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Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia)

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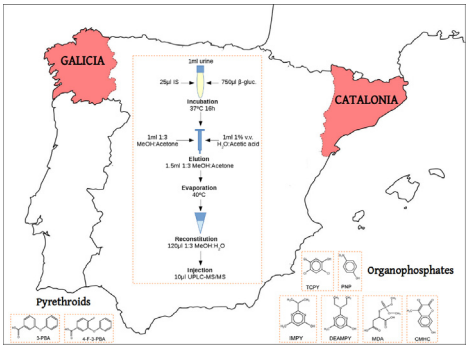
HIGHLIGHTS

- An UPLC-MS/MS method for analysis of urine organophosphate metabolites was developed.
- An UPLC-MS/MS method for analysis of human urine pyrethroid metabolites was developed.
- The use of synthetic urine afforded calibration straight lines with lower detection limits.
- Detection limits were in the range of 14–69 pg/ml.
- Organophosphate concentrations in farmworkers was twofold than in urban populations.

GRAPHICAL ABSTRACT



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ABSTRACT

Isotope dilution solid phase extraction UPLC-MS/MS has been used to develop a robust and rapid methodology for the determination of eight specific metabolites of organophosphate and pyrethroid pesticides in human urine. The use of methanol:acetone (25:75 *v*/v) affords an improvement in extraction efficiency in comparison to these individual solvents. The use of synthetic urine improves selectivity and limits of detection for the calibration straight lines. The method provides detection limits of 14–69 pg/ml and 18–19 pg/ml for the organophosphate and pyrethroid metabolites, respectively. Urine analyses of these metabolites in urban non-occupationally exposed individuals and farm workers shows that ingestion of these pesticides occurred in both populations. The concentrations of organophosphate pesticide metabolites in the latter were twofold than those from non-exposed populations.

1. Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture as well as for domestic and gardening use. They eliminate insects because of their strong potential to disrupt the brain and nervous system of these organisms. Unfortunately, this neurotoxic effect is not selective enough as to avoid damage to other non-target species, including humans (Barr, 2008). There is growing public concern on pesticide use not only for the negative impacts on wildlife and the environment but also for the potential adverse health effects on humans. OP and PYR pesticide exposure has been related to several

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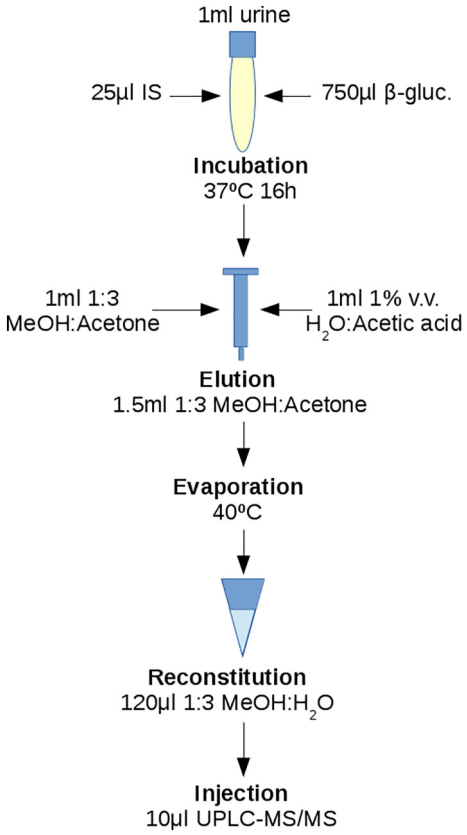

Fig. 1. Graphical representation of the extraction procedure.

health effects, including respiratory, digestive, reproductive and neurological problems, among others (Ye et al., 2013; Arcury et al., 2016; Llop et al., 2017).

Once in the human body, OP and PYR pesticides are typically metabolized and excreted in urine within 4–48 h after exposure, depending on the compound (Egeghy et al., 2011). Organophosphates are metabolized into dialkyl phosphates (DAPs) and specific compounds, including 3,5,6-trichloro-2-pyridinol (TCPY, the metabolite of chlorpyriphos), 4-nitrophenol (PNP, metabolite of parathion), malathion dicarboxylic acid (MDA, metabolite of malathion), 3-chloro-4-methyl-7-hydroxycoumarin (CMHC, metabolite of coumaphos), 2-isopropyl-6-methyl-4-pyrimidiol (IMPY, metabolite of diazinon) and 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY, metabolite of pirimiphos). For the most common pyrethroids, which include permethrin, cypermethrin, deltamethrin and esfenvalerate, all these pesticides are metabolized into one single compound, 3-phenoxybenzoic acid (3-PBA). Cyfluthrin pesticide is metabolized into 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Therefore, 3-PBA and 4-F-3-PBA can be used as a biomarker of the most common PYR pesticides (Barr, 2008; Ueyama et al., 2010; Egeghy et al., 2011).

