New Approach Methods (NAMs) in the science-based derivation of limit values – current and future role

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In recent years there has been increasing controversy at both national and international level about the necessity and human relevance of animal experiments, as well as the possibilities of dispensing with them entirely, also in connection with the derivation of limit values for hazardous substances. In some cases the view is expressed that animal experiments are already being replaced more or less completely by the use of so-called New Approach Methods (NAMs), or that this will happen in the near future. The MAK Commission cannot share this assessment at this stage, as a quantitative risk assessment requires *in vivo* data from human and/or animal studies that allow the identification of critical target organs, the consideration of complex multiple effects as well as the quantitative identification of the lowest adverse effect concentration. In fact, also NAMs include *in vivo* studies, studies on suitable cellular test systems and computer-based models. In all these areas, NAMs are capable of providing valuable additional insights regarding the mode and mechanism of action and therefore to a science-based risk assessment. They can help minimise animal testing, but they cannot replace it entirely. This assessment will be elaborated in the following.

The central task of the MAK Commission is to derive limit values for the workplace. This is a quantitative risk assessment that usually requires epidemiological data, human volunteer studies and/or results from animal experiments. In addition, mechanistic data and information is required from short-term tests such as genotoxicity studies based on *in vitro* tests. In order to be able to derive limit values, a very good data basis is required. If only insufficient substance-specific information is available, no MAK or BAT values can be established. In recent years, NAMs have been increasingly developed and discussed that might usefully supplement and possibly change existing toxicological risk assessments. In particular, promising possibilities are offered by the joint consideration and use of the various NAMs and conceptual approaches such as adverse outcome pathways (AOP).

Today, the term “NAMs” covers a broad spectrum of technologies and methods that are not necessarily limited to *in vitro* or *in silico* (see for example US EPA, EFSA, ECHA):

- Computer-based identification of critical structural features, so-called *in silico* methods (e.g. QSAR and read-across).

- *In chemico* methods (e.g. for the non-cell-based identification of reactive substances with potentially toxic properties).

- *In vitro* tests: Cell-based systems of varying complexity (2D and 3D cultures, including cocultures, organoids and organ-on-chip systems), usually of human origin and available for different organs; broadband screening methods (omics, cell painting methods); targeted high content screening (target molecules: receptors, enzymes).

- *In vitro* biokinetics: Modelling of the available intracellular concentration derived from the nominal concentration, taking into account various factors such as uptake by the cells, binding to proteins and lipids, and also binding to cell culture vessels and loss through evaporation.

- IVIVE (*in vitro* to *in vivo* extrapolation): Conversion of the available concentration defined on an *in vitro* basis at which an adverse effect was observed to the corresponding human-equivalent concentration using physiologically-based kinetics (PBK) models. The human equivalent dose is referred to as the "point of departure" (POD) for the risk assessment.

- Toxicological studies on "simple" organisms (e.g. zebrafish, fish embryo, *C. elegans*).

- Use of *in vitro* studies and investigations on simple organisms for the validation of read-across predictions.

- Computer models: Integration of multiple *in silico* and *in vitro* data streams using available databases (data integration approaches and AI).

It must be taken into account here that the establishment of some test systems such as organoids and organ-on-chip systems is highly complex; despite intensive research activity, standardisation and validation of the systems currently remain very difficult.

What potential is offered by the increased development and establishment of NAMs at present and in the future?

One particular potential offered by NAMs is in the area of identifying the toxic properties of a substance (hazard). For example, different test methods can be used to describe so-called molecular initiating (MI) and subsequent key events (KE) in AOPs. Mechanistic knowledge of the mode of action of a chemical has always been considered in the derivation of maximum workplace concentrations, and NAMs can provide a wealth of information and data to supplement this knowledge. Such substance information could be used in connection with the so-called read-across process, for example, in the risk assessment of related chemicals where insufficient substance-specific data are available for the respective chemicals in question. This procedure is already used in individual cases and could be significantly expanded in the future if the data base were broader and this data were made available on a curated basis.

