THE *IN VITRO* PERCUTANEOUS ABSORPTION OF RADIOLABELLED HEXABROMOCYCLODODECANE (HBCD) THROUGH HUMAN SKIN

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Abstract

Hexabromocyclododecane (HBCD) is a brominated flame retardant used in the manufacture of a range of domestic and consumer products. Despite the use of personal protective equipment, there remains the potential of dermal exposure to HBCD during manufacture and packaging of the material. In order to assess the likely human health risks associated with dermal occupational exposure to HBCD this *in vitro* study was undertaken to assess the rate and degree of absorption of [^{14}C]-HBCD following topical application of [^{14}C]-HBCD using human skin acquired from elective surgical procedures.

Following topical application of [^{14}C]-HBCD to human split-thickness skin *in vitro*, the absorbed dose and dermal delivery were 0.01% (0.06 µg.equiv./cm^2) and 1.35% (12.82 µg.equiv./cm^2) of the applied dose, respectively. At 8 h post dose, 34.62% of the applied dose was removed from the skin by washing and drying. At 24 h post dose, a further 28.76% was recovered in the 24 h skin drying and cell wash. Therefore, the dislodgeable dose was 63.37% of the applied dose. The stratum corneum contained a further 31.49% of the applied dose. The bulk of this (25.70%) was recovered in the first 5 tape strips. Since the bulk of the stratum corneum associated material was found in the first 5 tapes strips, this indicated that the [^{14}C]-HBCD was on the surface of the skin and that the stratum corneum was an efficient barrier to [^{14}C]-HBCD penetration.

Introduction

Brominated flame retardants are widely used in the manufacture of a broad range of domestic and consumer products in order to reduce the potential flammability risk. Hexabromocyclododecane (CAS No. 25637-99-4), also known as HBCD, is essential in flame retarding polystyrene foams that are used for buildings insulation, etc. To a minor extent, HBCD is also used to flame-retard textiles in upholstered furniture. HBCD is undergoing a risk assessment by the European Union in the frame of the EU Existing Substance Regulation (Regulation 793/93).

HBCD is a homogeneous mixture of three diastereomers (alpha, beta and gamma). The gamma is present at the highest percentage level of the three when manufactured. HBCD has very low solubility in most common solvents. Despite the use of personal protective equipment, the potential of dermal exposure to HBCD does remain, both during the manufacture of the material and packaging of the final product. In order to support the EU risk assessment of likely human health risks associated with such occupational exposure to HBCD this *in vitro* study was undertaken to assess the rate and degree of absorption of [^{14}C]-HBCD following topical application using human skin acquired from elective surgical procedures.

Materials and Methods

Human skin for use on this study was obtained from patients undergoing routine elective surgery at the Plastic Surgery Unit, St John's NHS Trust (Livingston, UK). Written, informed consent was obtained from all donors. Split-thickness skin membranes were prepared from the skin samples and were mounted into flow-through diffusion cells (Scott/Dick, University of Newcastle, UK). The skin surface temperature was maintained at *ca* 32°C throughout the experimental procedure. Receptor fluid (tissue culture medium containing bovine serum albumin, glucose, streptomycin and penicillin G) was pumped underneath the skin at a flow rate of *ca* 1.5 mL/h and a tritiated water barrier integrity test performed. All skin samples with a tritiated water permeability coefficient (k_p) less than 2.5 x 10^{-3} cm/h were accepted for use.

Analysis of the [^{14}C]-HBCD by HPLC with on-line radio-detection determined the radiochemical purity to be

ca 97%, with 8.24, 8.14 and 80.6% present as the alpha, beta and gamma forms, respectively.

The [^{14}C]-HBCD could not be applied to human skin as the dry powder as the mass to be applied (*ca* 640 µg, 1 mg/cm^2) could not be accurately dispensed. Therefore, [^{14}C]-HBCD was applied as a solution using acetone as the vehicle. Due to its low solubility, multiple applications (5 applications of 6.0 µL over a *ca* 15 min period) of [^{14}C]-HBCD in acetone were required to achieve the target application rate (*ca* 47 µL/cm^2). The acetone evaporated rapidly from the skin surface leaving behind the [^{14}C]-HBCD. A total of 9 skin samples from 7 donors were dosed with [^{14}C]-HBCD.

Following application of [^{14}C]-HBCD, absorption was assessed by collecting receptor fluid in hourly fractions from 0-8 h post dose (to replicate a typical period of operator exposure) at the end of which the skin surface was washed with a soap solution and dried with a tissue swab. The material washed from the skin (together with the pipettes used) and the tissue swabs were retained for analysis by liquid scintillation counting. Following the washing procedure the absorption of [^{14}C]-HBCD continued to be monitored by collecting receptor fluid in 2-hourly fractions until 24 h post dose. At 24 h post dose, the receptor fluid flow was stopped and the collection terminated. The skin was again dried with a tissue swab and the following terminal samples retained for analysis: receptor and donor chamber rinses, exposed and unexposed skin and the stratum corneum which was removed by successive tape stripping. All samples were analyzed by liquid scintillation counting.

The definitions have been used from OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In vitro* Method (2004).

Results and Discussion

A summary of the results is provided in the table below.

	Mean	SD
Target HBCD Application Rate (µg/cm^2)	1000	-
Actual HBCD Application Rate (µg/cm^2)	950	-
Dislodgeable Dose (µg equiv./cm^2)	600.90	114.17
Unabsorbed Dose (µg equiv./cm^2)	903.76	42.07
Absorbed Dose (µg equiv./cm^2)	0.06	0.04
Dermal Delivery (µg equiv./cm^2)	12.82	4.65
Mass Balance (µg equiv./cm^2)	916.58	40.36
Dislodgeable Dose (% Applied Dose)	63.37	12.04
Unabsorbed Dose (% Applied Dose)	95.31	4.43
Absorbed Dose (% Applied Dose)	0.01	0.00
Dermal Delivery (% Applied Dose)	1.35	0.49
Mass Balance (% Applied Dose)	96.67	4.25

Following topical application of [^{14}C]-HBCD to human split-thickness skin *in vitro*, the absorbed dose and dermal delivery were 0.01% (0.06 µg.equiv./cm^2) and 1.35% (12.82 µg.equiv./cm^2) of the applied dose, respectively. At 8 h post dose, 34.62% of the applied dose was removed from the skin by washing and drying. At 24 h a further 28.76% was recovered in the 24 h skin drying and cell wash. Therefore, the dislodgeable dose was 63.37% of the applied dose. The stratum corneum contained a further 31.49% of the applied dose. The bulk of this (25.70%) was recovered in the first 5 tape strips. Since the bulk of the stratum corneum associated material was found in the first 5 tapes strips, this indicated that the [^{14}C]-HBCD was on the surface of the skin and that the stratum corneum was an efficient barrier to [^{14}C]-HBCD penetration.

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