Mode of Action: Recent Developments, Regulatory Application and Future Work

21-22 February 2013, Vienna

Workshop Report No. 26

Co-organised by ECETOC and WHO-IPCS
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EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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Brussels, June 2013

ISSN-2078-7200-26 (print)
ISSN-2078-7219-26 (online)

ECETOC Workshop Report No. 26

European Centre for Ecotoxicology and Toxicology of Chemicals
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CONTENTS

1. EXECUTIVE SUMMARY

An ECETOC/ILSI RF/HESI workshop was held in 2009 to exchange views on conceptual approaches to the use of Mode of Action (MOA) in chemical risk assessment. A number of recommendations emerged, and a global Steering Group was convened by WHO/IPCS to co-ordinate implementation of an 'umbrella plan' of work, comprising experts from ECHA, EFSA, Imperial College, JRC, OECD, University of Ottawa, US EPA, ILSI/HESI, and ECETOC. The objectives of the 2013 workshop were to review progress that had been made since 2009. Specifically, these were to share experiences in applying the MOA approach in the regulatory environment, to identify any difficulties or 'roadblocks' that had arisen in applying MOA in the regulatory decision-making process, and finally to make recommendations for future work.

The first part of the Workshop focused on providing participants with information on the WHO/IPCS MOA Roadmap, the updated MOA Framework , regulatory application of the Framework, and, then, using case studies, highlighted how MOA can be used in chemical regulation together with some of the challenges encountered in applying the MOA approach - particularly in the US and EU.

In the second part of the meeting, the participants were divided into three breakout groups to explore how MOA can inform risk assessment, be used in defining testing strategies and what further research could be undertaken, including better integration of alternative methods. It was pointed out that the first essential step was problem formulation; only the amount of information and analysis of MOA necessary to answer the question under consideration was required.

Particular considerations were raised, and included:

- MOA being too elaborate and resource intensive.
- How to address multiple MOAs.
- Mutual acceptance of MOA.
- The role of 21st century technologies in MOA analysis.

Suggested areas of improvement included:

- More flexibility in the development and application of non-standard data by stakeholders and regulators.
- Better description of established MOAs in databases and their potential application as a basis to increase common understanding of their development and use.

Areas for future work included:

- Explore the extent to which key steps of known MOAs could be modelled in simpler organisms.
- For the development of data bases, look into the scope for building relevant tools, models and SARs, and in the documentation of normal physiological range and variability in adverse outcome pathways.
- Evolve the efficient and effective application of MOA in regulatory risk assessment, e.g. no guidance on the submission of data is currently available.
- Develop training with regard to the use of MOAs in various aspects of risk assessment and including its application in read-across.

These recommendations inform future work on MOA by the risk assessment community at large; they are, in particular, being considered by the WHO/IPCS Steering Group on MOA.

2. AIM OF THE WORKSHOP

Following an international workshop in 2009, a group of experts continued to oversee implementation of the recommendations made. The present workshop was co-organised by ECETOC and the World Health Organisation (WHO) – International Programme on Chemical Safety (IPCS), in the context of official collaboration between ECETOC and WHO, as an interim review:

- To present the WHO/IPCS guidance on mode of action/human relevance analysis revised to reflect increasing experience in integrating information from evolving technologies.
- To familiarise with and solicit input on envisaged implications for priority setting, risk assessment and testing strategies for both individual chemicals and groups, through consideration of case studies.
- To consider next steps for additionally addressing identified priorities in regulatory risk assessment such as category approaches.

Further background on Mode of Action (MOA) initiatives worldwide as well as on current and future developments were given in the Plenary Lectures.

3. PLENARY LECTURES

3.1 Introduction

3.1.1 Background to Global Initiatives Promoting the Use of 'Mode of Action'

Neil Carmichael
On behalf of ECETOC

Global initiatives to promote the use of mode of action (MOA) began around a decade ago and currently involve large numbers of scientists internationally (US EPA, 1996; Sonich-Mullin et al, 2001). In essence, MOA information on a chemical resulted from the use of good investigative science to ascertain the human relevance of findings in animal studies. This kind of information should form an essential and integral part of such activities as read-across, risk assessment and classification.

An ECETOC/ILSI RF/HESI workshop was held in 2009 to exchange views on conceptual approaches to the use of MOAs (Meek et al, 2003; 2008; Seed et al, 2005; ECETOC, 2006; Boobis et al, 2006; 2008). The presentations of case studies with both carcinogenic and non-carcinogenic MOAs were followed by breakout group discussions. Questions considered included: could an understanding of MOA lead to more accurate assessment of risk; what were the potential downsides of using MOA in hazard characterisation; what were the practical/operational steps that would make it easier to employ an MOA approach; how could understanding an MOA lead to a reduction in the use of animals in hazard characterisation.

An overview of the workshop views on using MOA information to improve regulatory decision-making was subsequently published (Carmichael et al, 2011). The recommendations included: establishing an expert working group to oversee the creation of a repository or reference database of agreed MOAs; continuing and extending training and educational programmes based on risk assessment practices using MOA; changing the present risk assessment paradigm to encompass early focus on hazard characterisation including MOA versus hazard identification; providing guidance on the generation of information during standard toxicity tests that could be of value in MOA analysis; developing predictive methods for MOAs, focusing on key events; making optimum use of information from human studies; agreeing and harmonising MOA terminology on a global level. These recommendations were helpful but not easy to put into practice.

As a consequence, WHO/IPCS held a Mode of Action Planning Meeting in London in October 2010, with the objective of harmonising approaches to the use of MOA in risk assessment from exposure to chemicals. At this meeting a global Steering Group was appointed to co-ordinate implementation of an 'umbrella plan' of work, comprising experts from ECHA, EFSA, Imperial College, JRC, OECD, University of Ottawa, US EPA, ECETOC, ILSI/HESI and WHO/IPCS. It was further agreed that ECETOC and WHO would organise an international workshop, i.e. this one in Vienna.

The objectives of this workshop were to review progress that had been made since 2009 in using MOA in chemical risk assessment. In particular, to share experiences in applying the MOA approach in the regulatory environment, to identify any difficulties or 'roadblocks' that had arisen in applying MOA in the regulatory decision-making process, and finally to make recommendations for future work.

3.1.2 WHO/IPCS Mode of Action Roadmap: Mode of Action Applications in Regulatory Toxicology

George Fotakis
European Chemicals Agency, Helsinki, Finland

The mode of action/human relevance framework has been developed in initiatives of the International Programme on Chemical Safety (IPCS) of the World Health Organisation (WHO) and the International Life Sciences Institute Risk Sciences Institute (ILSI-RSI) (Boobis et al, 2006; 2008). The framework continues to evolve as experience increases in its application to systematically consider the weight of evidence from traditional and evolving methods for assessing toxicity (Meek et al, 2013). In order to illustrate the iterative process whereby principles and concepts of mode of action analysis can be applied throughout human health risk assessment, a Mode of Action Roadmap has been developed integrating the WHO/IPCS Mode of Action framework within the process of human health risk assessment.

For an illustration of the Roadmap, see Meek et al (2013).

The Mode of Action Roadmap was created to provide a platform that enables risk assessors and managers to consider mode of action analysis to address risk management needs that can range from priority setting, risk assessment as well as integrated testing strategies.

The presentation showed the iterative process highlighting the potential of mode of action analysis within regulatory toxicology. This includes how mode of action analysis and the use of the WHO/IPCS framework are driven by problem formulation and how mode of action analysis can inform risk assessment, integrated testing strategies and targeted research.

3.1.3 Update of the WHO/IPCS Mode of Action Framework

M.E. (Bette) Meek (presented per audio link)
McLaughlin Centre for Population Health Risk Assessment
University of Ottawa

The World Health Organisation/International Programme on Chemical Safety mode of action/human relevance (MOA/HR) framework has recently been updated to reflect evolving experience in its application and to incorporate recent developments in toxicity testing at different levels of biological organisation and non-test methods (Meek et al, 2013). The modified framework is incorporated within an iterative roadmap, encouraging continuous refinement of problem formulation, mode of action based testing strategies and risk assessment. In this vein, mode of action considerations should inform further development of research strategies and data generation methods, as well as the development of biomarkers.

