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Estimating occupational exposure to the pyrethroid termiticide bifenthrin by measuring metabolites in urine

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Abstract

The control of subterranean termites in Australia is predominantly through the application of chemical barriers in the soil beneath and surrounding buildings. The chemicals used to repel or kill termites are the organophosphorus insecticide, chlorpyrifos, and the synthetic pyrethroid, bifenthrin. These are applied through surface sprays and subfloor injection by licensed pest control operators. To determine the exposure of these personnel to these pesticides it is most usual to measure airborne concentrations or dermal deposition rates. However, to support information obtained from these methods it is often appropriate to determine the amount of the chemicals absorbed, using biological monitoring techniques including measurement of the chemicals or their metabolites in urine. While there are effective techniques for the monitoring of chlorpyrifos exposure by measuring either the alkyl phosphate or trichloropyridinol metabolites, there have been no published reports of suitable methods to measure bifenthrin metabolites in urine. This paper describes an extraction and HPLC–UV method used to simultaneously measure the urinary excretion of 2-methyl-3-phenylbenzoic acid (MPA), a metabolite of bifenthrin, and 3-phenoxybenzoic acid (PBA), a metabolite of several other common pyrethroid insecticides, with a detection limit for each of 2.5 ng/ml. The paper also describes the pilot application of this method to a study of South Australian pest control operators handling bifenthrin. MPA ranged from 1.8 to 31.9 μg/g creatinine and PBA from 1.3 to 30.0 μg/g in the urine of pest control workers. MPA was detected in urine of control workers without bifenthrin exposure only at low levels (1.0–1.4 μg/g creatinine), but PBA was found in both at higher levels (1.2–61.1 μg/g creatinine).

Keywords: Pyrethroids; Bifenthrin

1. Introduction

Subterranean termites are responsible for significant damage to buildings in Australia. The application of chemical termiticide barriers has traditionally been the most commonly used method to protect buildings from termite infestation, and these are applied by spraying the entire area of soil beneath and around the perimeter of structural foundations either pre- or post-construction. The applied termiticide solution spreads through the soil, repelling termites or killing them on contact. Since 1995 organochlorines have been banned for use as termiticides in Australia [1], mainly due to concerns regarding environmental persistence and toxicity. The two insecticides currently approved for use in termite protection are the organophosphate chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)

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phosphorothioate, CAS No. 2921-88-2), and the synthetic pyrethroid ester, bifenthrin ((2-methyl[1,1′-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate, CAS No. 82657-04-3). These are each used for ~50% of termite treatments in South Australia. Consequently, the potential for occupational and environmental exposures to these insecticides is widespread.

Acute exposure to OPs causes inhibition of acetylcholinesterase in the brain, neuromuscular junction and peripheral nerves, characterised by central nervous system (CNS) effects and muscarinic and nicotinic effects in the periphery. Exposure to OPs is also a potential cause of longer term damage to the nervous system [2,3]. While plasma cholinesterase and erythrocyte acetylcholinesterase have been used to monitor the biochemical effects of exposure of Australian termiticide applicators to chlorpyrifos [4,5], there are methods available for the analysis of chlorpyrifos metabolites in urine [6,7].

In general, pyrethroids are much more effective against, or toxic to, a wider range of insect pests, and have a much lower mammal to insect toxicity ratio than their organophosphate, organochlorine, and carbamate counterparts [8]. Pyrethroids which lack a cyano moiety are classified as type I pyrethroids, and those which contain a cyano moiety are classified as type II pyrethroids [9], each of which exhibit distinct toxic syndromes in mammals [8–11]. Toxic effects are caused largely through inducing repetitive nerve activity, mediated through pyrethroid interaction with membrane sodium channels [8]. Adverse effects induced by pyrethroids are therefore a consequence of neuronal hyperexcitability [11]. Studies of workers spraying pyrethroids (deltamethrin, fenvalerate) reported increased symptoms including abnormal facial sensations (such as burning and tingling), dizziness, headache, nausea and fatigue, and loss of appetite, associated with exposure [12,13]. There have been no published reports on the human health effects of exposure to bifenthrin, a type I pyrethroid (Fig. 1), although it has been reported to cause tremor in a 52-week chronic oral toxicity study in dogs [14].

