the EU at volumes between 1-10 tonnes) are now under review (Annex VII, EU REACH) to address knowledge gaps under a precautionary approach. We question the necessity for animal tests to comply with the safety assessment requirements of these data poor substances, at least in the first instance, from an ethical position based on the current situation where animal data are not required. Thus, **alternatives to animal testing are urgently needed** to meet the purposes of hazard and risk assessment under UK REACH's protection aims by the most appropriate use of NAMs.

NAMs Advances Since 2012

Since the HSAC's 2012 statement:

- Major government-to-government initiatives have developed amongst regulatory agencies from North America, Europe, and Australasia including a programme that aims to Accelerate the Pace of Chemical Risk Assessment (APCRA) by NAMs.
- NAMs have also emerged as a major research and innovation funding priority under successive European Commission (EC) funding programmes, which have provided

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approximately €480 million in support of the development of alternatives to animal testing, in addition to at least €150 million in research and development efforts by industry.

- Among many funded projects by the EC, seven scientific projects began in 2021 that sum to €84 million, including the ASPIS cluster, consisting of three projects totalling €60 million.
- A new Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC) initiative began in May 2022, supported by a €400 million research investment from the EC and member states, which engage 200 agencies and affiliated partners across all member states and the UK towards PARC's mission to address current, emerging, and novel chemical safety challenges and enable the transition to next-generation risk assessment (NGRA).

These projects build on breakthroughs, many originating in the UK, including innovations in acquiring high-content and high-throughput data such as DNA sequencing that sparked the 'omics' revolution in biology and medicine, and the development of the UK's NC3Rs organization to support 3Rs science. Approaches that provide mechanistic data applicable to toxicology include the use of omics technologies such as a complementary combination of transcriptomics (revealing gene expression) with proteomics (revealing protein expression changes) and metabolomics (revealing biomolecular processes), to provide rich data on biological responses to chemical perturbation. RNA sequencing now routinely measures the levels at which all genes respond to chemicals, thereby providing comprehensive coverage of genes that signal chemical-induced responses including adversities.

Meanwhile, mass spectrometry is increasingly able to produce protein and metabolic profiles that illuminate, along with changes in gene expression, the toxicity-related biological activity pathways, identifying elements of the processes involved in an organism's response to chemical exposure. Interpretation of this information is aided by contemporary computational procedures, such as machine learning, to understand how the routes to adverse health outcomes caused by chemicals are due to the impact on gene and metabolic pathways. The recent advances in computational approaches and modelling are promoting development of more OECD Test Guidelines utilising NAMs, and significant advances have been achieved in the development of human-relevant in silico models and in vitro systems that are able to characterise chemical toxicokinetics and detect the perturbation of complex integrated functional processes, such as cardiac electrophysiology and contractility, inflammation, and kidney function.

With the UK now operating UK REACH independently of the EU, the opportunity has arisen to move into a position of leadership in utilising these innovations in environmental and human toxicology for chemical safety assessment. NAMs have already been embraced in the biomedical and healthcare sectors, within the OECD, and with the UK Food Standards Agency and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment developing a UK roadmap towards acceptance and

integration of NAMs in regulatory decision making. UK regulators have expressed a willingness to consider concrete proposals, with costings, for integrating NAMs into chemical safety assessment. Yet, the criteria for their evaluation and validation for regulatory use are often endpoint specific, which can limit the use of NAMs to only those that are predictive of health risks while there may be other regulatory objectives, such as obtaining information from NAMs that is protective of human health and the environment. Whilst the OECD has validated some approaches and whilst academic research underpins the science, the cost of implementation remains with industry. This cost is seen as an unnecessary risk, especially by smaller companies, as the information is considered as an addition to current regulation rather than an alternative and is widely expected to be rejected by regulators as an incomplete submission (in the absence of classical animal testing) regardless of the scientific and ethical basis.