For an illustration of the updated MOA/HR Framework, see Meek et al (2013).

The framework can be used as originally envisaged, where the outcome of chemical exposure is known, or in hypothesising potential effects resulting from exposure, based on information on putative key events in established modes of action from appropriate *in vitro* or *in silico* systems and other evidence. The implications of the considerable experience acquired in application of the framework in addressing documented (adverse) effects to inform the more limited knowledge base in these more predictive applications were addressed. This was illustrated in various case examples including the nature of information which demonstrates lack of human concordance and implications for subsequent dose-response analysis. The use of mode of action analysis in prioritising substances for further testing, in guiding development of more efficient testing strategies and in identifying critical data gaps and testing strategies in read-across was also illustrated.

In addition to clarifying terminology related to the essentially conceptually synonymous terms of mode of action and adverse outcome pathways, the Bradford Hill considerations have been additionally articulated as a basis to simplify their application in considering weight of evidence for hypothesised modes of action. Templates for extension of the species concordance table in the original framework to dose–response analysis and comparative assessment of weight of evidence and associated uncertainty for various modes of action based on the simplified Bradford Hill considerations have also been developed.

3.1.4 Contribution of New Technologies to Better Understand Modes of Action and Definition of Adversity in Toxicological Assays

Marjoke Heneweer[1]; Thomas Pfister[2]; Jane Botham[3]; Hans Ketelslegers[4]; Lauren K. Markell[5]; David Rouquié[6]; Winfried Steiling[7]; Volker Strauss[8]; Christa Hennes[9]

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Scientifically sound discrimination between adverse and non-adverse effects has always been a key element in the evaluation of toxicological results. ECETOC Technical Report No. 85 (2002) addressed this issue in terms of classical biochemical and histopathological endpoints. However, new technologies are now available to help in the exploration of toxicological MOAs and should contribute to the development of adverse outcome pathways (AOPs).

An ECETOC task force was established to review the current interpretation of adversity through literature and individual views. The aim is to formulate recommendations for the useful integration of these new technologies in toxicological testing. Additionally, the task force analyses the impact of such new data for the definition of adversity and its contribution to a better understanding of Modes of Action. Current learning was presented as a contribution to the discussion at the workshop and includes the observation that the use of 'omics technologies results in relatively high probability of identifying hazardous properties of which the relevance to human health risk is not fully understood (Thomas et al, 2012). A clear causal link between molecular effect and apical endpoint is required to increase confidence in a proposed MOA. The IPCS framework for analysing the relevance of a non-cancer mode of action for humans (Boobis et al, 2008) can assist in assessing this link. It also addresses the critical aspect of human relevance of proposed MOAs.

New technologies provide detailed information on molecular effects induced by chemical exposure, often at an early stage of an AOP. However, in such sensitive assessment the adversity of results has to be clearly distinguished from adaptive responses by well-defined criteria. The validation of new technologies with well-known reference toxicants against the molecular changes and apical adverse effects is essential for a meaningful translation of test results. Furthermore, information on dose-response, time-dependence and biological plausibility is key in such validation. In light of the Toxicity Testing in the 21st Century vision, scientifically sound validation programmes for new technologies such as 'omics should be a priority. In order for new technologies to be accepted by scientific and regulatory communities, the ability of new technologies to provide reliable *in vivo* hazard predictions needs to be further validated, by clearly linking them to studies currently required by regulations (e.g. animal tests).

3.2 Regulatory Application and Challenges

3.2.1 Overview on how Mode of Action is Used in a Regulatory Context: A US Perspective

Jennifer Seed (presenter) and Vicki Dellarco
Office of Chemical Safety and Pollution Prevention
US Environmental Protection Agency, Washington, USA

The Office of Chemical Safety and Pollution Prevention (OCSPP) within the US Environmental Protection Agency has a long history in the use of alternative methods to identify and prioritise information needs, and to estimate potential hazards of industrial chemicals, pesticide inert ingredients, and other chemicals where there are limited data. These tools have been applied as part of a weight-of-evidence approach to assess both ecological and human health hazards. Some of these tools have involved the incorporation of partial, but not complete, mode of action (MOA) information.

For the last decade, OCSPP has expanded chemical evaluations to incorporate the WHO/IPCS mode of action/human relevance framework. Most of the evaluations have focused on human health endpoints, and partial and complete modes of action have been incorporated into the regulatory programmes in a variety of situations. Partial modes of action have been used to advance the understanding of the relationships between chemical structure/properties and bioactivity in the development of predictive tools or expert knowledge systems. In addition, several regulatory programmes have developed prioritisation schemes that utilise a weight-of-evidence approach that incorporates a variety of existing information including physical-chemical properties, exposure, existing toxicology information, and partial mode of action information. This approach has been used to prioritise disinfection by-products, and recently was proposed to prioritise chemicals for endocrine screening.

The Office of Pesticide Programs is required to consider common MOA as the basis for cumulative risk assessments. Cumulative assessments are conducted for chemical categories that act by the same mode of action, and early key events are used to define the dose-response and potency of the members of the category as well as to characterise life stage susceptibility. Examples of cumulative assessments include the organophosphates (acetylcholinesterase inhibition), chloro-s-triazines (luteinizing hormone suppression), and pyrethroids (sodium channel alteration). The use of chemical categories for grouping industrial chemicals has primarily been based on physical-chemical properties to date, with a few examples of refinement through the incorporation of mode of action information.

OCSPP has also used MOA information for quantitative risk assessment. In some cases, the application of the mode of action/human relevance framework has led to conclusions of ‘no risk’ for some chemicals with animal responses due to MOAs that are not relevant to humans, and in other cases the information has been used to refine the quantitative assessment (e.g. dose-response). In addition, as quantitative linkages are established between key events and adverse outcomes, targeted testing aimed at early key events becomes scientifically supportable.

MOA information will continue to be incorporated into regulatory programmes and it is important to continue the development of new MOAs. However, given the number of potential MOAs, it will be important to focus on the regulatory endpoints of concern to prioritise their development.

3.2.2 Overview on how Mode of Action is used in a Regulatory Context: EU Perspectives

Brigitte Landesmann
European Commission, Joint Research Centre,
Institute for Heath and Consumer Protection, Systems Toxicology Unit, Ispra, Italy

An increased understanding of toxicological processes together with advances in toxicogenomics, bioinformatics, systems biology and computational toxicology has contributed to an on-going paradigm shift in regulatory toxicity testing and risk assessment, using systems- and pathway-based approaches. Substantial efforts are made worldwide to develop alternative solutions to *in vivo* (animal) toxicity tests for assessing human safety, as the Tox21 Consortium, the Center for Alternatives to Animal Testing (CAAT), the Human Toxicology Project Consortium (HTPC) and many others. WHO, especially in the context of the International Programme on Chemical Safety (IPCS), and OECD provided a framework for collecting, organising and evaluating relevant information on chemical, biological and toxicological effect of chemicals based on a Mode of Action (MOA) approach.

MOA frameworks have been incorporated in toxicological assessment guidance by the US EPA, the European Commission guidance for industrial chemicals, biocides and in classification and labelling and by the European Chemicals Agency guidance for REACH. The EU Cosmetic Products regulation (Directive 76/768/EEC), in particular, has been gradually phasing out the marketing of cosmetic products tested on animals.

Launched by the European Commission and the European cosmetics industry, the SEURAT-1 Research Initiative addresses the long term strategic target of 'Safety Evaluation Ultimately Replacing Animal Testing'. It started in 2011 with nearly 100 scientists from over 70 European organisations working together to find novel human safety testing solutions for repeated dose systemic toxicity.

