UNIVERSITY OF BIRMINGHAM

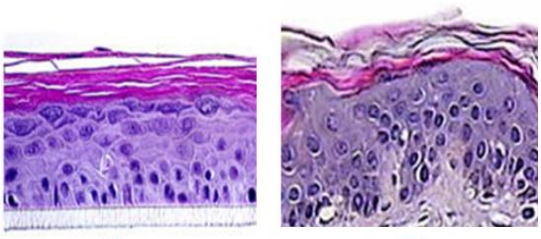
ADAPT

Assessment of Dermal AbsorPTion of organic flame retardant chemicals using 3D-*in vitro* human skin models (**ADAPT**)

ADAPT aims to fill a major research gap represented by the lack of information on human dermal absorption of organic flame retardants (FRs). These are a diverse group of chemicals widely applied in consumer products and building materials. However, they leach out from these products to the environment where their persistent, bioaccumulative and toxic properties are causing increasing concern. The ethical and technical issues associated with toxicological studies in human, combined with increasing restrictions on the use of laboratory animals and the substantial uncertainties associated with extrapolating data from animal studies to human are impeding current efforts for accurate risk assessment of hazardous chemicals. By delivering more effective alternative approaches to study the bioavailability of FRs, **ADAPT** will benefit public health, scientists and policy-makers taking informed decisions to minimize human exposure to hazardous chemicals within the context of legislative frameworks such as **REACH**.

This will be achieved via applying novel *in vitro* 3D-human skin equivalent tissues (3D-HSE) (Figure 1) to study the percutaneous bioavailability of various legacy and novel FRs present in indoor environment. The results of these experiments will be used to further understand the relationship between external human exposure to FRs and human body burdens of these contaminants.

Figure 1: H&E stained sections of (a) EPISKIN™ tissue and (b) excised human skin tissue.

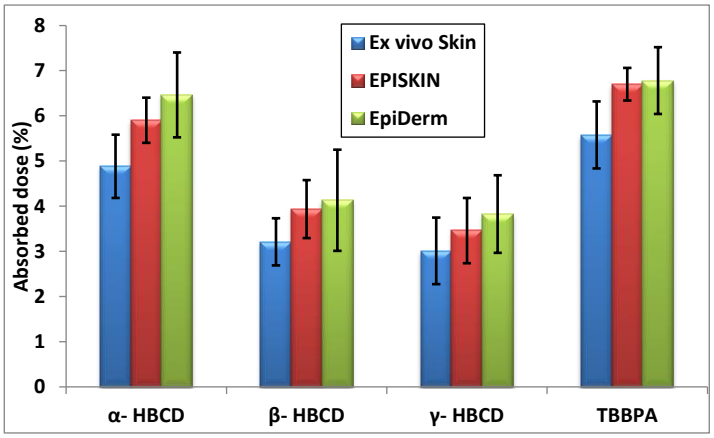


(a) (b)

In the first phase of **ADAPT**, a standard protocol was successfully developed to study the dermal absorption of FRs using *in vitro* 3D-HSE for the first time. The protocol involved multi-stage quality assurance/quality control (QA/QC) measurements including histological and physiological testing, membrane integrity assessment, positive and negative control groups, in addition to regular method blanks. The protocol complied with the current OECD (Organisation for Economic Co-operation and Development) guidelines for skin absorption testing and was applied to 2 existing 3D-HSE namely, EpiDerm™ and EPISKIN™. Results were validated against a viable human *ex vivo* skin model to evaluate the barrier function of the 3D-HSE for the studied FRs.

Results for two widely used brominated flame retardants (BFRs) , namely hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A) revealed no statistically significant differences ($P > 0.05$) between the absorbed fractions through 3D-HSE and human *ex vivo* skin at two exposure levels (Figure 2). However, it was evident that both 3D-HSE tissues were more permeable to the penetration of the studied FRs than human *ex vivo* skin.

Figure 2: Absorbed dose (%) of HBCDs and TBBP-A following 24h exposure of different *in vitro* skin models to 500 ng/cm^2 of the studied FRs.



