VNIVERSITAT DE VALÈNCIA

Facultat de Química

DESARROLLO DE NUEVAS ESTRATEGIAS ANALÍTICAS BASADAS EN LA ESPECTROMETRÍA DE MASAS DE ALTA RESOLUCIÓN (HRMS) PARA EL CONTROL DE PLAGUICIDAS Y SUS METABOLITOS EN LA ATMÓSFERA

TESIS DOCTORAL

ANTONIO LÓPEZ TOBARUELA

Dirigida por:

Dr. Vicent Yusà Pelechá

Dra. Clara Coscollà Raga

Tutor: Dr. Agustín Pator García

PROGRAMA DE DOCTORADO EN TÉCNICAS EXPERIMENTALES EN QUÍMICA

Valencia, Mayo 2018

TESIS DOCTORAL

DEPARTAMENTO DE QUÍMICA ANALÍTICA

PROGRAMA DE DOCTORADO: Cód. 3158. DOCTORADO EN TÉCNICAS EXPERIMENTALES EN QUÍMICA

LÍNEA DE INVESTIGACIÓN: CONTAMINACIÓN Y CONTROL DEL MEDIO AMBIENTE

TÍTULO: DESARROLLO DE NUEVAS ESTRATEGIAS ANALÍTICAS BASADAS EN LA ESPECTROMETRÍA DE MASAS DE ALTA RESOLUCIÓN (HRMS) PARA EL CONTROL DE PLAGUICIDAS Y SUS METABOLITOS EN LA ATMÓSFERA

NOMBRE: ANTONIO LÓPEZ TOBARUELA

DIRECTORES:

Dr. Vicent Yusà Pelechá

Dra. Clara Coscollà Raga

Tutor:

Dr. Agustín Pastor García

Valencia, Mayo 2018

A mis padres

A mi hermana

AGRADECIMIENTOS

En primer lugar, quiero dar las gracias y agradecer a mis dos directores de Tesis, tanto al Dr. Vicent Yusà como a la Dra. Clara Coscollà todo el apoyo recibido durante la realización de este proyecto.

Agradecer a Vicent el inculcarme su entusiasmo y pasión por la investigación científica, intentando siempre ir un paso más para lograr objetivos más entusiastas, aunque suponga una ruta más larga y difícil para conseguir los objetivos. Por otro lado me gustaría agradecer a Clara su confianza depositada en mí y el aprender con ella tanto sobre el mundo de los plaguicidas en la atmósfera.

Por supuesto, agrader a todos los compañeros del Departamento de Contaminantes Orgánicos del Laboratorio de Salud Pública: Anna, Carmen, Dolores, Eduard, Elvira, Julia, Lidia, Lucía, Maribel, Mari Cruz, Mertxe, Miguel Ángel, Olga, Pablo, Pepe Toni, Raquel, Sandra y Yovana por su apoyo, ayuda y comprensión, tanto en los buenos momentos como en los malos momentos.

También agradecer a mis amigos su paciencia conmigo durante todos estos años, sportando todos los “No puedo quedar que tengo faena”. Aun así, hemos sido capaces de disfrutar de muy buenos momentos.

Agradecer también a todas las personas, que de una forma u otra, han contribuido a la realización de esta Tesis Doctoral.

A mis padres, Lucía y Juan Ramón, agradecer el haber sido los dos pilares fundamentales de mi vida, por enseñármelo todo, creer y confiar en mí siempre, y aguantarme, que, en ocasiones, no es poco. Papá, aunque no hayas podido ver el final de esto, sé que todo el tiempo en el que hemos estado juntos has estado orgulloso de mí. También agradecer a mi hermana, Lucía, su apoyo, su confianza y su cariño. Ella siempre ha confiado en este “Loco” que un día decidió estudiar Química.

ÍNDICE

I. INTRODUCCIÓN

1. ABSTRACT/RESUMEN

El uso de plaguicidas en la agricultura, permiten aumentar el rendimiento de las cosechas, evitando pérdidas debidas a animales, plantas y microorganismos que pueden llegar a tener un efecto negativo sobre la producción agrícola. Así, debido a su uso intensivo en las prácticas agrícolas, los plaguicidas pueden contaminar los distintos compartimentos ambientales (agua, suelo, aire, etc). Actualmente, aproximadamente 500 sustancias activas están autorizadas por la Unión Europea para ser aplicadas a los distintos cultivos siguiendo la Regulación CE 1107/2009.

Una parte de los plaguicidas se emiten a la atmósfera durante y después de su aplicación. En la atmósfera, los plaguicidas pueden llegar a sufrir procesos de transporte y transformación, pudiéndose generar además nuevas sustancias como son los metabolitos o productos de transformación. Los plaguicidas se distribuyen en dos fases (particulada y gaseosa) según sus características fisicoquímicas y las características ambientales. Tanto los plaguicidas como sus posibles productos de transformación formados pueden presentar un riesgo para la salud, especialmente en aquellos grupos más sensibles, como es el caso de los bebés, las mujeres embarazadas o las personas con enfermedades crónicas.

La cromatografía líquida acoplada a espectrometría de masas de alta resolución se ha empleado para el análisis de plaguicidas en múltiples matrices. Sin embargo, esta técnica de análisis no ha sido aplicada en el análisis de plaguicidas en el aire ambiente. Esta Tesis Doctoral tiene como objetivos principales el desarrollo de metodologías adecuadas para el análisis de plaguicidas y metabolitos en el aire, evaluándose además el riesgo que existe para la población debido a la presencia de éstos compuestos en la atmósfera.

Esta tesis se compone de seis capítulos experimentales. En los cuatro primeros capítulos se implementan nuevas metodologías analíticas para el análisis de plaguicidas presentes tanto en la fase particulada como en la fase gaseosa, mientras que en los dos últimos capítulos se realiza una evaluación del riesgo a la exposición a la inhalación de plaguicidas en poblaciones de la Comunidad Valenciana y Francia.

En el primer capítulo se ha desarrollado una metodología analítica para el análisis retrospectivo (análisis post-target y análisis non-target) de metabolitos en filtros de fibra de vidrio PM10, creándose para ello una base de datos de unos 240 metabolitos de plaguicidas presentes en diferentes matrices.

En el capítulo 2 se han comparados técnicas de extracción de plaguicidas presentes en filtros PM10 empleando LC-HRMS: la extracción mediante MAE y la extracción mediante una cafetera Espresso, estudiándose las recuperaciones a distintos niveles y analizándose por cada método de extracción un total de 10 muestras reales, comparándose los resultados obtenidos.

En el capítulo 3 se ha desarrollado una metodología analítica para el análisis de la fase gaseosa de los plaguicidas (retenidos en los adsorbentes) mediante GC-MS/MS. Para ello, se ha seleccionado el adsorbente más adecuado para la retención de los plaguicidas, evaluándose tanto su capacidad de retención (retention capacity) como su

capacidad de colmatación (breakthrough). Además, se optimizó el equipo de cromatografía gaseosa, para obtener los mejores resultados posibles.

En el cuarto capítulo, se ha desarrollado una metodología analítica para el análisis de la fase gaseosa de los plaguicidas polares (retenidos en los adsorbentes) mediante UHPLC-HRMS. Para ello, se ha seleccionado el adsorbente más adecuado para la retención de los plaguicidas, evaluándose tanto su capacidad de retención (retention capacity) como su capacidad de colmatación (breakthrough).

En el quinto capítulo se ha evaluado la exposición a plaguicidas de diferentes poblaciones rurales, urbanas y remotas de la Comunidad Valenciana. Se han estudiado tres grupos de población diferentes (bebés, niños y adultos), estudiándose el índice de peligro individual (HQ) para cada plaguicida, el índice acumulado (HI, RPF) debido a la presencia de plaguicidas con características semejantes y el riesgo a exposición a cáncer (Cancer Risk).

En el sexto capítulo se ha la exposición a plaguicidas en una población rural de Francia (Región Centro). De igual forma que en el capítulo 5, se han evaluado tres grupos de población diferentes, estudiándose HQ, HI, RPF y riesgo a exposición a cáncer.

Esta serie de trabajos, tanto las metodologías analíticas desarrolladas como los estudios de evaluación del riesgo a la exposición a plaguicidas, pueden ayudar para posibles normativas futuras en el uso y restricción de plaguicidas en prácticas agrícolas.

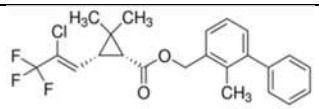
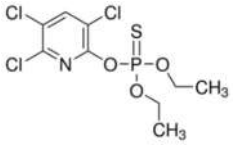
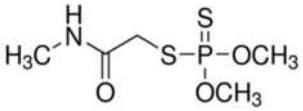
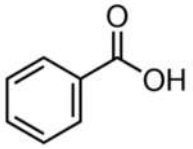
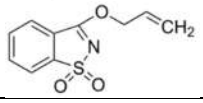
2. INTRODUCCIÓN

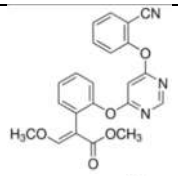
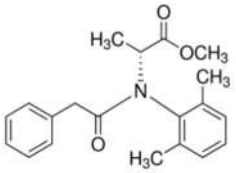
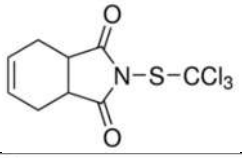
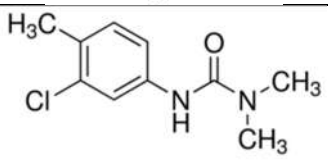
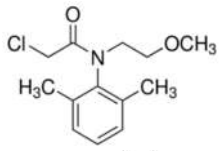
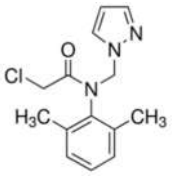
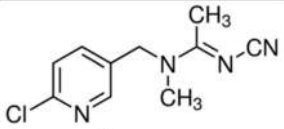
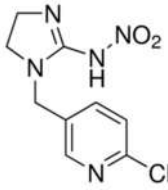
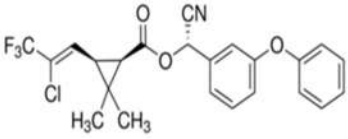
2.1. Plaguicidas: definición, clasificación, usos y riesgos.

