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Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

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ABSTRACT

New approaches are needed to assess the effects of inhaled substances on human health. These approaches will be based on mechanisms of toxicity, an understanding of dosimetry, and the use of *in silico* modeling and *in vitro* test methods. In order to accelerate wider implementation of such approaches, development of adverse outcome

Abbreviations: ADME, absorption distribution metabolism and elimination; AEP, aggregate exposure pathway; AOP, adverse outcome pathway; BAL, bronchoalveolar lavage; BEAS-2B, adenovirus-12 SV40 hybrid transformed, non-tumorigenic human bronchial epithelial cells; CFD model, computational fluid dynamics model; CxT, concentration x time exposure; d_{ae}, aerodynamic diameter; DAF, dosimetric adjustment factor; HBE cells, human bronchial epithelial cells; HEC, human equivalent concentration; IATA, integrated approach to testing and assessment; IVIVE, *in vitro* to *in vivo* extrapolation; KE, key event; LC_{50}, lethal concentration 50%; MMAD, mass median aerodynamic diameter; MIE, molecular initiating event; MPPD model, Multiple-Path Particle Dosimetry model; NICEATM, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods; NSAIDs, nonsteroidal anti-inflammatory drugs; NTP, National Toxicology Program; OECD, Organisation for Economic Co-operation and Development; PBPK model, physiologically based pharmacokinetic model; POE, portal of entry; PCLS, precision-cut lung slices; QSAR, quantitative structure-activity relationship; RDDR, regional deposited dose ratio; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; RGDR, regional gas dose ratio; RRDR, regional retained dose ratio; SAEC, small airway epithelial cells; TG, test guideline; TSE, target site exposure

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In silico
Ex vivo
Quantitative structure-activity relationships (QSAR)
Adverse outcome pathway
Aggregate exposure pathway
Dosimetry
Integrated approach to testing and assessment (IATA)
Risk assessment

pathways (AOPs) can help identify and address gaps in our understanding of relevant parameters for model input and mechanisms, and optimize non-animal approaches that can be used to investigate key events of toxicity. This paper describes the AOPs and the toolbox of *in vitro* and *in silico* models that can be used to assess the key events leading to toxicity following inhalation exposure. Because the optimal testing strategy will vary depending on the substance of interest, here we present a decision tree approach to identify an appropriate non-animal integrated testing strategy that incorporates consideration of a substance's physicochemical properties, relevant mechanisms of toxicity, and available *in silico* models and *in vitro* test methods. This decision tree can facilitate standardization of the testing approaches. Case study examples are presented to provide a basis for proof-of-concept testing to illustrate the utility of non-animal approaches to inform hazard identification and risk assessment of humans exposed to inhaled substances.

1. Introduction

Acute inhalation toxicity testing is conducted to characterize potential portal-of-entry (POE) effects (those that directly affect the respiratory system) and systemic toxicity hazards of substances that can be inhaled, including gases, vapors, and liquid (mist) or solid (dust) aerosols. Both the decision to conduct acute inhalation toxicity testing and the design of appropriate test systems are informed by an evaluation of a substance's physicochemical properties and other available information, which will indicate whether inhalation is a likely route of human exposure and the potential target tissues. Data from acute inhalation toxicity tests may be used to identify intrinsic hazard properties of chemicals or end-use products, hazard classification and labelling, or to inform risk management decisions. Depending on the approach used, these data may also help elucidate the mechanism through which a chemical causes toxicity or to select exposure levels for subsequent subacute and subchronic inhalation tests. Other applications of acute inhalation toxicity data include development of emergency response guidance levels to inform evacuation or re-entry decisions, setting short-term occupational exposure levels, and informing operational decisions of military personal facing chemical warfare threats (Jarabek, 1995a; US EPA, 2009).

