CAT-APP: category approaches and 'read-across' in regulatory programmes

A new approach to evaluating the toxicological effects of petroleum substances by making use of existing test data aims to reduce, and eventually eliminate, the use of animals for toxicity testing.

Toxicity testing in the 21st century

"It'll soon shake your windows, and rattle your walls, for the times they are a-changin'."[1]

Windows and walls of conventional regulatory toxicology testing strategies have been shaking and rattling since the publication of *Toxicity Testing in the 21st Century: A Vision and a Strategy*, more commonly referred to as Tox21c, by the US National Research Council in 2007 [1]. This proposed vision has fuelled the discussion and changed the perspective on conservative animal-based toxicology studies, driven by animal welfare considerations and the revolutionary advances made in the field of biotechnology over the past decades. The main aim of Tox21c is to take advantage of these technological breakthroughs and move away from a regulatory testing paradigm that is currently still based on vertebrate animal models, following the '3R' principle in toxicology testing: refinement, reduction and eventual replacement of animal studies for research purposes [2]. Although the publication of the Tox21c vision has contributed to major developments in the field of 'alternatives' (to animal testing) research, practical application—and regulatory acceptance—to replace current testing guidelines is still far away. The question is how to make best use of these available and developing technologies for a more short-term application in regulatory programmes, such as the REACH regulation[2].

Alternatives for animal testing under REACH

Currently, exposing an animal to chemicals including petroleum substances, following the OECD guidelines for testing of chemicals[3], to evaluate the potential associated toxicological effects is still considered the golden standard to comply with the REACH requirements regarding human health hazard information (i.e. health hazard 'end points', such as carcinogenicity). However, REACH tries to seek a balance between gaining an increased understanding of the potential hazard of a chemical and at the same time avoiding unnecessary animal testing. In other words, testing on an animal should be the last resort, and in order to keep the number of animal tests to a minimum the REACH guidance offers two ways to meet this goal: data sharing (e.g. joint chemical dossier submissions through a consortium of companies registering the same chemical) and alternative methods and approaches.

With regard to alternative methods, the main tool is 'read-across', i.e. using already available test data on a particular hazard end point of a substance to predict the properties of another, similar substance, instead of testing it again for the same end point. This sounds straightforward, and a useful historical toxicological database does indeed exist for petroleum products; hence, conducting an animal study to address an end-point requirement or data gap will, in many cases, be of questionable need. However, there is an additional complexity in applying this approach to petroleum substances: they are a prototypical example of highly complex UVCBs (substances of unknown or variable composition, complex reaction products and biological materials), which present enormous challenges for science-informed regulatory decision making. Although UVCBs are identified on global chemical inventories with unique Chemical Abstract Services (CAS) numbers and names, applying the similarity principle and evaluating their potential toxicity via read-across approaches remains challenging due to the chemical complexity and multiconstituent nature with largely unknown and variable composition. Read-across of petroleum substances within the REACH framework is typically done by grouping the individual substances into product categories with similar manufacturing processes, physical/chemical descriptors (including refining history, boiling point and carbon number ranges) and limited analytical chemical properties (such as hydrocarbon classes). However, category read-across approaches for (petroleum) UVCBs that are based solely on such broad similarity parameters are not considered to be sufficient under REACH.

[1] Written by Bob Dylan, ©1963,1964 by Warner Bros. Inc.; renewed 1991,1992 by Special Rider Music.

[2] Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency.