HSAC recommends DEFRA and other relevant agencies to:

- Update the UK Government position on animal tests with an additional commitment to implement NAMs for regulatory use (including timescales and the roadmap for implementation).
- Provide explicit, transparent, technology-agnostic criteria for a broad use of NAMs to be considered in chemical safety assessment.
- Develop policies that incentivise accelerated development of NAMs by the UK's private sector, for a positive impact on public/environmental health and the economic sustainability of its chemicals industry.
- Create and fund UK centres of excellence for the continuing development, training in and application of technology relevant to NAMs post-Brexit to supercharge UK research and innovation in regulatory science.

Establish a UK national reference laboratory for development and validation of NAMs to ensure uptake of technological improvements within the government and private sectors.

Benefits Of Early Adoption (of NAMs)

Transitioning now to chemical safety testing centred on NAMs would position the UK to receive the benefits associated with being an early adopter, including:

- An earlier move to an improved, protective framework providing wider societal benefits through human health and environmental protection.
- The robust application of the 3Rs provided by NAMs will offer the UK ethical standing and greater opportunity to take a leadership role in NAM implementation as societies continue to become more health and environmentally conscious.
- Technological opportunities for UK plc's science and services industries in a new NAMs market and in readiness for emerging export markets, along with delivering economic returns from past and current research and development investment.

It is the opinion of this committee that while there are risks associated with the UK being an early adopter of NAMs, the benefits from an effectively managed and mitigated implementation outweigh these risks.

Defining NAMs

The UK Committee on Toxicity of Chemicals (COT) defines NAMs as New Approach Methodologies including but not limited to high throughput screening and other in vitro assays, omics and in silico computer modelling strategies (for example Artificial Intelligence (AI) and machine learning) for the evaluation of hazard and exposure. This also advocates the Replacement, Reduction and Refinement (3Rs) approach to animal testing. Although correct, this definition focuses on the technologies involved. The utility of NAMs lies in their function of recording changes occurring within and provoked by **biomolecular interactions** (with chemical pollutants) that impact biology. These changes contribute to an understanding of AOPs; the AOP framework (supported by the OECD) enables mapping of the sequence and network of events from the molecular initiating event (such as interaction between a chemical and a specific cell receptor) through key events* at multiple levels of biological organization (molecular, cellular, organ, system) that ultimately result in an adverse outcome relevant to risk management.

In medical science, signals of these pathways (**biomarkers**) are widely used in public health management and in the diagnosis of a range of conditions including cardiovascular diseases, cancer, DNA damage, and oxidative stress. Some of these biomarkers are already being applied in the regulation of chemicals in assessments for endocrine disruption or liver toxicity, and the information provided by NAMs are increasingly able to link cause and effect to these and other adverse outcomes. Examples of pre-defined sets of toxicity biomarkers include the United States' National Toxicology Program's S1500+ reference gene panel, which is specific to human xenobiotic and stress response pathways, and the UK-led MTox700+ toxicity-related metabolic biomarker resource. These biomarker panels are already curated to be used as predictors of adversities that are relevant for chemical regulation.

In contrast to traditional animal testing approaches that seek to determine whether chemical exposure leads to apical endpoints such as reproductive failure and death, the value of NAMs to regulatory toxicology can be understood in terms of their ability to elucidate how chemicals, including as mixtures of unknown or variable composition, complex reaction products or of biological materials (UVCBs*), induce perturbations at a biomolecular level. Biomolecular responses may occur prior to, or even without ultimately causing, for example, reproductive failure or death, yet still result in health consequences of interest to regulators such as chronic organ disruption.

As more AOPs are mapped and NAMs technology further advances, more information will continue to become available on chemical MoAs and their associated hazards. Even if

some steps in this biochemical progression are not yet fully understood for a given hazard, NAMs can still be evaluated as methods of identifying molecular key events*: specific, measurable biological activity taking place along the path from exposure to adverse outcome.