Acute inhalation toxicity is defined according to the Organisation for Economic Co-operation and Development (OECD) as the totality of adverse effects caused by a test substance following a single, uninterrupted exposure over a period of < 24 h (OECD, 2009a). Acute inhalation toxicity data have historically been generated by exposing animals to single or multiple inhaled concentrations of a substance in a short period of time ($\leq$ 24, usually 4–6 h) and assessing the adverse effects. OECD and other authorities have issued various test guidelines describing methods to assess inhalation toxicity (US EPA, 1998; 40 CFR 799.9130, 2002; OECD, 2009b; OECD, 2009c; OECD, 2017b). OECD Test Guideline (TG) 403 (OECD, 2009b) and OECD TG 436 (OECD, 2009c) consider lethality as the primary endpoint, whereas evident toxicity is the primary endpoint in OECD TG 433 (OECD, 2017b). For these tests, acute inhalation toxicity may be expressed as a point estimate of the median lethal concentration (LC_{50}; the concentration that would be expected to cause death in 50% of animals during a 14-day observation period) (US EPA, 1998; OECD, 2009b); a probit analysis of exposure-response data based on multiple concentrations and exposure durations (concentration x time; CxT) (OECD, 2009b); a benchmark dose analysis (Vincent, 1995; Kulkarni et al., 2011); or a hazard-based classification into categories based on exposure to predetermined fixed concentrations (OECD, 2009c; OECD, 2017b). LC_{50} data generated from these tests are used to categorize and rank test substances based on lethality, often with little or no elucidation of the site or underlying mechanism of toxicity. Other acute assessment derivations currently based on *in vivo* data consider exposure durations spanning a range from 10 min to 24 h, designate various non-lethal severity categories, and consider clinical measures or endpoints (*e.g.*, developmental, reproductive) in addition to LC_{50} values (Vincent, 1995; OECD, 2016b; National Research Council, 2017; Hofmann et al., 2018). Developing non-animal approaches that leverage pathway-based mechanistic information will not only provide a predictive tool for establishing potential hazard, but will likely provide more information to the risk assessor than an LC_{50} or other *in vivo* observations.

Extrapolating animal data to predict human health consequences presents numerous challenges due to physiological, anatomical, and metabolic differences across species (*e.g.* dissimilar airway dichotomies, types and composition of cells, different bio-transforming enzymes, and physiological variations in breathing patterns and metabolic rates) (BéruBé, 2013). Data generated in these acute toxicity studies may not be appropriate or sufficient to predict and manage potential adverse effects in humans (Zbinden and Flury-Roversi, 1981; Balls, 1991; Chapman et al., 2010; Seidle et al., 2010). As various adverse outcome pathways (AOPs) following inhalation exposures are elucidated, the opportunity arises to develop human cell-based *in vitro* and *in silico* approaches to evaluate endpoints relevant to those AOPs. An AOP is a conceptual framework that organizes existing mechanistic evidence by connecting—*via* key event relationships—a defined molecular initiating event (MIE) on the cellular or subcellular level to subsequently occurring key events (KEs) at the tissue and organ levels that lead to an adverse outcome at the organism or population level (Villeneuve et al., 2014b; Villeneuve et al., 2014a). AOPs describe a series of essential, measurable events culminating in toxicity, and can be useful in delineating endpoints that can be assessed *in vitro*. These AOP-motivated *in vitro* approaches can then be used to inform interspecies extrapolation, assess target organ effects, and support a better understanding of how specific substances cause toxicity in humans (*i.e.*, providing mechanistic insight that goes beyond what can be gleaned from an LC_{50} value). While these approaches are yet to be accepted by global regulatory agencies, they represent a promising and emerging area of research.

The implementation of alternative approaches for the assessment of acute inhalation toxicity was the focus of a 2016 workshop co-organized by the PETA International Science Consortium Ltd. and the U.S. National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (Clippinger et al., 2018). This workshop was attended by government agencies, industry, academics, and non-governmental organisations interested in developing approaches that can replace or reduce the use of animals for acute inhalation toxicity testing. Experts in attendance at the workshop were tasked with developing a strategy to establish confidence in these approaches.

Working groups were formed to fulfill each of the workshop recommendations. One of these recommendations was to publish the current paper, a state-of-the-science review to:

1) Detail the mechanisms of acute inhalation toxicity of inhaled substances (gases, vapors, and dust/mist aerosols), and define relevant AOPs that could be used to inform the appropriate integrated testing and assessment approach;
2) discuss the influence of physicochemical properties (*e.g.*, pH, low volatility, gas category, and particle size) on the relevance of inhalation as a route of exposure or on the ability to generate a test atmosphere;
3) summarize factors influencing dosimetry as well as the potential for