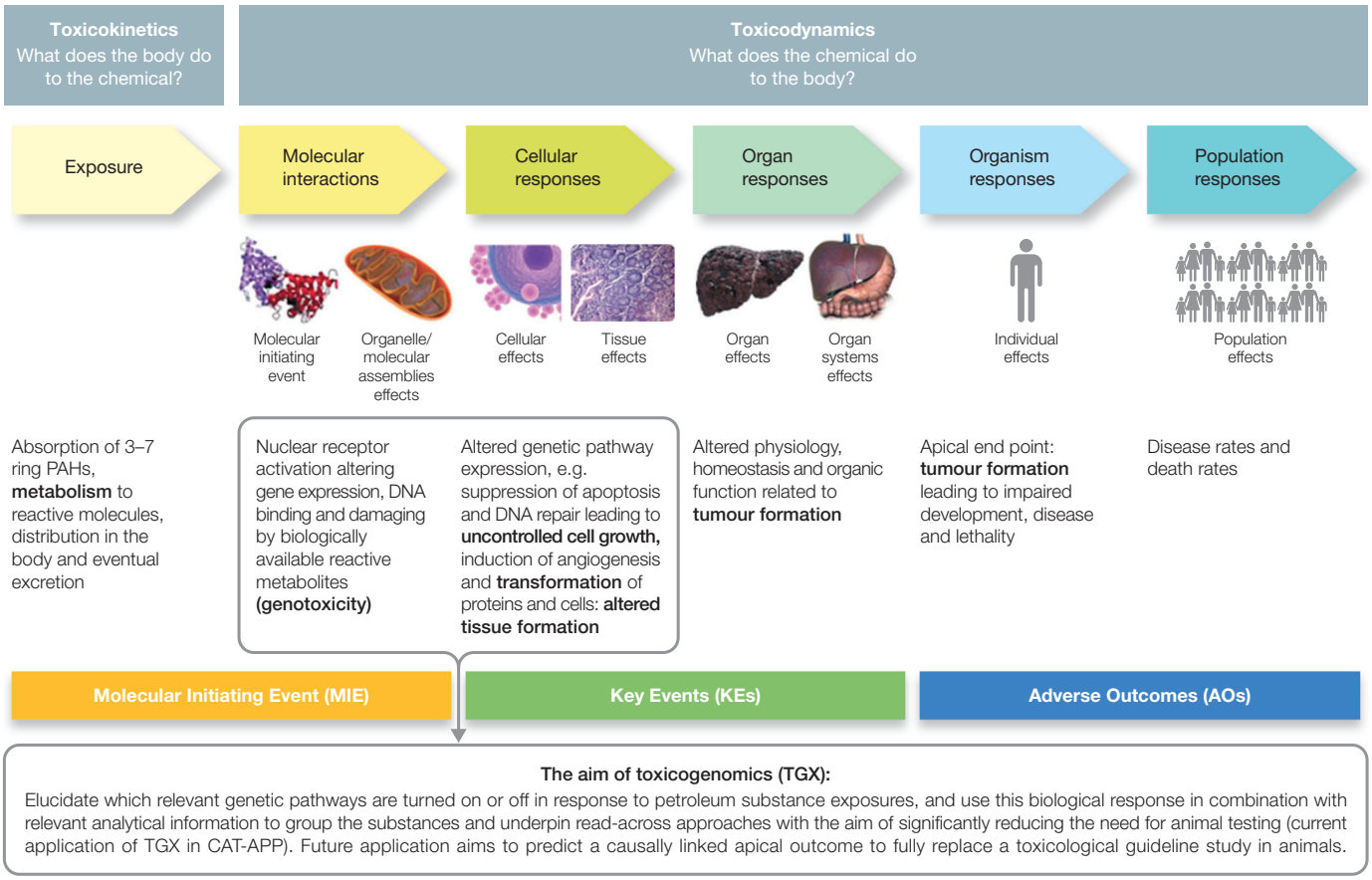
[3] More information on OECD testing guidelines can be found at www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

Therefore, with respect to animal welfare, and taking into account the high cost and relatively limited predictive power of the current animal guideline tests for determining human health end points, regulators and industry have a common interest in defining a process for (petroleum) UVCBs to ensure that there is no underestimation of hazards, while at the same time minimising or eliminating the use of animals in toxicology testing.

Mechanistic toxicology

The Tox21c vision aims to move away from studying observable outcomes in response to chemical exposure in whole animals 'in-vivo'[4], such as clinical signs and pathological changes indicative of toxicity at the level of a whole organism (a so-called 'apical end point'), towards predicting these adverse effects based on cellular or molecular events 'in-vitro'.[5] In the latter case, the events that take place during the period from initial exposure through to eventual toxicity can be observed to determine what the body actually does to a chemical (so-called toxicokinetics) and what that chemical does to the body (so-called toxicodynamics). This is illustrated in Figure 1, using carcinogenesis as an example. In mechanistic toxicology, underlying mechanisms leading to certain apical end points, such as tumour formation in the example of carcinogenesis shown here, are elucidated. Once these pathways of

Figure 1 Illustration of the basic concepts of an Adverse Outcome Pathway in mechanistic toxicology, using carcinogenesis as an example



[4] In-vivo: Latin for 'within the living', i.e. testing in a whole living animal.

[5] In-vitro: Latin for 'in glass', i.e. testing in components of an organism isolated from their normal biological context (organs, cells, subcellular components, molecules such as DNA, etc.).

toxicity are causally linked to the end point of interest—e.g. molecular markers for the formation of DNA damage linked to tumour formation in carcinogenicity—they can then be used to predict the toxicological end point, i.e. to answer the question, 'does the chemical trigger the pathway(s) leading to carcinogenicity or not'? This pragmatic approach would be used instead of conducting a full mandatory OECD guideline study for the same end point in which rats are exposed to a chemical on a daily basis for a time period of two years to observe tumour formation.