Monitoring of airborne pesticide, and the measurement of dermal deposition, provide evidence of the potential for chemical absorption. Biological monitoring of parent chemical or metabolites in urine is a

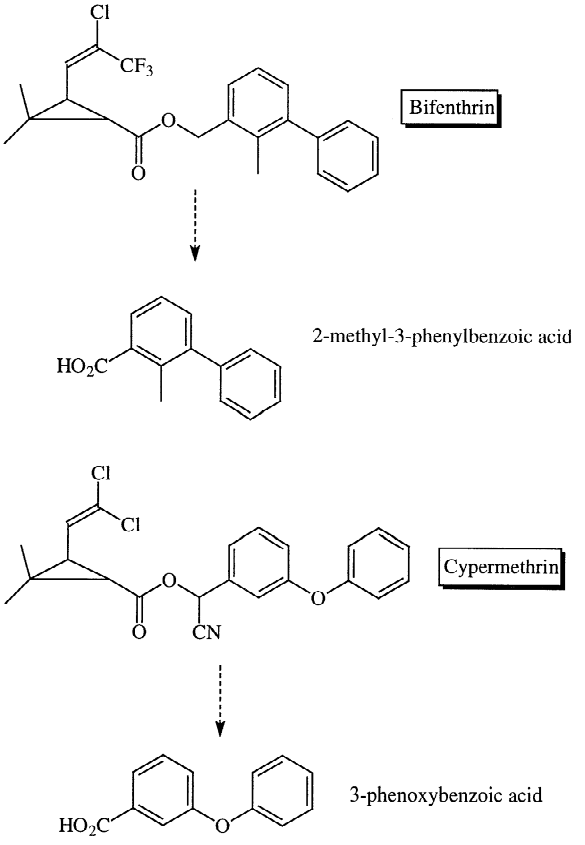

Fig. 1. Metabolism of bifenthrin and cypermethrin.

complementary approach that may reveal the influence of individual factors, including work practices, on absorption. There is currently no simple method for measuring the systemic uptake of bifenthrin. Major bifenthrin metabolites in rat plasma were reported as the parent compound, the hydrolysis product 2-methyl-3-phenylbenzylalcohol, and the further oxidised product 2-methyl-3-phenylbenzoic acid (MPA) [15]. MPA is a cleavage product analogous to 3-phenoxybenzoic acid (PBA), a metabolite of many synthetic pyrethroids including cypermethrin, deltamethrin, permethrin and fenvalerate [16–18]. While studies of human exposures to pyrethroids including cypermethrin have measured the urinary excretion of the cyclopropane acid moiety [19], this has been shown to represent 0.1% of the dermally administered dose of cypermethrin [20], and urinary PBA may be a better estimate of dermally absorbed cypermethrin [17]. Similarly, the

measurement of urinary MPA may be an effective marker of human exposure to bifenthrin. The aim of this project was to develop an HPLC method for the quantitation of worker exposures to termiticide containing bifenthrin.

2. Methods

All reagents were obtained from Sigma unless otherwise indicated

2.1. Subject selection

A total of 13 individuals, all male, were recruited for this study, and each subject gave informed consent to participate (Table 1). Of the nine termiticide applicators, eight were employed by the same pest control company, and one was self-employed. While all pesticide workers recruited are exposed to other pesticides, such as chlorpyrifos, inclusion criteria for subjects was the predominant use of bifenthrin. In South Australia, bifenthrin is used solely for the protection of buildings against termite infestation. Application modes included site pretreatment, where foundations were sprayed with termiticide prior to the pouring of concrete; crawl space treatments of existing homes, where applicators entered the space between the ground and the wooden floor to spray termiticide onto the ground; and subfloor treatment, where termiticide is pressure injected into the soil beneath concrete slab foundations of existing homes.

