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Review

The current status of exposure-driven approaches for chemical safety assessment: A cross-sector perspective

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ABSTRACT

For the purposes of chemical safety assessment, the value of using non-animal (*in silico and in vitro*) approaches and generating mechanistic information on toxic effects is being increasingly recognised. For sectors where *in vivo* toxicity tests continue to be a regulatory requirement, there has been a parallel focus on how to refine studies (*i.e.* reduce suffering and improve animal welfare) and increase the value that *in vivo* data adds to the safety assessment process, as well as where to reduce animal numbers where possible. A key element necessary to ensure the transition towards successfully utilising both non-animal and refined safety testing is the better understanding of chemical exposure. This includes approaches such as measuring chemical concentrations within cell-based assays and during *in vivo* studies, understanding how predicted human exposures relate to levels tested, and using existing information on human exposures to aid in toxicity study design. Such approaches promise to increase the human relevance of safety assessment, and shift the focus from hazard-driven to risk-driven strategies similar to those used in the pharmaceutical sectors. Human exposure-based safety assessment offers scientific and 3Rs benefits across all sectors marketing chemical or medicinal products. The UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) convened an expert working group of scientists across the agrochemical, industrial chemical and pharmaceutical industries plus a contract research organisation (CRO) to discuss the current status of the utilisation of exposure-driven approaches, and the challenges and potential next steps for wider uptake and acceptance. This paper summarises these discussions, highlights the challenges – particularly those identified by industry – and proposes initial steps for moving the field forward.

1. Introduction

The science of chemical safety assessment is on the verge of undergoing a paradigm shift, with the utility of non-animal approaches and mechanistic information on toxic effects being increasingly recognised. For sectors where *in vivo* toxicity tests continue to be a regulatory requirement and/or the only means to provide the evidence necessary for safety assessment, there has been a parallel focus on how to reduce animal numbers and refine studies (*i.e.* reduce suffering and improve animal welfare) and increase the value that *in vivo* data adds to the human or environmental safety assessment process (Burden et al., 2015, 2016; Sewell et al., 2016). In addition to other potential novel approaches, such as the identification of chemicals showing time-cumulative toxicity to allow further testing to be focussed on those that have a strictly dose-dependent toxicity (Tennekes, 2017), better understanding of chemical exposure is a key element necessary to ensure the transition towards successful utilisation of non-animal safety testing. 'Exposure' is used to mean both the applied, external exposure (in *in vitro* or *in vivo* models), and the internal or target site exposure. The need to understand and improve the use of exposure information in

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toxicity assessment has been described previously (Barton et al., 2006; Creton et al., 2009), and this need has become increasingly apparent in more recent years. For example, this has been highlighted within the RISK21 roadmap (Pastoor et al., 2014), the US National Institutes of Environmental Health's 2012–2017 Strategic Plan (NIEH, 2012), the US Environmental Protection Agency's ExpoCast initiative (EPA, 2017; Wetmore et al., 2015), and the recent National Academies of Science Report 'Using 21st Century Science to Improve Risk-Related Evaluations' (NAS, 2017); also see Burden et al. (2015).

Many of the non-animal approaches currently available provide information on the mechanism of action of drugs and chemicals. Although this information is useful for making hazard-based decisions and informing decision-making in product development programmes, without linking the *in vitro* toxicodynamic measurements to *in vivo* toxicokinetics (TK), the relevance to human exposure scenarios and risk assessment is limited. For example, it is not always clear how concentrations tested in *in vitro* assays relate to doses and exposure patterns that humans or environmental species (*in vivo*) would be exposed to in real-life situations. It can also be difficult to determine how much of the chemical applied to the *in vitro* model reaches the site ('internal') of action (*i.e.* measured *versus* nominal or applied concentrations). Therefore, without an understanding of exposure (both external and internal) to put the *in vitro* data into context, data from these studies and their relevance to toxicity endpoints can be misinterpreted.

An assessment of exposure is an integral part of safety assessment programmes in the pharmaceutical sector and the assessment of exposure allows bridging between toxicology studies in animals and clinical trials in humans. This enhances the value of the toxicological data generated, in terms of understanding the toxicity tests and in comparison with clinical data as part of the assessment of risk and safety in humans.

The value of exposure assessment is described through the ICH guideline (ICH, 1994) and the goals are summarised below:

The primary objective of the TK assessment is to describe the systemic exposure achieved in animals and its relationship to the dose, sex, species and the time course of the toxicity study. In order to achieve this, blood samples are taken at various time-points post dosing from animals on toxicity studies and the plasma/serum or blood concentrations of the test item or its metabolites are measured. TK information enhances the value of the toxicity studies by relating (i) the exposure to the test item to any toxicological findings and (ii) the exposure in animals to clinical data as part of the assessment of safety in humans.

