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ORIGINAL ARTICLE

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Dermal absorption of chlorpyrifos in human volunteers

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Abstract ***Objective***: The methods and results are described of a study on the dermal absorption of chlorpyrifos (CPF) in humans established via urinary excretion of the metabolite 3,5,6-trichloro-2-pyridinol (TCP). ***Methods***: Two dermal, single, doses of CPF were applied in two study groups (A and B) each comprising three apparently healthy male volunteers who gave their written informed consent. The clinical part of the study was conducted in compliance with the ICH Guideline and the EC principles of good clinical practice (GCP). An approximately 0.5 ml dilution of CPF in ethanol was applied to an area of ~100 cm^2 of the volar aspect of the forearm, resulting in doses of either 5 mg (A) or 15 mg (B) of CPF per study subject. Duration of dermal exposure was 4 h, after which the non-absorbed fraction was washed off. The following samples were collected at pre-determined intervals for the determination of either CPF or its metabolite TCP: dosing solutions, wash-off fractions and urine samples collected up to 120 h after dosing. ***Results***: A relatively large fraction of CPF (42%–67% of the applied dose) was washed off from the exposed skin area. Application of either 5 mg (A) or 15 mg CPF (B) resulted in the total urinary excretion of 131.8 μg (A) or 115.6 μg (B) of TCP 120 h after dosing. This indicated that 4.3% of the applied dose has been absorbed (A), while in group (B) no significant increase in urinary TCP (115.6 μg) was established. The latter indicates that an increase in the dermal dose at a fixed area does not increase absorption, which suggests that the percutaneous penetration rate was constant. Further, it was observed that the clearance of CPF by the body was not completed within 120 h, suggesting that CPF or TCP was retained by the skin and/or accumulated in the body. A mean elimination half-life of 41 h was established. ***Conclusion***: The results show that daily occupational exposure to CPF may result in accumulation of CPF and/or its metabolites, possibly resulting in adverse effects.

Keywords Pesticide · Percutaneous penetration · Urinary metabolites · Volunteers · Occupational exposure

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Introduction

Chlorpyrifos (*O*,*O*-diethyl-*O*-[3,5,6,-trichloro-2-pyridyl] phosphorothioate) is a widely used organophosphorus insecticide with numerous agricultural crop and urban pest control uses. Biologically, chlorpyrifos is a plasma cholinesterase inhibitor. In 1995 an estimated 4–6 million kg was used for crop protection and another 5–8 million kg was used for non-agricultural purposes in the USA (Hines and Deddens 2001). Daily, a large number of agricultural workers is exposed dermally and/or by inhalation to this substance. In humans, chlorpyrifos is moderately toxic (EXTOXNET 1996). Chlorpyrifos is metabolised rapidly, and the kidneys eliminate its principal metabolites. The major metabolite found in urine is 3,5,6,-trichloro-pyridinol (TCP). Field studies among applicators and residents (Hines and Deddens 2001; Krieger et al. 2001) have shown that exposure to chlorpyrifos may occur, due to its widespread use, outdoors as well as indoors. Inevitably, occupational workers are unintentionally and unavoidably exposed, which can be evidenced by the presence of

TCP in urine. Work activities and exposure levels of 41 termiticide applicators using chlorpyrifos resulted in a positive linearly correlated relationship of airborne chlorpyrifos levels and urinary TCP levels (Hines and Deddens 2001). In another study performed by Griffin et al. (1999, 2000) it was found that, on average, half of the applied amount of chlorpyrifos could be recovered from the skin surface but only 1% could be recovered as urinary metabolites. However, due to the in vivo nature of the experiment no information was obtained about the rate at which chlorpyrifos penetrated the skin (Griffin et al. 1999). Investigators from the same group (Griffin et al. 2000) studied the rate of dermal penetration using an in vitro human skin model. The major fraction of the topically applied amount of chlorpyrifos appeared to be retained in the skin. Only one pharmacokinetic study of chlorpyrifos in humans has been reported in which the blood and urine elimination half-life of TCP after high, oral and dermal doses of diluted chlorpyrifos was estimated (Nolan et al. 1984). The elimination half-life was calculated to be approximately 27 h. The objective of the present study was to determine the dermal absorption of chlorpyrifos in humans under controlled conditions and to establish the relationship between the dose of chlorpyrifos and its metabolite TCP excreted in urine after topical application of two doses of chlorpyrifos to a fixed skin area. The results will be helpful in establishing a biological monitoring strategy to be used in field practice and to assess the risk for agricultural workers more accurately.

