Toxicology Mechanisms and Methods

http://informahealthcare.com/txm
ISSN: 1537-6516 (print), 1537-6524 (electronic)

Toxicol Mech Methods, 2014; 24(1): 70–72
 DOI: 10.3109/15376516.2013.859193

informa healthcare

RESEARCH ARTICLE

In-vitro methods for testing dermal absorption and penetration of toxic gases

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Abstract

This technical note provides details of an experimental technique for *in-vitro* skin studies with atmospheric chemical challenge. There appear to be major evidence gaps in relation to dermal exposure of gases. We describe a modification of standard OECD protocols for an atmospheric delivery system which can be used to understand interaction of toxic gases and the skin. The system can be used to examine the mechanisms by which skin uptake occurs. Auxiliary components which allow for parameter variation such as temperature and relative humidity are also described. Methodology presented in this technical note uses examples of gas challenges (ammonia, chlorine) to illustrate its application to gases of differing physicochemical properties. This adapted protocol can be applied in the context of HAZMAT scenarios involving atmospheric toxic chemical release and dermal absorption potential under variable exposure conditions.

Keywords

Ammonia, chlorine, gas delivery, HAZMAT, skin

History

Received 12 August 2013
Revised 13 October 2013
Accepted 22 October 2013
Published online 20 November 2013

Introduction

There appear to be major evidence gaps in relation to skin-gas interactions, as well as emergency management advice for potentially exposed individuals (Gaskin et al., 2013). This technical note provides details of an experimental technique for atmospheric challenge *in-vitro* skin studies, and uses the examples of ammonia and chlorine, which are toxic gases of HAZMAT and security concern.

The existing protocols for *in-vitro* skin testing focus on liquid chemical rather than gas/vapour challenge. For the latter purpose, a modification of an existing method was used (OECD, 2004), in conjunction with a test atmosphere generator (Pisaniello, 1988).

The technique is applicable to scenarios where potential gas exposures are relatively brief and where no personal protection is worn other than street clothing.

Materials and methods

Overview of technique

The experimental approach addresses the kinetics and equilibria of skin absorption and penetration, and the moderating effects of environmental factors (e.g. temperature, humidity), and clothing. An overview of the technique can be described in three steps: (1) Substrate (skin sample) preparation, (2) Gas challenge, and (3) Post-exposure analysis of absorption and penetration. Figure 1 is a block diagram of the equipment setup.

Test cell

Jacketed 9 mm Static Franz diffusion cells housed in a stirrer unit (PermeGear™, Hellertown, PA) were used. Cells had a diffusion-available surface area of 0.64 cm^2 and utilized appropriate receptor fluid (e.g. physiological saline, pure water or 50% ethanol). The donor compartment of the cell was modified by a scientific glassblower for flow-through gas delivery to the surface of the skin (Gaskin et al., 2013). Of note, the central bar is positioned to ensure turbulence is created in the neck of the donor chamber and allow even distribution of gas flow over the surface of the skin/substrate. The side arm may be enlarged to accommodate real time monitoring of the receptor fluid, e.g. pH/ISE probe. Prepared skin epidermis (see Section ‘‘*Substrate preparation*’’) was mounted between the donor compartment and the receptor compartment, in this way acting as a barrier for the applied test chemical to permeate into the receptor fluid. Receptor fluid was kept in contact with the skin under-surface and continuously stirred with a Teflon-coated magnetic stirring

