A mode-of-action ontology model for safety evaluation of chemicals: outcome of a series of workshops on repeated dose toxicity

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Abbreviations

AO(P)(s), adverse outcome (pathway(s)); CE, Cosmetics Europe; ICCR, International Cooperation on Cosmetics Regulation; KE(s)(R)(s), key event(s) (relationship(s)); LRSS, Long Range Science Strategy; MIE, molecular initiating event; MIP-DILI, Mechanism Based Integrated Systems for the Prediction of Drug Induced Liver Injury; MoA(s), mode(s)-of-action; PBBK, physiologically-based biokinetics; QIVIVE, quantitative in vitro-in vivo extrapolation; QSAR(s), quantitative structure-activity relationship(s); RDT, repeated dose toxicity; SEURAT-1, Safety Evaluation Ultimately Replacing Animal Testing; TTC, threshold of toxicological concern.

Key words

Repeated dose toxicity, adverse outcome pathway, mode-of-action, ontology model.

Abstract

Repeated dose toxicity evaluation aims at assessing the occurrence of adverse effects following chronic or repeated exposure to chemicals. Non-animal approaches have gained importance in the last decades because of ethical considerations as well as due to scientific reasons calling for more human-based strategies. A critical aspect of this challenge is linked to the capacity to cover a comprehensive set of interdependent mechanisms of action, link them to adverse effects and interpret their probability to be triggered in the light of the exposure at the (sub)cellular level. Inherent to its structured nature, an ontology addressing repeated dose toxicity could be a scientific and transparent way to achieve this goal. Additionally, repeated dose toxicity evaluation through the use of a harmonized ontology should be performed in a reproducible and consistent manner, while mimicking as accurately as possible human physiology and adaptivity. In this paper, the outcome of a series of workshops organized by Cosmetics Europe on this topic is reported. As such, this manuscript shows how experts set critical elements and ways of establishing a mode-of-action ontology model as a support to risk assessors aiming to perform animal-free safety evaluation of chemicals based on repeated dose toxicity data.

1. Introduction

Evaluation of chemical safety to humans has drastically changed in the last decades. Historically, animal testing formed the basis for such risk assessment exercises. Driven by scientific and ethical reasons, however, there is a clear tendency worldwide to increasingly use animal-free methods for this purpose. This has been reinforced by a number of legislative changes over the past few years in the European Union, imposing a ban on animal testing for particular groups of chemicals, in casu in the cosmetics field (EU 2003 and 2009). Accordingly, the scientific community has been urged to develop animal-free methods for evaluating the safety of chemicals, being a research area that is gaining momentum. This has triggered a paradigm shift from classical toxicology, focusing on apical endpoints for toxicity in animal models, to predictive toxicology, relying on information on mechanisms of toxicity. In fact, contemporary safety evaluation of chemicals has become a multidisciplinary science, not only using (mechanistic) toxicological knowledge, but also considering data from a diversity of other areas, including epidemiology, (physico-)chemistry and bioinformatics. The optimal use of this diversity of information could be aided by a general practical framework, designated a mode-of-action (MoA) ontology model, for sound and reliable risk assessment. In this paper, such MoA ontology model is proposed for repeated dose toxicity (RDT) and is the result of a number of expert workshops organized by Cosmetics Europe (CE) in 2016 and 2017 in the context of its Long Range Science Strategy (LRSS) program. CE established the LRSS in 2016 as a follow-up of the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1) program (http://www.seurat-1.eu/). The LRSS equally supports the currently ongoing Eu-ToxRisk project, which is considered as the integrated European flagship program driving mechanism-based toxicity testing and risk assessment for the 21st century (http://www.eu-toxrisk.eu/) and that generates valuable RDT data. The LRSS aims to develop non-animal (in silico/in vitro) approaches, strategically combine them in a risk assessmentparadigm (Desprez et al., 2018), and support safety assessment and regulatory acceptance of these integrated non-animal approaches. Making the most of the comprehensive toxicological knowledge available and structure it in a transparent manner is a way to support non-animal safety assessment. The purpose of this particular initiative within LRSS is to generate a MoA ontology model as a tool relying on kinetics and systemic bioavailabity in order to bridge the gap between effects observed in high-dose animal studies and what may happen to humans considering realistic exposure scenarios. The MoA ontology model is expected to be used in the context of exposure-led safety assessment following the criteria of the International Cooperation on Cosmetics Regulation (ICCR) (Dent et al., 2018).