Materials and methods

Test substance and chemicals

Chlorpyrifos (CAS registry number 2921-88-2) with a purity of >99.2% was purchased from Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands. Methanol, HPLC grade, Rathburn Chemicals, was obtained via Brunschwig Chemie, Amsterdam, and The Netherlands. All other chemicals used were of the highest commercial grade available.

Subjects and study procedures

Six healthy male subjects participated in this study. They were recruited from the pool of volunteers at TNO Nutrition and Food Research. Candidate subjects were informed verbally on the study, about the aim, the study procedures, the constraints, the insurance cover and the financial compensation. In advance they received written information on the study that fully covered the oral information given. They underwent medical screening, which included a physical examination, an interview with the medical investigator, based on a completed health questionnaire, and a visual inspection of the application sites. All subjects participated with written informed consent. The demographic and anthropometric data of the subjects, on average, were: age 30.5 years (range 20–42 years), body weight 75.1 kg (range 67–81 kg), height 183 cm (range 180–188 cm). The study protocol was drafted in accordance with, and the study was conducted according to, the ICH Guidelines for good clinical practice (GCP) (ICH topic E6), adopted on 1 May 1996 and implemented on 17 January 1997, and the Principles of GCP for the conduct of clinical trials, according to the European Union Directive 91/507/EC. The study protocol was approved by the independent Medical Ethics Committee (METC-TNO). The clinical part of the study was conducted from 19–25 September 2000.

Study design

Six eligible subjects participated with informed consent in this study and were divided into two sub-groups, A and B. Application of the study substance was performed as described earlier (Meuling et al. 1991, 1993). In brief, an area of approximately 100 cm^2 (5.95×16.90 cm) was delineated at the volar aspect of one forearm of each volunteer; approximately 0.5 ml of a dilution of chlorpyrifos in ethanol was applied by pipette to this area, resulting in approximately 5 mg (group A) or 15 mg (group B) of chlorpyrifos per subject, and was spread uniformly with a glass slide. The amount to be applied was determined as the established concentration of the two test solutions multiplied by the volume of the applied test dilution minus the dose retained on the glass slide. Duration of the dermal exposure was 4 h, during which the coated area was not covered or occluded. To avoid accidental removal of the formulation due to contact with clothing all subjects were instructed to avoid any such contact. During exposure the subjects were confined to a designated room (exposure cabin) at constant temperature (T) and relative humidity (RH) ($T = 23°C$; RH = 50%), under constant medical surveillance by a nurse. Exposure was terminated by our washing off the amount of chlorpyrifos still present on the skin surface (see below).

Skin wash samples

At 4 h after the onset of exposure, we washed off any excess of the applied amount still present on the skin by wiping ten times with cotton wool rolls wetted with water. To avoid the possible influence of the skin absorption process due to extraction of the skin or penetration-enhancing effects by substances, we used water instead of a soap containing washing fluid. Five wash-off samples (two consecutive washes pooled per sample) were stored at 4–8°C for a maximum of 2 days, after which the determination of chlorpyrifos was initiated. A typical example of the results of the washing procedure is depicted in Fig. 1.

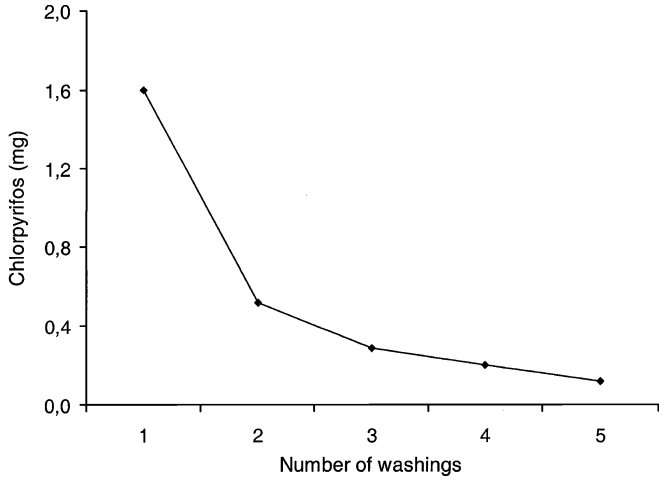

Fig. 1 Typical example of the results of the skin-washing procedure. Five wash-off samples (two consecutive wetted cotton wool rolls pooled per sample) were used