The four control subjects all worked for the same local government authority and reported the use of other pyrethroids (permethrin, deltamethrin), but not bifenthrin. These workers also reported the use of a range of herbicides, including glyphosate. Ages for all participants ranged from 32 to 55 years (mean±S.E.M.; 41±11.5 years), exposure was not controlled, and participants were asked not to modify their usual work practices or routine. All participants were provided with an information sheet regarding the study, a consent form, and a questionnaire to be completed on the same day as sample collection. Questionnaires were designed to obtain information such as personal details, smoking status, medication, diet, work practices, the use of personal and respiratory protective equipment (PPE, RPE), and a pesticide exposure history. The pesticide exposure history sought details on the number of pesticide applications in the preceding 14 days and the preced-

Table 1
Characteristics of termiticide applicators and control subjects

Subject	Age (years)	Insecticides used	Application type	No. bifenthrin applications		Average application time (min)	Bifenthrin use/week (l)	RPE
				14 Days	24 h			
S1	33	Bif, CP, +	PT, CS	20	3	40–120	25	HF
S2	–	Bif, CP, +	PT, CS, SF	30–40	2	10	2	HF
S3	33	Bif, CP	CS	0	0	5–15	2–4	HF
S4	38	Bif	PT	4	4	20	60	None
S5	43	Bif, CP, +	CS	50	4	15	5	HF
S6	41	Bif, CP	CS	40	10	15–30	2	HF
S7	53	Bif	PT	52	11	20–40	60	None
S8	46	Bif	PT	46	9	10–40	40–100	HF
S9	55	Bif	PT, CS, SF	60	10	5–10	6	HF
C1	36	Del, Per, +	Spray	0	0	NA	NA	None
C2	44	Del, Per, +	Spray	0	0	NA	NA	None
C3	36	Del, Per, +	Spray	0	0	NA	NA	HF
C4	32	Del, Per, +	spray	0	0	NA	NA	None

Bif, bifenthrin; CP, chlorpyrifos; CS, crawl space; Del, deltamethrin; HF, half-face respirator; NA, not applicable; Per, permethrin; PT, pretreatment; SF, sub-floor; +, other pesticides.

ing 24 h, the average application time, and the amount of bifenthrin concentrate used each week.

All subjects and controls were requested to provide three spot urine samples on the same day. Samples were to be collected just prior to starting work, in the afternoon or at the end of the work shift, and a final sample between 2 and 6 h after work. Urine samples were stored frozen until analysis.

2.2. Synthesis of 2-methyl-3-phenylbenzoic acid (MPA)

2-Methyl-3-phenylbenzyl alcohol (2-methylbiphenyl-3-methanol; Aldrich, Castle Hill) (2.2 g, 11.1 mmol) was added in one portion, with stirring, to potassium permanganate (2.7 g, 17.1 mmol) dissolved in acetone (30 ml) and water (20 ml). The reaction was exothermic, and after 1 h the acetone was removed in vacuo. The residual aqueous solution was treated with concentrated H_2SO_4 (8 ml) in water (50 ml) and sufficient sodium metabisulfite to dissolve the MnO_2. The acid was filtered and dried (2.2 g, 94%). It was obtained in white needles after crystallisation from aqueous ethanol, shrinks at 155°C, m.p. 157–158°C. The acid is very soluble in ethanol and chloroform.