With the new rapidly developing biotechnological tools that have become available in recent decades, mechanistic information can be generated in a cost-effective manner (i) at the cellular level, measuring physiological parameters in toxicity screens (e.g. evaluating cell functionality), as well as (ii) at the molecular level, measuring 'gene expression' changes in so-called 'toxicogenomic' screens (e.g. to observe which genes in which biological pathways are turned on or off—see Figure 1). Note that the word 'screens' is used here, meaning that in both cases short-term rapid assays are developed that are eventually thought to replace costly and time consuming animals studies. However, as indicated above, with the exception of a few prototypical examples that are currently being developed (also under the umbrella of Concawe's Health Management Group (HMG) for petroleum substances), in most cases the predictive power of these screens is not yet sufficient to fully replace a 'golden standard' animal study. The example of DNA damage leading to tumour formation is obviously overly simplified; in real life this, and other, apical events will depend on a highly complex network of numerous interacting pathways. Research efforts are currently ongoing, such as the Adverse Outcome Pathway knowledge base,[6] aiming to elucidate these pathways and especially to link them causally to eventual in-vivo end points. However, it will still take some time for this work to develop an approach that is suitable for practical application in regulatory programmes.

Chemical-biological read-across and CAT-APP

Considering the breakthrough developments that continue to be made in biotechnology and applied to mechanistic toxicology, as well as appreciating their current shortcomings, Concawe has developed the 'CAT-APP'[7] project—a strategy designed to make best use of the currently available data and screening tools for more realistic, short-term regulatory application. The fundamental principle of CAT-APP is to use the biological pathway information—not to predict toxicity, as described above—but instead to further underpin the similarity principle for grouping petroleum UVCBs. As mentioned on page 10, petroleum categories under REACH are currently based on limited similarity parameters such as boiling point information and other physical/chemical descriptors that define petroleum streams resulting from refining crude oil. The hypothesis is that these broad parameters drive the analytical chemistry of specific petroleum streams which, in turn, drive certain specific types of mammalian toxicity or more general biological responses. For example, certain high boiling point petroleum streams might contain polycyclic aromatic hydrocarbons (PAH) whereas low boiling point streams generally do not, hence these particular petroleum substances have the potential to show PAH-related toxicity. Thus, if it can be shown that specific petroleum substances defined by certain analytical chemical characteristics have similar biological behaviour because of this chemistry, while being distinct from other chemically different petroleum substances, this can provide a much more informed basis for grouping, and for eventually filling data-gaps, by so-called chemical-biological read-across of the toxicological data already available.

In this way the end-point requirement is fulfilled by 'indirect' prediction, supported by similarity in mechanistic biological responses, rather than by directly predicting the toxicological end point based on the mechanistic toxicity pathway.

[6] http://aopkb.org

[7] www.concawe.eu/cat-app-project

Over the course of 2017, a number of human cell lines representing various tissues, as well as functional cell models derived from so-called 'induced pluripotent stem cells', will be exposed to all Concawe registered petroleum substances. Subsequently, in response to these exposures, relevant mechanistic toxicology data will be generated at different levels, using several high-content toxicity screens (HCS), as well as more molecular types of information derived by evaluating gene expression changes using a state-of-the-art toxicogenomics-based approach (see section on mechanistic toxicology and Figure 1). All of these data will be collected and stored along with currently available relevant toxicological, physical/chemical and analytical chemical data in the CAT-APP database. Eventually, an overall integrative analysis of all available data types will define petroleum substance grouping and support chemical-biological read-across in a transparent and visually clear way. It is Concawe's aim that the indirect prediction of toxicological information to be included in Concawe REACH dossiers will significantly reduce the number of animal tests needed.

CAT-APP is expected to deliver its framework for application under REACH by mid-2018. However, initial results will become available and will be published by the end of 2017, among others on the CAT-APP[7] website which will be kept up to date with the latest developments—in addition to already-available background information on this project. A first pilot study presenting the basic principle of CAT-APP has already been published [3], and was acknowledged as one of the most innovative and impactful papers globally by the Society of Toxicology (SOT) in 2016.

The times are changing

This appreciation by the SOT is an initial indication of the impact that CAT-APP is expected to have on the way we look at the mammalian toxicology of petroleum substances. Over the coming years, the change in the field of toxicology research that started decades ago and received a boost with the publication of the Tox21c vision in 2007 will progress rapidly as a result of the major biotechnological advancements that are still ongoing. Concawe, as a credible and highly appreciated scientific organisation, will follow these developments closely and will continue to be involved. It is therefore inevitable that these developments will be a major factor in driving the research strategy of Concawe's HMG to a significant extent over the coming years. Already, several Tox21c-related projects are ongoing, such as the development of a stem-cell based screening assay for potential mutagenicity/carcinogenicity of petroleum products and mechanistic toxicity work to support our ongoing reprotoxicity studies under REACH, that are expected to be helpful in keeping our heads above the water in a challenging regulatory landscape.

We'll *"better start swimming or we sink like a stone, for the times they are a-changin'."*[8]

References

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2. Russell, W.M.S. and Burch, R.L. (1959). *The Principles of Humane Experimental Technique*. Methuen, London.
3. Grimm, *et al.* (2016). 'A chemical–biological similarity-based grouping of complex substances as a prototype approach for evaluating chemical alternatives.' In *Green Chemistry*, Issue 18, pp. 4407-4419.

[8] Written by Bob Dylan, ©1963, 1964 by Warner Bros. Inc.; renewed 1991, 1992 by Special Rider Music