The SEURAT-1 research strategy is based on generating and applying knowledge of MOA that will be used to design integrated test systems for associating a chemical with a selected MOA and possibly predicting effect levels. This information can support safety assessment processes and decisions.

Following this strategy two MOAs related to chronic liver toxicity have been elaborated based on literature research and following the OECD guidance and template. The purpose was to define and describe a MOA to a sufficient extent to facilitate conducting a feasibility study with human cells, with the aim of identifying relevant biomarkers *in vitro* which can be eventually used for *in vivo* predictions of selected types of repeated dose target organ toxicity in humans, based on complementary tools and test systems to be developed within the SEURAT cluster.

Notes from the discussion:

- Clarification of 'intermediate results', i.e. which ones are key for further measuring:
  Not all events that are found along the pathway initially are essential and necessary (key) for the adverse outcome – finally only key events should be included in the pathway description. To avoid confusion and following the OECD guidance, it has been agreed that all events that are mentioned in the AOP description are called key events.
- Need for different MOAs for different applications, essential to formulate the problem:
  AOP/MOA can be used for various purposes (e.g. understanding, classification, risk assessment) and different levels of detail are requested depending on the purpose. Also incomplete pathways might be useful for specific purposes.
  MOA development and MOA application are two different scenarios and are likely to require different experimental designs.
- Lots of data are available in relation to drugs and pesticides and they should be used:
  There was a discussion whether drugs are good references, because they are designed for a specific interaction (e.g. receptor-binding) at a certain concentration and therefore differ from pesticides which act non-specifically with a wide spectrum of interaction. There is a large amount of (human) data available for drugs, but not for pesticides.

3.2.3 Experience with Application of Mode of Action in Human Health Risk Assessment: Outcome of Survey

Carolyn Vickers and Kathy Hughes (presenter)
WHO/IPCS and Health Canada
Switzerland and Canada

Invitees to the present workshop were requested to complete a survey questionnaire on application of Mode of Action (MOA) in human health risk assessment. The survey aimed to gather information about how MOA is utilised by the chemical risk assessment community, for presentation and discussion at the workshop. Twenty-six completed questionnaires were received, reflecting a response rate of approximately 40 per cent. Four questions were asked, each with a check-box option plus a request to elaborate on the answer provided.

A little more than half of the respondents indicated that they consider MOA in the assessment-specific generation of data for human health risk assessment of chemicals, e.g. in targeted testing, non-test methods (QSAR, read-across, modelling) or other, either routinely (11 respondents) or occasionally (6). For 6 respondents this question was not applicable. Examples included: targeted testing; grouping of chemicals; development of new substances; and waiving of testing requirements.

All respondents for whom the question was applicable (24), currently consider mode of action in the assessment of data for human health risk assessment, e.g. in considering human relevance, human variation, species extrapolation, life stage effects, dose-response, combined exposures, or other, either routinely (16)

or occasionally (8). Uses included: human relevance; in classification, labelling and restriction as part of the criteria for carcinogens; and beginning to consider MOA in combined exposure assessments. A few respondents remarked that MOA was used, but not necessarily documented systematically.

Of the respondents engaged in research on chemicals (20), the majority use MOA (11 routinely, 4 occasionally). A variety of uses were provided, and MOA was described as both central to development of toxicity and disease pathways and underpins the Tox21 initiative.

More than half of the respondents (14) indicated that they had used MOA in another context/for another purpose. While some of the uses described may overlap with situations described above (e.g. design and interpretation of studies; identification of new molecules), a range of other uses was also offered, including: in public communication; in training and education; in the consideration of beneficial effects; and in the development of new studies to replace animal testing.

Overall, the responses received indicate that MOA has become an essential tool in the human health risk assessment of chemicals, and that it is now being employed in a wide range of applications.

3.3 Case Studies

3.3.1 Application of the IPCS MOA-HRF Framework in the Work of JMPR

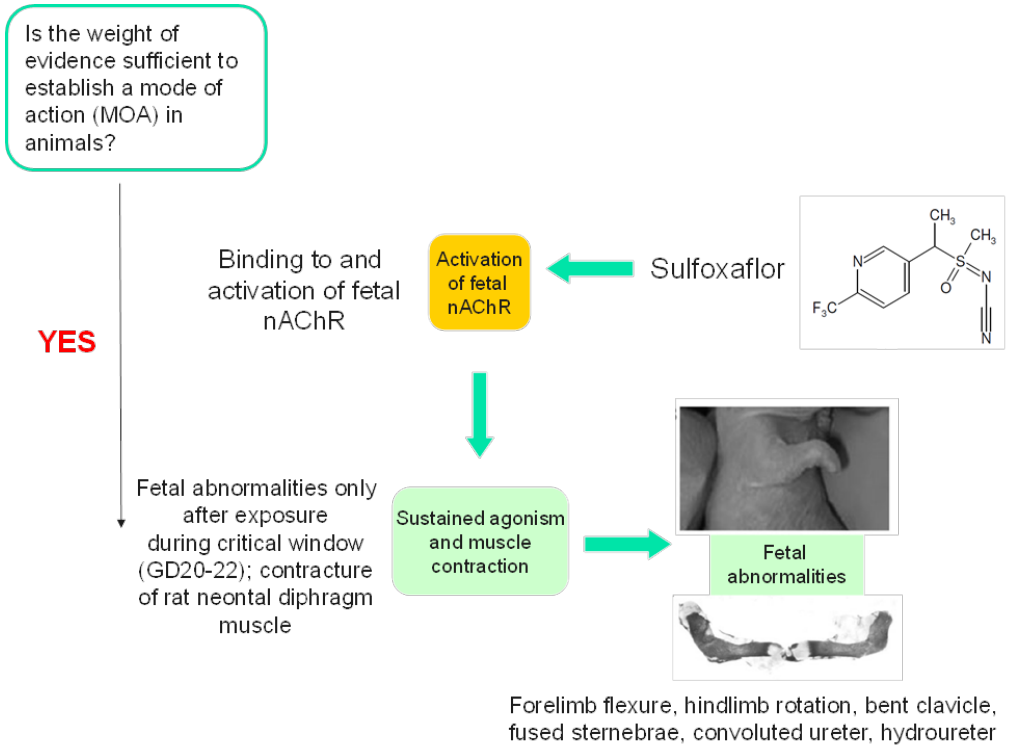
Alan R. Boobis
Centre for Pharmacology and Therapeutics
Imperial College London, UK

Risk assessment of pesticides is currently based largely on the results of studies in experimental animals. Exposure duration is from acute to lifetime and potential effects of concern include systemic toxicity, reproductive toxicity and carcinogenicity. The IPCS MOA-HRF framework for analysing cancer and non-cancer modes of action provides a systematic and transparent means of organising all relevant information in assessing the relevance of such effects for humans. To date, the framework has been most used in assessing MOAs for carcinogenesis. However, its application is becoming increasingly broad in scope and experience suggests that it is applicable to a wide range of endpoints. The IPCS framework first enables a coherent case to be made as to whether a mode of action can be established for a given tumour response or other adverse effect. If so, it may be able to exclude relevance of the MOA to humans, on the basis of fundamental qualitative differences in biology or profound quantitative differences in toxicokinetics or toxicodynamics.

An example of the former would be the induction of foetal abnormalities in the rat by sulfoxaflor, due to binding to an age- and species-specific nicotinic receptor (Figure 1), whilst an example of the latter would be the formation of thyroid tumours as a consequence of induction of hepatic glucuronidation of thyroid hormones. Whilst it may not be possible to dismiss the relevance of an effect, the framework can provide the basis for chemical-specific dose-response extrapolation. For example, due to the efficiency of p-glycoprotein (p-GP) in minimising the CNS (central nervous system) concentrations of emamectin in most

species, the risk of neurotoxicity in humans should be assessed from data obtained in p-GP expressing animals. Another example is the uncertainty factor used for carbofuran, due to neurotoxicity resulting from inhibition of acetylcholinsterase activity. Understanding the MOA led to a reduction in the uncertainty factor to 25, from the typical default value of 100. An appreciation of the MOA for glufosinate ammonium, involving inhibition of glutamine synthetase in the CNS, enabled a rational choice of metabolites to be included in the residue definition for the purposes of risk assessment, based on whether they were likely to share this MOA. The demonstration of a mode of action and establishing its relevance requires a reasoned argument based on demonstrable key events. Failure to provide an adequate case is a major reason for proposed modes of action for pesticides not to be accepted.