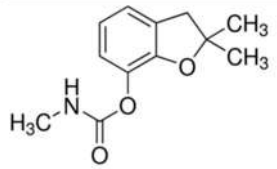
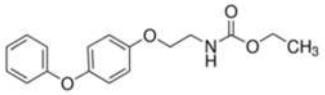
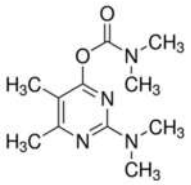
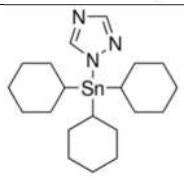
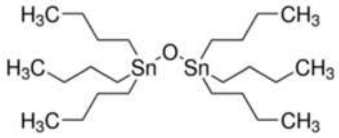
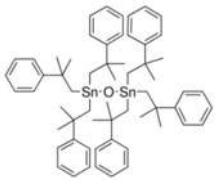
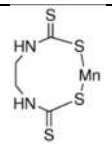
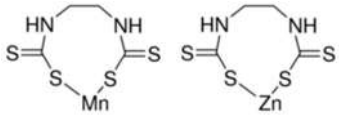
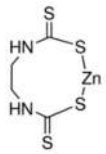
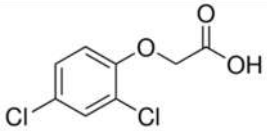
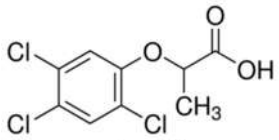
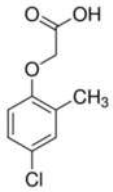
2.1.1. Definición y clasificación de los plaguicidas.

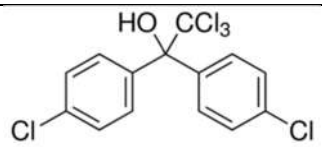
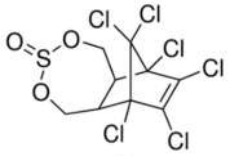
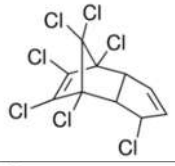
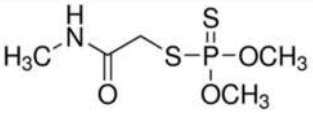
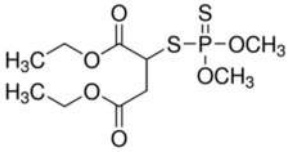
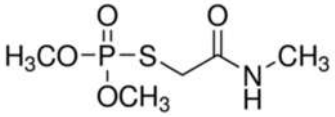
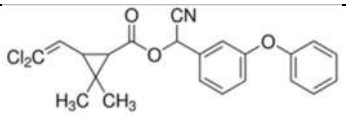
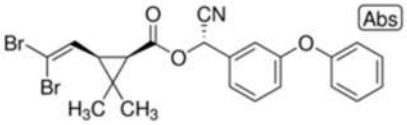
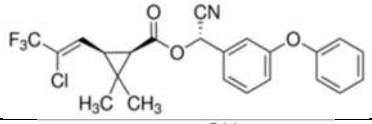
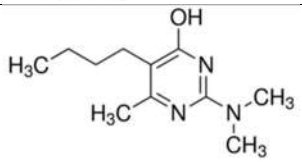
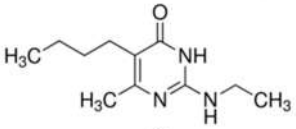
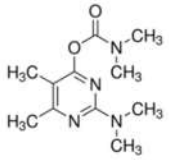
Según definición de la OMS, los plaguicidas son productos químicos que se emplean en la agricultura para proteger los cultivos contra los insectos, los hongos, las malezas y otras plagas, bien en la agricultura o bien para otras finalidades. Dependiendo de su función, los plaguicidas se clasifican en acaricidas, bactericidas, fungicidas, herbicidas, insecticidas, nematicidas y rodenticidas, principalmente. También pueden ser clasificados según su estructura química, dividiéndose principalmente en benzimidazoles, benzoilureas, carbamatos, derivados del estaño, ditiocarbámicos, fenoxiherbicidas, organoclorados, organofosforados, piretroides, pirimidinas y triazoles [1]. En la siguiente tabla (Tabla 1) se presenta una clasificación de los plaguicidas actualmente utilizados según su función en la agricultura y también según su estructura química, con ejemplos en cada uno de los grupos.

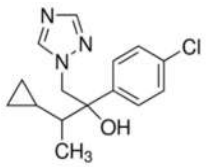
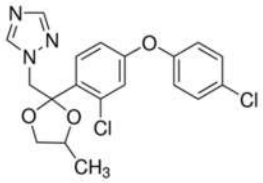
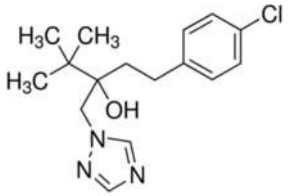
Tabla 1. Clasificación de los plaguicidas actualmente utilizados según su función y su estructura química

A. Según su función		
Grupo	**Plaguicida**	**Estructura**
Acaricidas	Bifentrina	
	Clorpirifos-etil	
	Dimetoato	
Bactericidas	Sulfato de aluminio	$Al_2(SO_4)_3$
	Ácido Benzoico	
	Probenazol	

Fungicidas	Azoxiestrobina	
	Benalaxil-M	
	Captan	
Herbicidas	Clortoluron	
	Dimetacloro	
	Metazacloro	
Insecticidas	Acetamiprid	
	Imidacloprid	
	Lambda-Cialotrina	

Carbamatos	Carbofurano	
	Fenoxicarb	
	Pirimicarb	
Derivados del estaño	Azociclotin	
	Óxido de tributiltin	
	Óxido de fenbutatin	
Ditiocarbámicos	Maneb	
	Mancozeb	
	Zineb	
Fenoxiherbicidas	2,4-D	
	Fenoprop	
	MCPA	

Organoclorados	Dicofol	
	Endosulfan	
	Heptacloro	
Organofosforados	Dimetoato	
	Malation	
	Ometoato	
Piretroides	Cipermetrina	
	Deltametrina	
	Lambda-Cialotrina	
Pirimidinas	Dimetirimol	
	Etirimol	
	Pirimicarb	

Triazoles	Ciproconazol	
	Difenoconazol	
	Tebuconazol	

2.1.2. Uso y consumo de plaguicidas en Europa, España y la Comunidad Valenciana.

En 2013, un total de 10,8 millones de explotaciones agrícolas existían dentro de los 28 países de la Unión Europea con una superficie global de 175 millones de hectáreas, suponiendo el 40 % de la superficie total y dando empleo a 9,5 millones de personas (el 92 % a tiempo total). El valor total de la producción agrícola anual fue de aproximadamente 164700 millones de euros en el año 2015 [2]. Por países, Francia fue el mayor productor agrícola (con un 18.3 % del total), seguido de Italia (13.4 %), Alemania (12.5 %) y España (11.8 %), que se situó en el cuarto lugar.

Las principales producciones dentro de la Unión Europea en el año 2015 han sido los cereales (317 millones de toneladas), la remolacha azucarera (102 millones de toneladas), hortalizas (principalmente tomates, cebollas y zanahorias) y frutas (manzanas, melocotones y naranjas).

El uso de plaguicidas es necesario en las técnicas agrícolas empleadas actualmente, ya que el rendimiento y la calidad de las cosechas pueden verse reducidos debido a diferentes plagas y enfermedades que pueden afectar a los cultivos. El ataque de plagas y enfermedades en los diferentes cultivos agrícolas ha aumentado en los últimos años debido al uso de variedades más productivas y plantaciones más sofisticadas. La gran expansión de los monocultivos y la globalización en el transporte de materias vegetales facilita la aparición de plagas [3]. Globalmente, existen unas 9000 especies de insectos y ácaros, 50000 especies de patógenos de las plantas y 8000 especies de malas hierbas que pueden dañar los cultivos. De la pérdida total de los cultivos, un 14 % se deben debido a las plagas de insectos y ácaros, un 13 % se deben a patógenos y otro 13 % se debe a malas hierbas [4].

Actualmente, unas 500 sustancias activas están autorizadas por la Unión Europea siguiendo la Regulación (CE) 1107/2009 [5] para su aplicación en los distintos cultivos. El 32 % de las sustancias autorizadas son fungicidas, el 25 % herbicidas y el 22 % insecticidas. Alrededor de 400000 toneladas de sustancias activas fueron consumidas en la Unión Europea (UE-28) en el año 2014, siendo el 44 % fungicidas, el 33 % de ellos herbicidas y el 5 % insecticidas. Los países donde se produjo un mayor consumo de plaguicidas en la UE fueron España (19.9 %), Francia (19.0 %), Italia (16.2 %), Alemania (11.6 %) y Polonia (5.9 %) [2, 6].

