AATEX **14**, Special Issue, 561-564
Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences
August 21-25, 2007, Tokyo, Japan

Systemic testing by the dermal route can be precluded by new non-animal percutaneous absorption strategies

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Abstract
Recent developments in the estimation and measurement of percutaneous absorption now make it possible to determine, using either *in silico* or *in vitro* prediction methods, whether a specific chemical will be absorbed through the skin. For chemicals where the exposure route of concern is primarily dermal, systemic toxicity tests may be avoided by first determining the dermal penetration potential, using these non-animal percutaneous absorption methods. This approach can still protect public and worker health while avoiding resource- and animal-intensive tests such as dermal reproductive or developmental testing. A case study used in the US EPA's High Production Volume Chemical Challenge Program is presented.

Keywords: percutaneous absorption, weight-of-evidence, QSAR

Introduction

The percutaneous absorption (PA) potential of a chemical is an important characteristic to estimate. For chemicals that come into contact with human skin either by accident or design, it is necessary not only to determine the potential effects on the skin itself, but to also determine whether the chemical can penetrate the skin. If a chemical permeates the epidermis, it can be absorbed into the vascular system and exert effects on other systems in the body.

Traditionally, estimation of PA was accomplished using various *in vivo* methods, which combined dermal application of the test chemical to an animal with metabolism and excretion measurement. Briefly, the animal is cannulized, the test material is applied to shaved skin for four hours, and excreted radiolabelled metabolites are analyzed, as are pertinent tissues after the animal is killed. This procedure is recorded in more detail in OECD TG 427 (OECD, 2004a). However, differences in skin thickness and permeability mean that many non-human *in vivo* studies can overestimate the absorption potential of a chemical in humans (Howes et al., 1996). This combined with animal welfare interests has led to the development of *in vitro* and *in silico* PA methods.

In vitro PA methods can use a variety of test materials, such as excised human or non-human animal skin, engineered skin construct, or a viable skin sample (Howes et al., 1996). In general, donor and receptor diffusion cells are used with skin positioned between and sampling is conducted at regular intervals from receptor cells. After the exposure period, remaining (unabsorbed) labeled test chemical and metabolites are measured by tape stripping, tangential skin slicing, or rubbing. This procedure is recorded in more detail in OECD TG 428 (OECD, 2004b).

In silico PA methods using Quantitative Structure Activity Relationships (QSAR) technologies are much more chemical-specific, as a model must be available for the particular chemistry of the test chemical, and PA information must be available for similar chemicals. A decision tree for the *in silico* PA process is shown in Fig. 1.

While PA measurement is a component of a number of different testing schemes, it is not usually conducted as part of the US Environmental Protection Agency's (EPA) High Production Volume Chemical Challenge Program (HPV Program). Instead, companies propose to fill "data gaps" from a list of screening-level tests, including toxicity tests, for chemicals manufactured or imported in quantities of 1 million pounds or more (Table 1). As part of the HPV Program process, test plans are reviewed by the EPA and the public. As the program progressed animal protection groups, namely the Physicians Committee

Table 1

ENDPOINT	GUIDELINE	ANIMALS[1]
Acute fish toxicity	OECD TG 203	40-120
Acute lethality-oral	OECD TG 425	3-10
Repeated dose-28/90 days	OECD TG 407/408	40-65
Combined repro/developmental screen	OECD TG 421	approx. 675
Combined repeated dose/repro/developmental screen	OECD TG 422	approx. 675

[1]Estimated number of animals consumed by following the OECD protocol.

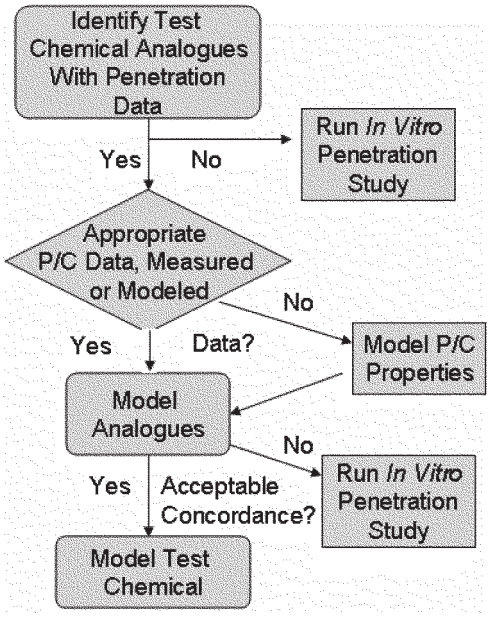

Fig. 1. Decision tree for determining whether to conduct an in vitro or in silico percutaneous absorption estimation method

for Responsible Medicine (PCRM) and People for the Ethical Treatment of Animals (PETA), reviewed test plans, submitted comments, and often contacted companies directly in order to suggest ways in which *in vivo* testing could be reduced or eliminated.