Urine samples

From the onset of the experiment the subjects collected all produced urine in various fractions, covering the periods from 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, and 96–120 h after the onset of exposure. Additionally, a pre-exposure sample had been collected. In total, nine samples per subject were collected, most of them at home. Subjects recorded, on the labels of the appropriate containers, the time of voiding. They were instructed to store the samples in a cool and dark place awaiting transportation to TNO. When the urine samples were received, voiding times and the amount of urine, by weight, were registered.

Analysis of chlorpyrifos

All materials used for application, i.e. the dosing solution, the glass slides and the cotton wool rolls for the skin wash, were analysed for chlorpyrifos. Chlorpyrifos was analysed by isocratic, reversed phase HPLC and UV-detection at 289 nm. Methanol was used for extraction of chlorpyrifos from the glass slides and the cotton wool rolls. The samples were analysed by a system consisting of an autosampler (Separations, Model Spark), a pump (Separations, Model-300), a UV-detector (Spectra-Physics, model SP 8490) and a column (Bio-Sil C18, 150 mm × 4.6 mm × 5 μm). The injected volume was 15 μl, and the flow rate was 1.0 ml/min, including water:acetonitrile 1:3 (v/v) as eluents.

The limit of detection (LOD) for chlorpyrifos was defined as noise (top–bottom) increased with three-times half noise (top–bottom), based on analyses of blanks. The LOD for chlorpyrifos from the cotton wool rolls and glass slides was 0.037 and 0.074 mg/l, respectively. The limit of quantification (LOQ) was defined as noise (top–bottom) increased with ten-times half noise (top–bottom), based on analyses of blanks. The LOD for the cotton wool rolls and glass slides was 0.088 and 0.177 mg/l, respectively. Between-day coefficients of variation for cotton wool rolls and glass slides were $< 6\%$. The mean recovery rates established (in fourfold) in spiked cotton wool rolls, wetted with water, at two levels of chlorpyrifos, low 0.58 mg and high 11.6 mg, were $> 92\%$ and $> 99\%$, respectively.

Analysis of TCP

The metabolite TCP was measured in urine by capillary gas chromatography and mass selective detection. To analyse the samples we used an autosampler (AS-2000) and a gas chromatograph (Trace-2000, Carlo Erba) and a Finnigan Trace-MS. One hundred microlitres of concentrated HCl was added per 3 ml urine. Subsequently, the samples were de-conjugated in a water bath at 90°C for 1 h. After being cooled to room temperature, 4 μl of the internal standard (γ-hexachlorocyclohexane) was added. Subsequently, 3 ml of hexane was added, and the samples were extracted for 15 min head-over-heels in a rotary mixer. Thereafter, the samples were centrifuged for 5 min at 5°C (ca. 2,100 *g*) after which the hexane layer was carefully removed. This extraction procedure was repeated once, and the two hexane fractions were combined. To the pooled hexane layers a small amount of Na_2SO_4 (anhydrous) was added by spatula. After the samples had been mixed and centrifuged for 5 min (ca. 2,100 *g*), the hexane layer was pipetted off and transferred to a glass tube. The hexane was evaporated under a gentle stream of nitrogen until dryness. Subsequently, 500 μl toluene and 5 μl (bis)-trimethylsilylacetamide (BSA) were added to the tubes to derivatise the samples (1 h incubation at room temperature). The sample was transferred to a 2-ml vial with a glass insert, capped and analysed. The gas chromatographic conditions were the following: column Zebron ZB-1, 30 m × 0.25 mm ID × 0.25 μm df, oven temperature, initial 90°C for 1 min then at 5°/min to 200°C for 5 min, then at 20°/min to 250°C for 5 min. Injection volume 1 μl, injector temperature 200°C. Type: splitless, split flow 50 ml/min, splitless time 1.0 min. During the analysis runs, selective ions were monitored (mass-ions 181 and 183 for γ-hexachlorocyclohexane and 254 and 256 for derivatised 3,5,6-trichloro-pyridinol, respectively). The LOD was determined as the average TCP concentration raised with three-times the standard deviation: 5.4 μg/l. For the LOQ the average concentration was taken raised with ten-times the standard deviation: 13.4 μg/l. The between-day coefficient of variation for the method was $< 6\%$.