Urine analysis is the simplest and least intrusive method for assessing human exposure to the aforementioned non-persistent pesticides. Previously published methods for the analysis of specific metabolites of OP and PYR pesticides in urine are based on both gas and liquid chromatography, and mainly using mass spectrometry techniques (Koureas et al., 2012). The concentrations of metabolites of these compounds in urine reflect the exposure levels of the individuals (Barr, 2008). Farmworkers and rural populations are in principle potentially more exposed to these pesticides than general populations (Arcury et al., 2007). However, the low concentrations of these metabolites in urine, currently in the order of ng/ml, and the large numbers of samples needed for epidemiological studies require robust, cheap and efficient analytical methodologies (Barr, 2008). In this context, the limits of detection (LD) are critical to discriminate for the presence of the analytes and for feasibility of study of high numbers of individuals and possible health effects (Currie, 1997; Koch et al., 2001; Ye et al., 2013).

In many of these epidemiological or population toxicity studies these limits are not only considered as analytical parameters but as reference for classification between individuals (Ueyama et al., 2010; Davis et al., 2013; Olsson et al., 2004; Barr et al., 2010; Koureas et al., 2012; Roca et al., 2014a, 2014b). Fulfilling the requirements for the use of detection limits following this approach requires extraction procedures adapted to the most representative conditions of real samples (Garí and Grimalt, 2010). In this context, interferences from human urine may increase limits of detection and distort calibration straight lines. Thus, the developed methodology must consider matrix effects and their variability. The use of synthetic urine instead of urine dilution may provide robust procedures to fulfill these requirements.

Accordingly, a new analytical methodology for the quantification of OP and PYR urinary specific metabolites has been developed in the present study. This method takes into account the variability of concentrations found in human urines from both general and highly exposed populations from rural or agricultural sites. The method is based on ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) and allows the quantification of eight biomarkers of several of these pesticides using only one ml of urine. It provides high precision and accuracy, and low detection limits to analyze these pesticide metabolites both in professionally exposed farmers and non-exposed general population.

2. Materials and methods

2.1. Standards, solvents and reagents

Standards of IMPY and TCPY were purchased from Sigma-Aldrich (Madrid, Spain), PNP from Supelco (Madrid, Spain), CMHC from Acros Organics (Geel, Belgium), DEAMPY, MDA, 3-PBA and 4-F-3-PBA from Dr. Ehrenstoffer (Augsburg, Germany). The isotopically-labeled

Table 1
Instrumental analytical data of the organophosphate and pyrethroid pesticide metabolites considered in the present study.

Acronym	Analyte	Q-SRM[a]	C-SRM[b]	Ion ratio	Collision energy	Cone voltage	Retention time
DEAMPY[c]	2-diethylamino-6-methyl pyrimidin-4-ol	182–154	182–84	1.3	20	40	4.65
IMPY[c]	2-isopropyl-6-methyl-4-pyrimidiol	153–84	153–70	1.9	20	40	5.05
MDA[d]	Malathion dicarboxylic acid	273–141	273–157	2.6	8	25	8.65
PNP[d]	4-nitrophenol	138–108	138–92	8.2	20	45	8.66
CMHC[d]	3-chloro-4-methyl-7-hydroxicoumarin	209–145	209–117	3.2	25	20	9.70
TCPY[d]	3,5,6-trichloro-2-pyridinol	196–196	198–198	1.0	7	10	11.28
3-PBA[d]	3-phenoxybenzoic acid	213–93	213–169	1.6	20	30	12.87
4-F-3-PBA[d]	4-fluoro-3-phenoxybenzoic acid	231–187	231–93	1.3	15	25	13.04

[a] Q-SRM: Quantification Selected Reaction Monitoring.
[b] C-SRM: Confirmation Selected Reaction Monitoring.
[c] Positive ion mode.
[d] Negative ion mode.