Figure 1: MOA for developmental effects of sulfoxaflor in the rat (from Rasoulpour et al, 2012)



3.3.2 Application of the Adverse Outcome Pathway for Skin Sensitisation

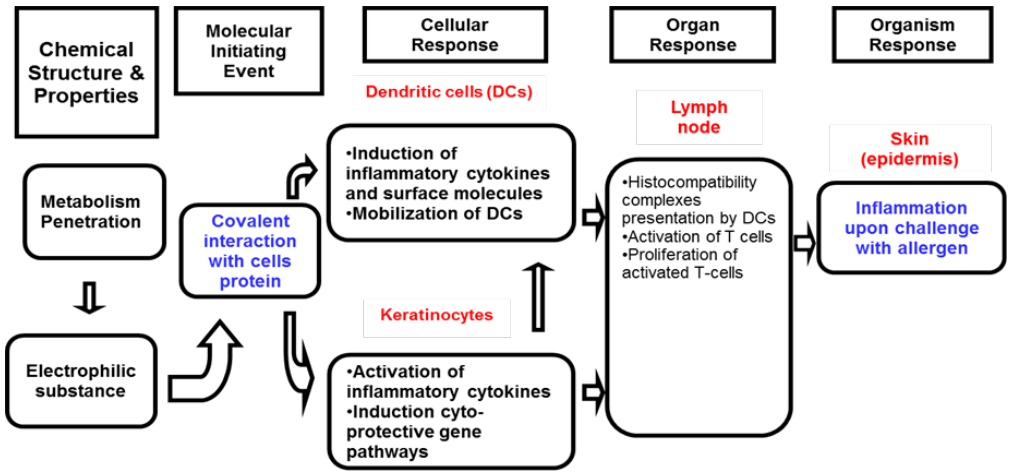
Joop de Knecht

Organisation for Economic Co-operation and Development (OECD), Paris, France

Adverse Outcome Pathways (AOPs) describe the linkages between a chemical interaction with a biological system at the molecular level and the biological effects at the subcellular, cellular, tissue, organ, and whole animal levels of observation. In this presentation the general concept of AOP was explained and exemplified with the AOP for skin sensitisation which recently has been published by the OECD. This AOP can be described in eleven steps of which four are recognised as key events: (1) the molecular interaction of a

chemical with skin proteins, (2) keratinocyte inflammatory responses and gene expression, (3) activation of dendritic cells, and (4) T-cell proliferation (Figure 2).

Figure 2: Adverse outcome pathway (Adapted from OECD, 2012)



By making the collective knowledge on the skin sensitisation process explicit in this way provides an invaluable theoretical framework to inform the work of the OECD Test Guideline Programme in the development, integration and validation of alternative methods. The AOP for skin sensitisation can be the basis for developing an integrated approach to testing and assessment for that hazard endpoint. The AOP for skin sensitisation is already implemented into the QSAR Toolbox and can be used to form categories by integrating knowledge of how chemicals interact with biological systems (i.e. the molecular initiating events) and *in vitro* and *in vivo* knowledge of the biological response, which may lead to the refinement, reduction and/or replacement of conventional *in vivo* testing for skin sensitisation.

3.3.3 Metabolomic Approach to Different Modes of Action of Amiodarone on the Thyroids in Rats

Volker Strauss

BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany

BASF and metanomics launched a project to predict toxicological risks by measuring metabolite profiles in rat plasma during a single repeated-dose screening study. For that purpose, a comprehensive database (MetaMapÒ Tox) has been established (van Ravenzwaay et al, 2012). About 300 metabolites were measured in plasma samples of Wistar rats after administration of about 500 pharmaceutical, chemical and agrochemical compounds for 7, 14 and 28 days. Sets of common metabolite level changes (metabolite patterns) were arranged to characterise several toxicological modes of action (MOAs).

Amiodarone, a class III antiarrhythmic drug with several known side effects in humans, was used to test which toxicological MOAs can be predicted with the MetaMap® Tox database (BASF unpublished data). The drug was administered to Wistar rats for four weeks and metabolite profiles measured on study days 7, 14 and 28. In a ranking list of MOA patterns which fit best to the profile of Amiodarone-dosed Wistar rats, direct (thyroid hormone syntheses inhibiting) and indirect (thyroid hormone decrease in the circulation) effects ranked among the top ten patterns.

Thyroid and liver were identified as main toxicological target organs of Amiodarone in rats. A direct inhibition of thyroid hormone synthesis as well as a peripheral thyroid hormone decrease can be assumed when comparing Amiodarone metabolite profiles with the MetaMap® Tox database patterns. This peripheral effect was found although a specified metabolite pattern detecting the known deiodinase inhibition of Amiodarone does not exist in the database up to now.

The Amiodarone metabolite profile matches well with the microsomal liver enzyme pattern in the database. When comparing the metabolites in detail, common metabolite regulations but also distinct differences can be found, assuming that Amiodarone is not a microsomal enzyme inducer although interfering similarly with the lipid metabolism.

This example demonstrates that metabolomics can be used to predict relevant toxicological MOAs even in drugs with several side effects in humans.

3.3.4 Human Relevance of Thyroid C-Cell Tumours Caused by GLP-1R Agonists in Rodents

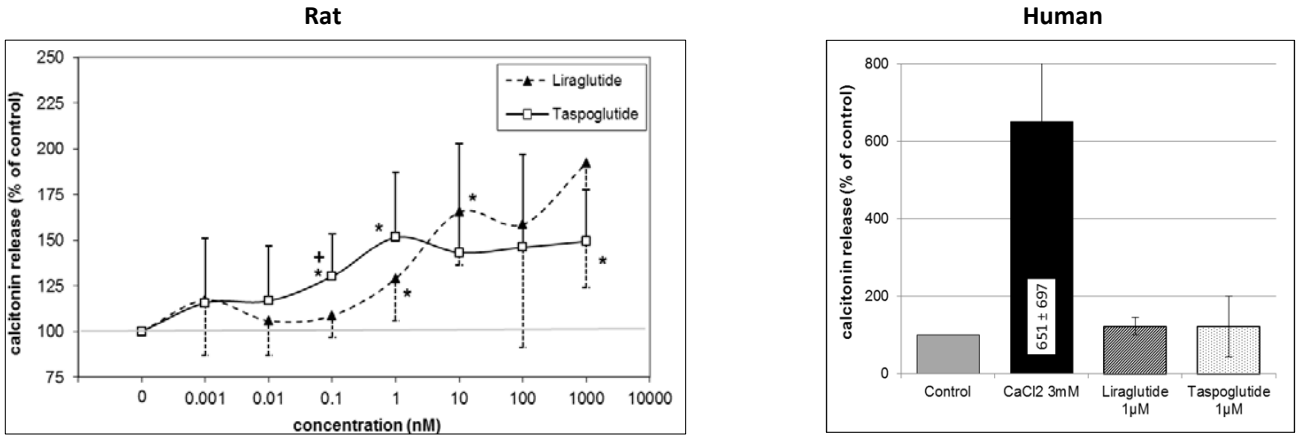
Thomas Pfister
Safety, Security, Health and Environmental Protection
F. Hoffmann-La Roche, Basel, Switzerland

Glucagon-like Peptide 1 (GLP-1) analogues were found to increase the incidence of thyroid C-cell hyperplasia and tumours in rodents (Bjerre Knudsen et al, 2010; EMA, 2006; 2009; 2011). The human relevance was and still is unclear. As peptides exert their biological effects via functional receptors, it is hypothesised that the proliferative effect in thyroid C-cells is due to a GLP-1 receptor (GLP1-R)-dependent mechanism. As C-cell proliferation has not been observed in monkeys, it is further hypothesised that the expression of GLP-1R is much lower in primates than in rodents. Therefore, the described C-cell proliferative lesions may not be relevant to primates including man. The aim of this work was to establish primary thyroid cell cultures of rat and human to evaluate the expression and function of GLP-1R in primary thyroid C-cells.