Results

Six male volunteers who gave informed consent were selected and divided into two groups (A and B). Two

doses of chlorpyrifos were applied, the concentrations of which were determined by HPLC analysis: 10.8 ± 0.07 mg/ml and 32.39 ± 0.39 mg/ml were used for groups A and B, respectively. Approximately 500 µl of each of these two dosing solutions was applied to the skin (100 cm^2) of the forearm of each of the three subjects and was uniformly spread with glass slides. In Table 1 the amount of test formulation applied is shown.

All six participants completed the study and none showed signs or symptoms of adverse dermal or systemic effects related to the applied dermal dose of chlorpyrifos. The net amount applied was calculated from the applied amount minus that recovered from the glass slides used to spread out the dosing solutions over the skin areas. This amounted to 5.39 ± 0.01 mg and 16.16 ± 0.01 mg for groups A and B, respectively.

Recovery of test formulation in skin wash

At 4 h after application of the test formulation the exposed skin area was washed by being wiped ten times with cotton wool rolls, wetted with water. In Table 1 the total fraction of recovered chlorpyrifos from the skin of each volunteer is shown and expressed relative to the applied dose. Subtraction of the amount of chlorpyrifos recovered in the skin wash from the amount applied to the skin yields the amount that is defined as the 'potentially absorbed dose' (PAD) by the body. This PAD is also expressed in Table 1.

Table 1 Recovered chlorpyrifos in skin wash samples

Subject	Applied net amount (mg)	Amount washed off		Potentially absorbed dose (mg)
		(mg)	Percentage of applied dose	
Group A				
01	5.38	1.54	28.6	3.8
02	5.39	2.56	47.4	2.8
02	5.39	2.68	49.8	2.7
Mean ± SD	5.39 ± 0.01	2.26 ± 0.63	42.0 ± 11.6	3.1 ± 0.6
Group B				
04	16.15	10.04	62.2	6.1
05	16.14	12.81	84.6	2.3
06	16.16	8.66	53.6	7.5
Mean ± SD	16.15 ± 0.01	10.50 ± 2.12	66.8 ± 16.0	5.3 ± 2.7

Concentration of TCP in urine samples

During the 120 h from the onset of exposure to chlorpyrifos, all urine produced had been collected in nine fractions (including a blank) per subject. The amount of each urine sample was determined by weighing (N.B. the density of urine was assumed to be 1 kg/l). The total amount of TCP excreted was determined through analysis of the urinary concentration of TCP multiplied by the total weighed amount of urine. In all urine samples

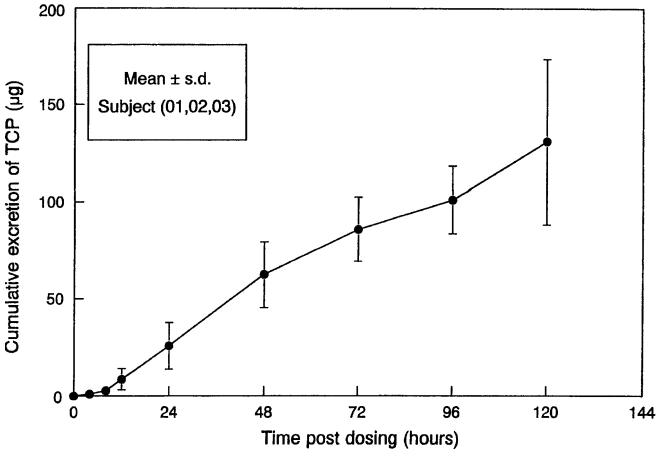

Fig. 3 Mean (± SD) cumulative urinary TCP (µg) excreted amount per urine sample collected (study group A)