En España, que cómo se ha comentado anteriormente fue el país de la UE que tuvo el mayor consumo de plaguicidas (19.9 %) durante el año 2014, el 49 % de los plaguicidas consumidos fueron fungicidas, el 19 % herbicidas y el 10 % insecticidas, siendo el resto (22%) otros productos fitosanitarios [2]. Dentro de España, según datos aportados por las propias Comunidades Autónomas [7], las regiones agrícolas con un uso más intensivo de plaguicidas fueron Andalucía (35.2 %), la Comunidad Valenciana (11.8 %), Cataluña (9.6 %) y Murcia (9.2 %).

La Comunidad Valenciana es una de las regiones con un mayor consumo de plaguicidas, debido a que es una región en la que la producción agrícola es una actividad con un peso muy importante. El 28 % de su superficie está dedicada a tierras de cultivo. Los principales usos agrícolas de la tierra se relacionan con la producción de cítricos (25 % de la superficie agrícola), frutales (20.5 %), olivar (14 %), viñedo (10.4 %), cereales para granos (6.9 %) y hortalizas (3.2 %), ocupando aproximadamente

660000 hectáreas [8]. Las principales producciones agrícolas en el año 2015 en la Comunidad Valenciana fueron cítricos (53 %), hortalizas (9 %) y frutales (6 %). La superficie de la agricultura ecológica en el año 2014 fue de 69209 Ha, lo que suponía el 10 % del total de la superficie de las tierras de cultivo presentes en la Comunidad Valenciana.

2.1.3. Exposición y efectos sobre la salud de los plaguicidas.

El impacto de los plaguicidas en la salud y el medio ambiente varía considerablemente de un plaguicida a otro, dependiendo de las características intrínsecas de la sustancia activa (toxicidad, persistencia, etc.) y del uso que se realiza (volumen aplicado, período de aplicación, método de aplicación, tipo de suelo, etc.). Así, la Unión Europea, a través de la Directiva 2009/128/CE [9] estableció un primer marco de actuación para alcanzar un uso sostenible de los plaguicidas. De esta forma, se establecieron una serie de medidas para reforzar la vigilancia, mejorar la formación y la información de los usuarios, implementándose también medidas específicas para el uso de plaguicidas. Todas las sustancias activas aprobadas por la Unión Europea no deberían presentar ningún efecto negativo en la salud de las personas y los animales ni tampoco en el medioambiente, siguiendo las condiciones autorizadas para su uso. A pesar de ello, el potencial efecto de exposición de los plaguicidas en la población general, y específicamente en los grupos más vulnerables, como pueden ser los niños y las mujeres embarazadas, es un asunto de especial importancia [10-11].

Las principales rutas de exposición a plaguicidas en los seres humanos son a través de la cadena alimentaria, el aire ambiente, el agua y el suelo [12]. Los plaguicidas, al introducirse en el cuerpo humano, se distribuyen a través del torrente sanguíneo y pueden ser excretados a través de la orina, del sudor y de la respiración [13]. La entrada de los plaguicidas en el organismo se producen a través de cuatro vías, principalmente: vía dérmica, vía oral, vía respiratoria y vía ocular. La toxicidad de los plaguicidas puede variar dependiendo de la vía de entrada (dérmica, oral y respiratoria). De forma general, el peligro de contaminación debido a plaguicidas aumenta con la concentración o con la dosis, teniendo en cuenta también la toxicidad del plaguicida en estudio [14].

2.1.3.1. Vía dérmica.

La exposición de plaguicidas a través de la vía dérmica es una de las vías más habituales de exposición a plaguicidas por parte de los aplicadores y agricultores [12]. La adsorción puede producirse como resultado del derrame y la pulverización en el momento de la manipulación o debida a una exposición continuada a residuos de plaguicidas [15].

2.1.3.2. Vía oral.

La exposición por vía oral se produce principalmente debido a la exposición crónica (dosis bajas generalmente) a través de la ingesta de alimentos y agua, siendo la dieta una fuente significativa de exposición a plaguicidas por parte de la población general [13].

2.1.3.3. Vía respiratoria.

Debido a la presencia de compuestos volátiles, la exposición a plaguicidas por vía respiratoria es elevada [16]. La inhalación de grandes cantidades de plaguicidas puede causar serios daños en la nariz, en la garganta y en los tejidos pulmonares [13]. El riesgo en la exposición a plaguicidas se reduce cuando éstos son aplicados mediante pulverización, formándose gotas, siempre y cuando no se usen concentraciones elevadas de plaguicidas [16]. Otro factor importante es el espacio donde se aplica, ya que la aplicación en lugares recogidos y pequeños aumenta el riesgo de inhalación.

2.1.3.4. Vía ocular.

El potencial de lesión en los tejidos oculares es elevado. Los plaguicidas granulares son especialmente peligrosos para los ojos en función del tamaño y peso de las partículas individuales [17].

Diferentes estudios epidemiológicos han investigado la relación entre la exposición a los plaguicidas y su posible efecto en la salud de los seres humanos y animales. Se ha sugerido que existe una relación entre la exposición a plaguicidas y diferentes enfermedades tales como alergias, asma, cáncer, diabetes, hipersensibilidad, problemas neurológicos y reproductivos o trastornos hormonales [18-19]. Además, existen evidencias de efectos negativos en la fauna cómo por ejemplo defectos de nacimiento en las aves, reducción de peso de nuevas aves, muertes súbitas, etc. [14, 20-21].

De forma general, en la tabla 2 se muestran algunos de los trabajos publicados donde se nos indican posibles riesgos en la salud debido a la exposición continuada a plaguicidas.

Tabla 2. Revisión de artículos relacionados con riesgos en la salud relacionados a la exposición de plaguicidas

Enfermedad	Plaguicida/s estudiado/s	Grupo de población estudiado	País	Referencia
Asma	Organofosforados	Madres y niños	EEUU	[22]
	Pendimetalina y aldicarb	Aplicadores	EEUU	[23]
	Exposición general a plaguicidas	Trabajadoras agrícolas	Sudáfrica	[24]
	Herbicidas	Agricultores	España	[25]
	Exposición general a plaguicidas	Granjeras	EEUU	[26]
Cáncer	Imidaclorpid	Ratones	Grecia	[27]
	Exposición general	Trabajadores agrícolas	Egipto	[28]
	Organoclorados	Mujeres	Túnez	[29]
	Imazetapir e imazaquin	Aplicadores	EEUU	[30]
	Organofosforados	Mujeres de aplicadores	EEUU	[31]
	Acetocloro y atrazina	Aplicadores/No aplicadores	EEUU	[32]
	Organoclorados	Mujeres sanas/con cáncer	España	[33]
	Exposición general a plaguicidas	Trabajadores sanitarios	Pakistán	[34]
	Exposición general a plaguicidas	Mujeres	Australia	[35]
	Plaguicidas residenciales	Mujeres entre 20-35 años	Brasil	[36]
	Imazetapir	Aplicadores	EEUU	[37]
	Herbicidas	Mujeres	EEUU	[38]
	Plaguicidas en general	Personas con tumores cerebrales	Francia	[39]
	Plaguicidas en general	Aplicadores en jardines	Italia	[40]
Diabetes	Organoclorados	Población global	Asia	[41]
	Piretroides	Aplicadores y personas no expuestas	Bolivia	[42]
	2,4,5-triclorofenoxiacético, dieldrin, fonofos, forato y paration	Aplicadores y parejas de aplicadores	EEUU	[43]
	Organoclorados y sus metabolitos	Población en general	Sáhara	[44]
	Organoclorados	Población diabética/ sin diabetes	EEUU	[45]
	Organoclorados	Población en general	EEUU	[46]
	Exposición general a plaguicidas	Mujeres embarazadas aplicadoras	EEUU	[47]
Efectos cognitivos	p, p'-DDE, trans-nonacloro y hexaclorobenceno	Hombres y mujeres de 70 años	Suecia	[48]
	Beta-hexaclorociclohexano, trans-nonacloro, oxiclordano, heptacloro	Hombres y mujeres de 60-85 años	EEUU	[49]
	3-PBA y cis-DBCA	Parejas madre-hijo	Francia	[50]
	Organofosforados	Niños hasta 7 años	EEUU	[51]
	Organofosforados	Niños hasta 9 años	EEUU	[52]
	Organofosforados	Niños hasta 7 años	EEUU	[53]
	Exposición general a plaguicidas	Trabajadores entre 42-57 años	Francia	[54]

Leucemia	Exposición general a plaguicidas	Madres antes, durante y después del embarazo	Global	[55]
	Exposición general a plaguicidas	Agricultores	Irán	[56]
Parkinson	Exposición general a plaguicidas	Población general	Holanda	[57]
	Exposición general a plaguicidas	Población general	EEUU	[58]
	Exposición general a plaguicidas	Aplicadores y agricultores	Francia	[59]
	Exposición general a plaguicidas	Pacientes de hospitales	EEUU	[60]
	Organofosforados	Población general	EEUU	[61]

2.2. Emisión, transporte y transformación de los plaguicidas en la atmósfera.

2.2.1. Emisión.

Existen diferentes fuentes de emisión a la atmósfera de plaguicidas tanto durante la aplicación de éstos como durante un tiempo posterior a la aplicación (tiempo post-aplicación). La primer fuente de emisión se produce durante la aplicación de los plaguicidas en diferentes actividades agrícolas, ya que una fracción de la dosis aplicada acaba depositada en zonas cercanas al área tratada (suelo y plantas, principalmente) y otra fracción (que suele oscilar entre un 20 % y un 30 %) se transfiere a la atmósfera (efecto deriva debido a la pulverización) [62]. Así, los plagucidas entran durante la aplicación debido a la deriva y a la volatilización que se produce durante la aplicación. El spray drift (la deriva debido a la pulverización) depende de diferentes factores tales como la volatilidad (a partir de su presión de vapor) y la viscosidad del plaguicida empleado, las técnicas de aplicación y equipamiento empleadas, las condiciones atmosféricas (temperatura, viento y humedad relativa) durante la aplicación o la habilidad del operador que realice el trabajo [63-64]. La deriva debido al viento sólo es relevante durante la aplicación a una distancia limitada desde el lugar de aplicación. Para largas distancias, la volatilización es el proceso de emisión predominante. Así, la presión de vapor es el elemento clave para determinar la posible volatilización del plaguicida [63].

