Assessment of potential (inhalation and dermal) and actual exposure to acetamiprid by greenhouse applicators using liquid chromatography–tandem mass spectrometry

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Abstract

New analytical methods based on liquid chromatography with electrospray tandem mass spectrometry (LC–MS/MS) have been developed and validated for assessing the exposure of greenhouse workers to acetamiprid. Both ambient (potential inhalation and dermal exposure) and internal dose (biological monitoring of urine samples) measurements were carried out. Potential inhalation exposure was assessed using Chromosorb 102 cartridges connected to air personal samplers. Potential dermal exposure was estimated by using whole body dosimetry. The measurement of actual exposure was done by analyzing the parent compound in urine samples of the applicators, after a solid-phase extraction (SPE) step. The methods showed a good accuracy (72–92%), precision (2–13%) and lower limits (few $\mu g \, l^{-1}$). The validated approaches have been applied to assess potential and actual exposure of agricultural workers spraying acetamiprid in greenhouses. The results shown the need to wear personal protective equipment (suits) in order to reduce the absorbed dose of acetamiprid.
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1. Introduction

Acetamiprid ((E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine), 135410-20-7 CAS number (Fig. 1), is a systemic and contact insecticide, belonging to the chloronicotine class, having a broad insecticidal spectrum [1]. It has a relatively low acute and chronic mammalian toxicity, with no evidence of carcinogenicity, neurotoxicity, mutagenicity or endocrine disruption [2]. However, considering that acetamiprid is being widely used, frequently as an important organophosphate pesticide replacement, there is potential health risk which should be evaluated by measuring occupational exposure to this compound. Obviously, metabolites of acetamiprid can be also used for exposure assessment. The problem is that there is no information available on the metabolism of the insecti-

cide in animals. While, there is information on the fate of acetamiprid in soils (6-chloro-pyridinyldimethylamine and 6-chloronicotinic acid) or plants (6-chloronicotinic acid and 6-chloropicolyl alcohol).

Human exposure to pesticides occurs both during application and afterwards when workers enter the treated area, via both the respiratory and dermal routes. Monitoring of airborne pesticides with a personal air sampling system, situated in the workers breathing zone during the spray application task, provides a measure of potential inhalation exposure. There are a range of sampling methods available, mainly involving the use of different sorbents such as Tenax, Chromosorb, Porapak R, Amberlite XAD-2 and XAD-4, polyurethane foam or resins [3–13]. On the other hand, sampling methods for potential dermal exposure are less complex, often involving the analysis of pesticide extracted from the coverall worn by workers during application. There are two traditional sampling methods; the patch and whole body dosimetry methods [7,14,15]. In addition, biological monitoring of pesticides in urine
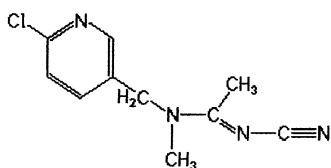
Fig. 1. Chemical structure of acetamiprid.

[7,16–19] is a complementary approach that may give an indication of the absorbed dose, which is influenced by factors such as protection by work clothing, rate of skin penetration, and the effect of ambient conditions on dermal absorption. Biological monitoring can be compatible with whole body dosimetry sampling method, when the workers own protective clothing is used as the dosimeter.

Monitoring of the exposure to pesticides requires analytical methods capable of performing determinations at trace levels. Gas chromatography (GC) in combination with several detectors: electron capture (ECD), nitrogen–phosphorus (NPD), flame ionisation (FID), flame photometric (FPD) and mass spectrometry (MS), have been used for determining pesticide residues in air, protective clothing and human fluids such as urine and blood for biological monitoring [3–5,7–13]. However, very few papers are available in the literature based on the determination of pesticides in air or protective clothing by high-performance liquid chromatography (LC) [6,13]. Those that do exist all use UV detection. No references have been found that use LC interfaced with mass spectrometry (MS) detection for the pesticide analysis in such matrices, although this is not the case for urine samples [16]. LC is a powerful tool for analyzing compounds of low volatility or thermal lability, and avoiding derivatization steps necessary in GC. The coupling of LC with MS presents a rich source of qualitative information from which component identity may be inferred with a reasonable degree of certainty. The use of tandem mass spectrometry (MS/MS) improves the selectivity of the technique with a drastic reduction of the background and without losing identification capability. In addition, MS/MS presents a good sensitivity, as other classical detectors (ECD and NPD) that lack of identification power.