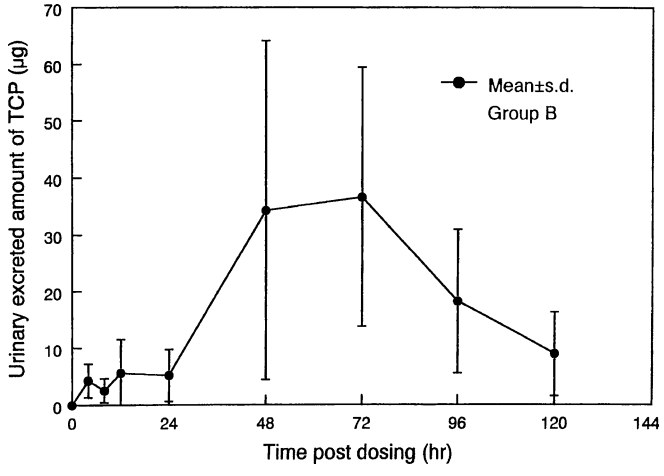

Fig. 2 Mean (± SD) amount of urinary TCP (µg) excreted per urine sample collected (study group B)

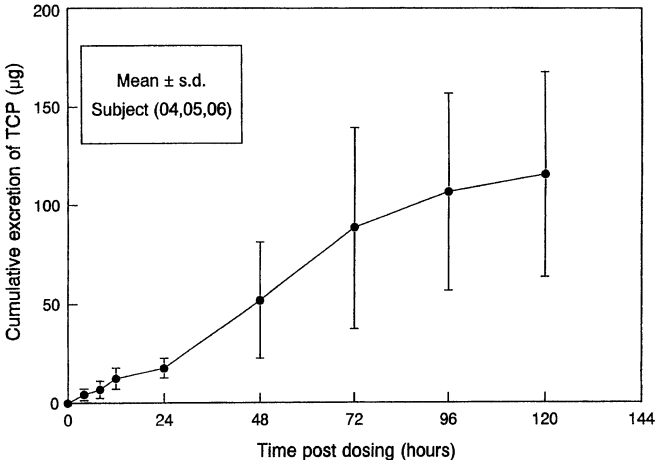

Fig. 4 Mean (± SD) cumulative urinary TCP (µg) excreted amount per urine sample collected (study group B)

up to120 h after dosing (except for one sample of subject 04) TCP levels above background could be established. The cumulative total amount of urinary TCP was calculated to be, on average, 131.8 ± 42.7 μg (range: 94.3–178.3 μg) and 115.6 ± 52.8 μg (range: 62.9–168.6 μg) for groups A and B, respectively. In Fig. 2 the individual cumulative urinary excreted amount over 120 h per subject in group B is shown. On average, the level of urinary TCP peaked in the 48–72 h sampling. Thereafter, the excreted amount per 24 h gradually decreased but was still above background level for the 96–120 h period.

Expressing the urinary excretion results as the (mean ± SD) cumulative excreted amount of TCP versus time for the two subgroups, we obtained the following values (Figs. 3 and 4).

Calculated absorbed amount of chlorpyrifos

Since, in this study, all urine produced per subject was collected, the total amount of TCP excreted over 120 h could be established and was used to predict the amount of chlorpyrifos absorbed by the body.

Taking the molecular weights of CPF (MW = 350.6) and TCP (MW = 198.4) and the excreted amount of TCP into account, we calculated the systemically absorbed amount of chlorpyrifos.

Results are summarised in Table 2.

Discussion

Although studies with animal and in vitro models may provide useful information with respect to the assessment of pesticide exposure, the most reliable approach to study pesticide exposure and percutaneous penetration is a human volunteer study (Meuling et al. 1991; Ross et al. 2000). Studies with human volunteers have the following advantages over animal studies: (a) daily practice situations can be simulated and (b) direct translation of the results is possible without extrapolation and the use of uncertainty factors. Additionally, subjects can be selected in such a way that a particular 'population at risk' can be studied in more detail. In the present study chlorpyrifos was applied to the skin (volar aspect of the forearm), and its absorption into the body was investigated. As the acceptable daily intake (ADI) of chlorpyrifos is 0.01 mg/kg b.w., it was estimated from literature on dermal absorption of chlorpyrifos that a single application of the dosing solutions to a skin area of 100 cm^2 would not result in systemic levels exceeding five-times the ADI. At 4 h after application the amount of chlorpyrifos that was recovered from the skin was determined. By subtracting this amount from the applied dose, we calculated the potentially absorbed dose of chlorpyrifos. The actual absorbed dose was calculated after determination of the levels of the principal metabolite, TCP, in urine collected up to 120 h after dosing.