HSAC recommends:

- A technology-agnostic definition of NAMs focused on the data they produce – mechanistic (for example biomolecular) information meeting acceptable standards on the modes of action (MoAs) by which chemicals perturb biology and are transformed through metabolism via alternatives to traditional mammalian animal methods.
- Adoption of NAMs, based on the above definition, into a regulatory strategy focused on assessment of toxicity pathways that benefits from an integrated approach to testing and assessment (IATA*).

Applying a Progressive NAMs Regulatory Framework

As more than £1 billion in global research efforts continue to produce data and insights that increase the certainty and precision of NAMs, going forward we recommend that NAMs be progressively incorporated into UK chemical regulation to first reduce, then refine and replace animal testing. Figure 1 illustrates a Progressive NAMs Regulatory Framework[2] by which to utilise NAMs as they advance in their capability to identify molecular key events (KE) and their biomarkers.

[2] E. Andrews, J. Colbourne, R. Lee, in preparation.

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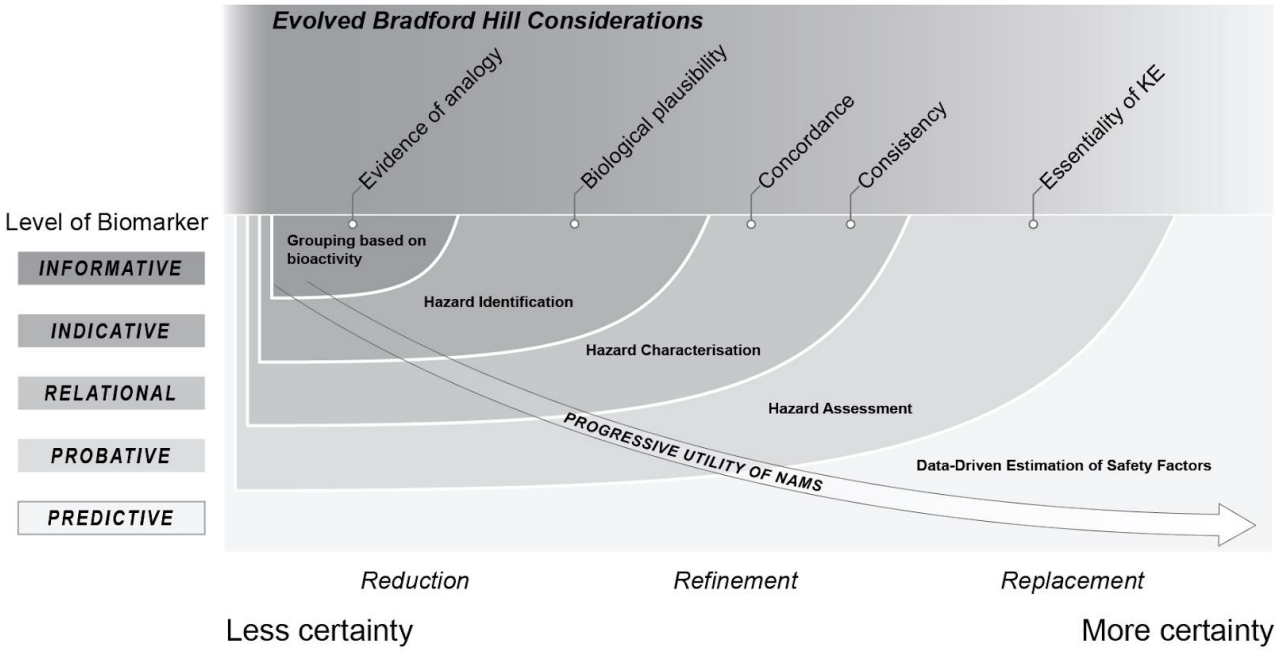

Figure 1 - Progressive NAMs Regulatory Framework (Colbourne et al., in preparation)

The application of NAMs for the purpose of providing information that is useful for the regulatory assessment of chemical safety is dependent on the level of certainty of the results yet is not restricted to data that are predictive of adversity. Biomarkers of chemical modes of action can also be informative, indicative, relational, or probative of adversity, which have varying levels of certainty in their findings as determined by evolved Bradford Hill (EBH) considerations for associating chemical exposures with health outcomes. As biomarker levels of certainty increase, so do their utility along a progressive order of NAMs-applications, beginning with grouping based on bioactivity. Progressive use of NAMs will increase certainty, based on increased data, and build towards more predictive positions for estimating safety and therefore contribute more strongly towards the replacement of animal studies. Examples of the application of the EBH criteria for development of NAMs are presented in Table 1.