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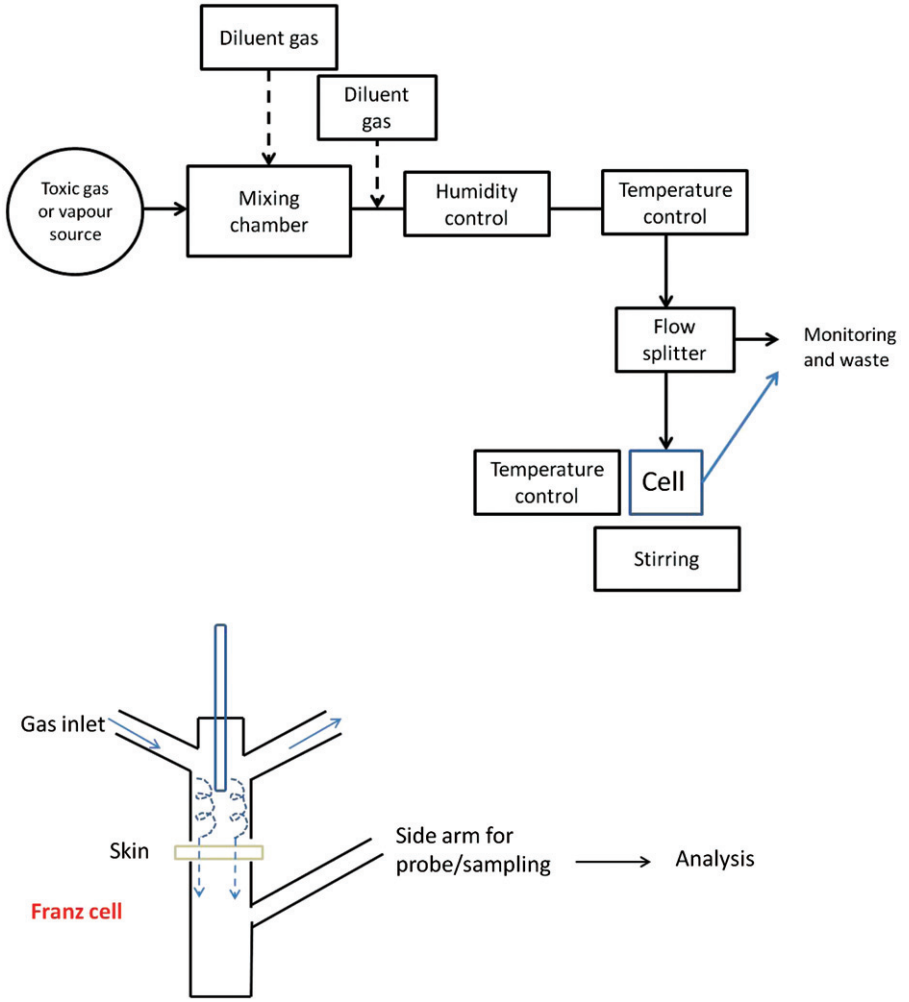
Figure 1. Block diagram of apparatus.



bar. After the set exposure period the Franz cell can be dismantled and the skin and complete volume of receptor fluid used for measurements of contaminant concentration in and through skin.

Substrate preparation

Various skin substrates may be used. However, the use of human skin samples is usually preferred for *in-vitro* studies of dermal absorption (Byford, 2009) as it provides data more appropriate to human *in-vivo* conditions. We used freshly excised human abdominal skin obtained from cosmetic reduction surgery. Epidermis was harvested from full thickness skin within one hour of excision from the donor. Subcutaneous tissue was removed and the epidermal layer was harvested from full thickness skin by heat treatment (Bronaugh et al., 1986; Davies et al., 2004; OECD, 2004). Pre-exposure skin electrical impedance (EI) testing was performed to provide a rapid assessment of barrier integrity *in-vitro* (Davies et al., 2004; Diembeck, 1999; Lawrence, 1997). For this purpose, a Tinsley LCR Databridge 6401 (Fasano & Hinderliter, 2004) was used set in Resistance (R), Parallel Equivalent (PAR) and 100 Hz modes. Skin EI may also be assessed post-exposure to toxic gas in order to assess changes in barrier integrity resulting from exposure.

Gas challenge

Toxic gases and vapours may be delivered directly from the source, for example a certified gas mixture cylinder. More commonly, it is diluted to test concentrations as required using a dynamic atmosphere generator and a diluent gas stream, typically purified air (Pisaniello, 1988). In our studies of ammonia and chlorine, certified gas mixtures were used (ammonia gas 2% in nitrogen and chlorine gas 1% in nitrogen; BOC, Australia) with diluent air. For HAZMAT scenarios, challenge gas concentrations should be physiologically relevant and appropriate for first responder guidance; for example the lowest lethal concentration (LC_{Lo}), the concentration immediately dangerous to life and health (IDLH), or other relevant concentrations (e.g. eye irritation). Short-term exposure times of 5–30 minutes were used, simulating HAZMAT incident exposures (Gaskin et al., 2013).