Application of 5 mg chlorpyrifos to ~100 cm^2 skin for 4 h resulted in a PAD of ~3 mg, and application of 15 mg to the same area resulted in a potentially absorbed dose of ~5 mg chlorpyrifos. This means that a threefold increase in the applied dose of chlorpyrifos to the same area resulted in not more than a 1.7-fold increase in the PAD. The dose actually absorbed, calculated from the levels of urinary TCP, was around 220 μg chlorpyrifos for both doses, indicating that a threefold increase in the applied dose did not result in increased absorption. Apparently, a relatively large fraction of chlorpyrifos is retained by the body, possibly in the stratum corneum. The results suggest that the percutaneous penetration rate is constant and was not affected by the increase in the dermal dose.

To date, two other human volunteer studies with chlorpyrifos have been reported. In a study by Nolan et al. (1984), on average, 416 mg chlorpyrifos (5 mg/kg b.w.) in an organic solvent was applied topically to an area of approximately 100 cm^2. In that study the exposure period was 12–20 h, dependent on the subjects' personal bathing/showering habits. No washed-off amount was established. It could be shown that 1.28% (± 0.83) of the applied dose was recovered in urine as TCP, and the elimination half-life in urine was calculated to be 26.9 h.

In a more recent study described by Griffin et al. (1999) approximately 28.6 mg of chlorpyrifos in water was applied to volunteers for 8 h on an area of ca. 78 cm^2 of the volar aspect of the forearm. After dosing,

Table 2 Absorbed amount of chlorpyrifos calculated relative to the applied dose and the PAD

Subject	Cumulative excreted TCP (μg)	Calculated equivalent chlorpyrifos (μg)	Applied dose CPF (mg)	Absorbed CPF relative to the applied dose (%)	Potentially absorbed dose (mg)
Group A					
01	94.3	166.6	5.38	3.10	3.8
02	123.0	217.3	5.39	4.03	2.8
03	178.3	315.0	5.39	5.84	2.7
Mean ± SD	131.8 ± 42.7	232.9 ± 75.5	5.39 ± 0.01	4.32 ± 1.39	3.1 ± 0.6
Group B					
04	168.6	297.9	16.15	1.73	6.1
05	63.0	111.3	16.14	0.69	2.3
06	115.2	203.5	16.16	1.26	7.5
Mean ± SD	115.6 ± 52.8	204.2 ± 93.3	16.15 ± 0.01	1.23 ± 0.52	5.3 ± 2.7

blood and urine samples were collected for 24 h and 100 h, respectively, and analysed for CPF and the sum of the alkylphosphate and alkyldithiophosphate metabolites of CPF. The results showed that approximately 1% of the applied dermal dose was recovered as the urinary metabolites, while, on average, 53% of the applied dermal dose was washed off after 8 h. The elimination half-life was calculated to be, on average, 41 h (range 27–88 h). The maximum concentration of urinary metabolites occurred, on average, at about 24–48 h after dosing. Clearly, the dose per skin area in the two reported studies differ widely; however, the relative absorbed doses were approximately equal. This is in contrast with the usual observation that the relative absorbed dose will decrease with increasing dermal doses [European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) 1993].

In order to investigate the dose–response relationship for realistic exposures, we applied, in the present study, approximately 5 mg/100 cm^2 and 15 mg/100 cm^2 to two groups of volunteers, resulting in doses of 0.067 mg/kg b.w. and 0.199 mg/kg b.w., respectively. The amounts applied in the present study were somewhat lower than those applied in the study by Griffin et al. (1999) and were much lower than those in the study by Nolan et al. (1984). The maximum concentration of urinary TCP peaked at approximately 48–72 h after dosing for both dermal doses. The percentage of the applied dose recovered in urine was established to be $4.32 \pm 1.4\%$ and $1.23 \pm 0.52\%$ for the 5 mg and 15 mg dosing experiment, respectively. These findings are in agreement with those reported by Nolan et al. (1984) and Griffin et al. (1999). In addition, the skin-wash results (53%) in this study are in line with our results (range 42–68%). Based on the results achieved in our study, an elimination half-life of, on average, 41 h (range 39–42 h) could be calculated, which is also in excellent agreement with the results from the aforementioned study.