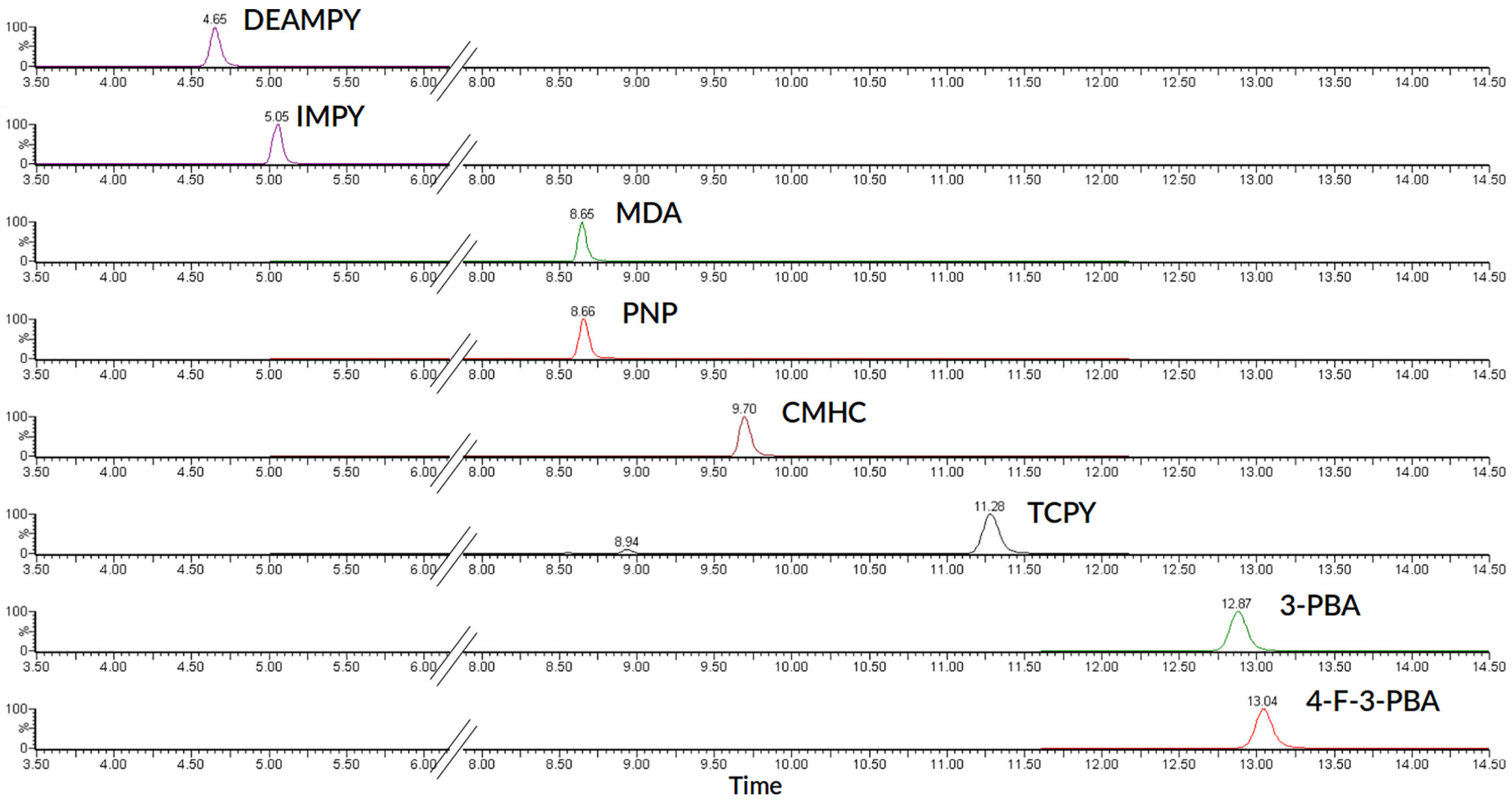

Fig. 2. Chromatogram of a synthetic urine extract showing the peaks of the analytes for selected ion transitions. The x-axis shows the eluting time. Acronyms in Table 1.

Table 2
Limits of detection, recoveries, repeatability and reproducibility of the analyses of the organophosphate and pyrethroid pesticide metabolites.

	LD (ng/ml)	Recovery (%)		Repeatability[a]		Reproducibility[a]	
		QCL	QCH	QCL	QCH	QCL	QCH
DEAMPY[b]	0.017	76	93	4.0	6.9	6.3	5.7
IMPY	0.014	74	97	6.7	6.0	11	11
MDA	0.069	74	73	4.7	6.6	16	17
PNP	0.017	95	87	5.7	2.9	11	8.5
CMHC	0.026	66	74	9.0	5.3	11	11
TCPY	0.020	70	78	5.3	4.4	11	11
3-PBA	0.018	94	84	3.1	2.3	9.7	5.5
4-F-3-PBA	0.019	100	96	3.0	2.3	6.9	6.5

[a] Coefficients of variation (%).
[b] Acronyms in Table 1.

standards were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents used were of analytical grade. Acetonitrile was from Panreac (Barcelona, Spain), methanol, acetone and water for HPLC were from Merck (Darmstadt, Germany), glacial acetic acid was from Scharlab (Barcelona, Catalonia, Spain), and sodium acetate anhydrous and β-glucuronidase type H-1 from *Helix pomatia* were from Sigma-Aldrich (Madrid, Spain).

2.2. Extraction procedure

The method developed here was based on previously reported procedures for the extraction of urinary insecticide metabolites but with substantial modification (Davis et al., 2013; Olsson et al., 2004). Prior to analysis, the urine samples were centrifuged and filtered. Then, one ml was introduced into 10 ml centrifuge tubes. 25 μl of a mixture of the available isotopically labeled internal standards was added. To hydrolyze possible glucuronide or sulfate conjugated metabolites, β-glucuronidase type H-1 from *Helix pomatia* with a specific activity of ~500 units/mg, was used. For a 10-batch sample, 7.50 ml of a buffer solution containing 33.3 mg of β-glucuronidase was used, giving a minimum of 990 units of activity per sample. The samples were incubated overnight at 37 °C and then extracted using solid-phase extraction (SPE). SPE cartridges (Oasis HLB 3 cm^3, Waters, Milford, MA, USA) were preconditioned with 1 ml of methanol/acetone (25:75 *v*/v) followed by 1 ml of HPLC H_2O containing 1% acetic acid. The sample was added and passed through the cartridge. Then the cartridges were washed with 500 μl of HPLC H_2O containing 1% acetic acid and dried for 20 min using vacuum. A solution containing methanol:acetone (25:75 *v*/v, 1.5 ml) was used for eluting the cartridge. The collected extracts were reduced to near dryness under a stream of pure nitrogen. Then, they were quantitatively transferred to chromatographic vials using 120 μl of methanol:water (25:75 *v*/v). An schematic view of the extraction procedure is shown in Fig. 1.