In this paper, we describe the development and validation of the analytical methodology, based on LC–MS/MS, for assessing the exposure of greenhouse workers to acetamiprid. Both potential (inhalatory and dermal exposure) and absorbed dose (biological monitoring of urine samples) measurements were carried out. Finally, the methodology was applied to evaluate the exposure to acetamiprid for two greenhouse workers.

2. Experimental

2.1. Reagents and materials

Standard of the acetamiprid pesticide was obtained from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Stock solution was prepared by weighing the appropriate amount and dissolving it in HPLC-grade methanol (200 mg l^{-1}) and stored in a refrigerator at 4 °C, where it was stable at least for 2 months. The working solutions were prepared by appropriate dilutions of stock solution in a mixture of methanol–water (50:50 (v/v)). The calibration curve consisted of seven different levels of concentration and the injections were made in triplicate. Replicate measurements are need to estimate whether the straight line model adequately fits the calibration data.

Chromatographic purity methanol, ethyl acetate and ammonium acetate, and highly purified water were obtained from Fisher Chemicals (Fischer Scientific, Loughborough, UK).

Granular Chromosorb 102, 60/80 mesh (125 mg) was packed in sampling tubes of 7.5 cm length and 0.5 cm diameter. The disposable coverall (Sontara) was worn by applicator as the protective clothing (and dosimeter) during treatment. Air sampling pumps model PCEX3KB, connected to the sampling tubes, working at a volumetric flow of 2 l min^{-1} were used.

A vacuum system VacMaster Sample Processing Station (International Sorbent Technology, UK), and C$_{18}$ cartridges (500 mg, 6 ml from Isolute International Sorbent Technology, UK) were used for the solid phase extraction (SPE) for the urine samples.

2.2. LC–MS/MS analysis

2.2.1. Liquid chromatography

Analysis was carried out with a Hewlett-Packard HP 1100 Series (Hewlett-Packard, Waldbronn, Germany) fitted with a Genesis C$_{18}$ reversed-phase column [150 mm length and 2.1 mm internal diameter (i.d.)] from Jones chromatography (Lakewood, Colorado, USA). Methanol (phase A) and a 10 mM aqueous ammonium acetate (phase B) were used as mobile phase with a constant flow-rate of 200 µl min^{-1}. The composition of the mobile phase, under gradient conditions, was as follows: initially 40% phase A and 60% phase B, 7 min linear gradient to 95% phase A and 5% phase B, and, finally, 9 min linear gradient to the initial conditions for subsequent analysis. The injection volume was 20 µl and the diverter valve was switched at the 3.5 min from injection to divert the matrix interferences.

2.2.2. Mass spectrometry

An API-2000 Perkin-Elmer-Sciex (Toronto, Canada) mass spectrometer was used. All measurements were carried out using the electrospray source in the positive mode. High-purity nitrogen was used as nebulizer and curtain gas at flow-rates of 40 and 50 l h^{-1}, respectively. The desolvation temperature was 350 °C, the ion source was kept at 120 °C. Capillary voltage was 5 kV and cone voltage 70 V. The collision energy was 30 eV. Table 1 shows the main parameters settings of the mass spectrometer.

Table 1
Retention time windows (RTW), calibration curves, accuracy, precision and lower limits of the method in the target matrices

	RTW	Calibration curve	Recovery (%; 2–20 μg l^{-1})	R.S.D. (%)	LOD (μg l^{-1})	LOQ (μg l^{-1})
Urine	6.61–6.77	$y = 7003x + 1742$	72–73	7–2	0.3	1
Suit	6.62–6.79	$y = 4200x + 3153$	88–92	12–9	0.3	1
Cartridges	6.60–6.80	$y = 3628x + 1879$	78–81	13–4	0.6×10^{-3}	2×10^{-3}

2.3. Sample extraction

2.3.1. Air samples extraction

Sampling sorbents (Cromosorb 102) 125 mg were extracted with ethyl acetate (20 ml) by sonication for 20 min. This operation was repeated three times with each sample. The final volume was reduced to dryness and re-dissolved in 10 ml of methanol:water (50:50 (v/v)), being ready for LC–MS/MS analysis.