La segunda fuente de emisión, posterior a la aplicación (tiempo post-aplicación), es la volatilización de los plaguicidas desde las plantas y desde el suelo a la fase gaseosa. Los principales factores que afectan a la volatilización de plaguicidas post-aplicación desde el suelo y las plantas son propiedades fisicoquímicas (presión de vapor, solubilidad, coeficiente de adsorción, reactividad,...), propiedades del suelo (contenido de agua, contenido de materia orgánica, densidad del suelo, pH, etc.), condiciones meteorológicas (temperatura del aire, velocidad del viento, humedad del aire) y actividades agrícolas (ratio de aplicación, tipo de formulación, etc) [64].

La tercera fuente de emisión de plaguicidas es la erosión debida al viento que provoca que se transporten partículas del suelo cargadas con plaguicidas (*soil tillage operations*) [65].

Seguidamente, se muestra un esquema donde se observan las tres fuentes de emisión principales de plagucidas a la atmósfera (ver Figura 1).

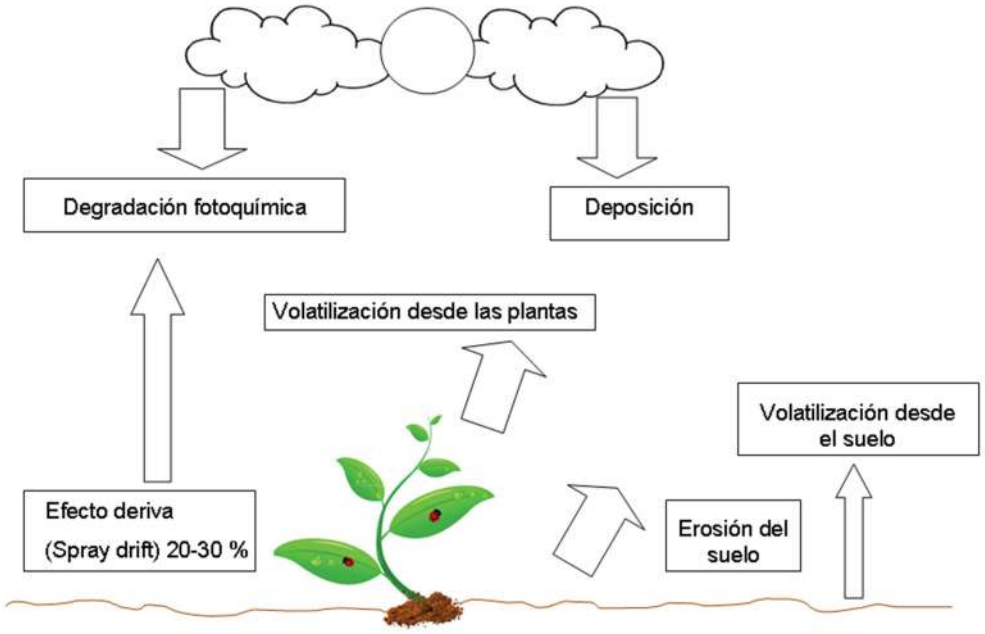


Figura 1. Fuentes de emisión de plagucidas a la atmósfera.

2.2.2. Transporte.

Una vez los plaguicidas entran en la atmósfera, éstos tienden a mezclarse uniformemente y a dispersarse en la zona baja de la troposfera, próxima a la superficie terrestre. En zonas próximas a la zona de aplicación, los plaguicidas desaparecen por procesos de transporte mientras que en zonas más alejadas los fenómenos más significativos son las transformaciones fotoquímicas, las deposiciones secas y húmedas y el transporte vertical a las capas más altas de la troposfera [66].

Después de la emisión y su distribución en la atmósfera, los plaguicidas pueden sufrir diferentes procesos como el transporte, la degradación o la deposición. A pequeña escala, la dispersión se produce poco tiempo después de la aplicación (entre unos pocos minutos y una hora) y está influida principalmente por la velocidad del viento. Consecuentemente, la mayor parte de la pérdida de los plaguicidas se produce por procesos de transporte a partir de los procesos de transformación y deposición de los plaguicidas. La capacidad de los plaguicidas para transportarse a distancias cortas o largas depende del tiempo en el que se encuentran en la atmósfera, relacionado con las propiedades de los plaguicidas y sus factores meteorológicos. A escala regional (por ejemplo, el transporte de largo alcance), el transporte vertical a capas altas de la atmósfera y la eliminación de procesos de intercambio como la transformación y la deposición seca y húmeda son factores influyentes [67-69]. Por tanto, los procesos de transformación y deposición son los dos procesos de pérdidas de plaguicidas en la atmósfera más importantes.

2.2.3. Transformación.

Los productos de transformación que se detectan en la atmósfera se producen tanto en la propia atmósfera como en otra superficie (agua, suelo) y son transportados posteriormente al aire ambiente a través de procesos de volatilización y erosión [70]. Las reacciones de transformación más habituales de los plaguicidas en el aire son procesos de fotólisis (luz solar) y oxidación con O_3, OH y NO_3 y radicales Cl [71-77]. Si las reacciones de los plaguicidas con los radicales en la atmósfera son rápidas (tiempos de vida medios cortos), es posible la evaluación del plaguicida y de su producto de transformación al mismo tiempo. La oxidación fotoquímica de los plaguicidas genera productos de oxidación que puedan llegar a ser más peligrosos que el propio precursor [78]. Todo esto constituye un gran número de plaguicidas y metabolitos presentes en la atmósfera que pueden causar un riesgo importante en la población [79].

Otra importante ruta de pérdida de plaguicidas en el aire es mediante deposición. La deposición se considera la vía de entrada de los plaguicidas desde el aire a la superficie terrestre. Tanto la deposición seca (en períodos secos) como la deposición húmeda (producida durante períodos húmedos), dependen de la distribución de los plaguicidas en el aire. Los compuestos presentes en la fase particulada están mayoritariamente presentes en la deposición húmeda. En cambio, los compuestos presentes en la fase gaseosa están más divididos entre la deposición seca y la deposición húmeda [70].

2.3. Distribución entre la fase gaseosa y particulada del aire.

En la atmósfera, los plaguicidas habitualmente utilizados en prácticas agrícolas se encuentran simultáneamente presentes en dos fases: en la fase gaseosa y en la fase particulada. La distribución entre ambas fases depende de las propiedades fisicoquímicas del compuesto (presión de vapor, constante de Henry o su diferente solubilidad en agua) y de factores ambientales (temperatura, humedad, concentración de partículas en el aire) [80]. Las fuentes de emisión y otros procesos influyen en la distribución de los plaguicidas en los diferentes tamaños de partículas: tamaño de partícula ultrafina (diámetro de partícula menor a 0.1 μm, tamaño de partícula fino (diámetros de partícula entre 0.1 y 1 μm) y tamaño de partícula grueso (diámetro de partícula superior a 1 μm) [81-84].

Existen diferentes modelos teóricos que han estudiado la distribución entre la fase gaseosa y particulada de los compuestos orgánicos semivolátiles. Dos de los modelos más empleados son los propuestos por Junge-Pankou (1987) [85], donde se relaciona la fracción de la fase particulada y la presión de vapor del compuesto, y más recientemente, el modelo del coeficiente de partición octanol-aire (K_{oa}) [86-87], que ha sido propuesto como un mejor descriptor de la adsorción. Para varios de los contaminantes orgánicos persistentes como PCBs, PCNs, OCPs y PAHs los resultados de adsorción obtenidos coinciden con los datos descritos por el modelo mediante la K_{oa}. Siguiendo el modelo descrito por Harner y Bidleman, en el modelo implementado posteriormente por Sufouglu et al., 2004, [88], la distribución potencial de los compuestos orgánicos semivolátiles en la fase particulada sigue la siguiente ecuación (ecuación 1):

$$Ø = (K_pC_{TSP})/ (1 + K_pC_{TSP}) \quad (1)$$

Donde Ø es el porcentaje de la fase particulada (fracción del compuesto en la fase particulada), C_{TSP} es la concentración total de las partículas suspendidas en el aire (μg m^{-3}) y K_p es el coeficiente de partición entre la fase gaseosa y la fase particulada. La constante de partición puede ser calculada empleando la siguiente ecuación (ecuación 2):

$$\log K_p = \log K_{oa} + \log f_{OM} - 11.91 \quad (2)$$

Donde $\log K_{oa}$ es el coeficiente de partición octanol-aire y el $\log f_{OM}$ es la fracción de la materia orgánica.