Primary thyroid cell cultures from rat and human showed physiological thyrocyte and C-cell responses upon stimulation with thyroid stimulating hormone (TSH) and Ca2+ ions, respectively. The use of qPT-PCR and bDNA (Panomics) analysis failed to demonstrate conclusively the expression of GLP-1R (mRNA and protein) on primary rat or human C-cells. Thus, the presence of GLP-1R was demonstrated indirectly by functional responses of the cultures upon stimulation with GLP-1R agonists. Two agonistic GLP-1 analogues elicited a

modest increase of calcitonin release and slightly induced calcitonin transcript expression in rat primary thyroid cell cultures, providing indirect evidence of the presence of functional GLP-1R on primary rat thyroid C-cells. On the other hand, no functional response to GLP-1R agonists was observed in human thyroid cultures (Boess et al, 2013; see Figure 3).

Figure 3: GLP-1R agonist induced calcitonin release in primary thyroid cell cultures (from Boess et al, 2013)



In conclusion, the lack of functional response of the human cultures adds to the weight of evidence indicating that human C-cells have very low levels or completely lack functional GLP-1R. These results strongly support the hypothesis that the GLP-1R agonist induced C-cell responses observed in rodents are not relevant to primates including human.

3.3.5 Tokicokinetics, Toxicodynamics and Cross-Species Aspects in Ecotoxicology

David Spurgeon

Centre for Ecology and Hydrology, Wallingford, Oxfordshire, UK

Ecotoxicologists aim to study effects of toxic chemicals on organisms at the population, community and ultimately ecosystem level. To achieve this aim, researchers need to utilise principals from both toxicology and ecology to support development of tools needed to conduct assessments that can support the conservation of ecosystem services. With a focus on investigating and ultimately predicting effects at the level of organisation above that of the individual, it can seem an anathema that ecotoxicologists should study such detailed processes as toxicokinetics processes and gene, protein and metabolite expression in response to exposure. Yet in some situations such work can be informative.

The toxicokinetics models that are used in ecotoxicology often take the relative simple forms of one or two compartment-based equations. This simplification is justified based on the small size of many of

the organisms considered and also the absence of physiological data in many cases. Even though toxicokinetic analysis may be restricted to simple models, these assessments can still be informative. For example, modelling of the relative rates of uptake and elimination of different chemicals can highlight cases where the results of time-bounded assays may fail to provide an accurate picture of long-term effects. Further, linking toxicokinetic to toxicodynamic hazard models can also inform on the potential physiological modes of action of chemicals and their association to the higher tier effects that drive ecological risk assessment (Baas et al, 2009).

Physiological measurements based on the exposure- and effect-based biomarkers that are regularly applied in mammalian toxicology have often shown that the modes of action of many common contaminants may be conserved between 'higher' and 'lower' species. Where more detailed knowledge is needed, the more comprehensive approaches of gene, protein and metabolite profiling can sometimes be useful (Spurgeon et al, 2010). These methods are, however, hampered to an extent by the absence of detailed physiological information for the many non-model species. As such they probably best serve as classification tools, rather than as a means of mode of action analysis. To address the absence of physiological knowledge, next generation methods are now allowing the sequencing of an increasing number and range of species genomes (Colbourne et al, 2011). Comparative analysis of this information can be seen as a fertile resource for future attempts to link the presence of known toxicological targets with species sensitivity (Gunnarsson et al, 2008). Identification of such physiological traits has the potential to complement on-going work on the role of ecological traits in determining species sensitivity.

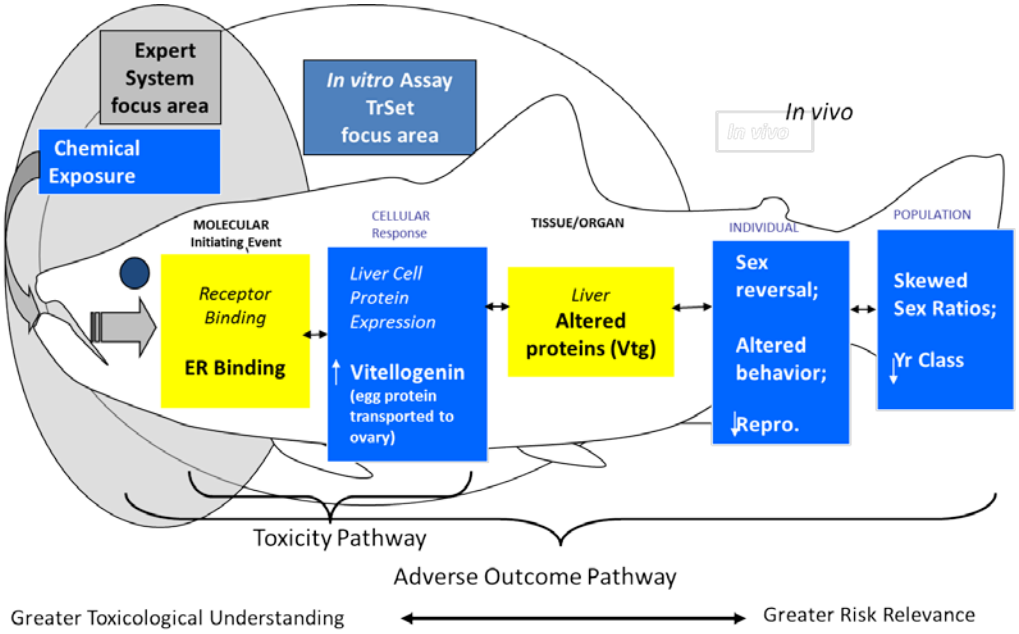
3.3.6 Mode of Action-Based Priority Setting: Drawing on Experience from the Ecological Domain

Patricia Schmieder and Vicky Dellarco (presenter)
US Environmental Protection Agency, Office of Research and Development
National Environmental and Effects Health Research Laboratory
Mid-Continent Ecology Division, Duluth, MN, USA

A brief review was provided of the basic concepts and context upon which an Effects-Based Expert System was built to predict oestrogen receptor (ER)-binding affinity for purpose of prioritising chemicals under the auspices of the US EPA Endocrine Screening Program. This expert system was reviewed by the US Scientific Advisory Panel in 2009 and 2013, and by the Organisation for Economic Cooperation and Development in 2009.

The ER-mediated mode of action (MOA) or adverse outcome pathway (AOP) was the conceptual framework utilised as the basis for developing this model. The AOP starts at the point of chemical-biological interaction or the molecular initiating event (MIE). The pathway proceeds through a series of additional key events that are relevant measures of effects associated with an observed adverse outcome of regulatory concern. The ER expert system is an example of how data generated from lower levels of biological organisation can be effectively used to develop an effects-based chemical category approach for chemical prioritisation (Figure 4).

