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Modeling and simulation of the mass transfer of volatile compounds in a membrane device for toxicity tests

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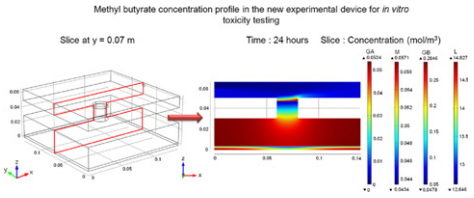
HIGHLIGHTS

- Toxicity tests of volatile organic compounds and loss during experimental studies.
- Novel membrane device for the experimental study of cell culture exposure to volatile compounds.
- Modeling and simulation of COV transfer.
- Relationships between COV transfer properties and the operating conditions.
- Determination of the best characteristics to avoid COV loss in the exposure chamber for a long period of time.

GRAPHICAL ABSTRACT



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ABSTRACT

A major problem when assessing in vitro the toxicity of volatile organic compounds is the loss of the tested chemicals during the course of experimental studies. This work presents a novel device for the experimental study of cell culture exposure to volatile compounds. The device is formed by different compartments separated by a porous hydrophobic membrane and allows for lengthy experiments without restricting cells from breathing. A mathematical model taking into account the mass and momentum conservation between the different device compartments has been built in order to predict the evolution of the volatile compound concentration. Theoretical results revealed a good match with experimental data and showed that the membrane surface, the volatile compound transfer properties and the operating conditions have a significant influence on the evolution of the volatile compound concentration in the liquid phase. Finally, the model proposed here is used to choose the best parameters related to both membrane structure and enclosure design so that the volatile compound can be maintained at a higher concentration for a longer period of time in the exposure chamber.

1. Introduction

Public opposition to animal testing and increasing concern over the safety of chemicals were significant social factors behind the implementation of a new regulatory framework in the European Union (EU) regarding chemical toxicity testing and consequently the encouragement to develop alternative methods. EU Directive 86/609/EEC (European Committee 1986) was the starting point for controlling animal experiments for scientific purposes based on the 3R principle (Refine, Reduce, Replace – defined by Russell & Burch in 1959), followed by the 7th Amendment to the EU Cosmetics Directive (EU Directive 76/768/EEC) that introduced an explicit ban on animal

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testing for cosmetic products and their ingredients, with cut-off dates in 2004, 2009 and 2013 respectively (Pauwels and Rogiers, 2004). In addition, the European Community (EC) Regulation 1907/2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemical substances (REACH) came into effect in the EU in June 2007. Like the EU Directive on protection of animals, it promotes the development of alternative methods according to the 3R principle and proposes integrated strategies that make maximum use of non-animal approaches, with refined and updated animal testing only being allowed as a last resort (Marx and Sanding, 2007).

Non-animal approaches consist of two major alternatives to in vivo animal testing: in vitro cell and tissue cultures (such as freshly harvested "primary" cells, tissues or organs; cell lines; stem cells and complex reconstructed tissue models) (Eisenbrand et al., 2002) and in silico systems such as computer structure–activity relationship (SAR) or Quantitative SAR (QSAR) models which predict the biological/toxicological properties of a substance based on its chemical structure and knowledge of similar structures, and expert systems programs that predict toxicological and metabolic activity (*e.g.* DEREK for Windows, METEOR and VITIC) (Marchant et al., 2008).

Besides human toxicology testing, the REACH law makes it mandatory to measure the impact of chemicals on living organisms and to conduct eco-toxicological tests which have the same constraints as in vitro human toxicological studies.

An in vitro test must be useful in quantitative risk assessment and it should be also capable of producing quantitative data from a dose–response relationship relevant to target species exposure. This information is crucial for quantitative risk assessment and involves knowing the chemical concentration throughout the exposure period (Rogers and McDougal, 2002).

Nevertheless, when the chemicals are volatile, their concentration varies in time due to their volatility. This is the case of volatile organic compounds (VOC) and many cosmetic ingredients which are volatile compounds with varying degrees of volatility and hydrophobicity. Moreover, losses can occur for highly volatile compounds, which render it difficult to evaluate the actual substance concentration at each moment of time during experimentation. Acquiring the dose–response data is therefore a challenge (Bakand et al., 2006). Two main approaches are usually employed to overcome these problems.

