DERMAL UPTAKE AND PERCUTANEOUS PENETRATION OF ORGANOPHOSPHATE ESTERS IN A HUMAN SKIN EX VIVO MODEL

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Introduction

Organophosphate esters (OPE) are used in a variety of products as plasticizers and flame retardants. They have been used alongside and as a replacement for PBDEs1,2 and possibly also for the “novel” BFRs (NBFRs) and some of the problematic phthalates. The toxicology of OPEs is best described for the chlorinated compounds, which are classified as CMR-compounds (carcinogenic, mutagenic, reprotoxic) and resultantly banned in e.g. toys in the EU2.

Dermal absorption has been increasingly discussed as an important exposure pathway for flame retardants and other lipophilic compounds, though very little data are available for OPEs. When looking at the physical-chemical properties, OPEs are expected to be absorbed to a much greater extent than e.g. PBDEs and hydrophobic NBFRs since OPEs have lower log Kow-values making them able to penetrate both lipophilic and hydrophilic barriers of the skin. Recently, Hoffman et al.4 found that triphenyl phosphate (TPHP) and tris(1,3-dichloropropyl) phosphate (TDCIPP) in hand wipes was significantly correlated with their metabolites in urine, indicating either dermal absorption or intake by hand-to-mouth exposure. In this study we address the dermal uptake and percutaneous penetration of OPEs, which can help disentangle exposure pathways.

Materials and methods

Dermal uptake and percutaneous penetration was studied in a Franz static diffusion cell system as previously described for brominated and chlorinated flame retardants3. In brief, human skin patches obtained from the department of plastic surgery were used (3 female donors aged 42-50 y; abdominal region, average skin thickness was 0.99 mm). The skin was mounted in Franz diffusion cells with an average exposed skin area of 2.64 cm² and 16.6 mL volume of the receptor chamber. The receptor fluid consisted of an aqueous solution of 0.9% NaCl, 5% bovine serum albumin, 40 mg/L hexamycin and Na2HPO4 buffer (to pH 7.4). The cells were kept in a water bath at 32 °C for 24 h prior to loading and the capacitance was measured to ensure barrier integrity. The OPEs were loaded in 500 µL ethanol (with 20% toluene); 1000 ng of each compound was added together with a positive control (vanillin). The OPEs included here are tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), tris(n-butyl) phosphate (TNBP), tris(2-butoxyethyl) phosphate (TBOEP), TDCIPP, and TPHP. Receptor fluid (1 ml) was sampled at 2, 8, 24, 48 and 72 h and analyzed for the positive control; the sampled volume was replaced with fresh receptor fluid. Cells were taken at 24 h (n=3), 48 h (n=5) and 72 h (n=4) for OPE analysis. At this point the barrier integrity was checked again, and the cells were discarded if it failed, thus the variation in number of cells. From each cell the following compartments were sampled for analysis: donor cell/skin surface wash, epidermis, dermis and receptor fluid as previously described4. The samples were stored at -20 °C until analysis.

The OPEs were analyzed by slightly varying methods depending on the matrix. All samples were spiked with the internal standards 13C-TPHP and d-TDCIPP prior to extraction. For donor/skin surface wash and epidermis the entire sample was extracted by adding 10 mL ethyl acetate and sonicated for 15 min, the supernatant was
transferred to a centrifuge tube and sonication was repeated twice with fresh solvent. The extracts were concentrated to approximately 0.5 mL using a SpeedVac Concentrator (Savant SPD121P, Thermo Scientific), transferred to autosampler vials and d-TCEP was added to measure recovery of internal standards. Dermis samples required further clean-up, thus they were extracted as described above, evaporated to 1 mL and cleaned on hand-packed Florisil columns (8 g) eluted with 50 mL ethyl acetate:acetone (1:1). The extracts were concentrated to approx. 1 mL, transferred to autosampler vials and recovery standard was added. For the extraction of the receptor fluid an aliquot of 10 mL was spiked with internal standards, 10 mL ethyl acetate was added, and the mixture was vortexed for 1 min and centrifuged for 5 min at 3000 RPM. The extraction procedure was repeated twice with fresh solvent. The rest of the procedure was identical to the other matrices. All extracts were analyzed by GC-MS using EI (5975C/7890A, Agilent Technologies) as previously described.

Results and discussion
Preliminary results revealed that all of the studied OPEs were found to migrate into the skin. However, their penetration profiles varied substantially (Figure 1). The transport of TCEP occurred quite rapidly and to such an extent that after 72 h the donor compartment was depleted and approximately 86% of the dose was recovered in the receptor fluid. Given the time resolution an exact lag-time cannot be estimated but is a little less than 24 h, as 9% of the TCEP is detected in the receptor fluid after 24 h and a steep increase is observed after that time. Lag-time is model specific and depending on e.g. skin thickness, comparably, the lag-time for the positive control substance in this model was 8 h (data not shown). TCIPP was also transported across the skin (Figure 1), first by building up in the dermis, and later appearing in the receptor fluid, however with a substantial fraction remaining in the dermis at 72 h. For the remaining four OPEs an accumulation in the skin was observed. For TNBP and TBOEP a smaller fraction was recovered in the receptor fluid after 72 h whereas for TDCIPP and TPHP the observed transport across the skin barrier was limited under the current study conditions.

The absorption profiles of the chlorinated OPEs were similar to those observed by Abdallah et al., who for a similar dose after 24 h found 28%, 25% and 13% being absorbed of TCEP, TDCPP and TDCIPP, respectively, though with much lower lag-times in their model. The existing data on dermal uptake of the non-halogenated OPEs is limited, but the markedly lower absorption for these corresponds well with their higher log Kow values, as was previously shown for highly hydrophobic NBFRs in the same ex vivo model.

Dermal uptake is a non-negligible exposure pathway for OPEs. Many of these compounds are found at high levels in indoor environments consequently dermal exposure from e.g. dust and materials may be substantial. In addition, dermal uptake directly form air may be of importance for the most volatile OPEs as has been observed for phthalates of similar vapor pressure and lipophilicity.
Figure 1. Distributions of OPEs between different compartments after 24h, 48h and 72h of exposure. Percent of total measured mass and SEM.
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