2.3. Measurement of urinary MPA and PBA

MPA and PBA were analysed using a modification of the reverse phase HPLC method of Mourot et al. [21]. Urine samples (10 ml) were added to glass tubes containing 100 μl HCl (4 *M*) and 2 ml chloroform. After mixing for 1 min, samples were centrifuged for 5 min at 500 *g*, and the organic phase transferred to a second glass tube containing 100 μl NaOH (4 *M*) and 1 ml water. After mixing for 1 min and centrifugation (500 *g*, 5 min), 0.5 ml of aqueous extract was mixed with 0.5 ml of HPLC mobile phase (acetonitrile:1% H_2SO_4; 1:1) and 20 μl was injected onto an ODS2-I (C_{18}) column (250×4.6 mm; SGE, Ringwood, Australia) protected with an ODS2 guard column (SGE, Ringwood, Australia) for isocratic HPLC analysis. HPLC was performed using a GBC system (GBC Australia, Regents Park, Australia) comprising an LC1650 Advanced Autosampler, LC1150 pump, and an LC1205 UV–Vis detector operating at 230 nm. Mobile phase flow-rate was 1.5 ml/min. Analyte peaks were sharp and symmetrical, and peak heights were compared with those of standard solutions of MPA and PBA in water that were carried through the extraction and analysis procedures.

2.4. Measurement of urinary creatinine

Creatinine was measured using the reversed-phase HPLC method of Huang and Chiou (1983) as modified by Muirhead et al. [22] in which 50 μl of clear urine was added to 2 ml of mobile phase ($NH_4H_2PO_4$ (20 m*M*):acetonitrile, 9:1). Of this, 10 μl was injected onto a column packed with Partisil-10 SCx (Whatman, Clifton, NJ, USA) protected by a C_{18} guard column (SGE, Ringwood, Australia). Samples were eluted at a flow-rate of 1.5 ml/min and sample detection was by UV absorbance (254 nm). Peak heights were compared with those of standard solutions of creatinine.

3. Results

3.1. Synthesis of MPA

The identity of synthesised MPA was characterised by NMR and mass spectroscopy. NMR spectra were determined on a Varian Gemini 300-MHz instrument. Shifts are relative to internal TMS. Assignments are supported by appropriate SCS, Hippo CNMRS prediction program, and 2D NMR HETCOR, and long range HETCOR mass spectra were determined using a Kratos MS25RFA mass spectrometer.

3.1.1. ^1H NMR ($CDCl_3$)

δ 8.02, dd, C_4-H: 7.48–7.24, m, Ar-H: 2.5, s, Ar-CH_3.

3.1.2. ^{13}C NMR ($CDCl_3$)

δ 174.08, COOH: 144.35, C_3: 141.76, C_1': 138.26, C_2:134.61, C_4: 130.62, C_6: 130.18, C_1: 129.65, $C_{3,5}''$: 128.48, $C_{2,6}''$: 127.44, C_4': 125.61, C_5: 18.83, CH_3.

3.1.3. MS

70 eV, found 212.0840, $C_{14}H_{12}O_2$ requires 212.08372: M^+ 212 (100%), 167 (74%), 166 (40.8%), 165 (55.3%), 152 (23.4%).

3.2. HPLC assay of MPA and PBA

The HPLC analysis of Mourot et al. [21] was modified to permit the rapid elution of both metabolites and their effective baseline separation, together with a sensitivity suitable to detect workplace exposures. PBA, with a retention time of ~7 min, was effectively separated from MPA (retention time ~9 min) with a detection limit of 2.5 ng/ml (signal:noise ~5:1). Fig. 2a,b shows representative standard chromatograms at concentrations of 25 ng/ml and 1 μg/ml. Retention times were unchanged for urine sample extracts and there were no interferences from endogenous compounds (Fig. 3). Standard concentration curves of both metabolites were linear over this quantitation range (Fig. 4a,b). The coefficients of variation (C.V.) were estimated from five each of the MPA and PBA calibration curves. Coefficients of variation were determined at the lowest and highest standard concentrations and at two concentrations between, and the average C.V. for each line and mean C.V. of five lines determined. The mean C.V. of analyses performed on the same day was below 3%, with replicate sample coefficients of variation below 2%. Mean inter-day C.V.s were ~14%. Extracted calibration curves were prepared for each batch of samples analysed.

