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Toxicokinetics of Pyrethroids in Humans: Consequences for Biological Monitoring

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Synthetic pyrethroids are among the most potent and effective insecticides available. Permethrin, cyfluthrin, and cypermethrin are successfully used in plant and storage protection, wood preservation, wool protection, disinfection, and for agricultural insect control. In addition, pyrethroids are also applied indoors in vector control and in public health.

Pyrethroids are acting on the axons in the peripheral and central nervous system of mammals and insects. Their neurotoxic effect is determined by a prolonged opening of the Na channel which evokes a repetitive nerve action associated with hyperactivity, tremor, ataxia, convulsions, and possible paralysis (Aldrige 1990). For humans, pyrethroids are much less toxic than other insecticides. The ADI values (acceptable daily intake) are 0.02 mg/kg body weight for cyfluthrin and 0.05 mg/kg body weight for permethrin and cypermethrin (FAO/WHO 1993). Nevertheless, a variety of reversible symptoms such as headache, dizziness, nausea, irritation of the skin and mucosa, and paresthesia have been reported after human exposure (He et al. 1988, 1989; Wieseler et al. 1998). In general, ester pyrethroids are rapidly detoxified in humans by hydrolysis, oxidation, and conjugation. After oral, inhalative or dermal intake, both acid and alcohol moieties of the pyrethroids are metabolized into carboxylic acids. The water-soluble metabolites and their conjugates are excreted with the urine by the same renal mechanisms the body uses to remove end products from intermediary metabolism. In addition, no accumulation of pyrethroids in tissues have been reported in the literature so far (WHO 1989).

For the risk assessment of pyrethroids, the design of field studies, and estimations of the extent of exposure, detail knowledge about pesticide elimination kinetics is of prime interest. Furthermore, since urinary elimination is an individual process, the calculation of e.g. elimination half-lives gives insight into the variability of human toxicokinetics. Volunteer exposure studies with cypermethrin have shown that its urinary metabolites are very usefull for the investigation of elimination kinetics in man (Eadsforth and Baldwin 1983; Eadsforth et al. 1988). Moreover, in oral and inhalative exposure studies with cypermethrin and cyfluthrin (Woollen et al. 1992; Leng et al. 1997) first-order elimination kinetics were observed. Validation of toxicokinetic models in field studies is an important contribution toward a correct risk assessment of xenobiotics. In this article, a simple one-compartment open model of urinary elimination kinetics is presented, and its relevance for the design of biological monitoring for pyrethroids in field studies is demonstrated.

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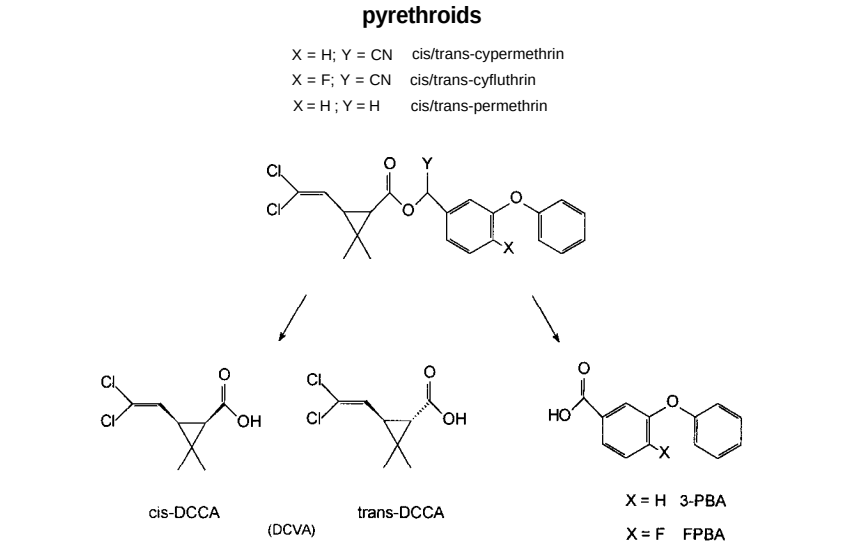

Figure 1. Relationship between structures of important pyrethroids and their urinary metabolites. The prefix cis or trans refers to the relative orientation of the dichlorovinyl and carboxylate groupings about the cyclopropane ring of the acid moiety.