The Dow Chemical Company (Dow) submitted its test plan for Commercial Hydroxyethylpiperazine (CHEP), a commercial mixture of 1,4-piperazinediethanol, piperazine, hydroxyethylpiperazine, and water, in 2004. The plan proposed a reproductive and developmental screen (OECD 421) by the dermal route as the primary route of exposure for CHEP. During post-comment discussions, it was proposed to first conduct an estimation of the PA potential of CHEP. Since CHEP is made up of chemicals from a structurally-related family (amines), we decided to conduct a QSAR estimation using the EPA DERMWIN™ model.

Materials and methods

DERMWIN™ was developed by EPA and Syracuse Research Corporation, and is available publicly as part of the EPISuite suite of programs (USEPA, 2006). It uses this equation:

$$\log K_p = -2.72 + 0.71(\log K_{ow}) - 0.0061(MW)$$

where K_P = dermal permeability coefficient, K_{ow} = octanol/water partition, and MW = molecular weight of the chemical.

Physico-chemical data for the components of CHEP and 10 structural analogs were obtained. In addition, existing experimental human data was obtained for the analogs from Brian et al. (2005), Patel et al. (2001), and Sun et al. (1996). The analogs were then modeled using DERMWIN™, and the experimental

Table 2

	Piperazine		Hydroxyethylpiperazine		Dihydroxyethylpiperazine		
INPUT PARAMETER	HAND	WHOLE BODY	HAND	WHOLE BODY	HAND	WHOLE BODY	Unit
Contact surface area	420	16900	420	16900	420	16900	cm^2
Skin permeability coefficient	0.0000748	0.0000748	0.0000239	0.0000239	0.0000071	0.0000071	cm/hr
Contact time	0.25	0.25	0.25	0.25	0.25	0.25	hr
Residue conc. in product	220	220	517	517	275	275	mg/cc
Dermal uptake	1.70E+03	7.00E+04	1.30E+03	5.20E+04	2.10E+02	8.30E+03	ug/event
Contact events per day	1	1	1	1	1	1	#/day
Total uptake	1728	69527	1297	52205	206	8296	ug/day
Mean body weight	65.4	65.4	65.4	65.4	65.4	65.4	kg
Average Daily Dose	0.03	1.06	0.02	0.80	0.00	0.13	mg/kg/day