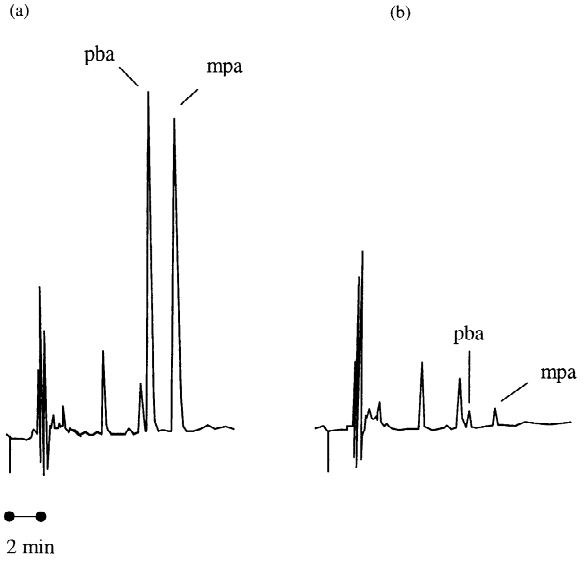

Fig. 2. HPLC traces of standard solutions of MPA and PBA; (a) 1 μg/ml and (b) 25 ng/ml.

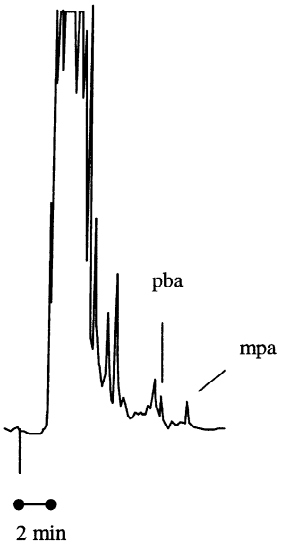

Fig. 3. HPLC trace of extracted urine sample from termiticide applicator indicating MPA and PBA at ~75 and 50 ng/ml, respectively.

3.3. Monitoring occupational bifenthrin and other pyrethroid exposure

Termiticide workers reported between 0 and 60 bifenthrin applications in the previous 2 weeks, with 0–11 applications in the immediately preceding 24 h (Table 1). Approximate use of bifenthrin concentrate ranged from 2 to 100 l/week, with average application times ranging from 5 to 120 min. All workers reported using personal protective clothing (overalls, boots and gloves) and all but two (S4 and S7) reported using half-face mask type respiratory protection.

MPA was found at measurable amounts (2.5 ng/ml) or greater in at least one urine sample from six out of nine termiticide applicators and three out of four controls (Table 2), although MPA was found in a higher range (1.8–31.9 μg/g creatinine) in 37% of samples from termiticide workers than in samples from controls (1.0–1.4 μg/g creatinine). Six of the control urine samples (50%) contained measurable PBA ranging from 1.2 to 61.1 μg/g creatinine.