MATERIALS AND METHODS

Chemically, pyrethroids like permethrin, cypermethrin, or cyfluthrin are esters of halo-substituted chrysanthemic acid (trans- or cis-DCCA) and specific alcohols (e.g., 3-phenoxybenzyl alcohol). For permethrin the chiral centers exist only in the acid moiety. An increase in potency was archieved in compounds like cypermethrin and cyfluthrin by introducing of a cyano group at the benzylic carbon atom of the alcohol moiety. Examples of this series of pyrethroids consisting of racemic mixtures of up to eight different stereoisomers (Figure1. In practice, commercial formulations are specified by the isomeric ratio of the active insecticides. For permethrin a trans to cis isomeric ratio of 1.5, for cypermethrin 1.0, and for cyfluthrin 1.4 are typical values of technical formulations and were used in this study. For indoor pest control, the pyrethroids were formulated as an emulsifiable concentrate (EC). All spray-emulsions for indoor knapsack spraying were prepared and applied according to manufacturer's instructions by healthy professional pest control operators (PCOs). The study protocol and written consent forms were reviewed and approved by a medical ethics committee.

For a determination of the cumulative elimination kinetics of cypermethrin and cyfluthrin, urine samples of two male pest control operators were collected at frequent time intervalls (12 h to 24 h) after work in the exposure-free time (over the weekend or holidays). The total trans- to cis-DCCA ratios were determinded in urine samples of five PCO's exposed to different pyrethroids. The urine was collected for up to 4 days after exposure. The effort involved in instructing workers to provide repeated urine collections is justified by the greatly improved accuracy of the resulting estimate of absorption of the insecticide.

To quantitate major pyrethroid metabolites (Figure 1), all samples were analyzed according to the GC/MS method described elsewhere (Kühn et al. 1996). The limit of detection (LOD) for all metabolites was 0.0025 µg in an urine sample of 5 mL (LOD: 5 µg/L) and the precision of the analytical method was 10 % relative standard deviation. In addition, the analytical method was fully validated and subjected to external quality assurance. Aliquots of urine samples were stored at -21° C until analyzed. The total volumes and the creatinine levels of urine samples were recorded. All urinary concentrations were corrected for analytical recovery. Linear regression analysis was performed using SigmaPlot (Jandel Scientific, Vers. 2.0). The linear relationship between two parameters was measured by the coefficient of determination (r^2) for four independent determinations at $p < 0.01$.

RESULTS AND DISCUSSION

The most common way to characterize the kinetics of xenobiotics has been to represent the body as consisting of a number of compartments, even though these compartments have no apparent physiological or anatomical reality. Although the design of more complex pharmacokinetic models are more desirable, then require physiological (e.g., blood flow rate, tissue volume) and biochemical parameters (e.g., rate of biotransformation in a particular tissues, concentration in blood). In the case of pyrethroids, such human parameters are inexact or unknown. In addition, human subjects are often unwilling to submit to repeated blood sampling. Fortunately, urinay elimination data can also be used to determine half-lives of xenobiotics.

The simplest case of a classical toxicokinetic is the one-compartment model. This model depicts the body as a fictitious homogeneous unit. The elimination of a pesticide or its metabolites whose disposition is described by such a simple model occurs by a first-order process; that is, the rate of elimination at any time is proportional to the amount of chemical in the body at that time. In the case of the pyrethroids, renal elimination of their metabolites is the major pathway of detoxification. The determined concentrations of metabolites in urine samples collected at that time t are multiplied by the urine volumes to give amounts excreted. Moreover, the eliminated amounts can be expressed either as detoxificated metabolites or as their corresponding pesticide equivalents. Conversion factor is the stochiometric ratio molecular weight pyrethroid to molecular weight metabolite. Thus, the amount of metabolized pesticide molecules A_t in the body and the amount of metabolites U_t in an urine collection interval1 are related to the total amount A_0 or U_∞ which will be recovered from all urine samples (Derendorf and Garrett 1987). The relation can be expressed as