Experimental variables such as temperature and relative humidity may be accommodated using auxiliary components (Pisaniello, 1988). For elevated temperatures, electrical heating tape wrapped around Teflon tubing can be used in order to maintain an injected gas stream temperature of 30–60 °C. The system minimises thermal losses by the use of foam insulation around the Teflon tubing. The heating tape temperature is controlled by a simmerstat or voltage regulator. For lower temperatures of 10–20 °C, a refrigeration unit

circulates chilled water around jacketed glass fittings (for the gas stream and also the cell). Humidification of the diluent gas is achieved by using a split stream saturator (Pisaniello, 1988). Temperature, humidity and pressure are monitored in the final gas stream, which is typically reduced from 10 L/min to 500 mL/min over the exposed substrate. The whole assembly is mounted in a fume cupboard.

Post-exposure analysis of absorption and penetration

Quantification of skin absorption and penetration requires a suitable analytical method. Ammonia and chlorine interactions with skin were studied by pH change, using standard calibration curves and relevant blanks. Receptor fluid was analysed to determine gas penetration, and following exposure, the amount of gas absorbed was assessed by rapid dismantling of the cell and extraction of the skin substrate in a vial.

Results and discussion

The system described allowed determination of how much chemical remained in (or on) and penetrated through skin under variable conditions and exposure times. Results for ammonia gas show very limited penetration through human skin exposed to 2000 ppm (LC_{LO}) for up to 30 min, and estimated to be 0.15 μg NH_4^+. In contrast, significant skin gas absorption was measured from five minutes onwards, mostly associated with the hydration layer. When human skin was challenged with chlorine gas at 500 ppm (LC_{LO}) no significant penetration was observed across all exposure time periods. However, as with ammonia, significant skin absorption was measured from ten minute exposures onwards. For these two toxic gases, undamaged skin appears to be a good barrier at the concentrations tested.

The experimental system may also be applied to assess the modifying effects of clothing types and personal products worn on the skin. For example, topical sunscreen could be applied to the skin (in accordance with Australian Standard 2604; Standards Australia, 1998), then mounted in to the test Franz cell for toxic gas exposure. Similarly, different clothing types could be mounted on top of the skin and exposed in the same way in combination.

The main limitations to the application of this test protocol can be related to the test substrate (human skin), the chemical entities used for challenge, and the quantitative analysis of chemical on skin, in skin and penetration through skin. The system does not represent functional skin, but rather the barrier properties of the epidermis. While we may simulate physiological circumstances, such as the presence or absence of sweat by the hydration film of saline solution, this may not adequately represent the physiological events that occur in exposed individuals. In particular, the model uses skin at a high level of hydration, whereas exposed skin may present a more dry surface. The relative humidity of challenge chemicals is also practically limited to approximately 80%. Similarly, the available temperature range in these experiments was restricted since gas delivery lines were not all jacketed. The nature of challenge chemical may restrict the range of concentrations available to be investigated, for example, the corrosive nature of strongly acidic and alkaline gases would disrupt the skin structure and lead to perforation. Some gases or vapours may be degraded under the conditions of humidity and temperature appropriate to maintain the integrity of the skin. The overt toxicity of some materials (such as hydrogen cyanide) requires the use of a fume cupboard or local exhaust ventilation system.

Ideally, measurements of skin absorption and flux should be specific to the parent chemical. This is not always possible. For reactive challenge chemicals, there may be complex skin interactions and degradation. In these cases, more sophisticated techniques such as GC-MS, or integrative techniques, such as radioactivity monitoring, may be used (te Brake et al., 2012).

In conclusion, we describe here an *in-vitro* testing protocol suitable for toxic gases and short-term exposure scenarios typical of HAZMAT incidents. The data gathered may be useful for risk-based decision making by first responders, particularly with regard to skin decontamination (Gaskin et al., 2013).

Declaration of interest

The authors wish to acknowledge the funding provided by the Australian Attorney General's Department, Department of Prime Minister and Cabinet, and the Combating Terrorism Technical Support Office, Department of Defence, U.S. Government under the counterterrorism funding scheme.

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