2.3. Instrumental analysis

Compound analysis was performed using Ultra-Performance Liquid Chromatography (UPLC Acquity H-class, Waters, Milford, MA, USA) coupled to a Triple Quadrupole Mass Spectrometer (XEVO-TQ-S, Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. The chromatographic separation was performed on a Betasil C18 column (100 mm × 2.1 mm, 3 μm particle size, Thermo Scientific, West Palm Beach, FL, USA). To extend the life of the column, one guard holder (2.1 μm and 3.0 mm id, Universal Uniguard Holder, Thermo Scientific, West Palm Beach, FL, USA) and a guard column of the same sorbent material (Thermo Scientific, West Palm Beach, FL, USA) were installed inline before the column.

The injection volume was 10 μl, at a flow rate of 0.3 ml/min. The column temperature was kept at 30 °C during the analysis. A gradient elution with a mobile-phase of acetonitrile and a mixture of HPLC H_2O with 1% acetic acid and 5% methanol was used for analysis. The gradient started with ACN/Mixture 2:98, increased to 20:80 in 4 min, then to 40:60 in another 3 min, to 50:50 at minute 14, and finished with 100% ACN at minute 16.5. During the following 3 min the column was cleaned with 100% ACN, adjusted to the initial conditions in minute 19.5, and finally equilibrated for an additional 2.5 min.

Total run time was 22 min. During this interval the MS acquisition parameters changed following three distinct timed segments. In the first, data were acquired in positive ionization mode, and the total run time was 5 min (from minute 3 to 7). In the second and third, data were acquired in the negative ionization mode, and the total run time was 17 min (5–12.3 min in the 2nd segment and 11.6–22 min in the 3rd segment).

The selected reaction monitoring (SRM) transitions for each compound are also reported in Table 1. The first and more abundant was used for quantification (Q-SRM) and the second for confirmation (C-SRM). Besides retention time, the relative abundances of these selected SRM transitions were used to identify the metabolites in the samples and to discriminate against possible coelutions. Thus, for ion ratios (IRs = Q-SRM/C-SRM) of the standards between 1 and 2, DEAMPY, IMPY, TCPY, 3PBA and 4F3PBA, the IRs of the samples should not differ by >20%. For the metabolites with IRs between 2 and 5, MDA and CMHC, the ranges in the samples should not be lower than 25% and for those with IR between 5 and 10, PNP, lower than 30%. The resulting separation and selective MS traces for the organophosphate and pyrethroid pesticide metabolites are shown in Fig. 2.

Data adquisition, data handling and instrument control were performed with Masslynx software version 4.1 (Waters Inc., 2008). This software included the MRM tool that generates small dynamic periods or segments of acquisition around the expected retention time of the analyte of interest. The algorithm optimizes the dwell time based on the number of transitions that are co-eluting.

2.4. Quality assurance procedure

Synthetic urine (Surine, Preserve Free, Sigma-Aldrich) was used for blanks, quality control (QC) materials and standard preparation. Blanks were analysed for every set of 10 to 15 urine samples and were used for measuring of the existing contamination of the laboratory environment, including the material and solvents. Two synthetic urine samples fortified with the analytes were prepared at low (QCL, 1 μg/l) and high (QCH, 10 μg/l) concentrations. Calibration straight lines were prepared by adding 25 μl of standard solutions at concentrations ranging from 2.5 to 800 ppb into 1 ml synthetic urine sample, yielding final concentrations of 0.06, 0.12, 0.24, 0.48, 0.95, 1.9, 2.4, 4.8, 9.5, 13.3 and 19 ng/ml in urine. Quantification was performed by isotopically-labeled internal standards.

In addition, the methodology was externally checked out by participation in rounds of the German External Quality Assessment Scheme since 2016 (GEQUAS, 2016), which include the organophosphate metabolites PNP and TCPY and the pyrethroid metabolite 3-PBA.

Table 3
Results obtained in the analysis of proficiency testing materials from the G-Equas program. Reference values and tolerance ranges provided by G-Equas are also shown.

		PNP[a]	TCPY[a]	3-PBA[a]
RV-57	Result A	29	5.4	2.3
	Ref. value A	25.7 [19.1–32.3]	6.3 [4.8–7.9]	2.1 [1.7–2.5]
	Result B	160	10	6.9
	Ref. value B	151.4 [132.2–170.6]	12.2 [9.7–14.8]	6.1 [4.8–7.3]
RV-58	Result A	16.7	3.4	1.3
	Ref. value A	16.2 [12.6–19.8]	4.6 [3.4–5.8]	1.1 [0.89–1.3]
	Result B	52	11	3.7
	Ref. value B	51.7 [42.1–61.3]	15.2 [11.6–18.9]	3.5 [2.9–4.0]

[a] Acronyms in Table 1.