2.3.2. Personal protective equipment (PPE) extraction procedure

For the whole body method the coverall was sectioned in nine pieces following the procedure described by Egea González et al. [20]. The pieces were extracted with a mixture of methanol:water (50:50 (v:v)) as follows: head and neck (250 ml), left and right arms (250 ml), chest (350 ml), back (350 ml), thighs/waist front (350 ml) and back (350 ml), lower left and right legs (250 ml). The pieces of coverall were placed in 1 l capacity bottles with the mixture of extraction solvent and placed for 30 min in an overhead shaker at 30 rpm. An aliquot of this extract was transferred to a vial which was used for the LC–MS/MS analysis.

2.3.3. Urine samples extraction

The urine extraction procedure was similar to that described by Cruz Márquez et al. [7]. A 3 ml volume of sample was passed through the C$_{18}$ cartridge previously conditioned under gravity conditions with 6 ml of methanol followed of 4 ml of distilled water. The cartridge was not allowed to dry in any of the stages during the procedure. Following this 2 × 4 ml of distilled water were passed through the cartridge as a clean-up step. The analyte was eluted with 3 ml of methanol. The solvent was evaporated down with nitrogen to half of the original volume (1.5 ml) without heating. Finally, 1.5 ml of water were added, so the final volume was 3 ml water:methanol (50:50 (v:v)).

2.4. Field trial

Occupational exposure to acetamiprid during its application in greenhouses is estimated by determining inhalation and dermal exposure and the concentration of acetamiprid in urine samples of applicators. Two similar applications were carried out by two different applicators (A1 and A2) in a flat-roofed greenhouse of polyethylene (200 μm thickness) and area 20 m × 35 m, with a height of 2.5 m, situated in Almería, (Spain). The crops were green beans of approxi-

mately 2 m height, and 1 m of inter-row distance. A1 wore PPE while A2 did not wear any protection. Both applicators carried a personal air sampler, sampling air at a flow rate of 2 l min^{-1}. In addition, another personal air sampling system was located outside of the greenhouse in order to assess the airborne concentration of pesticide in the area surrounding the greenhouse.

The applications were carried out using a high volume application equipment with three circular nozzles, operating at 25 bars of pressure. Approximately, 5000 m^2 were sprayed during 120 min, using 500 l of the spray tank in each case. The concentration of the pesticide in the tanks was 373 and 385 mg l^{-1}, respectively, for A1 and A2. Applicators sprayed following a similar application pattern, walking between the rows spraying one side of the crop, and returning along the same row spraying the other side of the row. One of the applicators (applicator A1) wore a Sontara coverall with a Tyvek underneath, while the other applicator (A2) wore a short-sleeved cotton T-shirt and shorts.

2.4.1. Sampling procedure

Following the applications, air sampling cartridges, and the suit of the first applicator (A1) were carefully removed avoiding accidental contamination of the different suit parts. Urine samples of applicators were taken during the following 48 h after applications. Samples of personal protective equipment, sampling tubes and urine samples were stored in darkness at −15 °C until analysis.

A field quality control procedure was established in order to ensure the integrity of samples during sampling, transport and analysis [21]. An aliquot of the tank mix was taken from the guns 10 min after starting each application. Six blanks of each sampling medium (coveralls, sampling tubes and urine) were taken before the applications. Three replicates of each media type were labeled as field blanks and stored in the same way as field samples. In order to obtain field quality control samples, the rest of blank samples were spiked as follows: three sampling tubes and three pieces of suit were spiked with 10 and 300 μl, respectively, of the spray tank liquid. Three aliquots of urine blank (sampled before application) were also spiked with acetamiprid standard solution.

Field blanks, field spikes and samples were stored in the same way and processed and analyzed in the same batch. The acceptability criteria assumed were: recovery rates of field spikes should be between 70 and 120% and relative standard deviation (R.S.D.) <20%; field blanks should not have any evidence of any contamination or sample decomposition; slopes of calibration curves should not differ more

than 25% of these obtained in validation studies and should fit to straight lines with $r^2 > 0.95$.