Siguiendo el modelo K_{oa}, los plaguicidas que presentan Koa inferiores a 8.6 se esperan que estén presentes mayoritariamente en la fase gaseosa (< 5 % en la fase particulada). Aquellos plaguicidas que presentan K_{oa} superiores a 10, se espera que estén presentes en la fase particulada por encima del 50 %.

Para los plaguicidas utilizados habitualmente (currently used pesticides, CUPs), que son plaguicidas más polares que los plaguicidas persistentes y organoclorados), hay pocos datos disponibles respecto a su partición entre la fase particulada y la fase gaseosa [89-90]. Además, Yusà et al., 2014 [91] mostró que muchos de los datos de distribución experimentales entre la fase particulada y la fase gaseosa están de acuerdo con el modelo de adsorción propuesto por Harner y Bidleman. Además, la partición entre la fase particulada y la fase gaseosa adquiere un papel importante en la deposición seca y húmeda de las partículas y es un proceso importante que afecta al modo y al transporte de los compuestos orgánicos semivolátiles (SVOCs) [63].

2.4. Métodos de captación de los plaguicidas en el aire.

Para detectar y cuantificar los niveles de plaguicidas que se encuentran normalmente en el aire ambiente (concentraciones bajas, en el orden de pocos pg m^{-3} a cientos de ng m^{-3}), se necesitan métodos de captación apropiados para alcanzar una sensibilidad adecuada. Las técnicas más comunes de captación están agrupadas en dos categorías principales: métodos de captación activos [92] y métodos de captación pasivos [93].

2.4.1. Métodos activos de captación.

Los captadores activos permiten la captación de los plaguicidas presentes tanto en la fase particulada como en la fase gaseosa mediante el bombeo de aire a través de un filtro seguido de un adsorbente sólido. En la siguiente figura (Figura 2), se presenta un esquema del sistema activo de captación de plaguicidas.

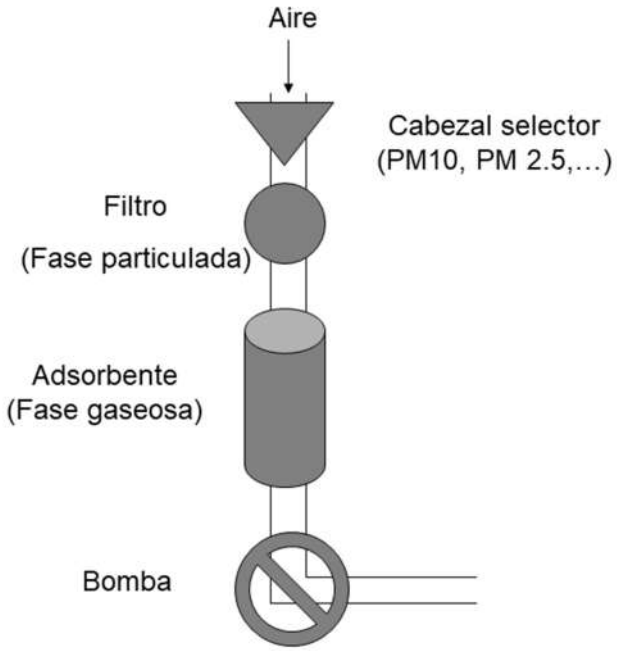


Figura 2. Esquema del sistema activo de captación de plaguicidas.

El aire puede ser muestreado mediante el uso de captadores de alto volumen (high-volume samplers, HVSs, 10-30 m^3 h^{-1}) o mediante captadores de bajo volumen (low-volume samplers, LVSs, < 10 m^3 h^{-1}). Debido a las bajas concentraciones en las cuales se encuentran los plaguicidas en el aire habitualmente, se necesitan bombear grandes cantidades de aire, por lo que es más habitual trabajar con captadores de alto volumen [94]. En la siguiente tabla (Tabla 3), se muestra una recopilación de artículos en los que se han empleado métodos activos para la captación de plaguicidas en el aire.

Tabla 3. Métodos de muestreo activos para la captación de plaguicidas en el aire

Flujo (m^3 h^{-1})	Tiempo de exposición (h)	Tipo de filtro (fase particulada)	Adsorbente (fase gaseosa)	Plaguicidas	Referencia
11-15	24	GFF, 30 cm Ø	XAD-2, 20g	11 plaguicidas multiclase	[68]
0.35	18-67	GFF, 9 cm Ø	PUF	OCPs	[95]
8.33	84	GFF, 10.2 cm Ø	XAD-2 , 25 ml, s-PUF	2 herbicidas	[96]
5.8-29.1	24	GFF, 30 cm Ø	XAD-2, 20 g	11 plaguicidas multiclase	[97]
34	24	QFF	XAD-2	OCPs	[98]
18.5	12	QFF, 10-5 Ø	XAD-2, s-PUF	23 plaguicidas multiclase+OCPs	[99]
8.33	84	GFF	XAD-2 , 25 ml, s-PUF	2 herbicidas	[100]
2.3-12.5	24/48	QFF	XAD-2	15 plaguicidas multiclase	[101]
34	24	QFF	XAD-2, 40 g	OCPs	[102]
1	168	GFF, 4.7 cm Ø	XAD-2	31 CUPs	[103]
42	24	GFF	PUF, 827 cm^3	OCPs	[104]
8.33	84	GFF, 10.2 cm Ø	XAD-2, 25 ml s-PUF	5 fungicidas	[105]
30	24	QFF	PUF	OCPs	[106]
12-48	24	GFF	XAD-2, 40 g	51 plaguicidas multiclase	[107]
10-15	24	GFF, 30 cm Ø	XAD-2, 20g	27 plaguicidas multiclase	[108]
12.5	168	GFF	XAD-2, 25 ml, s-PUF	10 herbicidas	[109]
4.6	24	QFF, 9 cm Ø (PM2.5-10)	–	OCPs	[110]
20.8	24	GFF, 10 cm Ø	PUF (Ø 8 cm x 7.5 cm)	24 OCPs	[111]
15	24	QFF	PUF-XAD-2	48 CUPs y persistentes	[112]
25.8	41-43	GFF, 25 cm	PUF-XAD2	12 plaguicidas	[113]
15	168	GFF, 10.2 cm Ø	XAD-2, 10g, s-PUF	40 plaguicidas multiclase	[114]
16	168	GFF, 10.2 cm Ø	XAD-2, 7 g + Tenax-TA, 7g , s-PUF	3 fungicidas	[115]
26	23	QFF, 78/516 cm^2	PUF	9 OCPs	[116]
13	24	QFF, 25 cm	PUF	OCPs	[117]
10-15	24	GFF, 30 cm Ø	XAD-2, 20 g	28 CUPs	[118]
21	24	GFF, 10 cm Ø	PUF	OCPs	[119]
12.5	168	GFF, 10.2 cm Ø	PUF/XAD-2	91 plaguicidas multiclase	[120]
15	266	GFF, 10.2 cm Ø	PUF	OCPs	[121]
12-18	24	QFF	PUF	OCPs	[122]
16	168	GFF, 10.2 cm Ø	XAD-2, 7 g + Tenax-TA, 7g , s-PUF	13 OP+ 13 productos de degradación	[123]
10-15	24	GFF, 30 cm Ø	XAD-2	Lindano+CUPs	[89]
10.4	24	QFF, 10.2 cm Ø	PUF, Ø 8.0 cm x 7.5 cm thick	20 OCPs	[124]
12.5	24	QFF	PUF/XAD-2	OCPs	[125]
6	4	GFF	-	OCPs	[126]
15	24	GFF, 9 cm Ø	PUF	OCPs	[127]
1	168	GFF, 4.7 cm Ø	PUF	56 CUPs	[128]
17	24	GFF	PUF-XAD-PUF	9 CUPs+ 6 OCPs	[129]

0.06	12	QFF	PUF	Paraquat	[130]
10-15	24	GFF, 30 cm Ø	XAD-2	71 CUPs	[90]
30/1	24	GFF, 4.7 cm Ø	XAD-2, XAD-4	16 plaguicidas multiclase	[131]
0.9	24	-	XAD-4	32 plaguicidas	[132]
7-12	24	GFF, 10.2 cm Ø	PUF	10 OCPs	[133]
0.042	24	GFF	PUF	OCPs	[134]
30	24	QFF, 15 cm Ø	-	42 CUPs	[135]
0.12	8	-	XAD-2	39 CUPs	[136]
0.9	24	-	XAD-4	32 plaguicidas	[137]
30	24	QFF, 15 cm Ø	-	40 CUPs	[138]
42	24	QFF	PUF	OCPs	[139]
0.12	24	-	PUF/XAD-2	4 plaguicidas	[140]
0.9	24	-	XAD-4	19 plaguicidas	[141]
30	12	QFF	PUF	OPs y piretroides	[142]
1	168	GFF, 47 cm	XAD-2	31 CUPs	[143]
18		QFF, 25 cm	PUF	15 OCPs	[144]
25	168	QFF, 15 cm	PUF	37 plaguicidas (OCPs+CUPs)	[145]
2.2-60	24-168	-	PUF	7 OCPs	[146]
30	24	QFF	PUF	17 OCPs+ 16 OPs+ 11 plaguicidas más	[147]
24	40-80	GFF, 25 cm	PUF	12 plaguicidas (OCPs +CUPs)	[148]

GFF=filtro de fibra de vidrio; QFF= filtro de fibra de cuarzo; CUPs= plaguicidas de uso habitual en la agricultura; OCPs= plaguicidas organoclorados; OP= plaguicidas organofosforados.