$$A_0 = A_t + U_t = U_\infty \quad \textbf{(1).}$$

The mathematical expression of this first-order process is given by

$$U_t = U_\infty (1 - e^{-(ket)}) \quad \textbf{(2).}$$

Equation (2) is an exponential equation where k_e is first-order elimination rate constant and t is the time of urine sampling.
The logarithm of equation (2) yields

$$\log (U_\infty - U_t) = \log U_\infty - (k_e t)/2.303 \quad \textbf{(3).}$$

Equation (3) has the general form of an expression describing a straight line where $\log U_\infty$ is the intercept, and $- k_e/2.303$ is the slope of the line.

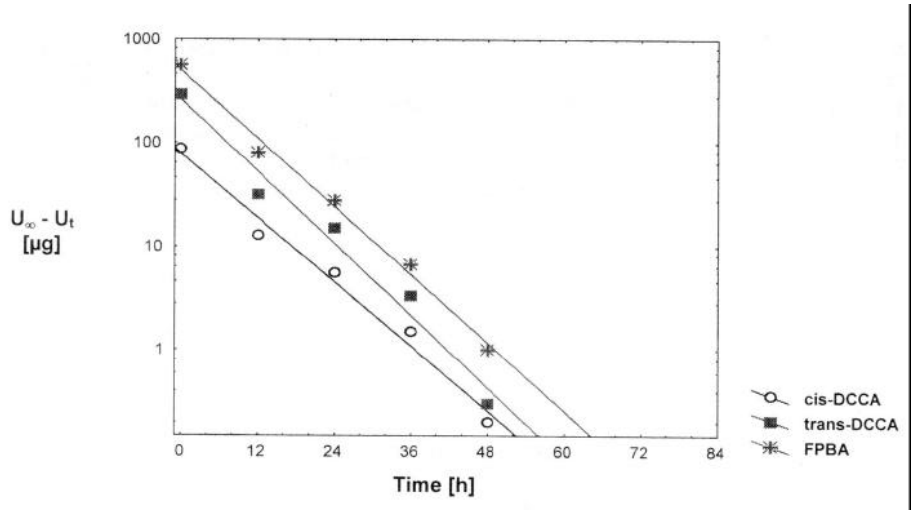

Figure 2. The Σ-minus plot of renal elimination of the cyfluthrin metabolites of a PCO

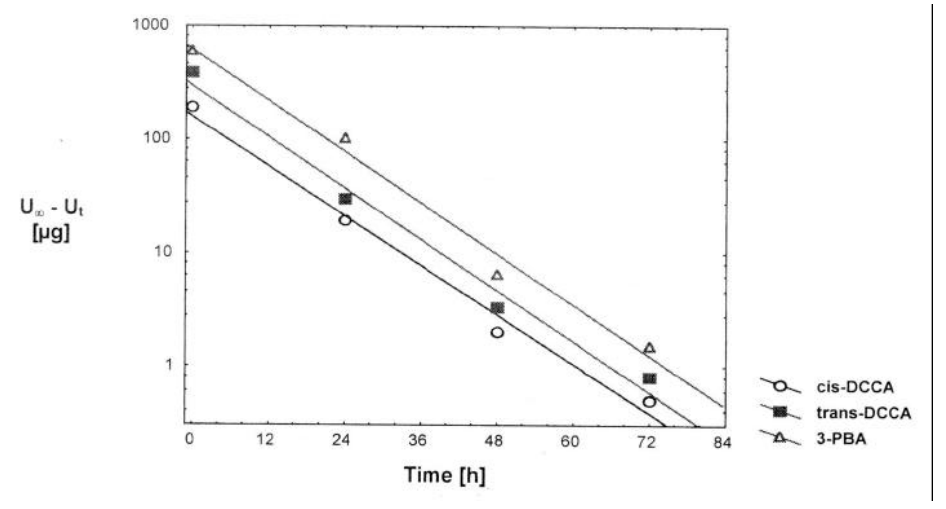

Figure 3. The Σ-minus plot of renal elimination of the cypermethrin metabolites of a PCO