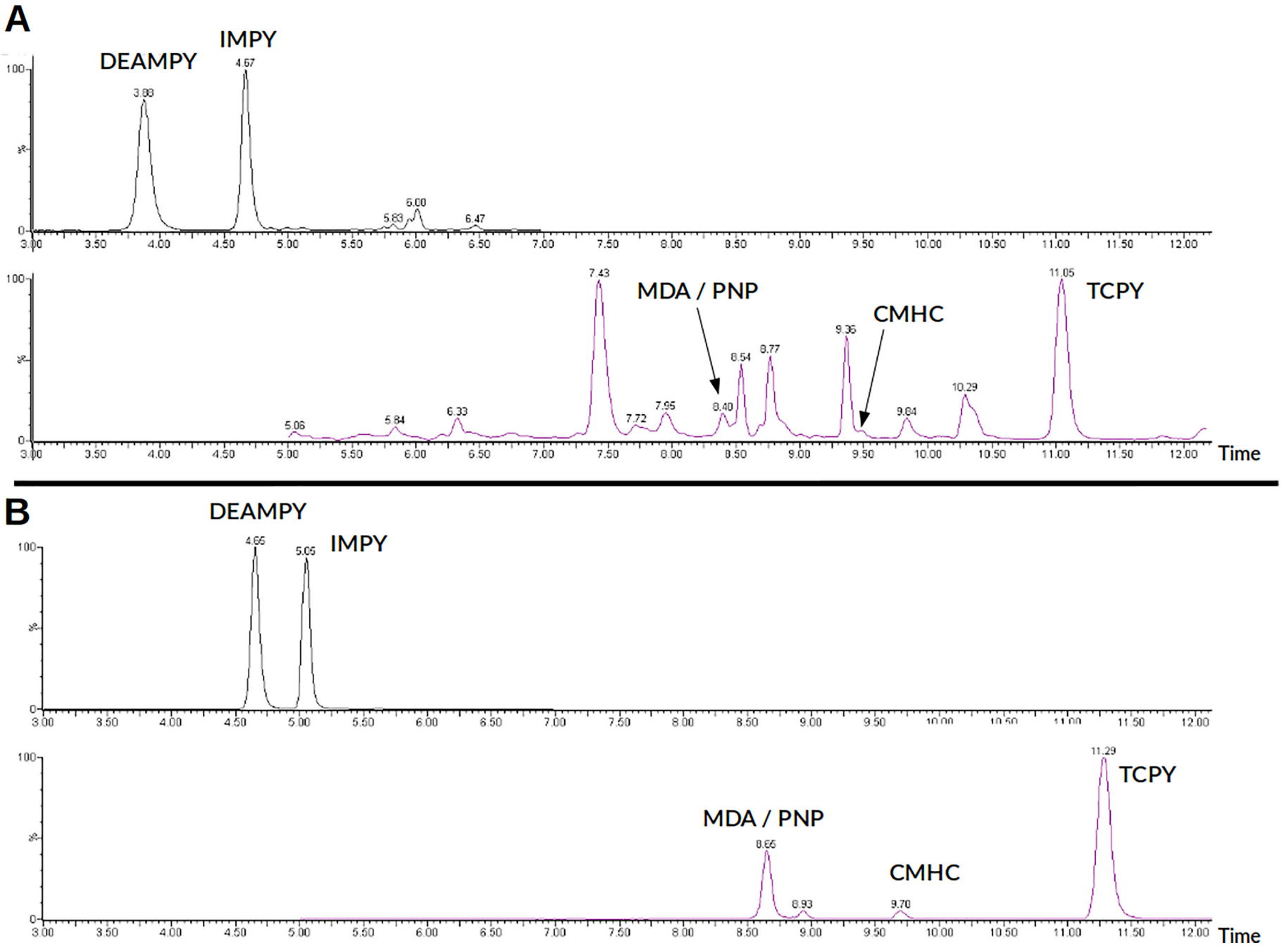

Fig. 3. TIC chromatograms of a real urine extract (A) and synthetic urine extract (B), for each of the three timed segments.

Limits of detection were calculated by the DIN 32645 methodology (equivalent to ISO 11843) using calibration straight lines. The calculation was carried out following previous descriptions (Massart et al., 1997) and implemented through chemCal package (Ranke, 2015) of the statistical software R (R Core Team, 2016).

2.5. Biological samples

Human urine samples ($n = 125$) from two adult Spanish populations in Catalonia and Galicia were analysed. One third of the cohort ($n = 48$) are farmworkers whose exposure levels to pesticides was presumably high, whereas the rest of the samples belonged to inhabitants from rural and urban areas located in Catalonia and Galicia. The samples were frozen within 4 h of collection and were stored at −20 °C until analysis.

3. Results and discussion

3.1. Method optimization

Precondition and elution of SPE cartridges with adequate solvents is a key step for the extraction procedure. The present optimized methodology used a mixture of methanol and acetone (25:75 *v*/v) for these steps (Fig. 1). Previously reported methodologies using SPE cartridges only used methanol (Olsson et al., 2004; Baker et al., 2004) or acetone (Davis et al., 2013), but not a mixture of both. In the present study, this mixture was found to be more effective for achieving better recoveries of certain metabolites, *e.g.* CMHC, 3-PBA and 4-F-3-PBA, without compromising the rest of the analytes. In addition, the same mixture was used for final vial reconstitution before injection (Davis et al., 2013).