Additionally, TK data can be used to inform dose selection for a subsequent study. For example, where TK data indicate that absorption limits exposure to the test item or its metabolites, the lowest dose which achieves maximum exposure should be used as the 'high dose' in the absence of other dose-limiting constraints. This is often referred to as saturation of exposure.

Current animal-based testing paradigms across sectors often *require* that doses used in toxicity studies exert toxic effects, even if these doses result in exposure far beyond that which is likely in human exposure scenarios or where there is no possibility of exposure due to the chemical's physicochemical properties. Such doses can result in internal concentrations that are several magnitudes higher than those predicted in humans. If realistic human exposure scenarios are considered during the dose selection for *in vivo* studies, there is the potential to avoid administration of unrealistically high (maximum tolerable dose (MTD) or test guideline limit) doses to animals. As well as avoiding irrelevant and misleading toxicities and associated discomfort to the animals, there are scientific benefits to incorporating TK/pharmacokinetic (hereon referred to as kinetic) measurements to animal studies, and the added advantage of maximising the information obtained from such studies (Terry et al., 2016). An exception is in the pharmaceutical sector where it is possible to use saturation of exposure and a mean exposure margin of 50 x clinical to define the high dose alongside more standard approaches of MTD or maximum feasible dose in general toxicity studies (ICH, 2009) and a mean margin of 25:1 rodent to human plasma exposure is acceptable in carcinogencity studies (ICH, 2008).

There is scientific value in applying the concept of human exposure-based safety assessment across all sectors marketing chemical or medicinal products. The NC3Rs convened an expert working group of scientists across the chemicals (agrochemical and industrial chemicals) and pharmaceutical industries, plus one CRO to discuss the current status of utilisation of exposure-driven approaches, and the challenges and potential next steps for wider uptake and acceptance, particularly within Europe. This was discussed across the areas of 1) the use of *in vivo* kinetic data to inform study design and support data interpretation; 2) prediction of internal human exposure to aid interpretation of data from *in vitro* assays, increase their human relevance and enable *in vitro* to *in vivo* extrapolation (IVIVE); and 3) the use of predicted human exposures and scenarios to determine data needs and inform study design.

§ Key points:

- Improved consideration of exposure is key to improved toxicology safety assessments.
- 'Exposure' includes both applied, external exposures and internal or target organ/site of action exposures, and is relevant for both *in vitro* and *in vivo* conditions
- For non-animal and increasingly mechanistic approaches to be applied, it is important to understand how the *in vitro* concentrations (at the site of action as well as nominal) relate to likely doses experienced by humans and environmental species.
- Better understanding of exposure can also be used to guide dose selection in *in vivo* studies, reducing the need to test molecules at unrealistically high doses.

2. The use of *in vivo* kinetic data to inform study design and support data interpretation[1]

Kinetic assessments can be used within animal toxicity studies to determine internal circulating concentrations of test chemical and/or metabolites. Traditionally, due to the volumes of blood required, sampling at various time points post-dose in order to generate *in vivo* kinetic data in toxicology studies has required the use of additional groups of animals referred to as satellite animals. However, increasing the generation of kinetic data in study types and sectors where this information is not currently available should not automatically lead to an increase in animal numbers used. For example, microsampling approaches are one way in which this can be overcome. The volumes of blood required (often 30 µl or less) allow sampling of main study animals to generate kinetic profiles using the same numbers of post-dose timepoints as would have been used in the satellite animals and this approach has been taken up in the pharmaceutical sector (see NC3Rs, 2017). Modern microsampling and analytical techniques are now available which have a low biological impact on the test animals (Chapman et al., 2014; Saghir et al., 2012; Powles-Glover et al., 2014; Prior et al., 2015; Mitchard et al., 2016) thereby providing the opportunity for kinetic assessments (including full kinetic profiles due to low volume needs) to become a routine and integral part of investigative and regulatory toxicology studies. TK profiles have also been generated from main study animals using other techniques, such as dried blood spots (Stove et al., 2012). The use of satellite animals is an alternative in the rare case that higher volumes are needed, although this obviously has a

[1] Note, the generation of animal kinetic data is not applicable to the cosmetics sector, where there are now geographical bans on the use of animal toxicity tests to inform safety assessment (*e.g.* EC, 2009b. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. In: *OJ L 342*: Official Journal of the European Union.)