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Capturing the applicability of *in vitro-in silico* membrane transporter data in chemical risk assessment and biomedical research

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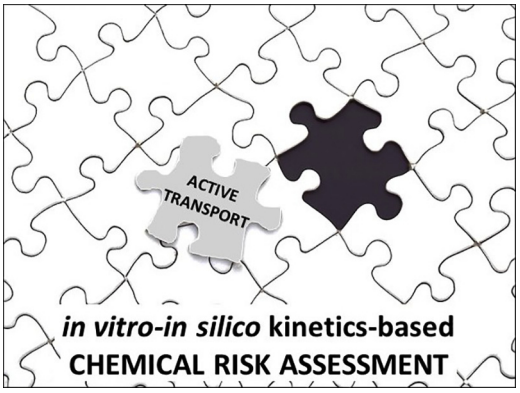
HIGHLIGHTS

- Membrane transporters (MTP) are key determinants of drug and chemical kinetics.
- MTP studies are recommended during drug development.
- MTP data is an essential puzzle piece for kinetics-based chemical risk assessment.
- Integrated *in vitro-in silico* methods increase confidence in animal-free MTP data.

GRAPHICAL ABSTRACT



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ABSTRACT

Costs, scientific and ethical concerns related to animal tests for regulatory decision-making have stimulated the development of alternative methods. When applying alternative approaches, kinetics have been identified as a key element to consider. Membrane transporters affect the kinetic processes of absorption, distribution, metabolism and excretion (ADME) of various compounds, such as drugs or environmental chemicals. Therefore, pharmaceutical scientists have intensively studied transporters impacting drug efficacy and safety. Besides pharmacokinetics, transporters are considered as major determinant of toxicokinetics, potentially representing an essential piece of information in chemical risk assessment. To capture the applicability of transporter data for kinetic-based risk assessment in non-pharmaceutical sectors, the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) created a survey with a view of identifying the improvements needed when using *in vitro* and *in silico* methods.

Seventy-three participants, from different sectors and with various kinds of expertise, completed the survey. The results revealed that transporters are investigated mainly during drug development, but also for risk assessment purposes of food and feed contaminants, industrial chemicals, cosmetics, nanomaterials and in the context of environmental toxicology, by applying both *in vitro* and *in silico* tools. However, to rely only on alternative methods for chemical risk assessment, it is critical that the data generated by *in vitro* and *in silico* methods are scientific integer, reproducible and of high quality so that they are trusted by decision makers and used by industry. In line, the respondents identified various challenges related to the interpretation and use of transporter data from non-animal methods. Overall, it was determined that a combined mechanistically-anchored *in vitro-in silico*

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approach, validated against available human data, would gain confidence in using transporter data within an animal-free risk assessment paradigm. Finally, respondents involved primarily in fundamental research expressed lower confidence in non-animal studies to unravel complex transporter mechanisms.

1. Introduction

Humans and ecosystems are continuously exposed to various environmental chemicals, such as pesticides, manufactured chemicals, cosmetics ingredients or food contaminants. Their potential toxicity is of public concern. However, clinical trials are not conducted for pollutants, as compared to drugs, depriving the toxicologists of human *in vivo* data to rely on. Currently, the safety assessment of environmental chemicals for regulatory purposes mainly involves animal testing. However, costs, scientific and ethical concerns have created the need to develop reliable, relevant and economically feasible tools based on alternative (non-animal) approaches. In 2010 the EU adopted Directive 2010/63/EU which updated the 1986 Directive 86/609/EEC on the protection of animals used for scientific purposes. The aim of the new directive is to anchor in EU legislation the principle of the Three Rs: Replace, Reduce and Refine the use of animals for scientific purposes. Under this Directive, the EURL ECVAM was established to contribute to the development, validation, and international recognition of alternative methods. In 2015 EURL ECVAM published a toxicokinetic (TK) strategy proposing kinetics as the cornerstone in an integrative *in vitro-in silico* risk assessment (Bessems et al., 2015). Kinetics determine what amount of an external exposure dose of a compound reaches the systemic circulation and the target organ(s) by providing essential information on the ADME processes (Coecke et al., 2013; Tsaioun et al., 2016). TK here defines kinetics of environmental toxicants in contrast to pharmacokinetics related to drugs. Despite the usefulness of TK information, there are only few legal requirements in EU chemicals legislation for the generation of TK data. However, the use of TK data to support the assessment of systemic toxicity is widely recommended in regulatory guidance and various scientific opinions (Bessems et al., 2015). TK data are proposed for use to evaluate cross-species differences, to waive specific *in vivo* studies when applicable, and to support the development of novel approaches in chemical safety assessment (Corvi et al., 2013; Prieto et al., 2014; Casati et al., 2013; Bessems et al., 2014; ECHA, 2011; EFSA, 2014). Moreover, TK data are valuable for the development of mathematical models, such as physiologically based kinetic (PBK) models and could increase the accuracy of *in vitro* fate models (Armitage et al., 2014; Fischer et al., 2017; Comenges et al., 2017).