The apparent first-order elimination rate constant k_e can be determined from the slope of the log $(U_\infty - U_t)$ versus time plot (Figures 2 and 3). The apparent renal elimination half-life $t_{½}$ can be calculated from the expression

$$t_{½} = \ln 2 \; / \; k_e \quad \textbf{(4).}$$

For compounds eliminated by a pseudo first-order kinetics, the time required for the urinary amount to decrease by one half is constant and independent from the absorbed dose. Nevertheless, excretion from the body by a first-order process is theoretically never complete. However, 97 to 99 % of a xenobiotic is eliminated during five to seven half-lives, which for practical purposes can be viewed as complete elimination. Therefore, it is necessary to collect urine for seven half-lives and not lose any samples. This method is called the C-minus plot, because the amount remaining to be excreted is calculated by deducing the amount excreted for each collection period from the total amount excreted during up to seven half-lives (Figures 2 and 3, Table 1).

Table 1. First order elimination half-lives ($t_{½}$) are obtained from linear regression analysis of Figure 2 and 3

cypermethrin	r^2	p	k_e [h^{-1}]	$t_{1/2}$ [h]
cis-DCCA	0.982	0.005	0.084	8.25
trans-DCCA	0.985	0.008	0.086	8.06
3-PBA	0.987	0.007	0.087	7.97
mean ± SD			0.086 ± 0.02	8.09 ± 0.14
cyfluthrin	**r^2**	**p**	**k_e [h^{-1}]**	**$t_{1/2}$ [h]**
cis-DCCA	0.982	0.001	0.119	5.82
trans-DCCA	0.974	0.002	0.131	5.25
FPBA	0.992	0.004	0.126	5.50
mean ± SD			0.125 ± 0.06	5.52 ± 0.29

Another method of determining the apparent elimination half-life of a compound from urine is to plot semi-logarithmically the rate of excretion dU/dt against the midpoint time of the successive collection periods.

In the one-compartment open model the mathematical expression of the rate of urinary excretion is given by

$$dU/dt = k_e A_o e^{-(ke\ t)} \quad \textbf{(5).}$$

In practice, the exact rate of excretion dU/dt can be estimated by measuring the amount of eliminated metabolites AU in urine samples of successive collection intervalls Δt. The time t of sampling is replaced by the midpoints t_{mid} of the collection intervall. The logarithm yields the linear expression

$$\log (\Delta U/\Delta t) = \log (k_e A_o) - (k_e t_{mid})/2.303 \quad \textbf{(6).}$$

Linear regression analysis can be performed on peak urinary excretion rate versus midpiont time (Derendorf and Garrett 1987). Again, the half-life can be estimated from the slope of the plot inserted in expression (4).

In contrast, this method does not require the collection of all urine samples to estimate U_∞ but its accuracy greatly depends on how often, and how meticulously, continuous sampling of urine is carried out. Examples of this method applied in pyrethroid exposure studies are given by Woollen et al. (1992) and Leng et al. (1997).

Previous volunteer studies with cypermethrin (Eadsforth and Baldwin 1983) have shown that on average 64 % (49 % of the cis- and 78% of the trans-isomer) of an oral dose (0.25 - 1 mg) were excreted in urine as their metabolites cis- and trans-cyclopropanecarboxylic acids (cis/trans-DCCA) in the subsequent 24 h period after dosing. In a second cypermethrin study (Eadsforth et al. 1988) subjects excreted after repeated oral intake (0.25, 0.75, and 1.5 mg daily for five days) 45 % of the cis-isomer and 72% of the trans-isomer dose in the subsequent 24 h period.