2. Definition and use of the mode-of-action ontology model

According to the English Oxford dictionary, an ontology is “a set of concepts and categories in a subject area or domain that shows their properties and their relation between them” (https://en.oxforddictionaries.com/definition/ontology). The National Center for Biomedical Ontology defines it as “a kind of vocabulary of well-defined terms with specified relationships between those terms, capable of interpretation by both humans and computers”. In safety assessment of chemicals, one approach used, RDT evaluation aims at detecting effects that may occur in an organism, which would be in relation to adverse effects, mainly organ toxicities, triggered by internal exposure to the chemical of interest. As such, many mechanisms are potentially involved. Therefore, and more specifically in the field of toxicology, the ontology definition regarding RDT would go beyond the notion of organized vocabulary and would not be a descriptive, but rather an active system that supports inference. Accordingly, such system would structure and organize toxicological knowledge. Indeed, it should cover adverse effects, including organ toxicities, and relate to MoAs of chemicals. In case of organ toxicity, this could imply the use of adverse outcome

pathways (AOPs) relying on key events (KEs). It should include the relationships between KEs and thus establish networks of AOPs. This comprehensive structure, with inclusion of MoA knowledge and RDT, is meant for prediction purposes on the toxicity of chemicals in relation to repeated exposure. It should be a support to answer the question "What are the MoAs likely to be triggered by a certain level of exposure and certain chemical features, and which pathways of toxicity are truly activated after that systemic exposure is confirmed?" The MoA ontology model would therefore also be a system with a chemical entry point that takes into account the fate of the chemical in the organism, and produce an outcome that would indicate whether the chemical is toxic or not and which organ(s) is (are) affected. Hence, exposure and kinetics elements should be equally incorporated in such system.

The envisaged MoA ontology model is much more than a passive structure that stores and groups toxicological data in an organized manner. It is rather a dynamic and active structure that integrates specific workflows, linked together, in view of supporting safety assessment. The defined workflows encompass (i) exposure/kinetics, notably estimation of likely internal dose, (ii) chemistry/chemical features, (iii) MoAs, if internal exposure and chemical properties are likely to trigger a known molecular initiating event (MIE) and the series of KEs at the organelle and cellular levels that lead to (iv) adverse effects and toxicities at the tissue, organ and organism levels (Figure 1). Each of these 4 workflows constitutes a pillar of the MoA ontology model, which in turn includes specific components and subworkflows described hereafter.

3. Structure of the mode-of-action ontology model

A variety of approaches could be proposed to establish a MoA ontology model. Among those is the definition of a number of pillars that support the model by providing critical data using a wide spectrum of tools. Pillar 1 hereby is the kinetic anchor that precedes the actual dynamic phase. Pillar 2 encompasses the chemical basis for the interaction of the compound with the biological target. Pillar 3 and pillar 4 underlie the upstream and downstream parts of the dynamic process by providing a mechanistic foundation and in vivo outcome, respectively (Figure 2).

3.1. Pillar 1: kinetics aspects

The adverse effect of a chemical is a function of the intrinsic properties of the compound and its ability to interact with a biological entity in the organism as well as the exposure scenario to the compound at the site in an organism where a toxic action may take place. Thus, if and when a chemical will have a toxic effect highly depends on the concentration-time profile of the compound at the target site. The first consideration of a chemical's toxicity therefore should be a description of the kinetic behaviour of the compound in the organism (Tsaioun et al., 2016), and hence represents pillar 1 in the MoA ontology model. This description comprises a number of aspects. The concentration-time profile at the target site is depending on the processes of uptake in the organism, the distribution over the body, the metabolism of the compound and the resulting formation of metabolites and the excretion processes from the body. In turn, these processes are to a high degree depending on the physico-chemical properties of the compound as well as on the properties of the organism. This implies that these processes, as part of the MoA ontology model, can be predicted and described in a high level of detail (DeJongh et al., 1997; Schmitt, 2008).