Various mobile phases and other parameters (flow rate, solvation and column temperatures) were tested in order to achieve a good separation and peak shape for all target metabolites, posing special attention to MDA, which usually involves high difficulties. Previous studies have reported high limits of detection and low levels of relative recoveries for this compound (Davis et al., 2013; Olsson et al., 2004; Roca et al., 2014a, 2014b). Common solvents, including methanol and acetonitrile, and modifiers such as acetic acid (at different percentages ranging from 0% to 5%), ammonium acetate and ammonium formate (both at 20 mM) were tested, in combination with different gradient, flow rates (ranging from 0.2 to 0.5 ml/min), solvation temperatures (500 °C and 600 °C) and column temperatures (30 °C to 50 °C). Finally, a mobile phase containing HPLC H_2O with 5% methanol and 1% of acetic acid was the one which provided the best peak shape for MDA without compromising the analytical performance of the rest of the pesticide metabolites. Solvation temperature was set at 500 °C, the columnn temperature at 30 °C and a flow rate at 0.3 ml/min.

3.2. Method validation

Limits of detection were calculated from the calibration straight lines following the IUPAC recommendations (Massart et al., 1997; Currie, 1997). These calibration straight lines provided good linearity for all the compounds ($R^2 > 0.99$). Limits of detection below 0.069 μg per litre urine for OP metabolites and between 0.018 and 0.019 μg/l for PYR metabolites could be achieved (Table 2). These values were lower than those reported in previous similar methodologies (Roca et al., 2014b; Davis et al., 2013; Olsson et al., 2004). In addition, calculation of the LDs according to this method provides values that are close to the real limits when using synthetic urine (see next subsection below).

Accuracy and precision were assessed from two concentration levels, 1 ng/ml and 10 ng/ml, QCL and QCH, respectively, using synthetic urine. Recoveries ranged between 66% and 100% for CMHC and 4F3PBA, respectively (Table 2). The repeatability and reproducibility coefficients of variance (CV) were lower than 20% in all cases and lower than 10% in the repeatability runs (Table 2).

Analysis of proficiency testing materials obtained from the G-Equas programme (GEQUAS, 2016) provided results within the range of 20% of the consensus values (Table 3).

3.3. Matrix effects

Matrix effects are common in urine analyses which are already observed as interferences in the calibration straight lines (Tudela et al., 2012; Deventer et al., 2014). Human urine samples diluted in water are generally used for the preparation of these calibration straight lines (Olsson et al., 2004; Davis et al., 2013; Roca et al., 2014a, 2014b). However, human urine composition varies greatly due to many different factors. In the present study, the use of synthetic urine has been observed to be the best method to solve these interfering matrix effects. This approach is also useful to account for possible contaminations when used for blanks. A total of 10 procedural blanks of synthetic urine were analysed. Overall, the concentrations of the analysed metabolites, when found in blank urines, corresponded to small contamination of the analytical process and did not biased the final results. The total ion chromatograms (TIC) of real and synthetic urine extracts previously fortified with the analytes of interest are compared in Fig. 3. As shown in this figure, real urine extracts present many peaks, specially in the second segment, which may interfere in the real signals of calibration straight lines for specific compounds, *e.g.* MDA, PNP, CMHC and TCPY, whereas fortified sythetic urine samples only show the peaks of the selected analytes.

3.4. Analysis of real samples

The application of this procedure to human urine samples from rural farmers and general population living in rural and urban areas shows that PNP (found in all samples analysed), TCPY (found in 95% of the samples) and DEAMPY (77%) were the most abundant OP metabolites, with median concentrations of 1.8 ng/ml, 1.1 ng/ml and 3.2 ng/ml, respectively (Table 4). None of the samples showed MDA, and a few of them (<5%) had detectable concentrations of IMPY and CMHC (Table 4). Concerning the PYR metabolites, 3-PBA was found in 81% of the samples (median 1.5 ng/ml) and 4-F-3-PBA

Table 4
Detection frequencies (DF, %) and median, mean and concentration ranges (ng/ml) of organophosphate and pyrethroid pesticide metabolites in urine of adults from Catalonia and Galicia.

	Total (n = 125)				Farmworkers ($n = 45$)				Rural & urban ($n = 80$)			
	DF (%)	Median	Mean	Range	DF (%)	Median	Mean	Range	DF (%)	Median	Mean	Range
DEAMPY[a]	77	1.1	2.2	nd – 18.8	82	1.7	2.9	nd – 15.6	74	0.81	1.9	nd – 18.8
IMPY	2	nd	0.24	nd – 14.2	2	nd	0.32	nd – 14.2	3	nd	0.20	nd – 13.7
MDA	0	–	–	–	0	–	–	–	0	–	–	–
PNP	100	1.8	2.9	0.059–16.0	100	2.3	3.9	0.25–16.0	100	1.3	2.3	0.059–14.8
CMHC	1	0.013	0.014	nd – 0.10	0	–	–	–	1	nd	0.014	nd – 0.10
TCPY	95	3.2	3.7	nd – 20.0	100	4.2	5.4	1.2–20.0	93	2.2	2.7	nd – 8.8
3-PBA	82	1.5	2.5	nd – 20.5	91	2.4	3.7	nd – 20.5	76	1.1	1.8	nd – 15.0
4-F-3-PBA	54	0.076	0.088	nd – 0.34	47	nd	0.084	nd – 0.26	58	0.079	0.091	nd – 0.34

[a] Acronyms in Table 1.

was found in half of the cohort (54%) with median concentrations of 0.076 ng/ml (Table 4).