The proposed Progressive NAMs Regulatory Framework makes use of the evolved Bradford Hill (EBH*) considerations for associating chemical exposures with health outcomes, which draw on the AOP model and toxicokinetics. As increasingly refined NAMs data progressively satisfy the EBH considerations (as outlined in Table 1), each level of association warrants an additional step in the hazard characterisation and assessment process. The set of warranted actions is cumulative, so that as NAMs data become more precise, thereby satisfying more of the EBH considerations, further steps can be taken along the regulatory path that reduces, refines, and replaces animal testing, while justification for previous steps is also strengthened, especially by embedding the NAMs into integrated approaches to testing and assessment (IATA)*.

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Table 1 - Applications for Evolved Bradford Hill considerations using NAMs

Aspect of association (EBH considerations)	Explanation	Level of biomarker	Regulatory applications
Analogy	Similar bioactivity responses are observed from exposure to chemically similar substances yet do not necessarily provide information about their MoA	Informative	Substantiating structure-based grouping hypotheses or de-novo grouping of substances for prioritisation or read-across
Biological concordance	Biomarkers of key events (KE) fit within recognizable pathways indicative of an adverse outcome	Indicative	Hazard identification
Temporal concordance and Dose-response concordance	KE biomarkers are occurring in a progressively correct order, including their relative magnitude of response	Relational	Hazard characterisation and exposure assessment
Consistency	KE biomarkers show replicable associations across species		
Essentiality of key events	Experimental knock-out of genes associated with KE biomarkers prevents adverse outcome; knock-in restores outcome	Probative / Predictive	Hazard assessment / Data-driven estimation of safety factors

In practical terms, NAMs application can reduce animal testing at the first level of biomarkers through MoA-based grouping where 'evidence of analogy' is provided from NAMs-derived 'informative biomarkers' demonstrating that observable effects (termed bioactivity*) are occurring in a similar manner in response to each chemical in the set. Importantly, the identity of informative biomarkers need not be known to facilitate grouping for prioritisation and/or read across.

At the next level, 'Indicative biomarkers' have the additional criterion that the sequence of biomolecular events is associated with a known outcome, meaning evidence of 'biological concordance' is established by linking the biomarkers with a MoA or known AOP.

The technology is already viable to identify 'informative' and, where AOPs are known, 'indicative' biomarkers. These include 'omics' technologies that measure a broad range of biomolecular responses to a test chemical and deliver meaningful data on the ways in which chemicals interact with, and perturb, biological systems. Recognising the need for

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standardisation and harmonisation of such NAMs data for regulatory use, the OECD EAGMST* launched a project to develop a reporting framework and guidance for generating and analysing Omics data, led by UK participants. As NAMs data advance and accumulate, providing greater certainty, NAMs are anticipated to refine animal testing in the near term – by guiding the tests to be conducted based upon biomolecular evidence that is indicative of adversity – and to be demonstrated as viable replacements for animal testing in the longer term. Adoption of the Progressive NAMs Regulatory Framework ensures not only that the aims of the 3Rs are realised, but also that those exacting standards are applied to data used in the hazard and risk assessment process, leading to better protections and a safer, more sustainable chemicals industry.

HSAC recommends:

- Adoption of the Progressive NAMs Regulatory Framework to enable use of NAMs according to their level of certainty and their association to regulatory endpoints, and evolution of their utility and confidence in the data by applying NAMs in actual regulatory practice.
- Setting of NAMs criteria for satisfying each biomarker level described in the Progressive NAMs Regulatory Framework, including through bolstering the UK's leadership roles in related activities at the OECD.