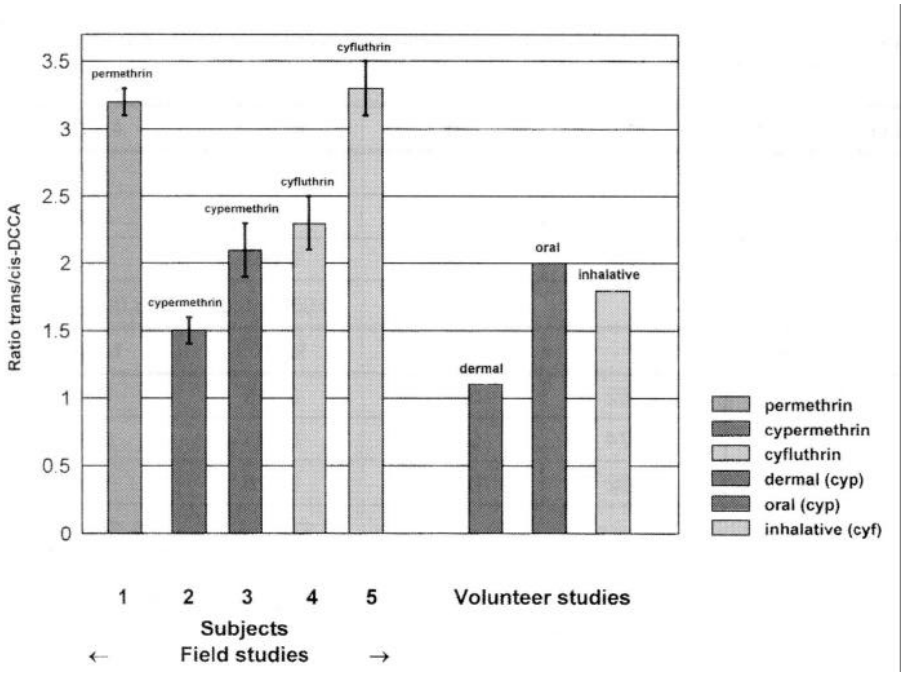

Figure 4. Comparison between of the total urinary trans- to cis-DCCA ratio for field and volunteer studies (mean ± SD, n = 4) following exposure to various pyrethroids. Values of volunteer studies were taken from literature (dermal and oral: Woollen et al. 1992, inhalative: Leng et al. 1997)

In contrast, after a single dermal application of 25 mg cypermethrin on the forearm only 0.1 % of the dosage was excreted within 72 h as urinary metabolites (Eadsforth et al. 1988). In another human study using a different design (Woollen et al. 1992), 36 % of the oraly administred cypermethrin (3.3 mg) and 1.2 % of the dermal dose (31 mg) were eliminated in urine as their metabolites. Peak urinary elimination rates occured between 8 h and 24 h after oral and between 13 h and 36 h after dermal dosing. The mean elimination half-lives of the total metabolites were 16.5 h (range: 11 - 27 h) after oral and 13.0 h (range: 8 - 22 h) after dermal exposure. In a volunteer study (Leng et al. 1997) with the cyfluthrin, 93 % of the metabolites were eliminated within the first 24 h after inhalative exposure of 160 µg/m^3. Peak excretion rates occured between 0.5 and 3 h after inhalation. The mean elimination half-lives of the metabolites were 6.9 h (range: 3.3 - 12.2 h) for cis-DCCA, 6.2 h (range: 2.9 - 11.6) for trans-DCCA, and 5.3 h (range: 3.1 - 6.5 h) for FPBA.

The results from our presented field study are consistent with the data obtained from the volunteer exposure studies described above. Cyfluthrin is eliminated slightly more rapidly than cypermethrin (Table 1) and linear regression analysis is adequate for the estimation of the half-lives (Figures 2 and 3). These findings supported the assumption that the excretion of structurally related ester pyrethroids from the human body (Figure 1) can be described by first-order kinetics.