Comparison of the population of farmworkers with the population of non-farmworkers living in rural and urban areas shows higher concentrations of DEAMPY (medians 1.7 *vs.* 0.81 ng/ml, respectively), PNP (2.3 *vs.* 1.3 ng/ml), TCPY (4.2 *vs.* 2.2 ng/ml) and 3-PBA (2.4 *vs.* 1.1 ng/ml) in the former (Table 4). These differences are consistent with occupational activity (Ye et al., 2013; Wang et al., 2016; EFSA, 2014). Farmworkers are directly exposed to these pesticides through inhalation, dermal contact and indirect ingestion (*e.g.* skin, eyes), through the manipulation of these substances when either mixing, loading and handling treated crops, or spraying and applying them into the fields (Egeghy et al., 2011; EFSA, 2014). A continuous exposure can occur if workers and operators do not undertake additional measures during and after work, including use of personal protective equipment (*e.g.* gloves, mask, glasses) or washing regularly contaminated clothing (Arcury et al., 2009; Farahat et al., 2011). However, the aforementioned median values show differences of two times, indicating that people not occupationally exposed to the use of these pesticides is also incorporating these compounds, probably as consequence of food consumption. This is consistent with previous studies in which OP and PYR metabolites were positively associated with higher intakes of fruits and vegetables (Llop et al., 2017). The studied pesticides are commonly used in agriculture, and some of them have been encountered in food products from European countries (EFSA, 2017). For instance, the OP pesticide chlorpyriphos (metabolized into TCPY in humans) is one of the most frequently found pesticides in plant products, and the one with higher number of quantifications exceeding the maximum residue levels (MRL) allowed by the EU legislation (EFSA, 2017).

OP and PYR pesticides are also employed as biocidals for domestic purposes, for household pets and gardening, among other uses (*e.g.* ornamental plants), and exposure of general populations through other non-food sources in rural and urban areas should not be underestimated. In addition, residents living in areas close to the application of pesticides may be at increased risk of exposure, in a similar way than occupationally-exposed individuals (EFSA, 2014).

The present study is the first comparing the metabolite OP and PYR concentrations among general and occupationally exposed populations in countries with strong agricultural activities such as those in Spain. The results show that both populations are generally exposed to pyrethroids and OP pesticides, including chlorpyriphos, pirimiphos and parathion.

4. Conclusions

The isotope dilution solid phase extraction UPLC-MS/MS method developed in the present study is adequate for the analysis of organophosphate and pyrethroid pesticide metabolites in urine samples from general and potentially highly exposed human populations. It allows the determination of eight target pesticide metabolites in urine with satisfactory sensitivity, accuracy and precision. The use of methanol:acetone (25:75 *v*/v) for extraction is more effective and provides better recoveries than these individual solvents. In addition, the use of synthetic urine improves significantly the method selectivity and limits of detection in the construction of the calibration straight lines. Detection limits in the range of 14–69 pg/ml were obtained. Metabolites of both pesticide groups were observed in both occupationally and non-occupationally exposed populations, the former showing twofold average concentrations of organophosphate metabolites than the second.

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References

Arcury, T.A., Grzywacz, J.G., Barr, D.B., Tapia, J., Chen, H., Quandt, S., 2007. Pesticide urinary metabolite levels of children in eastern North Carolina farmworker households. Environ. Health Perspect. 115 (7), 1254–1260.

Arcury, T.A., Grzywacz, J.G., Isom, S., Whalley, L.E., Vallejos, Q.M., Chen, H., Galván, L., Barr, D.B., Quandt, S.A., 2009. Seasonal variation in the measurement of urinary pesticide metabolites among Latino farmworkers in eastern North Carolina. Int. J. Occup. Environ. Health 15 (4), 339–350.

Arcury, T.A, Laurienti, P.J., Chen, H., Howard, T.D., Barr, D.B., Mora, D.C., Summers, P., Quandt, S.A., 2016. Organophosphate pesticide urinary metabolites among Latino immigrants: North Carolina farmworkers and non-farmworkers compared. J Occup Environ Med. 58 (11), 1079–1086.

Baker, S.E., Olsson, A.O., Barr, D.B., 2004. Isotope dilution high-performance liquid chromatography-tandem mass spectrometry method for quantifying urinary metabolites of synthetic pyrethroid insecticides. Arch. Environ. Contam. Toxicol. 46, 281–288.

Barr, D.B., 2008. Biomonitoring of exposure to pesticides. J. Chem. Health Saf. 15 (6), 20–29.

Barr, D.B., Olsson, A.O., Wong, L.-Y., Udunka, S., Baker, S.E., Whitehead Jr., R.D., Magsumbol, M.S., Williams, B.L., Needham, L.L., 2010. Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: national health and nutrition examination survey 1999–2002. Environ. Health Perspect. 118 (6), 742–748.