Acceptance of NAMs

To support the development of specific proposals for methodologies and protocols for integrating NAMs into chemical safety assessment, a set of criteria for NAMs must be established supported by certification that the proposed NAMs meet such criteria by UK national reference laboratories and/or a centre for the acceptance of NAMs data. Mechanism-focussed accepted NAMs will enable more informed, precautionary, and protective chemical safety assessments, initially by supporting bioactivity-based grouping for prioritisation and read-across, and as a strategy to reduce animal testing. The criteria must be sufficiently robust to meet the needs of, and provide assurance to, risk managers in accepting a NAM within the framework, and the acceptance criteria are technology agnostic to encourage the deployment and continuing advancement of all available NAMs technologies.

In line with the progressive modes-of-action-based NAMs framework and the set of criteria established as per the above recommendations, we propose the following criteria including work already done under the auspices of the OECD.

General Recommended Criteria for NAMs data

- Deliver information on bioactivity/chemical modes of action
- Be reproducible within and across laboratories, tested chemicals, and biological test systems
- Be independently and transparently peer-reviewed
- Be fully described, including their limitations and chemical domains
- Be reported in accordance with currently accepted templates
- Be shared via open access following FAIR principles of findability, accessibility, interoperability, and reusability

Additional criteria for Evidence of Analogy (supports grouping for prioritisation and read across)

- NAMs data provide information on the substances' likely shared modes of action, providing weight of evidence for a grouping hypothesis based on comparable patterns of bioactivity

Additional criteria for Evidence of Biological Concordance (supports hazard identification and previous steps)

- NAMs data can link observed bioactivity to known AOPs
- Application to available reference chemicals demonstrates known hazard identification, without precluding unknown hazards

Additional criteria for Evidence of Temporal Concordance, Dose-Response Concordance, and Consistency (supports hazard characterisation and previous steps)

- NAMs data can elucidate higher-order biological processes (for example cellular, organ, metabolic) in the order in which they occur and in response to differing levels of exposure
- NAMs data can compare responses across species, including humans
- NAMs data can elucidate toxicokinetic information

Additional Criteria for Evidence of Essentiality of Key Events (supports hazard assessment and previous steps)

- NAMs use forward and reverse genetics (gene knock-in and knock-out) to definitively implicate biomarkers of key events predictive of adverse outcomes

HSAC recommends:

- Adoption of criteria including those developed and accepted internationally and in use for the acceptance and application of NAMs data, in the progressive certainty regulatory framework.

Group First and Tiered Testing: An Example of Possible Early Adoption

Enshrined in UK-REACH is the legal obligation to implement the 3Rs. Whilst we emphasize that traditional animal safety testing does not necessarily achieve a high degree of certainty, and despite the extensive research investments described earlier in this brief contributing to rapid advancement toward delivering reliable evidence, NAMs do not yet satisfy regulatory conditions for fully replacing animal testing. However**, the opinion of this Committee is that the UK need not delay in reducing and refining animal testing using NAMs** by implementing the Progressive Regulatory Framework and technology agnostic NAMs criteria.

Given the current state of the science, we expect that NAMs will readily satisfy criteria for evidence of analogy to support grouping and prioritisation. Therefore, **we recommend that a tiered testing system be adopted**, in which NAMs are applied as a first tier of evaluation (thereby reducing the use of animals) and enabling all substances including UVCB* substances, to be grouped based on evidence of their shared MoAs. A much smaller number of group-representative chemicals may then be interrogated by 'higher-tier' animal testing, further reducing the use of animals by enabling positive read-across for the bio-mechanistically relevant groups.

