Application of IndusChemFate to metabolism and excretion of DEET following human dermal exposure

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Abstract

IndusChemFate is an open-source Physiologically-Based Pharmaco-kinetic (PBPK) modeling program that simulates metabolite excretion following oral, pulmonary and dermal exposures. It was developed by IndusTox Consult under the auspices of Cefic LRI for the purpose of facilitating monitoring of chemical biomarkers of male adults' environmental exposures. In the current study, IndusChemFate is used to simulate the fate of N,N-diethyl-m-toluamide (DEET) and two of its primary metabolites in the body and urine following *in vivo* dermal exposure to the semi-volatile organic compound, using independently derived human metabolic data from an *in vitro* study. While there is qualitative agreement between the *in vivo* case study and the model, quantitative disagreement appears to result from overestimates of amount of compound initially absorbed into the stratum corneum.

Introduction

Why DEET?

DEET is a common insect repellent, available over-the-counter in many products and formulations and used by about one-third of Americans each year (Selim *et al.,* 1995). DEET is of interest here because it could be a surrogate for potentially more toxic pesticides. The properties of DEET that make it an attractive test compound are its:

- Availability over-the-counter and low toxicity
- Amenability to urinary biomonitoring
- Relatively rapid excretion

DEET Metabolism

In the body, DEET is metabolized into N-ethyl-m-toluamide (ET, 7.6-25.5% of metabolites) and N,N-diethyl-m-hydroxymethylbenzamide (DHMB, 24-42.4%), among others (Selim *et al.,* 1995). This metabolism is a parallel process (Figure 1a), but because of constraints within IndusChemFate requiring serial metabolic processes, DEET metabolism is simulated as two individual processes as in Figure 1b.

Figure 1a. Metabolism in the body (simultaneous, parallel)

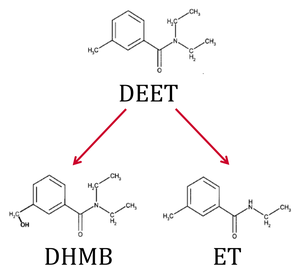
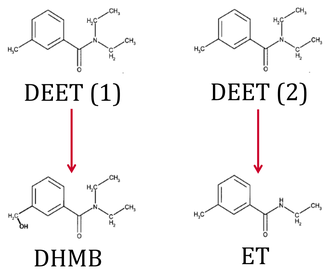


Figure 1b. Metabolism in the simulation (independent)



Adapted from Sudakin and Trevathan, 2003.

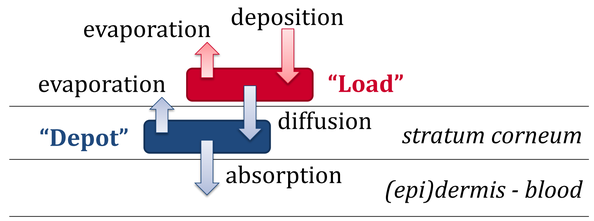
Methods

Dermal exposure to DEET was modeled in IndusChemFate (Figure 2) using the exposure conditions of Selim *et al.* (1995) with kinetic parameters based on Usmani *et al.* (2002). Physicochemical parameters were obtained from EPI Suite (US EPA, 2010).

IndusChemFate Model

- Runs in Microsoft® Excel
- Is open-source freeware (available for download at http://www.cefic-lri.org/lri-toolbox/induschemfate)
- Requires user-input physicochemical & kinetic parameters
- Estimates mass and concentration of compound and its metabolites in body tissues and fluids after oral, dermal and/or inhalation exposures

Figure 2. IndusChemFate Dermal Model



Adapted from Jongeneelen *et al.*, 2010a.

Selim *et al.*, 1995: *In Vivo* Exposure Conditions

Selim *et al.* exposed a 24-cm^2 section of the forearms of six volunteers to ~15 mg of DEET from undiluted technical grade compound. Forearms were washed at eight hours. Radiolabeled DEET was used to track the compound's metabolism and excretion. Subjects' urine was collected for five days.

Adapted Selim Conditions

	Value
Skin deposition, pure substance (mg/cm^2/hour)	6.25
Duration of skin exposure (hours)	0.1
Affected skin area (cm^2)	24

Usmani *et al.*, 2002: *In Vitro* Kinetic Parameters

In vitro enzyme kinetic assays incubated human liver microsomes (protein concentration of 1.5 mg/ml of buffered media) with DEET (1,000 µM); metabolites produced were analyzed using HPLC to determine kinetic parameters. The published V_{max} and K_m were adjusted to account for microsomal concentration in the liver compared to that in the media.

Adapted Usmani Parameters

	V_{max} (µMol/kg liver/hr)	Km (µMol/ L liver)
DHMB	31,000	1,800
ET	49,000	22,000

Results and Discussion

Sequential Metabolism

Individual metabolite modeling (as in Figure 1b) produces results different from when multiple metabolites are formed, which causes IndusChemFate to over-predict DEET in urine. Figures 3a and 3b show results of the independent metabolism simulations of DHMB and ET.