The first and most common approach is the utilization of sealable culture vessels such as cell culture flasks. In these systems, the volatile compound is dissolved in the culture media and the flask is kept sealed during the exposure period to maintain a constant concentration. However, the drawback of such a protocol is firstly, the decrease in the experimental duration to avoid hypoxic conditions and secondly, the adsorption of the chemical into plastics which affects the concentration level (Bakand et al., 2006; Mückter et al., 1998). Several researchers have used glass culture vessels to limit the chemical loss by adsorption into plastic vessels (McDermott et al., 2007; Rogers and McDougal, 2002). Furthermore, an enclosed glass chamber called Vitrobox™ has been built to keep the chemical concentration constant in the culture media by forcing in standard test atmospheres of a specific vapor concentration (Geiss and Frazier, 2001, 2002).

The second approach consists of using open systems so that lengthier experiments may be conducted. However this approach is not intended for volatile compounds since their uncontrolled evaporation leads to variations in concentration and so influences the results.

In recent years, advances in biomedical sciences have led to the construction of new experimental devices to develop in vitro models that mimic the exposure patterns in vivo. For instance, to test in vitro airborne contaminants, two novel direct exposure chambers are commercially available: the Cultex® system (Bakand and Hayes, 2010; Pariselli et al., 2009) and the horizontal diffusion chamber (Harvard Apparatus Inc., USA) that can be operated independently from the cell culture incubator.

In spite of all the progress made in developing these commercial systems, they are intended for in vitro models using nasal, pulmonary, corneal or dermal cells. Nevertheless, an in vitro assay may use different target species that are not necessarily human or vertebrate animal cells and tissues, but may also be plants, algae, aquatic invertebrates or their cell cultures as in the case of eco-toxicity studies. In addition, to the author's knowledge, there is no mathematical model in the literature to predict the concentration of the volatile compound over time inside these exposure systems. Such a predictive model added to an appropriate in vitro model may be of great help in describing the relationship between the chemical concentration and observed biological response(s).

This work therefore had two main objectives. The first was to design a novel experimental apparatus on which in vitro tests could be conducted for long periods without restricting the respiration of the species used and with better control of the volatile chemical concentration. The second was to develop a numerical model to simulate under non-steady state conditions the evolution of a soluble volatile compound concentration inside the device for both liquid and gas phases. The new device consists of two superposed compartments that communicate through a membrane. The lower compartment serves as an exposure chamber while the upper one simulates the incubator environment. The role of the membrane is to allow the transfer of oxygen and carbon dioxide in sterile conditions but to limit the loss of volatile compound vapors. As for the model, it includes the interfacial equilibrium partitioning and describes volatile compound mass transport in several in-series phases existing in the device: (i) diffusion through a stagnant liquid phase, (ii) diffusion through a stagnant gas phase, (iii) diffusion through the gas phase contained in membrane pores, (iv) convection and diffusion through the gas phase which simulates the incubator environment. The mathematical description is based on the species conservation and continuity equations and takes into account the geometry of the device. To validate the numerical model, two model volatile compounds with different hydrophobicities and volatilities were chosen. The purpose of this model was to simulate the system compartment under transient conditions in order to predict the evolution of the concentration of the volatile compounds during in vitro tests and to establish relationships between this evolution, volatile compound characteristics, membrane structure and enclosure design.

2. Membrane enclosure for toxicity tests

2.1. Design of the experimental device (Stoian et al., 2010)

The system consists of three main parts made of stainless steel and Teflon™; both materials are easy to sterilize using an autoclave or in a laminar flow hood under UV lightening. The three superposed parts form two compartments (Fig. 1). The lower compartment (B) represents the exposure chamber while the upper compartment (A) simulates the conventional culture conditions of a carbon dioxide incubator (5% CO_2, 100% relative humidity). The temperature was controlled by placing the device in an oven at 310 ± 1 K during the experiments. The upper compartment therefore comprises an inlet (1) and an outlet (2) in order to ensure a constant laminar flow ($Re \sim 4$) of a humidified gas mixture containing 95% air and 5% CO_2. The laminar air flow rate was chosen based on the same principle as the one used to design carbon dioxide incubators equipped with