2.4.2. Métodos pasivos de captación.

Un muestreador pasivo (passive air sampler, PAS) es aquel dispositivo que nos permite atrapar compuestos procedentes de la atmósfera sin la ayuda de una bomba externa, con una alta capacidad de retención para aquellos compuestos que se consideren prioritarios en la zona de aplicación. Dichos muestreadores permiten realizar muestreos en aquellas localizaciones donde los muestreadores activos no puedan ser prácticos (por ejemplo, sitios remotos sin acceso a electricidad) [149]. Sin embargo, los muestreadores pasivos sólo son capaces de captar la fase gaseosa de los plaguicidas y su tiempo de muestreo oscila entre unas pocas semanas y varios meses, tiempo significativamente más largo que el empleado utilizando los muestreadores activos.

2.4.3. Materiales empleados para la captación.

Para la captación de los plaguicidas presentes en la fase particulada se emplean filtros de fibra de vidrio (glass fiber filter, GFF) o filtros de fibra de cuarzo (QFF). Su diámetro varía entre los 4.7 y los 30 cm, dependiendo del captador empleado.

Para la captación de la fase gaseosa, se emplean diferentes adsorbentes sólidos convencionales tales como espumas de poliuretano (polyurethane foam, PUF), la resina polimérica hidrofóbica XAD-2, la resina polimérica XAD-4, Florisil C18 o nuevos adsorbentes como la resina XAD-7 o distintos tipos de nanotubos (Multi-Walled Carbon Nanotubes) con diferentes diámetros internos y longitudes. Seguidamente, se describen los adsorbentes empleados para la realización de esta Tesis Doctoral:

➔ La espuma de poliuretano (PUF) es un tipo de espuma generada para tapicería de muebles, almohadas y colchones. Es de color blanco y se transforma en amarillo en contacto con la luz. La espuma de poliuretano se ha empleado para el muestreo de OCPs y otros contaminantes prioritarios, como PCBs, PBDEs, etc. [103].

➔ La resina polimérica XAD-2 es la más empleada habitualmente para la captación de la fase gaseosa. Es un poliestireno hidrofóbico con una gran área de superficie (300 m^2 g^{-1}) que es capaz de interaccionar con los analitos básicamente a través de fuerzas de Van der Waals e interacciones π-π de los anillos aromáticos. La resina XAD-2 es un adsorbente universal, muy eficaz para la captación de herbicidas, fungicidas e insecticidas. Se suele emplear en solitario o formando sándwiches con las espumas de poliuretano.

➔ La resina polimérica XAD-4 se está empleando habitualmente para comparar la capacidad de retención con otros adsorbentes. XAD-4 es un adsorbente polimérico en forma de perlas. Es un polímero reticulado no iónico cuyas propiedades de adsorción provienen de su estructura reticular, su alta capacidad de superficie (800 m^2 g^{-1}) y la naturaleza aromática de su superficie.

2.5. Métodos de análisis para la determinación de los plaguicidas en el aire.

El análisis de plaguicidas en aire incluye tres etapas principales: la etapa de extracción, la etapa de purificación (clean-up) y la etapa de análisis. Es necesario realizar una optimización adecuada de cada una de estas etapas para realizar una determinación adecuada de los plaguicidas presentes en el aire ambiente (tanto en la fase particulada como en la fase gaseosa).

2.5.1. Etapa de Extracción.

La etapa de extracción es una de las etapas fundamentales del proceso analítico. Después del muestreo, los plaguicidas presentes en los filtros de fibra de cuarzo PM10 (fase particulada) y en los adsorbentes (fase gaseosa) deben ser extraídos para su posterior determinación.

La extracción sólido-líquido (liquid solid extraction, LSE) se ha empleado de forma habitual para la extracción de los plaguicidas procedentes de los filtros y adsorbentes, utilizando el disolvente orgánico apropiado para cada caso. La extracción mediante el método Soxhlet ha sido la primera elección habitual para la extracción de los plaguicidas muestreados con muestreadores activos, debido a las características que presenta (es un método eficaz y simple) [131, 133, 134, 139, 142]. La extracción mediante Soxhlet se puede realizar empleando un único disolvente orgánico, como acetona o diclorometano o bien una mezcla de disolventes como hexano-diclorometano, diclorometano-éter de petróleo, ciclohexano-acetona o hexano-acetona. Este método clásico de extracción tiene como principales desventajas el tiempo (6-24 horas) y el elevado consumo de disolventes (250-700 mL). El tiempo excesivo junto con la temperatura que se genera en el Soxhlet provoca la ruptura de la estructura de los plaguicidas polares térmicamente lábiles empleados en la agricultura moderna. Como

alternativa se ha utilizado la extracción con líquidos presurizados (pressurized liquid extraction, PLE) [150].

La extracción mediante líquidos presurizados obtiene recuperaciones comparables con los obtenidos mediante el método Soxhlet y otras técnicas en uso. PLE utiliza disolventes orgánicos a elevada temperatura (50-200 ºC) y alta presión (1000-2000 psi) para la extracción de contaminantes orgánicos procedentes de matrices medioambientales. PLE aumenta la velocidad del proceso de extracción, reduciéndose además el consumo de disolvente, pudiendo ser un proceso automatizado. Los principales parámetros para la optimización del método son la elección del disolvente, la temperatura empleada y el tiempo de extracción.

Otra técnica muy empleada en la etapa de extracción es la extracción asistida con microondas. La extracción mediante microondas permite la reducción del tiempo de extracción y la disminución del consumo de disolventes orgánicos, incrementando el rendimiento de extracción de los diferentes contaminantes estudiados procedentes de matrices medioambientales [151-152]. La extracción mediante microondas se ha empleado para la extracción de plaguicidas en diferentes matrices como el suelo [153-154], sedimentos [155] y alimentos [156-157].

La siguiente tabla (Tabla 4) muestra un resumen de diferentes trabajos anteriores en los que se han empleado distintos métodos para la extracción y el análisis de los plaguicidas en la atmósfera:

Tabla 4. Resumen de métodos propuestos para la extracción y el análisis de los plaguicidas en el aire

Plaguicidas	Extracción	Clean-up	Determinación	LOD (pg m^{-3})	Referencia
11 plaguicidas multiclase	Soxhlet, 12h, n-Hexano:DCM	HPLC, silica	GC-ECD; HPLC-UV	-	[68]
OCPs	Soxhlet 24h, PE	Silica	GC-ECD; GC-NI-MS	0.1	[95]
2 herbicidas	Soxhlet, 16h, acetona	Florisil	GC-ECD; GC-MS	40	[96]
11 plaguicidas multiclase	Soxhlet, 12h, Hx-DCM	Silica	GC-ECD; HPLC-UV	-	[97]
14 OCPs	Soxhlet 24h, Acetona:Hx	Silica gel	GC-ECD	-	[98]
23 plaguicidas multiclase	Soxhlet, 12 h, DCM:PE	Alúmina	GC-ECD	-	[99]
15 plaguicidas multiclase	Soxhlet, 24 h, nHx-DEE	_	HPLC-UV	70-13800	[101]
OCPs	Soxhlet, 24 h, acetona/hexano	Alúmina, Silica	GC	-	[102]
OCPs	Soxhlet, 16h, DCM	Extracción L-L, Florisil	GC-MS	0.1	[104]
44 OCPs	Soxhlet 24h, PE	Florisil+Alúmina (GPC)	GC-MS	0.24-9	[158]
OCPs	Soxhlet, 24 h, PE	Alúmina	GC-NI-MS	0.01-0.48	[159]
5 herbicidas	Soxhlet, 12h, acetona	Florisil	GC-MS	40	[105]
4 OCPs	Inmersión in DCM	Silica gel	GC-NI-MS	1.2-26	[160]
22 OCPs	Soxhlet, 24 h, PE/DCM	Alúmina	GC-NI-MS	0.002-0.13	[106]
19 OCPs	Soxhlet, 18 h, PE	Alúmina	GC-MS	0.7-1.3	[161]
51 plaguicidas multiclase	Soxhlet, 24 h, Hx-acetona	Florisil	GC-EI-MS	0.71-110	[107]
17 OCPs	Soxhlet 12h, n-Hx-DCM	-	GC-ECD	5.0-8.0	[108]
27 plaguicidas multiclase	Soxhlet, 12 h, DCM-Hx	_	GC-MS/MS	2.5-1250	[108]
10 herbicidas	Soxhlet, 8h, acetona	_	GC-MS; GC-MS/MS	5-0-25	[109]
OCPs	Soxhlet 24h, c-Hc:acetona	Alúmina	GC-ECD; GC-MS	0.005-0.1	[110]
24 OCPs	Soxhlet, 16 h, éter de petróleo	Neutral Al_2O_3	GC-ECNI-MS	0.1	[111]
48 CUPs y persistentes	→PUF: Soxhlet, 8 h, dietil/hexano (5/95) →XAD-2:Ultrasonidos, 2 x 10 mL de acetona/hexano	-	HPLC-DAD y GC-MS	14-140	[112]
9 plaguicidas multiclase	Soxhlet, 16h,	-	GC-MS	0.70-89	[113]
40 plaguicidas	Soxhlet, 12h, acetona	Silica gel; Florisil	GC-ECD; GC-MS	4.0-20	[114]
3 fungicidas	PLE, acetato de etilo	C18	GC-NI-MS	0.8-3.8	[115]
9 OCPs	Ultrasonidos, 30 min, n-Hx		GC-ECD	1.4-73	[116]
OCPs	Soxhlet, 48 h, DCM	Alúmina, Gel	GC-MS	-	[117]
28 plaguicidas	Soxhlet, 12h, Hx-DCM	-	GC-MS/MS	2.5-1250	[118]
12 OCs	Soxhlet, 16h, éter de petróleo	Neutral Al_2O_3	GC-ECD	0.09-9.1	[119]
91 plaguicidas	Soxhlet, 24h, DCM	-	GC-MS	1-100	[120]
12 OCs	Soxhlet, 8 h, Hx-acetona	Silica	GC-HRMS	-	[121]
OCPs	Diclorometano	Silica gel	GC-ECD y GC-MS	1	[122]
10 plaguicidas	Soxhlet, 18 h, éter de petróleo		GC-MS	0.4-13.6	[162]
10 OP	PLE, Eac	C18	LC-MS/MS	0.2-10	[123]
4 CUPs	Soxhlet, 12 h, Hx-DCM	-	GC-ECD	2.5-1250	[89]
20 OCPs	PLE, Hx-DCM-EE	Silica	GC-EI-MS	0.4-1.6	[124]
16 OCs	Soxhlet, 16h, Hx-DCM	Florisil	GC-MS	0.6-7.3	[125]
OCs	Ultrasonidos 30 mL	Florisil +	GC-MS	-	[126]