Currie, L.A., 1997. Nomenclature in evaluation of analytical methods including detection and quantification capabilities (UIPAC recommendations 1995). Anal. Chim. Acta 391, 105–126.

Davis, M.D., Wade, E.L., Restrepo, P.R., Roman-Esteva, W., Bravo, R., Kuklenyik, P., Calafat, A.M., 2013. Semi-automated solid phase extraction method for the mass spectrometric quantification of 12 specific metabolites of organophosphorus pesticides, synthetic pyrethroids, and select herbicides in human urine. J. Chromatogr. B 929, 18–26.

Deventer, K., Pozo, O.J., Verstraete, A.G., Van Eenoo, P., 2014. Dilute-and-shoot-liquid chromatography-mass spectrometry for urine analysis in doping control and analytical toxicology. TrAC Trends Anal. Chem. 55, 1–13.

EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products. EFSA J. 12 (10), 3874 (55 pp).

EFSA (European Food Safety Authority), 2017. The 2015 European Union report on pesticide resudies in food. EFSA J. 15 (4), 4791 (134 pp).

Egeghy, P.P., Cohen Hubal, E.A., Turve, N.S., Melnyk, L.J., Morgan, M.K., Fortmann, R.C., Sheldon, L.S., 2011. Review of pesticide urinary biomarker measurements from selected US EPA Children's observational exposure studies. Int. J. Environ. Res. Public Health 8, 1727–1754.

Farahat, F.M., Ellison, C.A., Bonner, M.R., McGarrigle, B.P., Crane, A.L., Fenske, R.A., Lasarev, M.R., Rohlman, D.S., Anger, W.K., Lein, P.J., Olson, J.R., 2011. Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. Environ. Health Perspect. 119 (6), 801–806.

Garí, M., Grimalt, J.O., 2010. Use of proficiency testing materials for the calculation of detection and quantification limits in the analysis of organochlorine compounds in human serum. Anal. Bioanal. Chem. 397, 1383–1387.

German External Quality Assessment Scheme for Analyses in Biological Materials (2016).

Koch, H.M., Hardt, J., Angerer, J., 2001. Biological monitoring of exposure of the general population to the organophosphorous pesticides chlorpyrifos and chlorpyrifos-methyl by determination of their specific metabolite 3,5,6-trichloro-2-pyridinol. Int. J. Hyg. Environ. Health 204, 175–180.

Koureas, M., Tsakalof, A., Tsatsakis, A., Hadjichristodoulou, C., 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol. Lett. 210, 155–168.

Llop, S., Murcia, M., Iñiguez, C., Roca, M., González, L., Yusà, V., Rebagliato, M., Ballester, F., 2017. Distributions and determinants of urinary biomarkers of organophosphate pesticide exposure in a prospective Spanish birth cohort study. Environ. Health 16, 46.

Massart, L.M., Vandeginste, B.G.M., Buydens, L.M.C., De Jong, S., Lewi, P.J., Smeyers-Verbeke, J., 1997. Handbook of Chemometrics and Qualimetrics: Part a, p. 200. Elsevier, Amsterdam.

Olsson, A.O., Baker, S.E., Nguyen, J.V., Romanoff, L.C., Udunka, S.O., Walker, R.D., Flemmen, K.L., Barr, D.B., 2004. A liquid chromatography-tandem mass spectrometry multiresidue method for quantification of specific metabolites of organophosphorus pesticides, synthetic Pyrethroids, selected herbicides, and DEET in human urine. Anal. Chem. 76 (9), 2453–2461.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL https://www.R-project.org/.

Ranke, J., 2015. chemCal: calibration functions for analytical chemistry. R package version 0.1-37 URL http://www.r-project.org, http://chem.uft.uni-bremen.de/ranke/?page=chemCal, http://cgit.jrwb.de/chemCal.

Roca, M., Leon, N., Pastor, A., Yusà, V., 2014a. Comprehensive analytical strategy for biomonitoring of pesticides in urine by liquid chromatography-orbitrap high resolution mass spectrometry. J. Chromatogr. A 1374, 66–76.

Roca, M., Miralles-Marco, A., Ferré, J., Pérez, R., Yusà, V., 2014b. Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. Environ. Res. 131, 77–85.

Tudela, E., Muñoz, G., Muñoz-Guerra, J.A., 2012. Matrix effect marker for multianalyte analysis by LC-MS/MS in biological samples. J. Chromatogr. B 901, 98–106.

Ueyama, J., Saito, I., Kamijima, M., 2010. Analysis and evaluation of pyrethroid exposure in human population based on biological monitoring of urinary pyrethroid metabolites. J. Pestic. Sci. 35 (2), 87–98.
Wang, L., Liu, Z., Zhang, J., Wu, Y., Sun, H., 2016. Chlorpyrifos exposure in farmers and urban adults: metabolic characteristic, exposure estimation, and potential effect of oxidative damage. Environ. Res. 149, 164–170.
Waters Inc., 2008. http://www.waters.com/waters/en_US/MassLynx-Mass-Spectrometry-Software-/nav.htm?cid=513164&lset=1&locale=en_US&changedCountry=Y.
Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2013. Occupational pesticide exposures and respiratory health. Int. J. Environ. Res. Public Health 10 (12), 6442–6471.