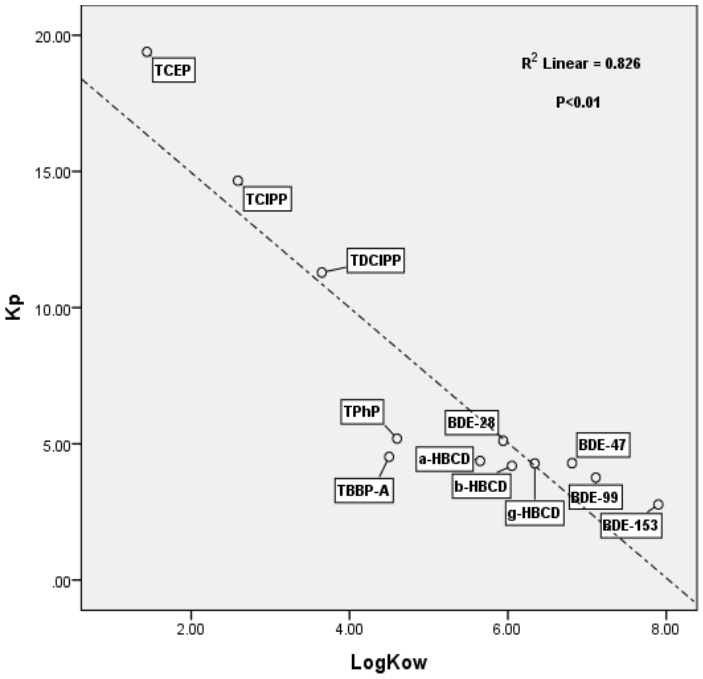
The standardized *in vitro* protocol was then applied to study -*for the first time*- human dermal absorption of a wide variety of FRs using 3D-HSE. The studied compounds included different

polybrominated diphenyl ether (PBDE) congeners with various degrees of bromination in addition to 3 widely applied chlorinated organophosphate FRs (PFRs).

Due to the novelty of this work and the wide variation of physico-chemical properties of the studied FRs, we optimised and applied a mathematical model to fit the generated data. The model allowed for identification of the linear absorption range and estimation of important compound-specific kinetic parameters including the dermal flux (J_{ss}, ng/Cm^2.h), the permeability constant (K_p, cm/h) and lag time (t_{lag}, h). These parameters can be further applied to provide valuable information on human dermal absorption of the studied FRs under different exposure scenarios for risk assessment purposes.

Moreover, **ADAPT** provides first insights on the factors affecting human dermal absorption of FRs. Our results revealed a significant negative correlation between the permeability constant of the studied FRs and their log K_{ow} values (Figure 3). Investigation of the influence of exposure vehicle revealed enhanced dermal penetration of FRs in the presence of surfactants (20% Tween 80). This is potentially significant within the context of human exposure owing to the presence of natural surface active agents in the human skin surface film (SSFL, sweat/sebum mixture). Therefore, we designed and applied a physiologically-based *in vitro* test to study the influence of the SSFL and topically applied cosmetics on the dermal bioaccessibility of several FRs present in indoor dust. We used a synthesized human SSFL (more than 50 components) with varying sweat/sebum composition under physiological conditions. Results revealed the composition of the SSFL and the presence of certain topically applied cosmetics (e.g. sun block, moisturizing cream and shower gel) to be important factors affecting the dermal bioaccessibility of FRs following contact with contaminated dust.

Figure 3: Correlation between K_p and log K_{ow} values of the studied FRs.



We also investigated the influence of hand-washing on the dermal absorption of FRs over 24h exposure. Our results indicate that hand-washing can reduce the overall dermal absorption of the studied FRs, albeit to varying degrees depending on the compound specific physico-chemical properties.

Finally, we applied a single compartment pharmacokinetic model to estimate the absorbed doses of different FRs following dermal contact of UK adults and toddlers with contaminated dust using different exposure scenarios. Results indicate that toddlers are at higher risk due to increased dermal uptake of FRs than adults.

To conclude, **ADAPT** delivered an effective, sustainable and reproducible alternative approach to animal testing for studying human dermal absorption of hazardous chemicals. The innovative application of 3D-HSE provided novel insights and valuable information on the bioavailability of several FRs via the dermal route and the factors influencing this process. This will allow for more accurate risk assessment studies of these hazardous chemicals.