The first element of the description of the kinetics pillar in the MoA ontology model is the relationship between external exposure and internal exposure. The absorption process of a compound is a function of the properties of the chemical as well as of the ability of the absorbing tissue to transport the compound. Apart from the ability to predict absorption on the basis of

physico-chemical properties, information on the process can also be gained by quite a number of experimental non-animal models (Heylings et al., 2018; Hubatch et al., 2007).

Proper quantification of absorption may yield important information in terms of the MoA ontology model, while it may allow an estimation of the maximal possible systemic available compound. If this amount is very low, it may allow the application of the internal threshold of toxicological concern (TTC) (Ellison et al., 2019; Partosch et al., 2015).

The second element of the description of the kinetics pillar in the MoA ontology model is the distribution of the compound in the organism. Estimating the distribution and hence the concentration-time profile of compounds is a function of the physico-chemical properties as well as of the properties of the different organs and tissues. Since the blood stream is the main transport route through the organism, the partitioning between blood and the tissues is the determining factor. In this respect, special attention needs to be paid to the existence of special barriers (Prieto et al., 2004), including the role of transporters (Notenboom et al., 2018).

The third element of the description of the kinetics pillar in the MoA ontology model is the metabolism of the compound. This is an important determinant for the change in concentration of the compound to which one is exposed. Typically, the biotransformation system, consisting of a wide variety of enzymic reactions, will lead to compounds with a lower lipophilicity, thereby facilitating excretion. However, this might also yield compounds with a higher reactivity towards tissues. While metabolism is a critical issue and although exceptions exist, many isolated non-animal systems are not well equipped with the physiologically-relevant biotransformation systems. Moreover, the role of different tissues in biotransformation is differing widely, and this leads to concentration-time patterns differing considerably in the body (Coecke et al., 2006). However, for estimating the metabolic profile on the basis of experimental non-animal models, a proper estimation of these profiles is possible and this estimation needs to be part of the MoA ontology model.

The fourth element of the description of the kinetics pillar in the MoA ontology model is excretion. The most important tissues contributing to excretion of compounds from the organism are the kidney, liver, gastrointestinal tract, lungs and to a lesser extent the skin. As holds for the other 3 elements critical for the description of the kinetics pillar in the MoA ontology model, the physico-chemical properties of the chemical as well as the transporter functions of the tissues dictate the velocity of excretion.

In order to obtain a comprehensive picture of the concentration-time profile of the compound and its metabolite(s) at possible target sites, the use of physiologically-based biokinetics- (PBBK) models is of paramount importance (Bessems et al., 2014). PBBK models consist of a physiologically-relevant description of an organism, a set of parameters describing the fate of the chemical under study and a set of differential equations. Software handling the simultaneous solution of these equations ideally results in the estimation of the concentration of the compound and metabolites after any exposure scenario, at any time at any place in the organism. The quality of the estimates depends on the ability to collect the appropriate parameters to be used in the PBBK models. These parameters may be either estimated on the basis of the physico-chemical properties of the compounds or measured in non-animal methods.

A possible adverse effect of a chemical is to a great extent depending on the concentration-time profile of the compound at sites of action. Kinetic modelling is an important tool to quantify such profiles (Bouvier d`Yvoire et al., 2007). A first use in the MoA ontology model can be the estimation of the possible internal exposure in tissues given a certain external exposure scenario, which will be

of use in determining the need to pay attention to those tissues with high concentrations in appropriate in vitro systems. Another use may lie in the combination with MoA knowledge for the compound. Concentration-time profiles may give answers to the question as to whether a high-peak compound or prolonged exposure is the determining factor in a compound's toxic profile. This is of particular interest in RDT or extended exposure scenarios. Kinetic modelling is equally of great promise in the interpretation of any in vitro toxicity studies for their meaning in a risk assessment setting. It allows the integration of in vitro data in a quantitative in vitro-in vivo extrapolation (QIVIVE), thus linking this to the external exposure (Blaauboer, 2010; Yoon et al., 2015).