Another aspect that is currently the subject of intense debate, also in connection with the EU's new chemicals strategy, is the aspect of Safe and Sustainable by Design. In concrete terms, this means that toxicological aspects are considered even before new chemicals are placed on the market or new materials are designed, taking into account the entire life cycle of the relevant products. Comparable to the detection of mutagenic effects using the Ames test, particularly critical properties could be identified at an early stage in this way, and the relevant chemicals or materials replaced with toxicologically less critical substances or components. Such procedures are already routinely used in the chemical and pharmaceutical industry, for example to exclude substances with questionable toxicological profiles in the early phase of active substance development.

In recent years, considerable progress has also been made in the field of so-called omics methods, which are used to assess complex biological reactions of chemicals and which can be used to establish toxicity profiles and further elucidate mechanisms of action; these are already successfully applied when it comes to determining the comparative properties of nanomaterials. Omics methods are also valuable tools to provide more in-depth information on animal experiments, e.g. by means of transcriptome or proteome studies at the single cell and tissue level or using *ex vivo* tissue sections. A prerequisite for the optimum use of the results is good accessibility and usability of the relevant data; one example is the ToxCast/Tox21 Dashboard database which is operated by the US Environmental Protection Agency (US EPA). All in all, the establishment of NAMs is a very dynamic process and it can be expected that numerous methods will be refined in future; in addition to hazard considerations, (a) toxicokinetic aspects will be increasingly incorporated, and (b) further approaches for quantifying dose-response relationships will be generated in the area of *in vitro* methods. NAMs already make a significant contribution to the so-called 3Rs principle in animal experiments, the objective of which is to replace such experiments (Replacement), reduce the number of animals (Reduction) and reduce the suffering of the animals (Refinement) to a level that is strictly necessary.

How are these methods already included in the assessments, limit value derivations and classifications carried out by the MAK Commission?

The general prerequisite and starting point for the derivation of limit values is the identification of the most sensitive toxicological endpoint *in vivo*, i.e. in humans or in animal experiments, providing the results from animal experiments are plausibly transferable to humans. This includes both the toxic properties of a substance (hazard) and the risk, which also takes into account the reaching of critical concentrations in the target organ. Possible interspecies differences in toxicokinetics and dynamics are likewise considered here. The MAK Commission already uses additional data from NAMs – where available – in order to assess the relevant substances as comprehensively as possible. For several years now, data from so-called PBPK (Physiologically-Based Pharmacokinetic) models have been used for individual substances to analyse internal exposure to the toxic substance, taking into account physiological and biochemical differences between species. For example, for the assignment of the pregnancy group of acetone, the main metabolite of 2-propanol, a PBPK model has been applied to estimate the internal exposure for foetuses based on comparative analyses between the parent substance 2-propanol and its metabolite acetone. Conversely, based on internal exposure and the relevant modelling, an air limit value for lead and its inorganic compounds was recently derived at which the critical internal exposure for the majority of workers (95th percentile) is not exceeded.

Another example is the use of ToxCast/Tox21 Dashboard data provided by the US EPA to establish the hazards and for the use of AOPs of individual substances. These data provide information on a wide variety of receptor interactions that may indicate an endocrine or liver-toxic effect, for example. Furthermore, it is foreseeable that promising NAMs will develop in the relevant areas/endpoints of “sensory irritation” and neurotoxicity testing. Such data have so far only been used to clarify or confirm mechanisms of action, however. Exceptions here are cosmetics, where animal testing is no longer permitted for toxicological assessment of the ingredients and the product; animal testing is now also being largely replaced in the identification of sensitising effects. In all other areas, quantitative *in vitro* to *in vivo* extrapolation