	hexano/acetona	Na_2SO_4			
18 OCs	Soxhlet, 24h, Hx-dietil éter	Silica	GC-ECD	1.6-30	[127]
56 CUPs	PLE, DCM	-	GC-MS; LC-MS/MS	1.32-6.5	[128]
9 CUPs + 6 OCs	DCM	-	GC-MS	0.6/24.5	[129]
Paraquat	Extracción con centrifuga y HCl	-	HPLC-PDA	33333	[130]
71 CUPs	Soxhlet, 20h, Hx-DCM	-	GC-MS/MS	7.32-230.22	[90]
16 CUPs	Ultrasonidos, 10 min, isooctano	-	GC-MS	0.053-88.53	[131]
OCs	Soxhlet, 16h, acetona-tolueno	Cartuchos extracción SPE	HRGC/HRMS	0.13-1.00	[134]
32 plaguicidas y 5 productos de transformación	Extracción con acetato de etilo	-	HPLC-MS y GC-MS	1000-222000	[132]
10 OCs	Ultrasonidos/Soxhlet; 30min/24h; DCM/PE	Alumina, Silica	GC-ECD	0.71-2.13	[133]
42 CUPs	MAE, Eac	GPC	GC-MS/MS	1.32-39.47	[135]
39 CUPs	Extracción con CS_2	-	GC-MS/MS y GC-NPD	20.8-62.5	[136]
32 plaguicidas y 5 productos de transformación	Extracción con acetato de etilo		HPLC-MS y GC-MS	1000-222000	[137]
40 CUPs	MAE, acetato de etilo	-	LC-MS/MS	6.5-32.5	[138]
12 OCs	Soxhlet, 24h, DCM	Florisil	HRGC/HRMS	0.091-0.380	[139]
4 plaguicidas	Ultrasonidos	-	LC-MS/MS	0.15-5 ng/muestra	[140]
CUPs	MAE, acetato de etilo	-	LC-HRMS	6.5	[163]
9 CUPs	Soxhlet, 48h	Alúmina-silica	GC-MS	6.2-11.8	[142]
19 plaguicidas	Extracción con acetato de etilo	-	GC-MS, LC-MS	148-1755	[141]
31 CUPs	PLE, acetonitrilo	-	GC-MS	1-947	[143]
10 plaguicidas	Soxhlet, éter de petróleo, 24 h	-	GC-MS	0.3-20	[164]
15 OCPs	Soxhlet, 48 h, DCM	Alúmina, silica	GC-MS	0.15-0.24	[144]
37 plaguicidas (OCPs+CUPs)	Soxhlet	Silica gel	GC-MS/MS, HPLC-MS/MS	14-55	[145]
9 OCPs	Soxhlet, 200 mL hexano/acetona, 24 h	Alúmina	GC-MS	0.02-1 pg /muestra	[165]
7 OCPs	Soxhlet, 60 min, DCM	-	GC-MS/MS	-	[146]
20 OCPs	Soxhlet, 24h, éter de petróleo	Albúmina-sulfato sódico	GC-MS/MS	-	[166]
17 OCPs+ 16 OPs+ 11 plaguicidas más	PLE, hexano	-	GC-MS	300-10000	[147]
8 OCPs	Soxhlet, 60 min, DCM	Silica	GC-MS/MS	0.035-0.005 ng	[167]
12 plaguicidas (OCPs +CUPs)	Soxhlet, 24 h, DCM	Alúmina, gel	GC-MS/MS	0.001-0.01	[148]

2.5.2. Etapa de clean-up.

Las muestras de aire pueden llegar a contener cantidades significativas de otros compuestos. Durante la etapa de extracción, estos compuestos (mayoritariamente de

naturaleza orgánica) pueden ser extraídos juntos a los plaguicidas y pueden llegar a interferir en su detección y cuantificación. Este hecho produce que, de forma general, sea necesaria una etapa de clean-up.

La extracción en fase sólida (SPE) es una de las técnicas de purificación más empleadas en el análisis de muestras de aire (ver Tabla 4). Los adsorbentes más utilizados habitualmente son el Florisil (silicato de magnesio sintético), la alumina, el gel de sílice y una combinación de ambos [96, 133, 139, 143]. Debido a la polaridad de éstos, sólo los extractos en disolventes no polares (hexano, isooctano), pueden pasar a través de la fase estacionaria. Si se realiza una elución mediante disolventes con incremento de polaridad se puede realizar una elución fraccionada en función de los analitos a estudiar. Los cartuchos de fase reversa C-18 permiten la purificación de plaguicidas más polares, como es el caso de los herbicidas organofosforados y sus productos de transformación [123] y diversos fungicidas [115].

La cromatografía de exclusión molecular (GPC) nos permite la separación de los analitos en función de su tamaño. Esta técnica es la más adecuada para eliminar ceras y grasas. Esta técnica se ha empleado para la purificación de CUPs y de plaguicidas organoclorados [135, 158].

Antes de decidir si se realiza la etapa de purificación, es necesario estudiar de forma exhaustiva la matriz de estudio (exaltación o supresión de la señal). Cuando se emplean detectores selectivos como en el caso de la espectrometría de masas, en muchas ocasiones no es necesaria la etapa de purificación. El estudio del efecto matriz es el parámetro que nos indica la necesidad de esta etapa [118, 134, 138, 163, 168].

2.5.3. Análisis.

En esta Tesis Doctoral se han analizado los plaguicidas tanto por cromatografía gaseosa como por cromatografía líquida, acopladas ambas a espectrometría de masas. A continuación se explica la evolución que han presentado ambas técnicas en el análisis de plaguicidas.

2.5.3.1. GC-MS.

Hasta la década del 2000, el análisis de plaguicidas en el aire se centraba en el estudio de plaguicidas persistentes analizados por cromatografía de gases acoplada a detectores convencionales, tales como detectores de captura electrónica (ECD) y detectores de nitrógeno-fósforo [88, 104, 106]. Para ello, era necesario que los plaguicidas estudiados fueran volátiles y presentaran una estabilidad térmica. Los principales plaguicidas estudiados eran los plaguicidas orgánicos persistentes (POPs), incluyendo entre ellos varios organoclorados (OCPs) tales como el aldrin, el cis y el trans-clordano, el DDT y sus productos de transformación, el dieldrin, el heptacloro, HCB y mirex), los cuales se siguen analizando mediante cromatografía gaseosa. La incorporación de la espectrometría de masas ha ido sustituyendo a detectores más convencionales, siendo actualmente el detector más empleado en el análisis de plaguicidas. Posteriormente, la incorporación de técnicas más selectivas y sensibles a partir de la aparición del acoplamiento MS/MS nos ha permitido el desarrollo de métodos multirresiduo.

2.5.3.1.1. Analizador de Trampa Iónica.

En esta tesis doctoral, el analizador de masas empleado acoplado a la cromatografía gaseosa ha sido la trampa iónica (Figura 3).

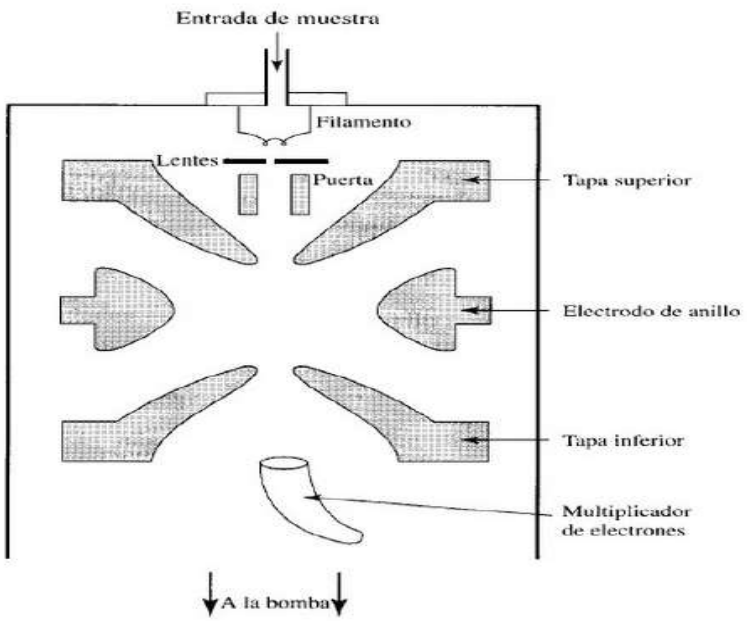


Figura 3. Esquema general de la Trampa Iónica.