3.2. Pillar 2: chemical aspects

Pillar 2 relates to the use of chemistry to drive understanding of toxicological effects and to form relationships with other chemicals within the MoA ontology model. A number of aspects of chemistry need to be considered to implement chemistry as a pillar in a the MoA ontology model, especially as it may form the basis of computational approaches, namely (i) the correct representation of chemical structures, (ii) understanding of physico-chemical properties and their relationship to toxicology and biokinetics, (iii) an appreciation of the structural basis of toxicity and metabolism in terms of molecular structure, (iv) development of relationships with other similar molecules through techniques, such as read-across and quantitative structure-activity relationships (QSAR). Taking each consideration in turn, whilst it may sound trivial, the essential starting point for any chemistry-related aspect of the MoA ontology model is the correct definition of chemical structure. Thus, a minimum requirement is the need for appropriate structural identifiers to be available for chemistry. For a single substance, this would be an unambiguous definition of structure, including consideration of stereochemistry, such as potential isomerism and tautomerism. This is achieved ideally by the use of some description of chemical structure. Previously, SMILES strings, which may be insensitive to isomerism, and InChi Keys have been applied for this purpose. An important aspect to bear in mind is the definition of chemical structure that will be appropriate for use in toxicological databases as well as being interoperable with other computational systems.

Another key component for the definition of chemical structure is the identification of significant impurities, especially those that may be relevant to the toxicological endpoint being considered. A MoA ontology model should also be flexible enough to define and identify mixtures, registered multicomponent substances, unknown or variable composition, complex reaction products or biological materials and even natural products. To cope with these complexities, and the other requirements for chemistry, a robust and flexible chemoinformatics structure and platform is required.

The understanding of physico-chemical properties is a vital component of the MoA ontology model. Particular elements to this are the characterization of a compound's hydrophobicity and solubility, ionisation, volatility, stability and reactivity. These are some, if not the majority, of the key properties that affect distribution of a compound throughout the target species, and hence the potential toxic effect and potency. The collation of measured or estimated values for the logarithm of the octanol-water partition coefficient, aqueous and lipid solubility, acid dissociation constant, vapour pressure and Henry's Law constant is commonplace, and these properties should be captured through the chemoinformatics platform. Information on stability and reactivity is more disparate, but valuable in terms of understanding toxicity. In chemico methods, thus abiotic assays to measure chemical reactivity, may be of great use in this respect. As well as forming a valuable source of information in their own right, physico-chemical properties may form the input to computational models for biokinetics, distribution and toxicology. These properties will also assist in

the extrapolation of points-of-departure from in vitro or high-throughput assays to in vivo exposures as well as for reverse dosimetry.

Chemical structure is intimately linked to toxicological effect and/or metabolism, and this can be used for advantage within a MoA ontology model. It is well established that specific molecular subfragments can be associated with toxicity, such that an overall assessment of an effect may be made. An excellent example includes the creation of chemical classes or categories for developmental and reproductive toxicity (Wu et al., 2013). The basis for this is that a particular chemical structure or, more commonly, substructure, is associated with toxicity, as this drives the interaction with the biological molecule(s), or maybe the site of metabolism. Hence, once chemical structure is known, analysis of possible toxic substructures and metabolic sites can be undertaken and used as valuable supporting information. The concept fits well into how in silico models are perceived to relate to AOPs, with structural chemistry being a key component in the modelling of the MIE (Cronin et al., 2017; Cronin and Richarz, 2017). With regard to RDT, one of the possibilities relates to understanding organ level effects. Taking liver toxicity as an example, much work has been undertaken on individual adverse effects, such as general hepatotoxicity (Hewitt et al., 2013), phospholipidosis (Przybylak et al., 2014) and steatosis (Mellor et al., 2016). However, an overarching in silico profiler is required for organ level effects. Once established, such a computational approach could be implemented in a robust chemoinformatics platform enabling the knowledge to be applied further to new chemicals and to assist in building weight-of-evidence for existing chemicals (Yang et al., 2018).