(QIVIVE) will gain in importance particularly in connection with future developments towards animal-free *in vitro* and *in silico* methods. These models should make it possible to use *in vitro* data to simulate concentrations in plasma or in specific target organs/tissues of an organism and estimate at which dosages effective *in vitro* concentrations are reached *in vivo*.
All in all, the MAK Commission engages very closely with the current possibilities offered by using NAMs and also conceptually with future application possibilities; for this purpose, high-ranking international researchers have been involved in recent years to report on and discuss the current status of possibilities and limitations in the use of NAMs. Focus areas here have included the possibilities of omics methods to differentiate between genotoxic and non-genotoxic activities of chemicals, and the usefulness of information from the ToxCast/Tox21 Dashboard to assess the hazard potential of chemicals.
Several members of the MAK Commission are actively involved in the development and establishment of NAMs to address specific questions, for example concerning comparative toxicity profiles for nanomaterials, the establishment of test systems for neurotoxicity, sensory irritation effects and sensitising effects, as well as for the application of an *in vitro* test battery for developmental neurotoxicity in the regulation of plant protection products.
It is highly likely that a sensible combination of these methods will lead to a situation in the future where, for example, effective read-across limit values can be established for many more substances. At all levels, however, traditional toxicological data are currently still used as training sets to validate NAMs. One of the greatest difficulties of NAMs at present is the quantitative extrapolation of *in vitro* to *in vivo*. There are also challenges involved in comparing *in vivo* rodent studies with *in vitro* studies that are mainly based on human cell systems. In addition, there are uncertainties in the assessment of combined test systems and the definition of the relevant and sufficient extent of testing. For this reason, integrated testing strategies have only been presented for a small number of toxicological endpoints such as skin sensitisation and irritation. In terms of developmental neurotoxicity, too, the *in vitro* test battery was used by the EFSA in a case study in the context of IATA (Integrated Approaches for Testing and Assessment). Nonetheless, the objective of a comprehensive assessment of new, toxicologically still poorly investigated substances, especially for endpoints such as chronic toxicity, reproductive toxicity and carcinogenicity, is still a long way off.

In summary, from the point of view of the MAK Commission, the establishment of NAMs is currently – and probably in the near future, too – not yet far enough advanced to be used reliably on their own for the purpose of quantitative risk assessment and limit value derivation, replacing animal testing entirely, for example. There is still a considerable need for research here, including both the establishment of suitable test systems and their validation. However, NAMs already offer a useful and valuable supplement to the more conventional toxicological research methods in some areas.

Statements from other organisations:

EPA: USEPA (2021) New Approach Methods Work Plan (v2). U.S. Environmental Protection Agency, Washington, DC. EPA/600/X-21/209

ECHA: European Chemicals Agency (2016) New Approach Methodologies in Regulatory Science; Proceedings of a scientific workshop, Helsinki, 19-20 April 2016. ECHA-16-R-21-EN. https://doi.org/10.2823/543644

EFSA: Escher SE, Partosch F, Konzok S, Jennings P, Luijten M, Kienhuis A, de Leeuw V, Reuss R, Lindemann K-M, Hougaard Bennekou S (2022) Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment. EFSA Supporting publication 2022: EN-7341. https://doi.org/10.2903/sp.efsa.2022.EN-7341

IARC: Samet JM, Chiu WA, Cogliano V, Jinot J, Kriebel D, Lunn RM, Beland FA, Bero L, Browne P, Fritschi L, Kanno J, Lachenmeier DW, Lan Q, Lasfargues G, Le Curieux F, Peters S, Shubat P, Sone H, White MC, Williamson J, Yakubovskaya M, Siemiatycki J, White PA, Guyton KZ, Schubauer-Berigan MK, Hall AL, Grosse Y, Bouvard V, Benbrahim-Tallaa L, El Ghissassi F, Lauby-Secretan B, Armstrong B, Saracci R, Zavadil J, Straif K, Wild CP (2020) The IARC Monographs: Updated Procedures for Modern and Transparent Evidence Synthesis in Cancer Hazard Identification. J Natl Cancer Inst 112: 30–37. https://doi.org/10.1093/jnci/djz169

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