A multicentre ring trial study for in vitro dermal absorption of 14C-Caffeine according to OECD test guideline 428

Main author: Styliani Totti (University of Surrey)

Co-authors: Felix Kluxen, Frank Toner, Leanne Page, Kathryn Webbley, Rajendra Nagane, Clair Valentine, John Kendrick, Robert Mingoia, Christine Whitfield, Jeanne Dorange Bernal, Edgards Felkers, Jeanne Domoradzki, Basma Darraji, Steven McEuen, Philip Fisher,

INTRODUCTION

Dermal absorption of chemicals is a key factor in non-dietary human risk assessment with agrochemicals. In vitro dermal penetration studies are conducted to determine a suitable fraction, the relative dermal absorption value, which is the fraction that reaches the systemic compartment surrogate, based on the applied dose. Today, most regulatory studies for pesticide registration purposes are conducted according to the in vitro Organisation for Economic Co-operation and Development test guideline (OECD TG) 428 with human skin and radioactive-labelled material.

The methodology has significantly evolved from its status at the time of OECD guideline development and is under continuous further development. To assess the robustness of the methodology some limited inter-laboratory ring-trials have been published but comparing different methodologies and not necessarily conducting state-of-the-art fully OECD guideline compliant studies. A ring trial exploring the dermal absorption potential of 14C-Caffeine was investigated in six laboratories under GLP conditions using the OECD TG 428-compliant in vitro assay with flow-through cells and dermatomed human skin.

METHODOLOGY

The ring trial was conducted according to a common protocol, test item and skin source. A semi-automatic design of experiments for the assessment of inter-laboratory and intra-laboratory variability was followed for the skin donor distribution across the laboratories in order to fulfil the specific study requirements and facilitate the statistical data analysis. Skin samples from the same donor have been distributed across 3 laboratories. 1-Methyl -14C-Caffeine at a dose concentration of 4 mg/mL in Phospate-Buffered Saline (PBS) vehicle was applied to human abdominal dermatomed (300-400 μm thickness) skin membranes. The study was conducted in flow-through diffusion cells. The exposure period to caffeine lasted 8 hours, following a skin wash and the experiment was completed after 24 hr. Each laboratory performed two independent experimental runs in order to additionally assess the intra-laboratory repeatability.

RESULTS

The data show very similar recovery in the various investigated compartments of the assay between laboratories, repeats and donors, which demonstrates that the assay can be robustly and reliably performed. A low intra-lab variability was demonstrated in all 6 laboratories participating. Further, a low inter-lab variability was notable in five out of six laboratories resulting in mean caffeine absorption estimates of 3.93±2.96 % of the applied dose (4 mg/mL) and a mean overall compound recovery of 99.03±2.11 %. In the remaining laboratory presumably due to a Covid-19 driven shipment delay of the skin samples, the skin quality may have been affected since the receptor fluid recovery was substantially higher and to a certain degree did correlate with skin integrity parameters.

DISCUSSION

Overall, the ring trial's results demonstrate that the OECD TG 428-compliant in vitro assay can be robustly and reliably performed in different laboratories. Skin absorption results for caffeine did not present any statistically significant variability, with minor differences in the results across the laboratories (5/6) and indicate the robustness of the methodology and the positive impact of strictly controlling the variables in the study. Understanding the variability in the in vitro dermal absorption assay with human skin will continue contributing to the design and conduct of appropriate regulatory studies, and adding further considerations to the existing OECD dermal guidance notes. This will result in greater confidence in the data utilised from these assays in risk assessments by companies and regulators alike.