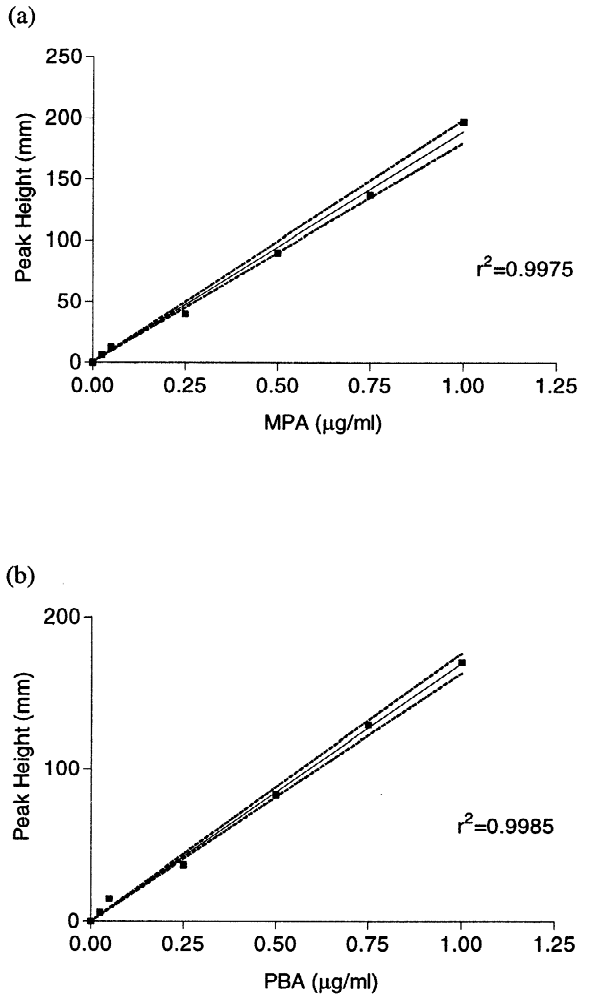

Fig. 4. Calibration standard curves of (a) MPA and (b) PBA (with 95% confidence intervals).

Seven termiticide applicators had measurable PBA in at least one urine sample, ranging from 1.3 to 30.0 μg/g creatinine.

4. Discussion

While bifenthrin and chlorpyrifos currently share the market for termite treatments in Australia, a perception within the pest control industry in South Australia that the pyrethroids are less acutely toxic than OPs may lead to reduced care and vigilance in bifenthrin application. An independent and objective method for measuring bifenthrin exposure, such as the measurement of its metabolites in urine, would be beneficial in providing evidence of insecticide absorption in individual workers. The levels of exposure determined in this way may then be related to work patterns and practices in order that appropriate interventions may be implemented as necessary. This paper describes the simultaneous measurement of urinary MPA and PBA as indicators of exposure to bifenthrin and other pyrethroid insecticides. The analytical method is simple, rapid (HPLC run time is ~13 min) and sufficiently sensitive to detect MPA and PBA at 2.5 ng/ml, with sensitivity potentially able to be increased by increasing the extracted urine volume.

Worker exposures to bifenthrin estimated from self report of treatment activity appeared to yield inconsistent results. For example, worker S6 reported 40 bifenthrin applications in the previous 2 weeks with ten applications in the preceding 24 h, yet reported only 2 l of concentrate used per week. Similarly subject S4 reported four applications in the previous 14 days (all in the preceding 24 h) but reported 60 l of concentrate per week. While it is possible that these figures may reflect the scale of pesticide applications performed, it is possible that the estimates of use provided by termiticide applicators are inaccurate and may distort assessments of their personal insecticide exposure, since a survey of Australian termiticide applicators found that they underestimate their risks of exposure to insecticide [23].

In this study, most workers reporting frequent bifenthrin application in the previous 24 h to 2 weeks (9–11 applications in 24 h or 40 or more applications in 2 weeks; subjects S6, S7, S8, S9) or heavy bifenthrin concentrate use (over 60 l/week; S4) were generally found to have at least one urine sample containing measurable MPA. Subject S3, who reported bifenthrin use, also indicated that more chlorpyrifos was used than bifenthrin, and that no bifenthrin had been applied in the previous 2 weeks. This worker yielded no measurable MPA in urine. MPA was absent or found in only low amounts in all four control subjects. This suggests that the assay has the potential to discriminate between workers with high and low exposures to bifenthrin. Further work is in progress to determine the relationships between