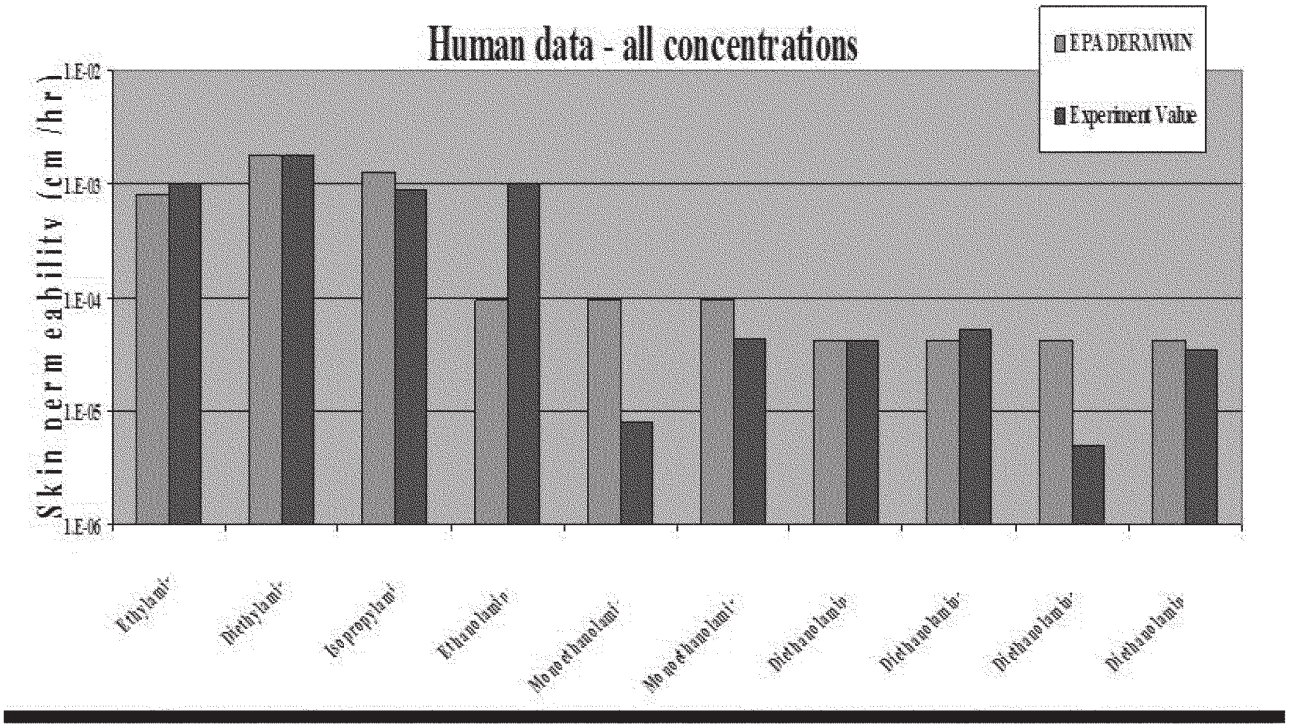

Fig. 2. Comparison between DERMWIN™ and experimental estimated skin permeability coefficient for chemicals structurally analogous to CHEP (ethylamine, diethylamine, isopropylamine, ethanolamine-1, monoethanolamine-2, monoethanolamine-3, diethanolamine-1, diethanolamine-2, diethanolamine-3, and diethanolamine-4).

and modeled data were compared to check for model applicability to the amine family of chemicals.

A satisfactory concordance justified modeling the PA of the CHEP components, and this was performed accordingly. The dermal permeability coefficient for each component was then used to calculate the estimated dermal uptake using this equation:

dermal uptake (mg) = K_p (cm/hr)*conc (mg/cc)*contact time (hr)*contact area (cm^2)

from Paustenbach (2000).

Results

Results of the comparisons of experimental and modeled PA data for the 10 analogs are shown in graphical form in Fig. 2. Ratios (experimental: modeled) ranged from 0.10 to 10.5 and averaged 1.71.

Modeled PA for the three chemical CHEP components (water was not modeled) is shown in Table 2. It is estimated that the total absorption of CHEP, with a whole body exposure for 15 minutes, would be ~2 mg/kg/day.

Discussion

The DERMWIN™ model accurately predicted human experimental data for the chemical analogs of CHEP. When DERMWIN™ was used to predict the PA potential for CHEP, conservative exposure values demonstrated a lack of significant systemic exposure potential.

The pertinent factors support the conclusion not to conduct a stand-alone dermal reproductive and developmental toxicity study. For the component predicted to have the highest absorbed dose, piperazine (1.06 mg/kg/day) a complete Screening Information Data Set exists. In addition, CHEP is produced and consumed within a closed system and workers use personal protective equipment.

Here we show that that principles of risk assessment, weight-of-evidence, and intelligent toxicology can help avoid dermal systemic toxicity testing while continuing to protect public and worker health. In this case, a combined reproductive and developmental toxicity screen was avoided, resulting in significant savings of animal lives, time, and money.

With the acceptance and continued use of *in vitro* and *in silico* approaches to measure percutaneous absorption, investigators and regulators should consider whether prerequisite PA testing can be utilized to avoid dermal systemic testing, including for acute, sensitizing, sub-chronic, and chronic endpoints, in other cases or screening programs.

Acknowledgements

We would like to thanks Dr. William Stott of Dow Chemical and Jessica Sandler of People for the Ethical Treatment of Animals for assistance with implementing the strategy and preparing the presentation.

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