2.5. Validation

Linear dynamic range, precision, recovery, lower limits and selectivity were evaluated for the analytical methodology developed. Also, the stability of spiked urine samples was studied.

For linear dynamic range, the calibration samples were prepared by appropriate dilution of the stock solution in blank matrix extract obtained in methanol–water (50:50 (v/v)). The concentrations of the standards within the linear portion was between 1 and 500 μg l^{-1}.

Within-day precision and recovery were assessed using spiked blank samples at two concentration levels, 20 and 200 μg l^{-1}. Replicated ($n = 5$) samples were all run and the R.S.D. and recovery values were calculated for each.

Lower limits of detection (LOD) and quantitation (LOQ) limits were determined as the lowest acetamiprid concentration injected that yielded a signal-to-noise (S/N) ratio of 3 and 10, respectively, when the quantification ion was monitored.

Tandem MS is described as an example of a technique selective [22]. The presence of potential interferences in the chromatograms from the analyzed samples was monitored by running control blank samples in each calibration. The absence of any chromatographic components at the same retention times as target pesticide suggested that no chemical interferences occurred.

3. Results and discussion

Until now, some analytical methods have been published for determining acetamiprid in foods by LC with UV [23] or MS [24] detection or GC–MS/MS [25]. There are also LC methods for structurally related compounds such as imidacloprid [26–28]. Both, LC methods for acetamiprid need a clean-up step and analysis time more than 13 min. The GC method does not need a clean-up step but the analysis time is more than 9 min. The LC–MS/MS method described in this work is simple (no clean-up) and fast (analysis time lower than 7 min).

3.1. Optimization of the MS/MS conditions

Ionization and fragmentation conditions were optimized for acetamiprid by continuous flow injection of a pure standard solution of 5 μg ml^{-1} in methanol:aqueous ammonium acetate (50:50 (v/v)). The best response was obtained by electrospray and using positive ionization mode. Full scan mass spectra were recorded in order to select an ion parent taking into account the mass-to-charge ratio (m/z) and the relative abundance. Once, the precursor ion was selected, the capillary voltage, cone voltage, collision cell entrance

potential (CEP), collision energy (CE) and collision cell exit potential (CXP) were optimized in order to obtain the highest response of the selected ion. In the positive mode acetamiprid was detected as $[M + H]^+$, while only poor ionization was observed when analyzing it in the negative mode. The parent ion (223.1) and the product ion (126.1) were selected for quantification. Other parameters of the mass spectrometer were DP (declustering potential) 70, FP (focussing potential) 350, CEP 11.82, CE 30 and CXP 10. With these conditions, a LC–MS/MS analysis of a standard solution of acetamiprid was carried out and the MS/MS spectrum obtained was stored. This was used as reference spectrum for confirmation purposes.

Retention times windows (RTWs), defined as the retention time averages ± 3 standard deviation of the retention times when 10 samples were analyzed, was used for the positive identification of acetamiprid. Therefore, acetamiprid was searched by its RTW and confirmed with the reference MS/MS spectrum.

3.2. Performance parameters of analytical methods

3.2.1. Potential inhalation exposure

The linearity of the method was tested by analyzing spiked extracts of uncontaminated Chromosorb 102 sorbent (matrix-matching calibration) over the range 1–500 μg l^{-1}. Excellent linearity was found in the concentration range tested, both using area and height peak, with determination coefficients higher than 0.99. The internal standard method was not used for calibration purposes, because the method is always under control and, the addition of an internal standard is not always useful and it will increase imprecision [29]. In addition, and based in our experience, LC methods are not so imprecise as gas chromatographic methods or atomic spectroscopic methods. Our method is short (<7 min), has high quantitative validity, precision and sensitive, so the need for internal standard is diminished.

The LOD and LOQ considered as the concentration yielding a signal-to-noise ratio of 3 and 10, respectively, shows the good sensitivity of the LC–MS/MS method, allowing the quantification of 2 ng m^{-3} of pesticide in air (considering a sampling time of 8 h at 2 l min^{-1}). Table 1 shows these results, as well as the retention time window (RTW) range.