Figure 4: ER-mediated reproductive impairment adverse outcome pathway (from Schmieder et al, 2004)



The *in vitro* assays used in developing the training set data for the model included measurement of ER-binding as the MIE in the pathway with confirmation of the binding activity at a higher level of biological organisation, the tissue level, using a liver slice assays for vitellogenin induction. Placing the assays in the context of an AOP provided greater confidence for making the linkages between chemical activity and the MIE used to develop the structure activity relationships. A large inventory of thousands of chemicals could be effectively covered by chemical extrapolation techniques through careful selection and generation of training set data for the types of chemicals and chemical properties found in the regulatory inventory of interest. Thus a minimal amount of testing in relatively inexpensive and rapid techniques could effectively be used to identify other chemicals in the broader inventory that might launch the ER AOP. The *in vitro* assays were developed and optimised to ensure confidence in the mechanistic association between the attributes of a chemical and its interaction with the biological target within AOP. The training set chemicals were tested up to solubility or cytotoxicity to provide confidence that a chemical is unlikely to initiate the ER AOP, if the *in vitro* assay training set data shows no binding activity. This was an important aspect of the training set data given that most chemicals in the inventory of interest are unlikely to even show activity for the AOP and those that may be active are likely to be of low binding affinity. The use of the MOA/AOP concept as well as the OECD QSAR Validation Principles promoted the transparency of the model (collected information, i.e. assays used and chemicals tested, and relevance to the chemical predictions) and usefulness of the system for the intended purpose (predictions provided for the regulatory inventory of interest).

4. BREAKOUT GROUP SESSIONS

The Workshop participants discussed specific questions under three broad themes. The following feedback was given from the discussions in the breakout groups. The questions to address in each of them had been communicated together with the programme prior to the Workshop.

4.1 Breakout Group I: Informing Risk Assessment

Moderator: Djien Liem
Rapporteur: Vicki Dellarco

Other members of the breakout group were:
Diane Benford; Christine Bjørge; Alan Boobis; Jane Botham; Adam Doane; Michelle Embry; George Fotakis; Helmut Greim; Kathy Hughes; Sharon Munn; James Plautz; Alan Poole; Winfried Steiling; Tokuo Sukata; Flavio Zambrone.

Problem Formulation

- The problem formulation is an important initial step in the risk analysis process where the issues and questions for the risk assessor are defined and a plan for the assessment is developed. At this stage, it may become clear whether MOA could be useful.
- There needs to be a dialogue between risk assessors and risk managers on the importance and usefulness of MOA within the risk assessment.
- The tier (see updated IPCS roadmap) where to apply MOA depends on the decision making context:
  - Hazard-based approach (e.g. classification & labelling).
  - Priority setting.
  - Risk-based approach:
    - Consider exposure in the problem formulation stage.
    - Need to make sure to relate the MOA to a specific internal (tissue) dose.
  - Targeted testing.

Improved Communication

- The benefits / utility of a MOA approach need to be better communicated (what is the value?):
  - How does MOA fit more broadly into risk assessment?
  - What is the range of applications and endpoints for MOA outside of the current use; need to generate examples of how else MOA can be used (e.g. priority setting, grouping, read-across, targeted testing)?
  - Distinction between hypothesised versus established MOA.
  - MOA enables a more explicit characterisation of uncertainty.

  - MOA is not a specific body of information or dataset, but a way of assimilating knowledge and laying out evidence in a transparent way, i.e.
    - It allows evaluating implications beyond empirical observations (e.g. could predict life stage sensitivity based on knowledge of key events).
    - It can help to be more selective about the necessary toxicity studies (targeted testing).
- Increase dialogue among different communities:
  - Between the assessment community and the regulated communities up-front – this cannot always be face to face.
  - Better dialogue is needed between researchers and the assessment community (they should both inform each other).
- Publication and database of MOAs:
  - Provide a forum to share partial MOAs that would encourage the research community to flesh out MOAs.

How to better communicate

- Where possible, encourage a culture shift to increase interactions among different parties (e.g. assessment community and regulated community) in a transparent manner.
- Demonstrate the added-value of MOA in different ways.
  - Write-up examples from this workshop in a very succinct way that could help to illustrate the benefits and application of MOA.
- Develop an information sheet on MOA – a flyer, one-pager:
  - Written in a non-technical way
  - Reference the full papers
  - Distil down the key points of why MOA is valuable ('elevator speech').
- Press release from WHO on the revised MOA Framework once it is published.
- Look at recently published risk assessments – if MOA had been used, how would it have helped; this might be a case study or an example that could be used in training.
- Outreach to broader stakeholder community (including non-technical community):
  - Discuss the various benefits of MOA (general public benefit: public health protection, availability of safe products, and effective use of resources).
- Better communicate the various initiatives – MOA vs. AOP and IPCS / OECD, etc.
  - Potentially hold a joint workshop between the various organisations.
- Give presentations on different applications of MOA (e.g. symposia).

Capacity Building / Training / Education

- Need to make sure to include the broader non-technical stakeholder community (including the NGO community).
- Training should be made available to the broader community of MOA 'users' (e.g. information users and generators, toxicologists, exposure assessors, epidemiologists, risk assessors).
- Need to reach out to the risk management community.

- Utilise virtual, remote training to increase availability.
- MOA training should be more holistic – include all aspects of the MOA roadmap and broader application to risk assessment.
- Basic training in toxicology, exposure and risk assessment is necessary as a foundation before MOA training will be useful.
- Participants of this workshop can help to increase knowledge / understanding of the MOA Framework within their organisations.

4.2 Breakout Group II: Testing Strategies / Assessment-Specific Data Generation

Moderator: Peter Chan
Rapporteur: Jack Dempsey

Other members of the breakout group were:
John Bucher; Neil Carmichael; Claire Davies; Joop de Knecht; Marianne Dybdahl; Kevan Gartland; Marjoke Heneweer; John McManus; Chris Money; David Spurgeon; Kurt Straif; Marianne van der Hagen; Kimberly Wise; Clemens Wittwehr.

Elements of MOA analysis in support of integrated testing strategies and to complement the use of non-testing methods

- All elements of MOA analysis are important. Depending on the question posed, the emphasis of the MOA analysis and the parts of the framework used can vary. Toolbox rather than framework?
- Clearly formulating the problem to be addressed is critical. The MOA process can be used for any/all of the common processes of screening, priority setting, classification or risk assessment. Insofar as any of these processes involve hazard characterisation, the gathering of data necessary for MOA analysis should be an integral part of that hazard characterisation step.
- An intelligent testing strategy will include MOA analysis to minimise time and resource costs.
- Sponsors of a chemical may use MOA analysis to argue against the need for further testing if the MOA for an adverse effect is not relevant to humans, or to direct research towards the key data gaps in characterising the hazard.
- Regulators may require MOA analysis to increase confidence in regulatory decisions particularly for data-poor chemicals.
- Regulators may also require a testing strategy that does not include MOA analysis. This is seen as counterproductive and indicates a need for more/better dissemination/education about the central role of MOA analysis in hazard characterisation.
- Regulators and sponsors use MOA in different paradigms: regulators should use it to understand findings/results in submitted data; sponsors should use MOA to decide on testing strategies to achieve minimal dataset required for submission to regulators.

Use of MOA for building chemical categories and use of read-across in risk assessment

- All elements of MOA analysis are important.
- Again, defining the question requiring answering is critical and will determine the data or analysis needs - it must be fit for purpose. MOA is expected to be an essential part of the read-across process. It requires more data than the initial grouping based on simple structural or physiochemical similarities. A major caveat is that a single MOA developed for a chemical or chemical group may not provide regulators with the required certainty (as an example the multiple MOAs for the carcinogenicity of PCBs).
- MOA can help reduce animal testing by refining the knowledge gaps and uncertainties to allow targeted testing.

Potential limitations of the current regulatory testing paradigm in using MOA concepts for hazard/risk assessment

- Distinguish between paradigm vs. practice vs. available guidelines.
- If the paradigm is based on best science then it should include MOA analysis as part of an integrated strategy. The practice however, is frequently based on adherence to current testing requirements/guidelines.
- Participants felt that:
  - MOA gets few mentions in chemical testing legislation, and this needs to be addressed.
  - The drive to reduce animal testing may hinder attempts to introduce new test requirements such as MOA analysis.
  - 'Slavish' adherence to current test requirements (whether on the part of the sponsor or regulator) hinders intelligent data generation such as the inclusion of non-required clinical tests that might address MOA in a short-term study.
  - OECD should publish all available MOAs (AOPs) with links to the supporting *in vitro* studies.