Table 2
Urinary MPA and PBA in termiticide applicators and control subjects

Subject	Urinary MPA concentration (μg/g creatinine)			Urinary PBA concentration (μg/g creatinine)		
	am	pm	Post-shift	am	pm	Post-shift
S1	ND	ND	ND	11.8	1.3	9.3
S2	ND	ND	1.8	2.3	ND	ND
S3	ND	ND	ND	30.0	12.2	8.2
S4	ND	31.9	ND	ND	ND	6.0
S5	ND	ND	ND	ND	ND	2.3
S6	22.2	4.4	23.2	ND	7.1	12.3
S7	ND	2.0	ND	3.9	11.4	15.9
S8	13.3	ND	ND	ND	ND	ND
S9	3.8	7.8	6.2	17.3	2.3	3.9
C1	ND	ND	ND	ND	61.1	ND
C2	ND	ND	1.0	ND	30.2	18.6
C3	ND	1.4	ND	1.2	2.2	ND
C4	ND	ND	1.2	ND	ND	19.2

am, pre-shift (or morning); pm, afternoon or end of shift; post-shift, 2–6 h post-shift; ND, not detected (<2.5 ng/ml).

worker exposure to bifenthrin and the time course of the appearance of MPA in urine.

While the level of chemicals in urine samples may be considered to represent an integrated estimate of the exposure of workers over the immediately previous hours or days, depending on the elimination half-life of the chemical concerned, the use of spot urine samples is fraught with difficulties. Not least of these is the problem of standardising urine output for flow and dilution effects. In this case we have chosen to express MPA and PBA relative to creatinine, as this is a widely used standardising technique that we have used in many previous studies. Creatinine concentrations of samples collected here ranged from 0.5 to 3.2 g/l, generally within the acceptable range that is not over-diluted or highly concentrated [24].

A more critical sampling parameter is the time between exposure and urine sampling. The levels of MPA and PBA in pre-work morning samples may have included some proportion of metabolites that had persisted since pesticide exposures earlier in the week. The final sample, potentially collected up to 6 h after exposure, may have been an under-representation of the ''true'' exposure as an unknown amount of metabolite would have been eliminated in the intervening period. The end of shift sample may be a more representative time for sampling, but would underestimate exposures if these had occurred only during the morning. It would be preferable to collect 24-h samples in any further biological monitoring studies of these workers. Alternatively, several urine samples throughout each day of a work week would indicate the time course of appearance of metabolites and indicate the most appropriate sampling regime for a more precise application of this method or the identification of a biological exposure index for bifenthrin.

No airborne or dermal exposure data were collected in this study, with which to compare biological monitoring data. Concurrent studies of termiticide applicators during a single application of chlorpyrifos in South Australia and Western Australia revealed pesticide deposition on overalls up to 40 $\mu g/cm^2$, on adsorbent pads under overalls up to 8 $\mu g/cm^2$, and up to 73 mg (total) on cotton undergloves worn beneath protective gloves or gauntlets [4]. It is likely that since application practices were very similar for chlorpyrifos and bifenthrin formulations, bifenthrin exposure levels, expressed as ml/cm^2, are likely to be similar to those of chlorpyrifos. Chlorpyrifos is applied as a 22/1000 dilution of concentrate at 450 g/l, while bifenthrin is applied as a 1/200 dilution of concentrate at 100 g/l. Hence, based on the observations relating to chlorpyrifos above, we would suspect that dermal exposures to bifenthrin would be of the order of 2.0 and 0.4

$\mu g/cm^2$ on overalls and absorbant pads, respectively, and over 3.5 mg beneath protective gloves.

Since PBA is a metabolite common to several pyrethroids (including cypermethrin, permethrin, and deltamethrin), the simultaneous measurement of PBA may be used to indicate exposures to other pyrethroids in the workplace, to domestic and garden exposures to products containing them, or through diet. This may be useful in studies of symptom prevalence, where a contribution to symptoms may be attributed to either workplace or home exposures.

In conclusion, this technique provides a rapid and robust method of monitoring exposure of termiticide applicators exposed to the pyrethroid bifenthrin.

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