In the literature, the results of a dermal application study are frequently reported as 'percentages of the applied amount excreted in urine'. Nevertheless, it has been stated that expression of absorption data as a percentage of the applied dermal dose is hardly applicable, since these percentages will increase with decreasing dermal doses (ECETOC 1993).

The PAD, defined as the applied amount minus the amount washed off after a certain exposure period, is a more reliable way to express and compare results (Meuling et al. 1991, 1993).

It was observed in our study (Figs. 2, 3 and 4) that the urinary excretion of TCP was not complete within 120 h after dosing, which means that the clearance of chlorpyrifos by the body had not been completed. This suggests that chlorpyrifos, or its metabolite TCP, may have been retained by the skin and/or had been accumulated in the body. Although both Nolan et al. (1984) and Griffin et al. (1999) reported urinary elimination half-lives of 26.9 h and 41 h, respectively, neither of them concluded that chlorpyrifos may be retained by the skin or may be accumulated in the body. Even Nolan et al. (1984) concluded that "based on the kinetics following a single dermal dose, chlorpyrifos and its principal metabolite, TCP, have a low potential to accumulate in man on repeated exposure". In general, it takes five half-lives to reach nearly complete clearance ($> 96.9\%$) of a substance from the body. For a half-life of approximately 41 h this indicates that it will take more than 8 days before a complete clearance will be achieved. In this respect it is also important for it to be realised that the calculation of the systemically absorbed dose of chlorpyrifos after dermal application, using the amount of urinary TCP, is bound to lead to underestimation, since the clearance was not completed and no corrections were made for incomplete excretion or metabolism (Feldman and Maibach 1974). The latter correction factor can be established only by an i.v. administration study that follows urinary excretion.

On the basis of the results of an in vitro percutaneous penetration study (Griffin et al. 2000) it has been concluded that the majority of a dermal dose of chlorpyrifos (56–66%) was still present at or in the surface of the skin 24 h after application, and that chlorpyrifos could be retained in a skin reservoir (stratum corneum) and, therefore, may be released over a longer period. These findings are in line with our results and support the conclusion that chlorpyrifos may be retained by the skin. After removal from the skin surface, chemicals remaining within the skin can become systemically available (Reddy et al. 2000). However, the fraction of chemical in the skin that eventually enters the body depends on the relative rates of percutaneous transport and epidermal turnover (i.e. stratum corneum desquamation). A number of investigators have dealt with this phenomenon with regard to assessing the risk of chemicals more accurately (Reddy et al. 2000; Jung et al. 2003; Yourick et al. 2004). From an occupational point of view exposure to pesticides occurs frequently and, in certain periods, more or less on a daily basis; thus, repeated exposure to a substance is common practice. With respect to chlorpyrifos, the reported long half-life may play a crucial role when health risks for workers have to be assessed by biological monitoring via urinary excretion. Interpretation of these results may not be a straightforward process due to the influence of possible accumulation of the substance under investigation. Therefore, the setting up of a sampling strategy for biological monitoring is warranted, with precautions taken to avoid repeated exposure during the sampling period.

Conclusions

It is concluded from this study that:

- A relatively large amount of chlorpyrifos was washed off the skin after 4 h of application, at an average range of 42%–67% of the applied dose.

- Only relatively small amounts of dermally applied chlorpyrifos penetrated the skin: 4.3% (A) and 1.2% (B) when 5 mg and 15 mg were applied, respectively, for 4 h, which was evidenced by urinary TCP levels.
- An increase in the dose applied (from 5 mg to 15 mg) to a fixed area did not increase skin penetration, suggesting that the percutaneous penetration rate was constant.
- A mean urinary elimination half-life of 41 h was established.
- Urinary excretion of TCP was not complete within 120 h after dosing, which means that the clearance of chlorpyrifos by the body had not been completed.
- The data indicate that chlorpyrifos or its metabolite, TCP, was retained by the skin and/or accumulated in the body.
- For risk assessment purposes possible accumulation of chlorpyrifos and/or its metabolite, TCP, has to be taken into account.

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