Membrane transporters are well-recognized key determinants of kinetics, affecting the ADME processes of various endogenous and exogenous compounds (Klaassen and Aleksunes, 2010; "The Transporter Book", 2017). The *in vivo* importance of transporters is demonstrated in several animal species, including knockout or mutated mice, as well as by genetic variants (polymorphisms) in humans (Klaassen and Hong, 2008). Furthermore, clinical data, -omics studies and non-invasive imaging on healthy volunteers or patients provide considerable information on the *in vivo* role of many transporters (Yee et al., 2010; Kusuhara, 2013). Besides *in vivo* studies, a plethora of *in vitro* methods exist to measure active transport. The most widely used are either membrane-based assays (including ATPase and vesicular transport assays) or cell-based systems such as cell lines, polarized and/or transfected with one or multiple transporters, providing information on specific transporter(s) interaction as well as animal or human primary cells representing rather holistic barrier models as their transportome profile is more similar to that found *in vivo* (reviewed in "The Transporter Book" 2017). Furthermore, hepatocytes, either suspended, plated or sandwich-cultured, are arguably one of the most valuable tools available to study drug metabolism and transport (Riley et al., 2016). Recently, more complex *in vitro* systems, such as organoids, 3D or co-culture are also exploited by transporter scientists (Zhang et al., 2017). In combination to experimental studies, several computational models are applied to gain deeper insight into transporter-substrate interaction or to integrate transport data at a systemic level. *In silico* models of transporters and transport processes range from quantitative structure-activity related relationship (QSAR), pharmacophore modelling and docking to PBK models and integrative platforms such as SimCyp, PKSim or GastroPlus, among others, as well as machine learning tools (Pajeva and Globisch, 2009; Ekins et al., 2015; You et al., 2015; Ekins, 2016; Kim et al., 2017). In recent decades, the pharmaceutical field has placed considerable effort to study transporters affecting drug disposition, therapeutic efficacy and adverse outcomes. Besides identification of substrates, inhibitors and inducers of transporters, drug-drug interaction (DDI) mediated by transporters are also intensively studied as they are a major cause of modulation of drug efficacy and toxicity. In 2010, an International Transporter Consortium (ITC) was formed (i) to identify transporters of clinical importance often called "drug transporters" and (ii) to discuss the appropriate methodologies to characterize drug-transporter interactions (Giacomini et al., 2010; Zamek-Gliszczynski et al., 2013; Brouwer et al., 2013). The ITC presented seven consensus transporters of clinical relevance, referred as the "ITC7": MDR1, BCRP, OATP1B1-1B3, OAT1-3 and OCT2 (Giacomini et al., 2010). Then MATEs, Bsep and MRPs have been highlighted as additional transporters of emerging importance (Hillgren et al., 2013). The ITC recommendations have led several drug regulatory agencies to publish guidance documents on the evaluations of transporter implications in ADME processes and in DDI when developing new drug, in particular the European Medicines Agency (EMA) (EMA, 2012), the US Food and Drug Administration (FDA) (US FDA, 2012) and the Japanese Ministry of Health, Labour and Welfare (MHLW) (MHLW, 2014). Revised guidelines were released last year (2017) by the FDA including principally new *in vitro* guidance and adding MATE1 and MATE2-K transporters as well as time dependence of transporter inhibition studies in the requirements (US FDA, 2017). Not all transporters have to be investigated in all cases. The choice of experiments to be performed largely depends on the pharmacokinetic properties of the compound. To support scientists with the choice of relevant transporter studies, decision trees represent a central part of the regulatory recommendations.

With an increased focus on TK, it has been shown more recently that besides pharmaceutical compounds, membrane transporters also interact with various environmental contaminants, such as pesticides, manufactured chemicals, food contaminants and metals (Leslie et al., 2005; Tachampa et al., 2008; Van Herwaarden and Schinkel, 2006; Epel et al., 2008; Fardel et al., 2012; Wilks and Tsatsakis, 2014; Chedik et al., 2018a, 2018b). Expressed at key physiological barriers of the body, uptake and efflux transporters may modulate the systemic and intracellular concentrations of chemicals and hence directly impact their degree of toxicity (Leslie et al., 2005; Fardel et al., 2012). Furthermore, transporter-mediated interactions, polymorphisms or disease-related change in transporter function could cause a significant alteration in the intracellular concentration and consequent toxicity (Schuetz et al., 2014). These aspects have been discussed in several international workshops and conferences, that brought toxicologists, biologists and computational modelling experts together, and the role of active transport has been highlighted as a critical puzzle-piece of information for chemical risk assessment (Bessems et al., 2014; Paini et al., 2017a; Paini et al., 2017b). In this context, EURL ECVAM created and disseminated a survey entitled '*Use of membrane transporter data and knowledge for chemical*