NIOSH guidelines [30] were followed in order to evaluate the retention efficiency of Chromosorb 102. One hundred twenty five milligram of sorbent were spiked at three concentration levels (30, 60 and 100 ng of acetamiprid), and air, at a flow rate of 2 l min^{-1} for 30 min, was pumped through the cartridge. The sorbent cartridges were extracted and the pesticide analyzed by LC–MS/MS. Recoveries were always higher than 78% with a R.S.D. lower than 13% (Table 1).

3.2.2. Potential dermal exposure

Matrix-matching calibration was also used in order to quantify coverall samples. Good linearity was found in the

Table 2
Potential dermal exposure levels

Description	Concentration ($ml\,h^{-1}$)	Concentaration* ($mg\,cm^{-2}\,h^{-1}$)	Concentaration** ($mg\,cm^{-2}$)
Head and neck	12.6	0.0031	0.006
Left arm	7.2	0.0010	0.002
Right arm	24.6	0.0037	0.007
Chest	12.3	0.0009	0.002
Back	9.9	0.0008	0.002
Thighs/waist front	9.0	0.0010	0.002
Thighs/waist back	7.8	0.0008	0.002
Lower left leg	15.9	0.0026	0.005
Lower right leg	13.8	0.0022	0.0.05
Total	113.1	0.0015	0.032

concentration range of $1–500\,\mu g\,l^{-1}$, with determination coefficient above 0.99. Table 2 summarizes the values obtained for the lower limits (LOD and LOQ), recovery rates and precision of the analytical method. They were obtained by spiking six pieces of Sontara at two concentration levels, 20 and $200\,\mu g\,l^{-1}$, respectively. After extraction and analysis, recoveries higher 88% and precision values lower than 12% were obtained in both cases (Table 1).

3.2.3. Absorbed dose measurements

The analysis of the residues of acetamiprid in the urine of both applicators, A1 and A2, was used as a measure of actual exposure.

Calibrations were carried out spiking blank extracts of uncontaminated urine samples (Fig. 2). Good linearity of the response was found at concentrations ranging from 1 to $500\,\mu g\,l^{-1}$, with determination coefficient higher than 0.99. Lower limits were similar to those obtained for the analysis of suits, with no sample concentration being necessary (Table 1).

For the recovery study, 10 aliquots of 3 ml of uncontaminated urine were spiked with acetamiprid at two concen-

tration levels (20 and $200\,\mu g\,l^{-1}$). Satisfactory results were found in both instances, with recoveries higher 72% and R.S.D. values lower than 7% (Table 1).

Finally, a stability study was performed in order to assess the stability of acetamiprid in urine samples during sampling, transport, storage and processing. The study was performed in darkness at room temperature, at fridge temperature (4 °C) and at −18 °C, spiking urine samples at medium-level concentration, $200\,\mu g\,l^{-1}$. Recovery rates of such samples were similar to those obtained during the method validation. This showed that acetamiprid was stable for the first 3 days of storage at room temperature, although on samples stored in the fridge temperature and at −18 °C, were found to be stable during the whole period of the study.

3.3. Exposure levels of applicators

Potential dermal exposure was assessed for the applicator A1 wearing the coverall. The results are expressed as milliliter of spray tank deposited on the suit per hour of application. The coverall was sectioned in nine pieces (Table 2) to obtain information of the distribution of the contamination over the body. The upper body was found to be most exposed, with almost 59% of the total potential dermal exposure. The right arm (the one holding the spray gun) was the most contaminated section with a rate of $24.6\,ml\,h^{-1}$. Such distribution of the contamination between upper and lower body parts is a consequence of agronomic factors such as the crop density and height, and also the effect of the small drop size, which tends to favor a uniform distribution of the spray over the body [31,32]. The Tyvek underneath showed a contamination level of 7% of the amount found on the outer Sontara. The area of greatest penetration being the elbow of the right arm, due to the movement of the arm during the application. Fig. 3 shows, as an example, the analysis of extracted lower right leg section of the Sontara suit.