A further component of the chemistry pillar of a MoA ontology model is the ability to support interpolation of effects to related chemicals. The ontology, and especially the definition of relevant chemistry, provides a suitable means of defining similarity to group-similar chemicals and allows for read-across of effects. Due to its mechanistic basis, the ontology has the capability to provide evidence directly to support a similarity hypothesis. In addition, the integration of biokinetics into the MoA ontology model enables the effect of change in chemical structure to be evaluated. Both the need for better weight-of-evidence for mechanistic effects as well as consideration of biokinetics, which are achievable within a MoA ontology model, are recognized needs to increase the acceptability of read-across for data gap filling (Schultz and Cronin, 2017; Schultz et al., 2019). Other in silico approaches that are appropriate to be integrated within a MoA ontology model are QSARs that maybe developed across chemical groups and assist in the implementation of the MoA ontology model, thereby rendering it a practical tool.

3.3. Pillar 3: mechanistic aspects

Pillar 3 of the MoA ontology model relates to the mechanisms driving the toxicological apical endpoint. The framework that is nowadays used to capture the mechanistic scenario underlying toxicity is embedded in, but not limited to, the AOP concept. An AOP refers to a conceptual construct that portrays existing knowledge concerning the linkage between a single MIE and an adverse outcome via a linear series of KEs at a biological level of organization relevant to safety assessment (Ankley et al., 2010). Although conceptually very similar, the scope of an AOP is broader compared to the MoA, as it can go up to the population level. Furthermore, while the MoA tends to be chemical-specific and takes into account kinetic aspects, such as metabolism, AOPs are chemical-agnostic in that they describe a toxicological process from a purely dynamic and biological perspective. Thus, an AOP can be ultimately associated with any chemical that is bioavailable at the relevant site of action and that has the specific properties to activate the associated MIE (Becker et al., 2015; Burden et al., 2015; Edwards et al., 2016; Perkins et al., 2015; Villeneuve et al., 2014a and 2014b). Each AOP comprises 2 fundamental modular components, namely KEs and key event

relationships (KERs). A KE represents a measurable change in a biological state that is essential, but not necessarily sufficient, for progression from the MIE to the adverse outcome. A KER defines a causal relationship between a pair of KEs, establishing one as upstream and one as downstream. It provides the scientifically plausible and evidence-based foundation for extrapolation from an upstream cause to a downstream effect, and thus for using KE information as indicators of adverse effects. Furthermore, a KER may reflect linkages between a pair of KEs that are either adjacent or non-adjacent in an AOP allowing the possibility to integrate parallel and interdependent processes within a single AOP (OECD, 2016 and 2017; Villeneuve et al., 2014a and 2014b).

Evaluation of newly developed AOPs includes consideration of the so-called tailored Bradford-Hill criteria. The Bradford-Hill criteria have been initially introduced to determine causality of associations observed in epidemiological studies (Hill, 1965). In the last few years, they have been adopted to assess AOPs, albeit in a more tailored format. In rank order, these tailored Bradford-Hill considerations include biological plausibility, essentiality and empirical support. While the former and the latter are considered for each KER individually, essentiality of the KEs is scrutinized in the context of the overall AOP. Each of these tailored Bradford-Hill considerations is subjected to weight-of-evidence analysis, whereby confidence should be judged as strong, moderate or weak for each of the KEs, KERs and the AOP as such, based on the availability of documentation and/or empirical support (Becker et al., 2015).

A major AOP resource includes the AOP knowledge base, introduced in 2014 by the Organization for Economic Cooperation and Development, the Joint Research Centre of the European Commission, the US Environmental Protection Agency and the US Army Engineer Research and Development Centre. One of the modules of the AOP knowledge base is the AOP Wiki, which provides an open-source interface that serves as a central repository for qualitative AOPs (OECD, 2016). At present, the AOP Wiki contains about 280 AOPs at different levels of maturity and development for a plethora of toxicological endpoints, including those relevant to RDT (http://aopkb.org/). It should be stressed, however, that most, if not all, of these AOPs are individual constructs, with a single MIE and adverse outcome. Although valuable, such individual AOPs are merely pragmatic units of development and evaluation. For real-world applications, including integration into a MoA ontology model, AOP networks, considering multiple MIEs and apical endpoints, are the actual eligible tools.