Additional practical/operational steps to facilitate more predictive MOA-based testing strategies

- Participants felt that we need to 'spread the word' about the importance of MOA analysis. The fact that MOA analysis is good science and has long been used by regulators and chemical sponsors and researchers needs to be emphasised.
- Participants suggested that chemical sponsors should be encouraged to submit more fulsome data packages based on good science rather than adherence to regulatory requirements. The lack of specific mention of the need for MOA analysis in regulations, much less the exact requirements for an MOA is a hindrance to progress or adoption of MOA more broadly.
- How to make the requirements for an 'adequate' MOA analysis clear? Training? Publications from OECD, WHO and regulatory agencies?
- Develop a template for reporting key events or dissemination of existing templates.

- A library of case studies e.g. further development of AOP/MOA for skin sensitisation or any endpoints. The inclusion of a 'domain statement' or caveat regarding the prototypical chemical fulfilling the MOA leaves the possibility that similar chemicals may not fit the MOA exactly (or may additional MOAs?).
- Include an analysis of the cost efficiencies of using MOA in the case studies?

4.3 Breakout Group III: Research, Including Better Integration of Alternative Methods

Moderator: Helen Håkansson
Rapporteur: Martin Wilks

Other members of the breakout group were:
Rémi Bars; Margaret Butler; Kevin Chipman; Samuel Cohen; Karel de Raat; Jean-Lou Dorne; Cliff Elcombe; Bruno Hubesch; Brigitte Landesmann; Lauren Markell; Stewart Owen; Thomas Pfister; Jennifer Seed; Benjamin Smith; Volker Strauss; Russell Thomas.

Use of current and future research in the application and development of MOA

- Research using new technologies (e.g. omics, HTS) and mathematical tools (e.g. QSAR, BMD) alone or in combination with existing animal databases have the potential to help elucidate chemical- as well as disease-related MOA/AOPs. This has already been used for cancer endpoints, but other areas such as reproductive and developmental toxicology endpoints and pathways need more attention, including e.g. receptor-mediated and metabolic pathways.
- Research using new technologies can also help in grouping and read-across attempts.
- Reference groups in toxicology (controls) can, on their own, provide physiological information of high relevance to toxicological interpretation and assessment activities such as:
    - What is the range of 'normal' variability? This applies to *in vivo* and *in vitro* situations
    - Better understanding of compensatory mechanisms (adverse vs. non-adverse effects), repair mechanisms, epigenetics, etc.
- Exposure analysis data on their own and in combination with effect data from experimental as well as monitoring data (from human and wildlife) should be promoted and will help to establish margin-of-exposure (MOE) information for assessment procedures.
- Research aiming at multidisciplinary problem solving is needed; to this end integration between toxicology and other research fields (e.g. including endocrinologists, immunologists, epidemiologists) needs to be promoted.

There was general agreement that basic research should be encouraged, as should be the publication of negative results.

Tools that could be developed towards providing better integration of alternative methods in the use of MOA in risk assessment

The breakout group defined alternative methods as 'non-animal' except when using ecotoxicological data (involving for example zebra fish, *C. elegans*, *C. leavis* and *drosophila*) in human health risk assessment.

- This should not be focused on technology platforms, but on scientific approach: use the method that is appropriate to the question:
    - Maximise species concordance
    - Focus on key events leading to MOA
- Better *in vitro* models: there is a need to concentrate on temporality and dose response when extrapolating alternative methods to human *in vivo* situation:
    - Allow for 'chronic exposure' *in vitro*
    - Extrapolate dose from *in vitro* to *in vivo* situation
- Knowledge base – further development of effectopedia and other bioinformatic tools; tools to structure information for database upload (also of interest are IPCS documents such as 'Principles and methods for the assessment of chemicals in food' - EHC 240 (IPCS, 2009).
- Quantitative MOA could be used for exploring the scientific relevance of uncertainty factors.

Contribution of MOA in the design of new (risk) assessment methods

MOA could be used:

- To provide information on relevant adverse criteria, thus leading to less extensive testing being required;
- to refine existing data bases [e.g. Cramer classification];
- as a screening tool in prioritisation of chemicals;
- in the development of targeted testing [e.g. replacement of some *in vivo* studies];
- in the development of chemical specific assessment factors;
- to enable read across to be focussed on biology rather than on chemical structure;
- to assess species sensitivity distribution for cumulative risk assessment.

Development of more predictive and efficient MOA-based approaches in risk assessment

- Training and education; courses, seminars and practical work on cases etc. Promote that academic scientists and others, engage more in MOA analysis as a research activity including publications.
- Increased awareness about existing tools and templates among all potential end-users e.g. through publications, active conference participations.
- Encourage better use of existing, currently unpublished data (think about incentives to publish or integrate into existing databases).
- Increased development and use of internet based discussion fora (web-based tools e.g. linked to eChem portal).
- Periodic revision and further development of technical guidelines and guidance documents.

5. CONCLUSIONS AND RECOMMENDATIONS

The Mode of Action concept had been developed initially from the use of good science to explore the human relevance of certain findings in animals, rather than as a tool for problem solving. Unfortunately there was a body of thought that MOA had to be enormously complicated to contribute anything. This needed to be countered and the concept broadened to demonstrate its usefulness in other contexts. In essence, MOA was a way of thinking that could be applied to many situations. The first essential step was problem formulation; only the amount of MOA necessary to answer the question under consideration was required. IPCS had developed a comprehensive framework but, depending on the nature of the question, it was not always necessary to follow the stepwise approach outlined there.

Particular considerations

Particular concerns had been expressed in the break-out groups in relation to the MOA being too elaborate and resource intensive to be widely used for most chemical risk assessments. The question of multiple MOAs had been discussed but not resolved. With many compounds there was more than one MOA and this was clearly an area that would need further consideration in the future. The question of mutual confidence had also been raised, with reservations being expressed that MOA data were not accepted or taken into account by many of the regulators. This might be due to the fact that - in contrast to standard toxicity endpoints - specific scientific knowledge is often necessary to assess the validity and relevance of MOA data. In relation to this, the on-going need to build bridges and develop communication, between those with and without experience of using the MOA approach, had been highlighted in the breakout groups. With regard to 21st century technologies, these still needed to find an agreed role to be useful and efficient in MOA analysis. Further work was clearly needed to build confidence in their interpretation. It was called into question whether, as was often suggested, the combination of new technologies and comprehensive databases would replace animal models any time in the near future.

Areas of improvement

Areas of improvement related to both the regulators and the regulated! In essence there was a need for the regulators to be more flexible in their approach to non-standard data and in their readiness to enter into a dialogue on problem solving. Toxicology was an investigative science and this should be recognised. The prescriptive approach of the 1981 OECD guidelines for the testing of chemicals had, unfortunately, tended to turn this into a repetitive process with ‘yes’ and ‘no’ answers. The guidelines did little to encourage thinking of what was happening in the tests and what it meant for human safety. More creativity was needed in using the MOA, and the regulated community would need to clearly explain the use of MOA in a way that would make the overall package easier to understand by the regulators who had to evaluate the data.

Areas worth pursuing

With reference to areas that were worth pursuing, more invertebrate genomes were being published and the extent to which key steps of known MOAs could be modelled in simpler organisms was one area for future investigation; the use of simpler organisms in tests would contribute towards animal replacement. Alternative methods did not necessarily involve the use of cell cultures. MOA could also contribute towards providing a better prediction of effects of potential concern for the environment.

With regard to the development of data bases, a significant effort had already been made. However, there was further scope for building relevant tools, models and SARs, and in the documentation of normal physiological range and variability in pathways.

A more effective role for MOA in regulatory risk assessments should be progressed. Although in principle there was an acceptance that MOA would be taken into account, it was not evident to what extent this was in fact done. In particular there was no guidance with regard to the submission of data. While there was general agreement that regulatory submissions should be based on the best available science, concern was expressed that some regulations were framed in a way which made the integration of non-standard methods challenging.