Concerning inhalation exposure, pesticide air concentration in the breathing zone was found to be similar in both
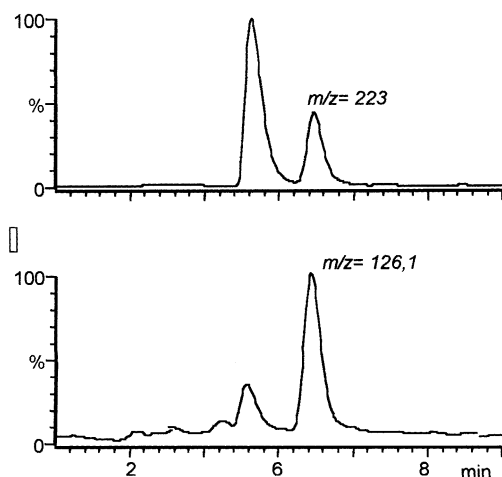

Fig. 2. LC–MS chromatogram of acetamiprid from a urine sample of the worker without suit (concentration: $145\,ng\,ml^{-1}$).
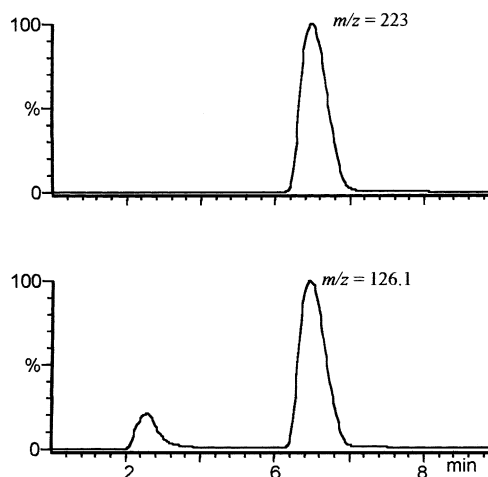

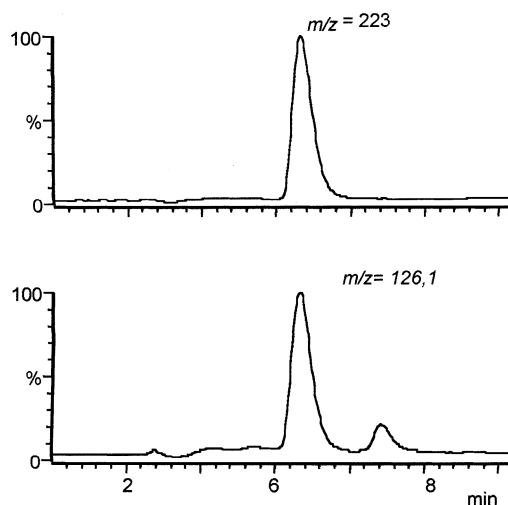Fig. 3. LC–MS chromatogram of acetamiprid obtained from the analysis of the suit (lower right leg section).

Fig. 4. LC–MS chromatogram of acetamiprid obtained from the analysis of the sampling sorbent of applicator B.
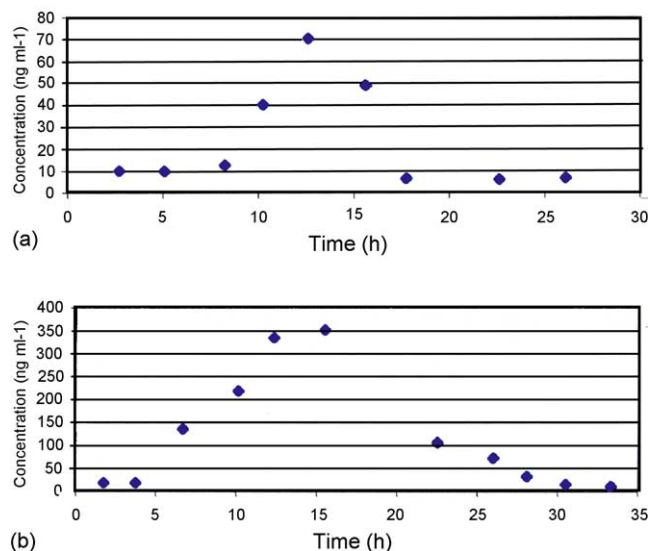

Fig. 5. Results of the biomonitoring in urine for: (a) worker with suit and (b) worker without suit. The points in the graph correspond to the time (h) that has passed since the application.

applications, with 14.4 and 13.8 mg m^{-3}, respectively, for applicators A1 and A2. Fig. 4 shows a chromatogram of acetamiprid after extracting the cartridge located in the breathing area of the applicator B. This is considered to be a high concentration in the air of the greenhouse, which is explained by the small droplet size produced by the application equipment, together with a high ambient temperature (37 and 35 °C in each application) and a high relative humidity (around a 90% during the applications). The amount found outside the greenhouses was also high, 4.5 and 3.7 mg m^{-3} in each application, which means that a high amount of pesticide reaches the outdoors environment, and may pose a risk for bystanders.