3.4. Pillar 4: toxicological aspects

Pillar 4 implies the toxicology cornerstone of the MoA ontology model, including available animal testing data and, if relevant and present, human epidemiological and clinical data. Even more than for the other 3 pillars of the MoA ontology model, the focus of the toxicological aspects is dictated by the nature and intended use of the chemical under investigation. For some cosmetic ingredients, liver and kidney have been previously identified as potential toxicity targets, albeit upon oral administration of high doses to rodents (Vinken et al., 2012). This was based on combined listing of toxicity endpoints as described in available animal testing reports, which is a major source of input for this aspect of the MoA ontology model. In particular, morphological, histopathological and biochemical parameters can be used to feed the toxicology pillar of the MoA ontology model. Thus, typical clinical manifestations of chronic liver toxicity include fibrosis, hepatitis, steatosis and cholestasis. Steatosis is characterized by a fatty liver and associated accumulation of lipids in histopathological testing. Furthermore, serum levels of alanine and aspartate aminotransferases, triglycerides and cholesterol are increased in liver steatotic subjects. By contrast, a cholestatic liver is typically yellowish and shows several necrotic areas upon histopathological examination. This is accompanied by high quantities of alkaline phosphatase, gamma glutamyl transferase and bilirubin in serum.

Chronic kidney pathology refers to the permanent loss of a large percentage of functional nephrons. Chronic kidney disease is diagnosed by graded decreases in glomerular filtration rates accompanied by microalbuminuria. As holds for liver injury, histopathological manifestations of kidney injury may reflect the tissue type being injured. Injury to the podocytes of the glomerulus can be observed by podocyte effacement in minimum change disease and by gross aberrations of glomerular architecture in focal segmental glomerulosclerosis. Injury to the tubular cells, which is often paralleled by inflammation and recruitment of circulating immune cells, is referred to as tubulointerstitial nephritis. Kidney injury can also be caused by acellular chemical precipitation in the tubular lumen, which can lead to tubular obstruction, epithelial injury and interstitial inflammation. However, kidney injury can equally be much more subtle and occur in the absence of histopathological changes, as in the case of the Fanconi-like syndrome, which is featured by polyuria, glucosuria, aminoaciduria, hyperuricosuria, hypophosphatema and hyperchloremia (Heidari et al., 2017; Klootwijk et al., 2015).

In general, histopathological and clinical chemistry parameters for assessing toxicity, either general or organ-specific, and disease are routinely used in clinical settings. In addition, diagnosis of toxicity can also be achieved by physical examination of patients. In recent years, a plethora of novel biomarkers has been introduced to allow more specific and early detection of toxicity, such as (epi)genetic and -omics-based indicators (Vinken et al., 2013). Such human-based information, which can be found in published papers, public databases or reports, constitutes a valuable source of input for the toxicology pillar of the MoA ontology model complementary to animal data. A noteworthy example in this context includes the Mechanism Based Integrated Systems for the Prediction of Drug Induced Liver Injury (MIP-DILI) consortium (https://www.mipdili.eu).

4. Application of the repeated dose toxicity mode-of-action ontology model

The RDT MoA ontology model is meant to be an applied tool to support risk assessors during safety evaluation of chemicals. On scientific aspects and outputs, the RDT MoA ontology model includes the necessary broad mechanistic coverage, but has the advantage to include elements of exposure, kinetics and chemistry. As such, the RDT MoA ontology model is a flexible tool, as it has the capacity to implement several exposure scenarios. Indeed, there are numerous targets in relation to RDT, and there are several ways of listing them, such as based on organ or origin (Table 1), or based on cellular structure and function (Table 2). From the technical perspective, the RDT MoA ontology model is broadly applicable because of its 4 generic pillars, transparently connected to each other, and since any chemoinformatics platform can be readily implemented. Both these scientific and technical aspects render the RDT MoA ontology model a robust tool that provides coherent outputs across several users. Thus, the same chemical with a given exposure should provide the same output irrespective of the user or the platform used. Moreover, the RDT MoA ontology model is a supporting tool in RDT evaluation that provides a frame for weight-of-evidence-based safety assessment, since it can, and should, be used together with other non-animal approaches, such as the (internal) TTC or read-across (Desprez et al., 2018). In this regard, in TTC exploration, exposure considerations overlap and link with pillar 1 of the RDT MoA ontology model, and can be used to confirm that being far below the TTC cut-off actually does not activate MoAs of concern, and thus no RDT effect is triggered. Conversely, when being above the TTC cut-off, the RDT MoA ontology model can help to prioritize potential MoAs of concern, and possibly trigger additional in vitro testing to move towards a more ab initio approach (Berggren et al., 2017).