Figure 3a. Urine Concentration, DEET and DHMB

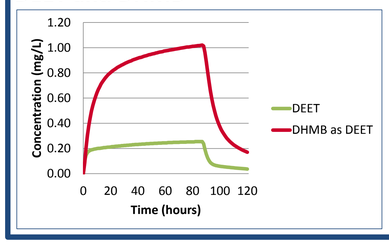
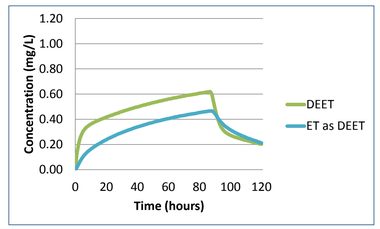


Figure 3b. Urine Concentration, DEET and ET



Comparison to Selim *et al.* Case Study

While the maximum excretion rate in IndusChemFate reasonably matches the Selim *et al.* data (Figure 4a), the cumulative mass of metabolites excreted (Figure 4b) is greater for the model than for the observations of Selim *et al.* due to a sustained higher rate of excretion.

Figure 4a. Rate of Excretion, Comparison of Selim and Modeled

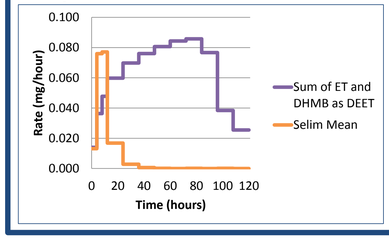
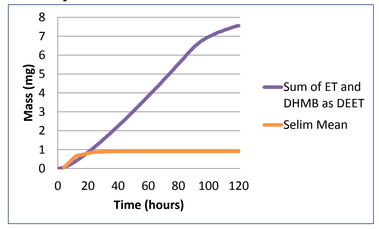


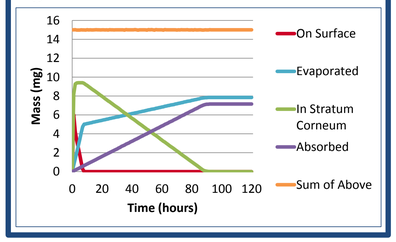
Figure 4b. Cumulative Excretion, Comparison of Selim and Modeled



IndusChemFate Mass Balance

Surface load evaporation, deposition in the stratum corneum (SC) and absorption of DEET are the various components contributing to the mass balance of the IndusChemFate simulation (Figure 5). The model predicts that the DEET moves very quickly from the surface of the skin to the SC, where it remains and creates a source for absorption through the 90th hour of observation. This large deposit in the SC is not supported by the Selim *et al.* study and accounts for the continuing high rate of excretion seen in Figure 4a.

Figure 5. Mass Balance, Modeled



IndusChemFate Limitations and Concerns

Although IndusChemFate has been shown to be effective at modeling exposure to volatile organic compounds (Jongeneelen *et al.,* 2010b), it is less fitting for this case study because it:

- Cannot model simultaneous, parallel metabolic processes
- Overestimates amount of compound initially absorbed into stratum corneum
- Has no provision for washing of skin surface
- Is oriented toward continuous rather than the batch exposures typical of laboratory studies

Acknowledgements

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References

Jongeneelen F, D Huizer, and W ten Berge (2010a). "IndusChemFate, A PBTK-model in MS-Excel applicable for worker and consumer exposure to multiple chemicals: User manual, version 1.5." IndusTox. <www.cefic-lri.org>. Accessed 6 Aug. 2010.

Jongeneelen F, D Huizer, and W ten Berge (2010b). "Simulation of blood and urine levels with a newly developed generic Physiologically Based Toxicokinetic (PBTK) model." Poster to be presented at: ISBL Symposium; 6-8 Sept. 2010; Helsinki, Finland. (Available for download at http://www.cefic-lri.org/lri-toolbox/induschemfate)

Selim S, RE Hartnagel, TG Osimitz, KL Gabriel, and GP Schoenig (1995). "Absorption, Metabolism, and Excretion of *N,N*-Diethyl-*m*-toluamide Following Dermal Application to Human Volunteers," *Fundamental and Applied Toxicology* 25(1): 95-100.

Sudakin, D.L., and W.R. Trevathan (2003). "DEET: A Review and Update of Safety and Risk in the General Population" *J of Toxicology* 41(6): 831-839.

US EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.00. United States Environmental Protection Agency, Washington, DC, USA.

Usmani KA, RL Rose, JA Goldstein, WG Taylor, AA Brimfield, and E Hodgson (2002). "In Vitro Human Metabolism and Interactions of Repellent *N,N*-Diethyl-*m*-toluamide," *Drug Metabolism and Disposition* 30(3): 289-294.