Specific actions which were considered worth continuing included the development of training with regard to the use of MOAs in risk assessment and read-across. It was considered that the WHO/IPCS Steering Committee should remain in existence to address the issues raised at the Workshop, and appropriate training was recognised as part of their activities. Increased visibility of the MOA approach at an international level was another area that had been highlighted, with a need to extend this to countries such as India and China.

ABBREVIATIONS

AOP	Adverse outcome pathway
BMD	Benchmark dose
CNS	Central nervous system
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria
ER	Oestrogen receptor
EU	European Union
GD	Gestation day
GLP-1	Glucagon-like Peptide 1
GLP-1R	GLP-1 receptor
HESI	Health and Environmental Sciences Institute
HTS	High throughput screening
ILSI RF	International Life Sciences Institute - Research Foundation
ILSI-RSI	ILSI - Risk Sciences Institute
IPCS	International Programme on Chemical Safety
JMPR	Joint Meeting on Pesticide Residues
JRC-IHCP	Joint Research Centre - Institute for Health and Consumer Protection
MIE	Molecular initiating event
MOA	Mode of action
MOA-HR	Mode of action – Human relevance
MOA-HRF	Mode of action – Human relevance framework
MOE	Margin of exposure
nAChR	Nicotinic acetylcholine receptor
NGO	Non-governmental organisation
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-Operation and Development
QSAR	Quantitative structure-activity relationship
PCB	Polychlorinated biphenyl
p-GP	p-glycoprotein
REACH	Registration, evaluation, authorisation and restriction of chemicals
RIVM	Dutch National Institute for Public Health and the Environment
SAR	Structure activity relationship
SEURAT	Safety evaluation ultimately replacing animal testing
TF	Task Force
UF	Uncertainty factor
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

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APPENDIX 1: WORKSHOP PROGRAMME

DAY 1, MORNING

08:30 – 09:00	*Registration*	
		Workshop Chair: **Neil Carmichael**, on behalf of ECETOC

INTRODUCTION

09:00 – 09:10	**Welcome**	**Richard Brown / Alan Poole** WHO-IPCS / ECETOC
09:10 – 09:30	**Background t o Global Initiatives Promoting the Use of Mode of Action**	**Neil Carmichael** on behalf of ECETOC
09:30 – 09:50	**WHO/IPCS Mode of Action Roadmap: Mode of Action Applications in Regulatory Toxicology**	**George Fotakis** ECHA
09:50 – 10:10	**Update of the WHO/IPCS Mode of Action Framework**	**Bette Meek** University Ottawa
10:10 – 10:30	**Contribution of New Technologies to Better Understand Modes of Action and Definition of Adversity in Toxicological Assays**	**Marjoke Heneweer** Shell and ECETOC Task Force
10:30 – 10:45	**Questions and discussion**	
10:45 – 11:15	*Coffee break*	

REGULATORY APPLICATION AND CHALLENGES

11:15 – 11:45	**Overview on how Mode of Action is used in a Regulatory Context**	
	- A US Perspective	**Jennifer Seed** US EPA
	- EU Perspectives	**Brigitte Landesmann** JRC-IHCP
11:45 – 12:00	**Experience with Application of Mode of Action in Human Health Risk Assessment: Outcome of Survey**	**Carolyn Vickers / Kathy Hughes** WHO-IPCS / Health Canada
12:00 – 12:15	**Questions and discussion**	
12.15 – 13.30	*Lunch*	

DAY 1, AFTERNOON

CASE STUDIES

13:30 – 13:45	**Application of the IPCS MOA-HRF Framework in the Work of JMPR**	**Alan Boobis** Imperial College, London
13:45 – 14:00	**Application of the Adverse Outcome Pathway for Skin Sensitisation**	**Joop de Knecht** OECD
14:00 – 14:15	**Metabolomic Approach to Different Modes of Actions of Amiodarone on the Thyroid in Rats**	**Volker Strauss** BASF
14:15 – 14:30	**Human Relevance of Thyroid C-Cell Tumours Caused by GLP-1R Agonists in Rodents**	**Thomas Pfister** F. Hoffmann-La Roche
14:30 – 14:45	**Toxicokinetics, Toxicodynamics and Cross-species Aspects in Ecotoxicology**	**David Spurgeon** Centre for Ecology and Hydrology
14:45 – 15:00	**Mode of Action-Based Priority Setting: Drawing on Experience from the Ecological Domain**	**Patricia Schmieder / Vicki Dellarco** US EPA
15:00	*Coffee*	

15:00 – 17:00 BREAKOUT GROUP DISCUSSIONS

Introduction to breakout groups — **Neil Carmichael**

BG I: Informing Risk Assessment

Moderator: Djien Liem, EFSA

Rapporteur: Vicki Dellarco, US EPA

In addition to what has been presented under 'Regulatory Application and Challenges':

- Do you have other examples of where knowledge of MOA (i.e. toxicokinetic and toxicodynamic key events) has informed qualitative/quantitative concordance of species and dose-response extrapolation, read-across and combined exposures?
- Are there other suggestions for, or examples of, how knowledge on MOA has been integrated to develop more predictive approaches?
- What additional approaches/guidance would be required to increase understanding of the importance of MOA in informing qualitative/quantitative concordance of species, dose-response extrapolation, read-across and combined exposures?
- What are additional practical/operational steps that would facilitate uptake of MOA concepts in priority setting/risk assessment?

DAY 1, AFTERNOON (CONT'D)

BREAKOUT GROUP DISCUSSIONS (*cont'd*)

BG II: Testing Strategies / Assessment-Specific Data Generation

Moderator: Peter Chan, Health Canada

Rapporteur: Jack Dempsey, Australian Office of Chemical Safety

- Which elements of MOA analysis support the building of integrated testing strategies and how can they be used to complement the use of non-testing methods?
- Is knowledge of MOA essential for the building of chemical categories and use of read-across methodology in risk assessment? If so, which elements are important?
- Are there limitations of the current regulatory testing paradigm that do not allow sufficient use of MOA concepts in hazard/risk assessment?
- What are additional practical/operational steps that would facilitate more predictive MOA-based testing strategies?

BG III: Research, Including Better Integration of Alternative Methods

Moderator: Helen Håkansson, Karolinska Institutet

Rapporteur: Martin Wilks, Swiss Centre for Applied Human Toxicology

- What current research can be used for applying MOA and what additional research might be useful?
- What tools could be developed that would provide for better integration of alternative methods within MOA analysis in risk assessment?
- How does MOA help in the design of new assessment methods?
- What are additional practical/operational steps that could facilitate better integration of research advances in developing more predictive and efficient MOA based approaches in risk assessment?

17:00 – 17:30 **In plenary:**
Brief interim reports from breakout groups: ***Rapporteurs***

19:00 – 22:00 *Workshop Dinner at "Brasserie"*

End of Day 1

DAY 2

09:00 – 10:30	**Breakout group discussions**	
10:30 – 11:00	*Coffee break*	
11:00 – 12:00	**In plenary:**	
	Reports from breakout groups:	***Rapporteurs***
12.00 – 13.00	*Lunch*	

WRAP-UP

13:00 – 14:30	**Mode of Action Knowledge Base (MoA-KB)**	
	- Overview	
	- MoA-Wiki, current version	**Clemens Wittwehr/Brigitte Landesmann**
	- Effectopedia, current version	JRC-IHCP
14:30 – 15:00	**Conclusions**	Workshop Chair: **Neil Carmichael**

Close of Workshop

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www.ecetoc.org
D-2013-3001-228

Established in 1978, ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) is Europe's leading industry association for developing and promoting top quality science in human and environmental risk assessment of chemicals. Members include the main companies with interests in the manufacture and use of chemicals, biomaterials and pharmaceuticals, and organisations active in these fields. ECETOC is the scientific forum where member company experts meet and co-operate with government and academic scientists, to evaluate and assess the available data, identify gaps in knowledge and recommend research, and publish critical reviews on the ecotoxicology and toxicology of chemicals, biomaterials and pharmaceuticals.