Actual exposure of workers was also obtained by analyzing up to 10 urine samples collected from each applicator, during the approximately 30 h after the applications. The lack of excretion data for acetamiprid in humans made it necessary to collect all the urine samples in a period of time long enough to collect all of the excreted parent compound. Fig. 5 shows the results obtained. Acetamiprid was not detected in the blank samples taken before the applications. In both applicators, the amount of acetamiprid in urine samples increased, reaching a maximum around 13–15 h after the applications performed by the protected and unprotected applicators, respectively. The unprotected worker shows a higher concentration level of acetamiprid in all the samples, with a maximum difference of about five times the found in the urine of the protected worker. After reaching the maximum concentration of acetamiprid, the non-detected level was reached again approximately 28 h (the applicator A1) and 33 h (the applicator A2), after the applications. These results suggest that the metabolism of acetamiprid is not dependent of the absorbed dose (higher for the applicator without coverall), because, as it is shown in the Figs. 1 and 2, the time to reach the maximum level of concentration is similar as are the excretion curves. Biomonitoring, also shows the

differences in the actual exposure of workers due to the use of PPE, the main route of exposure in the case of the protected worker A1 was inhalation, while for applicator A2 both dermal and inhalation routes were important. This confirms that the main exposure route of agricultural workers is the dermal route, despite the fact that in conditions such as those described in this paper, the inhalation route can also be an important route of exposure for the applicator.

4. Conclusions

A LC–MS/MS method was developed and validated in order to assess the human exposure to acetamiprid during field applications in greenhouses. Potential dermal and inhalation exposure was evaluated using the whole body dosimetry method and Chromosorb 102 cartridges connected to personal air samplers. The concentrations of the pesticide in the air of the breathing zone during the field applications were >13 μg m^{-3}. This contributed towards the total absorbed dose of the applicator A2, as measured by determining the amount of acetamiprid in urine samples. The maximum concentration in urine was found to be about 70 ng ml^{-1}. Potential dermal exposure was measured with the whole body dosimetry method, indicating a contamination rate of 113 ml h^{-1}. Distribution of contamination of the applicator showed that the upper body-parts are the most exposed especially the right arm, chest and head–neck. Inhalation is the principal source of exposure for the applicator A2 as shown by the amount of pesticide found in his urine samples. For both applicators, the highest acetamiprid concentration in urine was found 13–15 h after the beginning of the application task in the experiment, while the zero (non-detected) level was reached around 28 h after the applica-

tions. The highest levels of acetamiprid in urine were found in the samples from the applicator who did not wear a coverall during the application. These data reinforce the need to wear personal protective equipment, including a respirator for the application of pesticides such as acetamiprid in greenhouses.

Acknowledgements

References

[1] C.D.S. Tomlin, The Pesticide Manual: a World Compendium, 12th ed., British Crop Protection Council, Surrey, UK, 2000.

[2] http://www.epa.gov/opprd001/factsheets/acetamiprid.pdf.

[3] L. Elflein, E. Berger-Preiss, K. Levsen, G. Wünsch, J. Chromatogr. A 985 (2003) 147–157.

[4] O. Briand, M. Millet, F. Bertrand, M. Clément, R. Seux, Anal. Bioanal. Chem. 374 (2002) 848–857.

[5] J. Castro, R.A. Pérez, E. Miguel, C. Sánchez-Brunete, J.L. Tadeo, J. Chromatogr. A 947 (2002) 119–127.

[6] J. Demel, W. Buchberger, H. Malissa Jr., J. Chromatogr. A 931 (2001) 107–117.

[7] M. Cruz Márquez, F.J. Arrebola, F.J. Egea González, M.L. Castro Cano, J.L. Martínez Vidal, J. Chromatogr. A 939 (2001) 79–89.