When NAMs-derived biomarkers are described within known AOPs, satisfying criteria for evidence of biological concordance, refining the use of animals may be achieved by focusing the higher-tier tests on the predicted adversities. Such a strategy enables a streamlined approach of categorising chemicals according to their MoAs, and, when applied to the approximately 9,500 low-tonnage substances (Annex VII, UK-REACH) will enhance the understanding of their hazards, about which little is currently known. Such a strategy will also accelerate the evaluation of substances as groups for further regulatory action, such as authorisation and restriction, and aid in avoiding regrettable substitutions. Importantly, chemical groupings are scientific hypotheses based on the best available science. Similarly, the characterisation of the bioactivity profile of chemicals using NAMs, and the MoA-driven grouping strategy discussed above would facilitate the implementation of mechanistic considerations in the hazard and risk assessment of chemical mixtures.

HSAC recommends:

- Implementation of a ‘Group First’ tiered testing strategy using NAMs to reduce animal testing.
- Implementation of a chemical grouping strategy using NAMs, minimally based on evidence of analogy, according to the Framework and associated NAMs criteria.

Conclusion

By implementing the Progressive Regulatory Framework and associated NAMs criteria, UK regulators can facilitate proposals for immediately applicable NAMs approaches with numerous benefits, including advancing understanding of thousands of chemicals that remain largely untested, supporting the 3Rs, and building toward a robust body of mechanistic, biochemical knowledge concerning the connection between chemical structure and biological response.

As a result of over ten years of investments since HSAC published a statement on the UK’s use of animals in chemical testing, we now conclude that the science is sufficiently mature, offering opportunities for the UK to demonstrate global leadership in utilising its technologies, many of which originate with UK innovators, that have not yet been widely adopted in regulatory toxicology. Focussed work remains to be done to accelerate the standardisation and harmonisation of NAMs, requiring a UK strategy that may include a UK validation Centre (a Reference Laboratory), such as EURL ECVAM in the EU and NICEATM in the USA, for the uptake of the developing science, ensuring the benefits of former, existing, and future science investments are more fully realised through their application. Adoption of explicit criteria for NAMs data that are technology agnostic within a progressive regulatory framework is the essential first step in moving toward regulatory integration of NAMs, which is expected to ultimately reduce testing costs while improving the safety and sustainability of the chemicals industry.

Appendix 1 – Concept Definitions

AOP: Adverse Outcome Pathway is a framework that describes the sequence of causally linked molecular, cellular and higher-order key events required to produce a toxic adverse effect when a biological system is exposed to chemicals.

EBH: Evolved Bradford Hill considerations extend nine aspects of causal association that explore how we can know that an event of sickness or injury might be related to an environmental feature. Bradford Hill's (1965) original considerations relied on epidemiological data. Evolved Bradford Hill (EBH) considerations are aligned with advances in both toxicity testing and emergent fields such toxicokinetics drawing on the Adverse Outcome Pathway (AOP) framework.

IATA: Integrated Approaches to Testing and Assessment use diverse sources of information to conclude on the toxicity of chemicals, including information obtained from the scientific literature supplemented with newly generated data (for example using NAMs) to fill data gaps. These are developed around specific regulatory needs.

Key events: A key event is a measurable change in a biological system (that is compared to a control). It is considered essential to, but not necessarily sufficient for progression to an adverse outcome.

MoA: Mode of Action describes a biologically plausible series of key events leading to an effect. When this effect results in an adverse outcome, the MoA is an AOP.

NAMs: New Approach Methodologies are essentially cruelty-free methods that can enhance the assessment and regulation of hazardous chemicals, by improving the performance and reliability of toxicological testing, based upon an understanding of the chemical MoAs, and providing appropriate protection levels for human health and/or the environment.

OECD EAGMST: The Organisation for Economic Co-operation and Development Extended Advisory Group on Molecular Screening and Toxicogenomics.

Precautionary principle: Principle 15 of the 1992 Rio Declaration states that "where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation".

UVCB: Substances of Unknown or Variable composition, Complex reaction products, or Biological materials (UVCB) are materials that cannot be represented by single unique structures of formulas (for example complex mixtures of variable sized polymers).

WoE: Weight of Evidence is an approach where there may be sufficient weight of evidence from several independent sources of information leading to the

assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion.