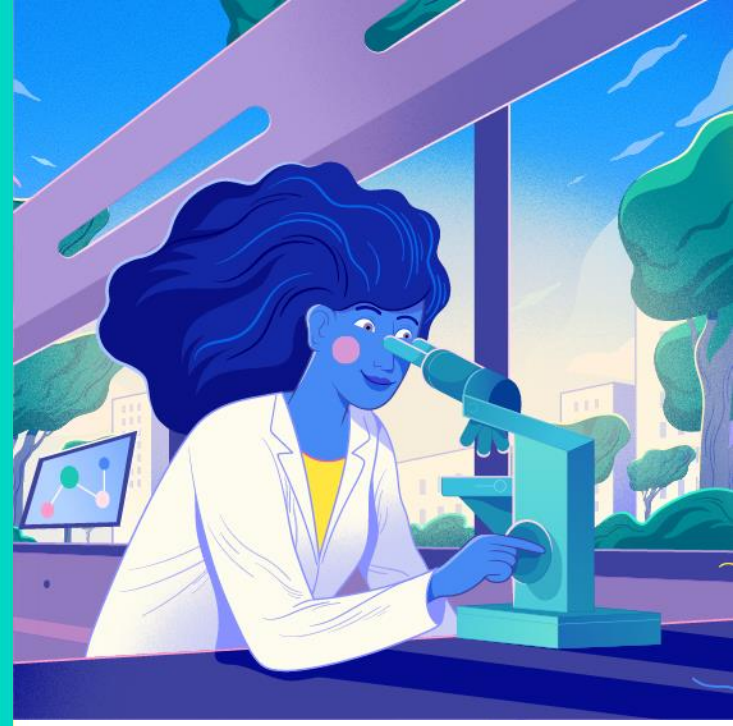
Measuring esterase activity in human skin S9, a tool to refine consumer safety risk assessment



24th Reid Bioanalytical forum
The Cambridge Belfry
13-16th June2022

Unilever

Presentation outline

- Background
- Design of the assay
- Results and challenges
- Integration into *in silico* model

07/11/2022

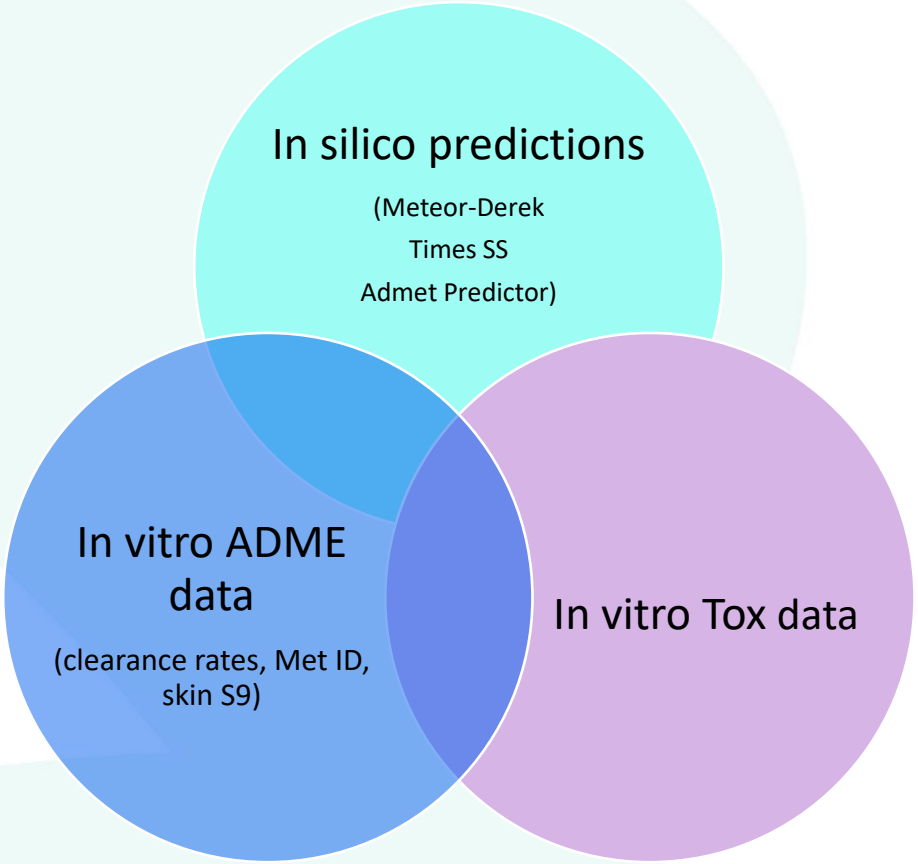
Background: Assessing ingredient &product safety without animal testing

Next Generation Risk Assessment (NGRA)

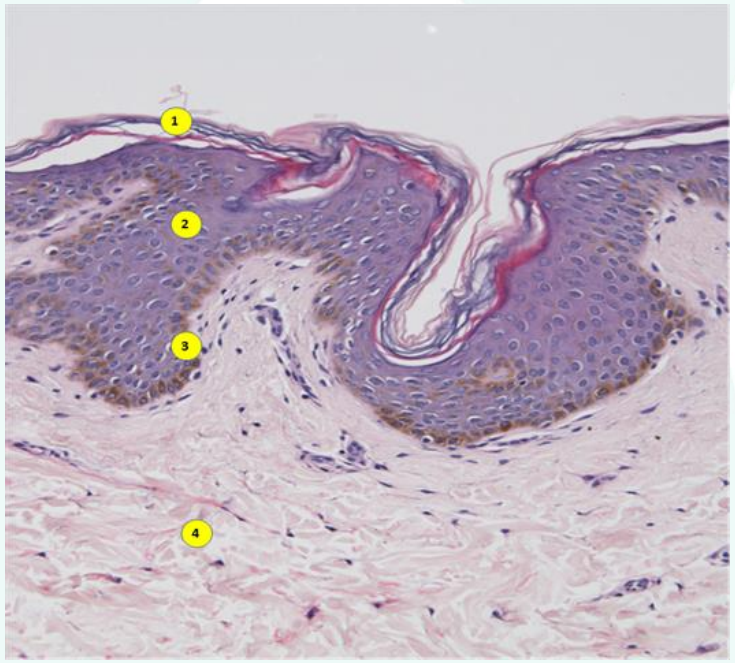
Is it safe to include x% of chemical y in product z?

Unilever

Background: Metabolism considerations



Mixed source of information for Risk Assessment

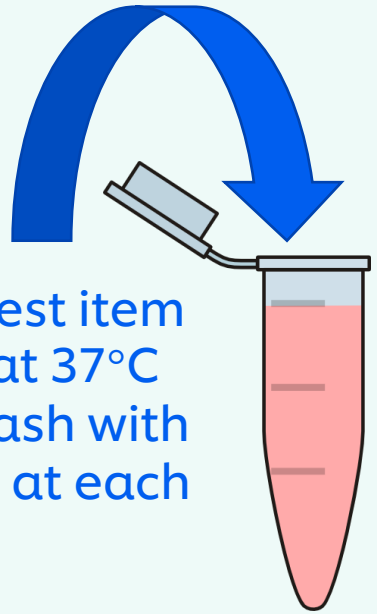


Skin structure depicting 1) the *stratum corneum*, 2) the epidermis, 3) the *stratum basale* (high concentration in melanin), 4) the dermis. Image provided by H. Minter, SEAC, Unilever.

Human skin is a complex organ for which metabolism assays are not standardised as well as they are for liver

Design of the assay: Human skin S9

Enzymatic activity decreases quickly in human skin. Preparing S9 as soon as possible helps maintaining some activity (Phase II enzymes)[1]. Esterase activity is well maintained in S9[2] Co-factors are not required for esterases in skin [3].



- Skin S9+ Test item
- Incubate at 37°C
- Protein crash with acetonitrile at each time point

An easy design

still requires

- Skin S9 has lower activity than liver S9. S9 amount increased (50µL S9, 50µL test item)
- Negative control: S9 needs boiling at 95°C for 20min to deactivate fully

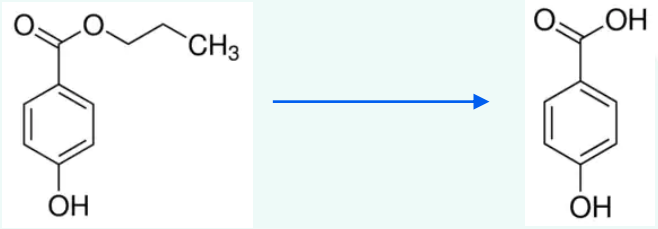
a few tweaks

[1] Spriggs S et al. A study of inter-individual variability in the Phase II metabolism of xenobiotics in human skin. Toxicol Lett. 2018 Aug;292:63-72.
[2] Phenyl acetate esterase and MTT reduction as markers for enzyme stability in human skin discs in vitro. Leanne Page, Caitlin McArthur, Frank Toner, Clive Roper and Jonathan Welch In Vitro Sciences, Charles River Laboratories, Edinburgh, UK
[3] Lester C et al, Metabolism and plasma protein binding of 16 straight- and branched-chain parabens in in vitro liver and skin models. Toxicology in Vitro, Vol 72, 2021

Unilever

Design of the assay: What to test to validate hypothesis?

Positive control for esterase, relevant for skin: propyl paraben



ELSEVIER

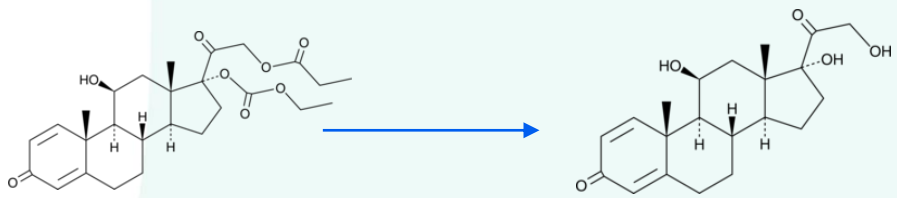
Toxicology in Vitro
Volume 72, April 2021, 105051

Metabolism and plasma protein binding of 16 straight- and branched-chain parabens in in vitro liver and skin models

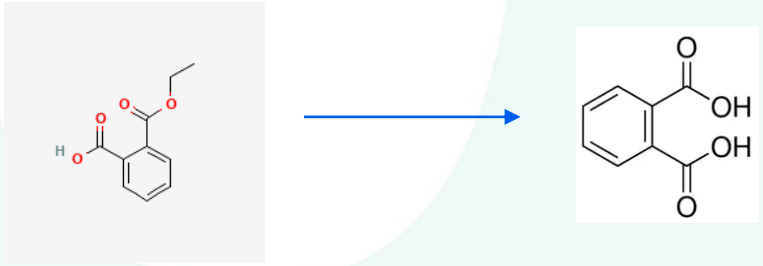
Cathy Lester [a, 1], Nicola J. Hewitt [b], Ursula Müller-Vieira [c], Manuela Mayer [c], Corie Ellison [a], Hélène Duplan [d], Camille Genies [d], Carine Jacques-Jamin [d], Eric Fabian [e], Ian Sorrell [f], Daniela Lange [g], Andreas Schepky [g], Sébastien Grégoire [h]

Ethyl Nicotinate

Prednicarbate (anti-inflammatory)



Monoethyl phthalate



Unilever

Design of the assay: LC-MS/MS analysis (Waters TQ-XS)

Most compounds :

Acquity BEH C18 (50 x 2.1 mm, 1.7µm particle size) column from Waters. Temperature 40 °C. 0.1 % formic acid in water (mobile phase A) and 0.1 % formic acid in acetonitrile (mobile phase B). Flow rate 0.5 mL/min. 5 min gradient.

Exception:
Monoethyl Phtalate/Phthalic acid

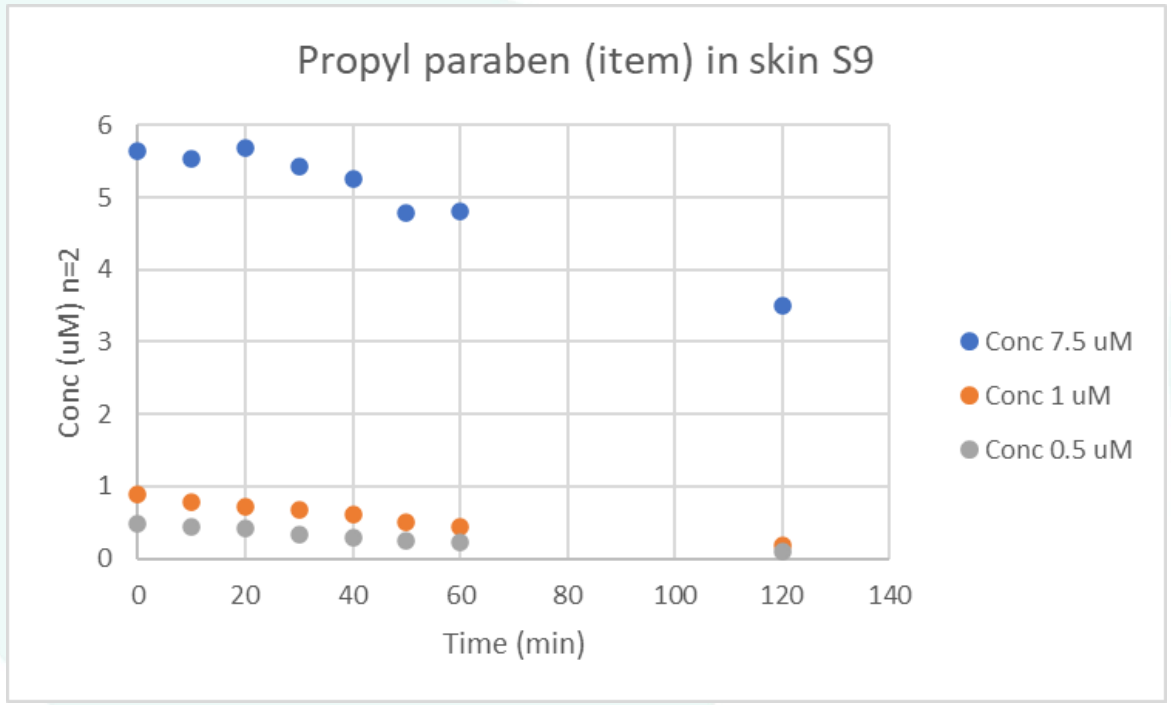
Acquity HSS PFP (100 x 2.1 mm, 1.8µm particle size) column from Waters. Gradient same as above.

Test item ID	Parent mass (Da)	Daughter mass (Da)	Cone voltage (V)	Collision energy (eV)
Propyl paraben	(negative ion) 179.03	(negative ion) 92.09	22	22
4-hydroxybenzoic acid	(negative ion) 136.90	(negative ion) 93.00	24	12
Monoethyl phthalate	194.97	148.89	14	12
Phthalic acid	(negative ion) 164.97	(negative ion) 120.95	2	10
Ethyl nicotinate	151.97	123.89	14	16
Nicotinic acid	123.97	52.76	6	36
Prednicarbate	489.35	381.24	38	12
Prednisolone	361.13	147.00	28	30

7 standards covering the range 0.1-10µM

Unilever

Results: The dilemma with 4-hydroxybenzoic acid



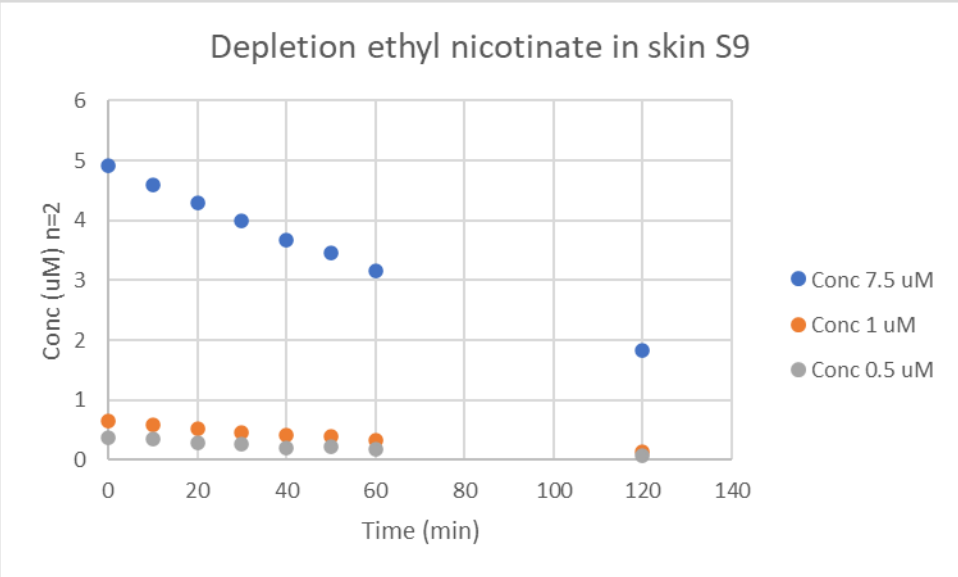
All samples contained 2-3µM of 4-hydroxybenzoic acid in final dilutions, including blanks (boiled S9).

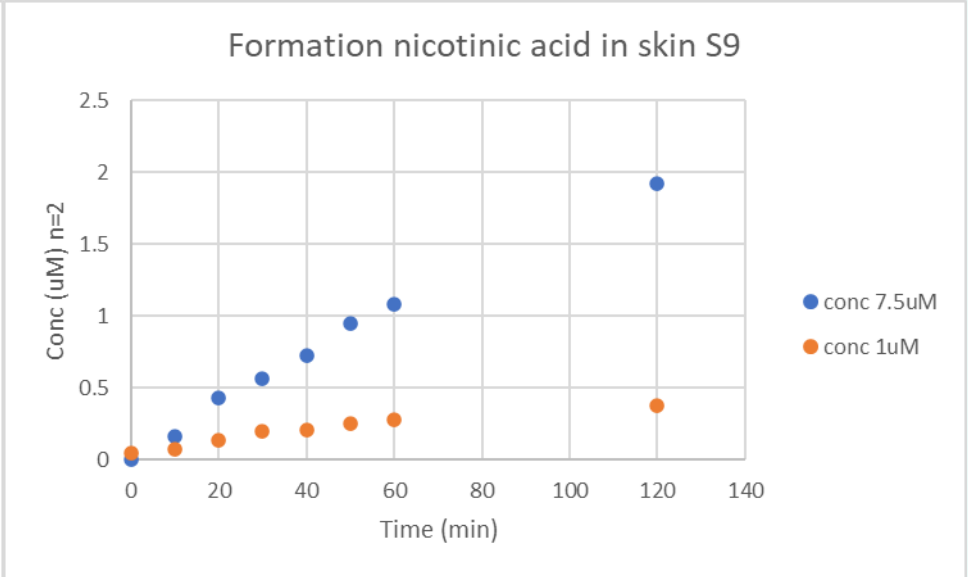
Formation of 4-hydroxybenzoic acid was "masked" by up to 30µM of it being already present in the purchased S9. Could not confirm if a paraben was used as a preservative during S9 preparation.

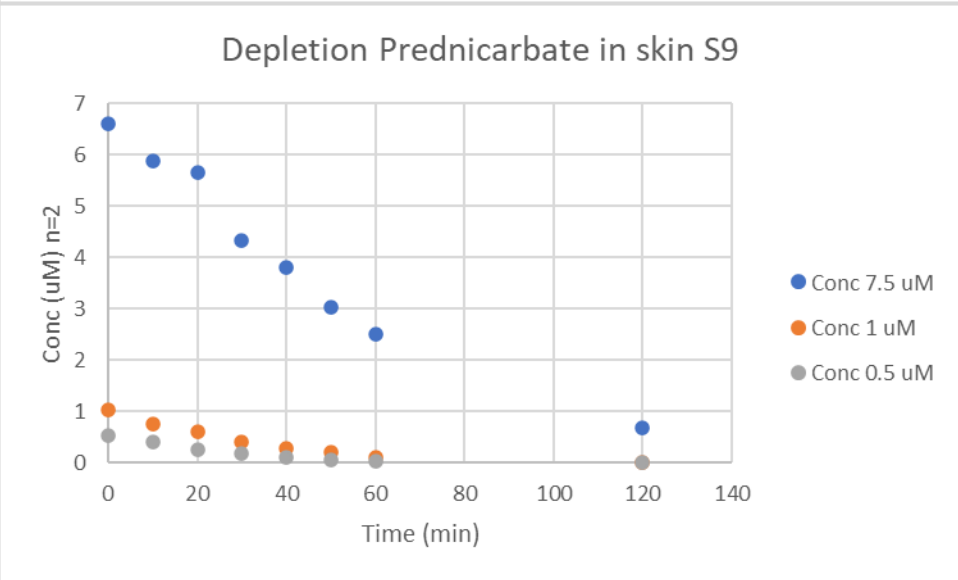
Concentration (µM)	Half-life method 1 (min)	Half-life method 2 (min)
7.5	180.4	166.8
1	57.6	52.5
0.5	53.4	50.8
Concentration (µM)	**$Cl_{int,\ in\ vitro}$ (half-life method 1)**	**$Cl_{int,\ in\ vitro}$ (half-life method 2)**
7.5	0.727	0.874
1	1.443	1.617
0.5	1.660	2.010

(Ln [conc % t=0]) plotted as a function of time. The slope and intercept were determined.
Half-life was calculated by two methods.
Method one: x= (y- intercept)/slope, where x is the half-life in min and y is Ln(50). Method two: t1/2 = -0.693/slope

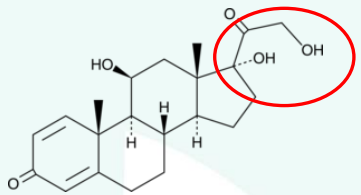
Results: When things go as expected and when they don't







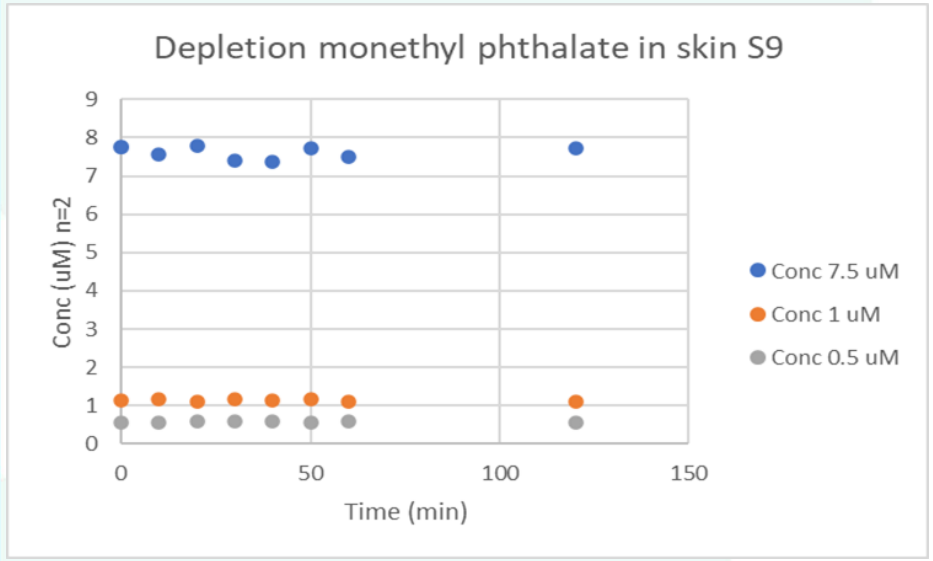
No Prednisolone? Only slightly at highest concentration and time point.
With hindsight Prednisolone is a double alcohol of prednicarbate.



Should we have monitored the formation of one alcohol as well?

Results: When things go as expected and when they don't

Monoethyl phthalate (in itself a metabolite of diethyl phthalate, present in some plastic products) did not metabolise in our assay. No depletion of parent and no formation of phthalic acid



Trying for an explanation?

1) This probe is not sensitive to carboxyesterase 2 (major form found in the skin) but carboxyesterase 1 (found in liver) and the three types of esterases found in humans differ a lot in their specificity.
2) This probe is not sensitive in humans, but works fine in bacteria!

environmental microbiology reports

sfam society for applied microbiology

Minireview | Full Access

Phthalate hydrolase: distribution, diversity and molecular evolution

Mousumi Bhattacharyya, Suman Basu, Rinita Dhar, Tapan K. Dutta

First published: 23 November 2021 | https://doi.org/10.1111/1758-2229.13028 | Citations: 1

Unilever

How do we use the data?

The half-life (t1/2) and in vitro intrinsic clearance (CLint, in vitro) can be used by PBPK modelling to refine clearance rate predictions for the full body.

Integration into a bespoke *in silico* human skin model is also an option

ELSEVIER

Toxicology in Vitro

Volume 63, March 2020, 104746

Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products

Thomas E. Moxon, Hequn Li, Mi-Young Lee, Przemyslaw Piechota, Beate Nicol, Juliette Pickles, Ruth Pendlington, Ian Sorrell, Maria Teresa Baltazar

Springer Link

RESEARCH PAPER | Published: 11 November 2020

In Silico Simulation of Simultaneous Percutaneous Absorption and Xenobiotic Metabolism: Model Development and a Case Study on Aromatic Amines

Lucy Coleman, Guoping Lian, Stephen Glavin, Ian Sorrell & Tao Chen

Pharmaceutical Research **37**, Article number: 241 (2020) | Cite this article

443 Accesses | **2** Citations | Metrics

Thank you

Team members:

David Glasspool
Richard Cubberley
Suzanne Martin
Hequn Li
Stephen Glavin
Georgia Reynolds

Any Questions?

Unilever

07/11/2022