[8] S. Herceg Romanic, B. Kranthacker, Bull. Environ. Contamin. Toxicol. 64 (2000) 811–816.

[9] R.A. Hallama, E. Rosenberg, M. Grasserbauer, J. Chromatogr. A 809 (1998) 47–63.

[10] J.L. Martínez Vidal, F.J. Egea González, C.R. Glass, M. Martínez Galera, M.L. Castro Cano, J. Chromatogr. A 765 (1997) 99–108.

[11] F.J. Egea González, M.L. Castro Cano, J.L. Martínez Vidal, M. Martínez Galera, J. AOAC Int. 80 (1997) 1091–1097.

[12] E.R. Kennedy, M.T. Abell, J. Reynolds, D. Wickman, Am. Ind. Hyg. Assoc. J. 55 (1994) 1172–1177.

[13] M.J.M. Jongen, J.C. Ravensberg, R. Engel, L.H. Leenheers, J. Chromatogr. Sci. 29 (1991) 292–297.

[14] G. Chester, in: P.B. Curry (Ed.), Methods of Pesticide Exposure Assesment, Plenum Press, New York, 1995.

[15] J.J. van Hemmen, D.H. Brouwer, Sci. Total Environ. 168 (1995) 131–141.

[16] J.M. Pozzebon, W. Vilegas, I.C.S.F. Jardim, J. Chromatogr. A 987 (2003) 375–380.

[17] P.A. Smith, M.J. Thompson, J.W. Edwards, J. Chromatogr. B 778 (2002) 113–120.

[18] K. Wittke, H. Hajimiragha, L. Dunemann, J. Begerow, J. Chromatogr. A 755 (2001) 215–228.

[19] H.M. Koch, J. Angerer, J. Chromatogr. B 759 (2001) 43–49.

[20] F.J. Egea González, M.L. Castro Cano, J.L. Martínez Vidal, C.R. Glass, M. Cruz Márquez, Chromatographia 50 (5/6) (1999) 293–298.

[21] J.L. Martínez Vidal, A. Garrido Frenich, F.J. Egea González, Trends Anal. Chem. 22 (2003) 34–40.

[22] J. Vessman, R.I. Stefan, J.F. Van Staden, K. Danzer, W. Lindner, D.T. Burns, A. Fajgeli, H. Müller, Pure Appl. Chem. 73 (2001) 1381–1386.

[23] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, J. Agric. Food Chem. 50 (2002) 4464–4467.

[24] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, J. Agric. Food Chem. 51 (2003) 2501–2505.

[25] M. Mateu-Sánchez, M. Moreno, J.J. Arrebola, J.L. Martínez Vidal, Anal. Sci. 19 (2003) 701–704.

[26] J.M. Bonmatin, I. Moineau, R. Charvet, C. Fleche, M.E. Colin, E.R. Bengsch, Anal. Chem. 75 (2003) 2027–2033.

[27] A. Garrido Frenich, F.J. Egea González, J.L. Martínez Vidal, P. Parrilla Vázquez, M. Mateu Sánchez, J. Chromatogr. A 869 (2000) 497–504.

[28] A.R. Fernández Alba, A. valverde, A. Agüera, M. Contreras, S. Chirón, J. Chromatogr. A 721 (1996) 97–105.

[29] I.D. Wilson, Observations of the usefulness of internal standards in the analysis of drugs in biological fluids, in: E. Reid, I.D. Wilson (Eds.), Methodological Surveys in Biochemistry and Analysis, vol. 20, Royal Society of Chemistry, Cambridge, 1990, pp. 79–82.

[30] E.R. Kennedy, T.J. Fishbach, R. Song, P.M. Ellner, S.A. Shulman, Guidelines for air sampling and analytical method development and evaluation, NIOSH, Technical Report, US Department of Health and Human Services, Cincinnati, OH, 1995.

[31] A. Garrido Frenich, P. Aguilera, F.J. Egea González, M.L. Castro Cano, M. Martínez Galera, J.L. Martínez Vidal, Environ. Monit. Assess. 80 (2002) 51–63.

[32] J.L. Martínez Vidal, F.J. Egea González, A. Garrido Frenich, M. Martínez Galera, P.A. Aguilera, E. López Carrique, Pest. Manage. Sci. 58 (2002) 784–790.