During the series of CE LRSS workshops addressing the RDT MoA ontology model, the general expert opinion was that a pilot version of the RDT MoA ontology model could be released by focusing on certain priorities. Indeed, for proof-of-concept purposes and testing the specific applicability of the

proposed RDT MoA ontology model for the safety evaluation of cosmetic ingredients, particular attention could be paid to liver toxicity. In this respect, a screening of RDT data present in safety evaluation reports issued by the Scientific Committee on Consumer Safety between 2000 and 2009 revealed the liver as a potential target of toxicity for cosmetic ingredients based on animal studies using oral gavage. The inflicted hepatotoxicity hereby is mainly manifested as steatosis and cholestasis (Vinken et al., 2012). A plethora of data are already available for populating the different pillars of the RDT MoA ontology model for these 2 specific types of liver toxicity without necessitating the need for large-scale additional experimentation. In order to assess its generic utility, the robustness of the RDT MoA ontology model can be challenged in a further step by application to other adverse effects and targets organs of RDT as well as to other chemical areas, such as the pharmaceutical, food and biocide industries. It can be anticipated that upon thorough evaluation of the RDT MoA ontology model, a tool will be delivered for direct implementation in chemical risk assessment yielding accurate and reliable prediction of human safety without using experimental animals.

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Conflict of interest

The authors report no declarations of interest.

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Figure and Table legends

Figure 1: Functioning of a repeated dose toxicity ontology based on exposure and kinetics, chemistry, modes-of-action and organ toxicity elements in view of supporting safety assessment (AOP, adverse outcome pathway; MIE, molecular initiating event; MoA, mode-of-action).

Figure 2: Proposal of a repeated dose toxicity mode-of-action ontology model. The model relies on 4 pillars providing critical information on kinetics (pillar 1), chemo-biological interaction (pillar 2), mechanisms (pillar 3) and in vivo outcome (pillar 4). Pillars 2-4 spread over different levels of biological organization (ADME, absorption/distribution/metabolism/excretion; AO, adverse outcome; KE(R), key event (relationship); MIE, molecular initiating event).

Table 1: Representative targets at the organ level (not exhaustive).

Table 2: Representative targets at the cellular level (not exhaustive).

Figure 1

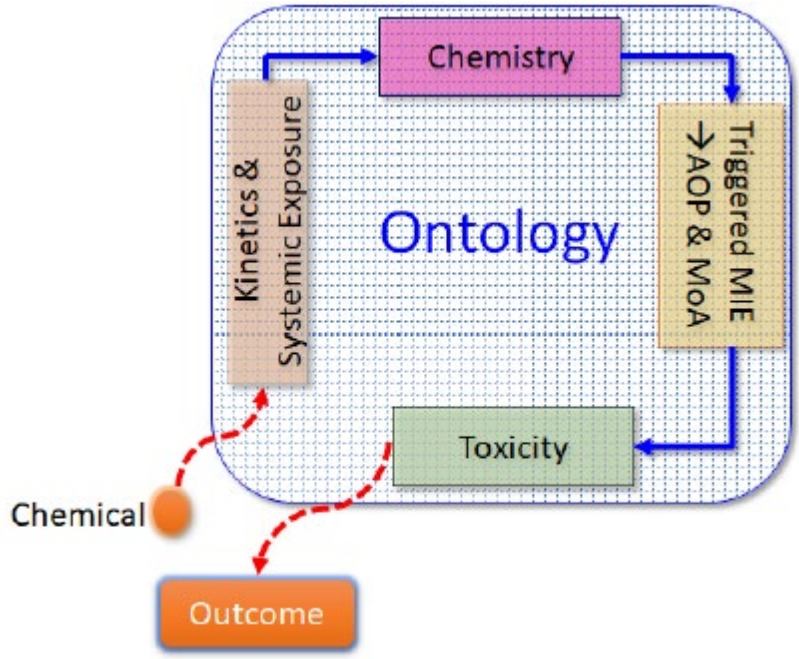
Chemistry
Kinetics & Systemic Exposure
Ontology
Triggered MIE →AOP & MoA
Toxicity
Chemical
Outcome