Previous studies of human pyrethroid exposure demonstrated a marked difference in metabolic patterns following oral and inhalative versus dermal

absorption. Following oral and inhalative intake, the ratio of trans- to cis-DCCA was approximately 2 : 1 (Woollen et al. 1992; Leng et al. 1997) whereas after dermal application an average trans- to cis-DCCA ratio of 1.0 : 1.2 was observed (Woollen et al. 1992). Figure 4 shows the urinary metabolite ratios in field studies with five PCO's exposed to different pyrethroids. The pyrethroid emulsions were sprayed indoors on average for 3 to 5 h. The PCO's wore protective suits but no special protective masks during spraying. In all cases the total urinary amount of trans-DCCA was always higher than cis-DCCA. The results suggest that oral and inhalative intake are major routes of absorption. Other field studies with cypermethrin and cyfluthrin confirm that dermal intake may not be the main route of absorption (Woollen 1993; Leng et al.1997). These findings are maybe explained by the special method of application which involved knapsack spraying at head height leading to potentially higher oral-inhalative exposure. Consequently, the use of protective suits is only effective to minimize skin exposure.

Once the profile of the metaboilites is established and cumulative elimination is completed, an estimation of the absorption of the pesticide is possible. The equation used for the conversion from metabolite into absorbed pyrethroid equivalents was derived by Woollen (1993):

$$P_{\infty} = (U_{\infty} M_1 / M_2)\ 100/R \quad \textbf{(7)}$$

where P_{∞} [mg] is the total amount of absorbed pyrethroid by the body, M_1 is the molecular weight of the pyrethroid, M_2 is the molecular weight of the metabolite, U is the total amount [mg] of this metabolite in all urine samples collected, and R [%] is the average recovery of the exposure pathway in volunteer studies. In the case of the PCO exposed to cypermethrin (M_1 = 415 mg/m*M;* isomeric trans/cis-ratio 1.0), the elimination profile of the metabolites shown in Figure 3 (C-minus plot) indicates a significant oral intake (U_{∞}: trans-DCCA > cis-DCCA, ratio of trans/cis-DCCA = 2). From oral volunteer studies with this pesticide an average recovery ranging from 36 - 64 % has been observed. A suitable metabolite for an estimation of the total absorbed pesticide amount is 3-PBA (M_2 = 214 mg/m*M*), since this compound is derived from cis- and trans-cypermethrin. After seven half-lives (57 h), the renal elimination of cypermethrin metabolites can be considered as complete with U_{∞} of 3-PBA of 0.59 mg (2.8 µ*M*). All data inserted into equation (7) gave an estimated amount of absorption P_{∞} ranging between 1.79 mg (R = 64 %) and 3.18 mg (R = 36 %) total cypermethrin equivalents. An estimate of the absorbed amount P_{∞} of the single isomere trans-cypermethrin based on trans-DCCA (U_{∞} = 0.38 mg) ranged between 1.20 mg (R = 64 %) and 2.13 mg (R = 36 %). Assuming that no cis-to-trans conversion occurs, the total absorbed amount of cis- and trans-cypermethrin (1:1 pesticide mixture) responsible for such a metabolic profile is approximately twice as much (2.40 mg - 4.26 mg). For cypermethrin, a comparison with the individual daily ADI of 3.5 mg 170 kg body weight shows that this exposure is limited.

However, these calculations should not be considered as exact determination of an actual intake since the predicted amount of absorption P_{∞} largely depends on the choice of R. In addition, dermal absorption of pyrethroids with its small values of urinary metabolic recovery (R < 1 %) can lead to unexpected high levels of exposure. There is a difference between the topical applied dose (adsorption)

and the fraction which is able to pass through the skin barrier or is subjected to cutaneous metabolism (Kao and Carver 1990). This is supported by recovery of significant amounts (> 53 %) of non-absorbed cypermethrin from frequent washings after dermal application (Eadsforth et al. 1983). Furthermore, considerable difficulties exist also in predicting the extent of inhalative absorption of pyrethroids from urinary metabolic profiles. In addition, especially in environmental medicine, other sources of oral exposure (e.g., hand to mouth contact, pyrethroid residues on food) must also be considered (Woollen 1993). As a consequence for field studies, simple calculations based on equation (7) are only suitable for an accurate assessment of pyrethroid absorption if oral intake is the major route of exposure.

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