Figure 2

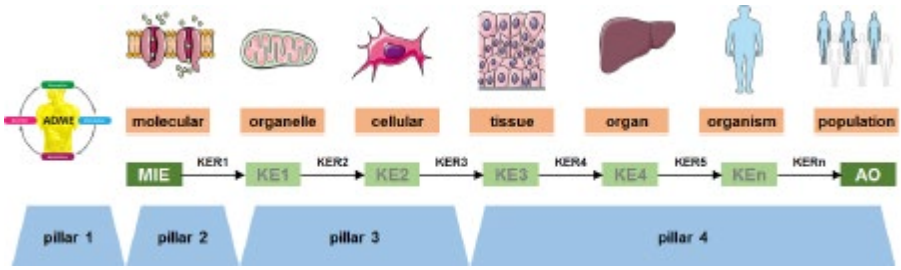
ADME
molecular
organelle
cellular
tissue
organ
organism
population
MIE
KER1
KE1
KER2
KE2
KER3
KE3
KER4
KE4
KER5
KEn
KERn
AO
pillar 1
pillar 2
pillar 3
pillar 4

Table 1

Liver
Bile salt export pump
Bile duct obstruction
Peroxisome proliferator-activated receptor alpha/gamma activation
Nuclear hormone-receptor activation
Mitochondrial activity
Immunotoxicity
Biotransformation enzyme induction
Transforming growth factor beta signalling/fibrosis
Kidney
Intercellular connections: tight junctions (paracellular transport) and adherens junctions
Renal transport: organic-anion and cation transport and megalin/cublin uptake
Renal metabolism: gamma-glutamyl transferase and beta lyase
Oxidative phosphorylation, leading to decreased energy production and transport shut-down
Podocyte injury and glomerular defacement
Immune reactivity with glomerular basement
Crystallization events and tubular obstruction
Lung
DNA damage
Particle-induced toxicity
Oxidative stress
Alveolar integrity
Mucus production/composition
Heart
Ion channels
Innervation
Mitochondria
Cellular communication
Cyto-architecture

Cell-to-cell adhesion
Circulation
Coagulation/thrombocytes
Hemolysis
Blood cell count
Complement system
Immune system
Histamine/mastocyte cells
Activation of death receptors
Activation of cytokine receptors
Cell cycling
Cyclophilins
Muscle
Innervation
Mitochondria
Actin/myosin system
Central nervous system
Ions channels
Transmitter receptors
Microtubule inhibitors
Acetylcholine inhibition
Guidance receptors
Neurotransmitter/receptor turn-over
Mitochondrial inhibition
Sensory system
Cilia
Mitochondria
Retinal cells
Vasculature
Endocrine system

Hypothalamic-pituitary-adrenal axis
Thyroid signalling
Estrogenic/androgenic signalling
General homeostasis
Microtubules
Carbohydrate metabolism/Krebs cycle/oxidative phosphorylation
Cytoskeleton
DNA repair
Epigenome
Cell cycle/death effectors
Cell-cell and cell-extracellular matrix contacts

Table 2

Cellular structures/compartments
DNA
RNA
Endoplasmic reticulum
Lipids/cholesterol
Proteins
Low molecular weight molecules
Membranes
Mitochondria/peroxisomes
Endosomes/lysosomes
Cellular functions
Death
Cell division
Respiration/energy production
Transcription/translation
Polarity
Epigenetic stability
Metabolic capacity
Communication with system
Membrane potential
Ion and osmolyte homeostasis
Cellular integrity
Tissue-specific cellular function
Receptors
Nuclear receptors

Plasma membrane receptors
Enzyme receptors
Transcription factor receptors
Structural proteins
Movement/motility
Secretion
Autophagy
Enzymes and signalling molecules