La trampa iónica consiste en dos electrodos colectores idénticos situados en los extremos (tapa superior e inferior), junto con un electrodo anular en la parte central. Los dos electrodos son placas de acero inoxidable con bordes interiores en forma de parábola. El electrodo en forma de anillo circular también es de acero inoxidable con bordes parabólicos. Los tres componentes están separados entre sí mediante anillos espaciadores de cuarzo-nitrilo, creando un hueco donde los iones están atrapados y controlados. Al electrodo anular se le aplica un potencial de radiofrecuencia variable mientras que los dos electrodos colectores (superior e inferior) están conectados a una toma de tierra. Los iones con un valor de m/z adecuado circulan en una órbita estable dentro de la cavidad que está rodeada por el anillo. Cuando se incrementa el potencial de radiofrecuencia, las órbitas de los iones más pesados llegan a estabilizarse, mientras que las de los iones más ligeros se desestabilizan, produciéndose su colisión con la pared del electrodo anular. La trampa presenta 3 dimensiones, a diferencia de un analizador simple o triple cuadrupolo, que presenta únicamente dos dimensiones.

Existen dos variables que controlan los iones presentes en la trampa: el radio desde el centro del electrodo en forma de anillo hasta el borde interior (r_0) y la distancia desde el centro de la trampa hasta cada uno de los electrodos superior e inferior (z_0).

Hay cuatro etapas que se pueden aplicar en todas las operaciones básicas que se realizan en la trampa: selección, aislamiento del ion, excitación del ion y expulsión.

La primera etapa siempre es la selección del ion. Todos los iones, independientemente de su masa, son capaces de entrar en la trampa iónica y ser retenidos. Posteriormente, se producen una serie de procesos de manera controlada, de forma que la monitorización de su masa es posible, antes de producirse su expulsión de la trampa y dirigirse hacia el sistema de detección.

En el caso más sencillo, la realización de un escaneo completo (full scan), todos los iones son seleccionados y posteriormente expulsados, obteniéndose como resultado un espectro que nos muestra el número de iones de cada masa que han entrado en la trampa.

En el caso de realizarse el control a un único ion (SIM, single ion monitoring), todos los iones son seleccionados, pero durante el tiempo que estos se encuentran atrapados en la trampa, los voltajes son modificados para seleccionar un único ion, reteniendo únicamente el ion de interés y eliminando el resto. Cuando se ejecuta la etapa de expulsión, el único ion que se muestra es el ion específico seleccionado.

En el caso de aplicar MS/MS, se seleccionan todos los iones y posteriormente se aísla un único ion (de igual forma que se realiza en el modo SIM). Después, posteriormente, se aplica un voltaje determinado para excitar el ion seleccionado, se fragmenta y se obtienen los iones productos que son enviados al sistema de detección. El proceso realizado presenta la misma sensibilidad que el modo SIM, pero nos aporta más datos cualitativos, ya que nos indica la huella dactilar espectral del ion fragmentado.

Para cualquier otro modo, como SRM (Selected Reaction Monitoring) o fragmentaciones a más nivel (MS^n), se repiten las etapas de aislamiento y excitación de los iones hasta llegar al ion final deseado, que es expulsado y enviado al sistema de detección [169].

2.5.3.2. LC-MS.

El incremento del uso de plaguicidas más polares y menos estables térmicamente, tales como los herbicidas, carbamatos, triazinas o ácidos fenóxidos [170], ha promovido el empleo de técnicas basadas en la cromatografía líquida. El empleo de la cromatografía líquida acoplada a espectrometría de masas presenta una gran selectividad y sensibilidad. Así, el uso de la espectrometría de masas de alta resolución acoplada a la cromatografía líquida (LC-HRMS), permite adquirir cientos de compuestos debido a su elevada sensibilidad y selectividad combinado con un alto poder de resolución (> 50000 FWHM) mediante la medida de la masa exacta en modo full-scan [171]. El potencial de esta herramienta nos permite el desarrollo de estrategias analíticas que combinan: i) análisis target (análisis de los plaguicidas específicos prioritarios para los cuales existe una disponibilidad de patrones de referencia y para los cuales se conoce su masa exacta, su tiempo de retención, sus fragmentos específicos y su perfil isotópico); ii) análisis post-target (suspected screening) (análisis de plaguicidas no prioritarios y conocidos, construyendo para ello una base de datos que contenga la masa exacta del ion molecular y sus fragmentos experimentales específicos [172-175]; iii) análisis non-target (búsqueda de compuestos desconocidos, bien utilizando la relación entre la fragmentación y la degradación o bien mediante el empleo de métodos propios de la metabolómica que implican la adquisición de señales en alta resolución, el

procesamiento de los datos analíticos (filtrado, detección de picos, alineamiento, etc.) con softwares específicos como MzMine [176] y la elucidación molecular frente a bases de datos como ChemSpider [177] o PubChem [178], confirmando posteriormente con patrones de referencia [179].

2.5.3.2.1. Espectrometría de masas de alta resolución tipo Orbitrap.

En esta tesis doctoral el analizador de masas empleado acoplado a la cromatografía líquida ha sido la espectrometría de masas de alta resolución (HRMS) tipo Orbitrap. A continuación, se explican las características generales que presenta, siendo adecuadas para el análisis de plaguicidas.

2.5.3.2.1.1. Descripción general y componentes.

El equipo funciona del siguiente modo: con posterioridad a la separación cromatográfica de los analitos mediante HPLC, los iones (positivos o negativos) formados previamente en el extracto de la disolución entran en el espectrómetro de masas a través de la fuente de ionización (electronebulizador) donde los iones en disolución pasan a iones en fase gaseosa. Seguidamente, a través de una serie de lentes y mulitpolos, los iones entran en la C-trap (trampa de iones curvada) donde son almacenados hasta su entrada en paquetes de iones al analizador (tipo Orbitrap). Dentro del Orbitrap, los iones giran orbitalmente alrededor del electrodo central del analizador de masas, en función de la frecuencia de la oscilación armónica axial que presentan y mediante una transformada de Fourier, se determina su valor m/z. Además, de forma opcional, los iones pueden pasar por una celda de colisión HCD donde se produce su fragmentación después de la aplicación de un potencial, dando lugar a los fragmentos iónicos que podrán ser determinados posteriormente en el Orbitrap. A continuación, se presenta un esquema general (Figura 4) del espectrómetro de masas tipo Orbitrap, indicándose sus partes principales:

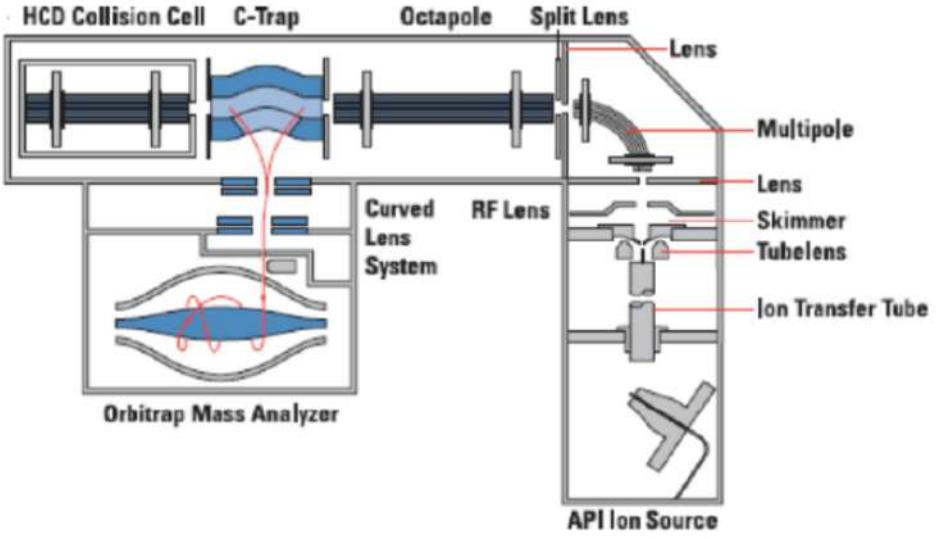


Figura 4. Esquema general de un espectrómetro de masas de alta resolución Orbitrap-Exactive ™.

Seguidamente, se describen de forma detallada cada uno de los componentes del analizador de masas y su funcionamiento:

2.5.3.2.1.1.1. Fuente de ionización.

En el Orbitrap Exactive ™, la interfase entre el HPLC y el analizador de masas está compuesta por la fuente de ionización, que permite la generación de iones tanto por electronebulización (Electrospray), como mediante Ionización Química a presión atmosférica (APCI, Atmospheric Pressure Chemical Ionization).

El proceso de ionización mediante electronebulización favorece el paso de iones formados previamente en disolución a iones en fase gaseosa (ver figura 5).

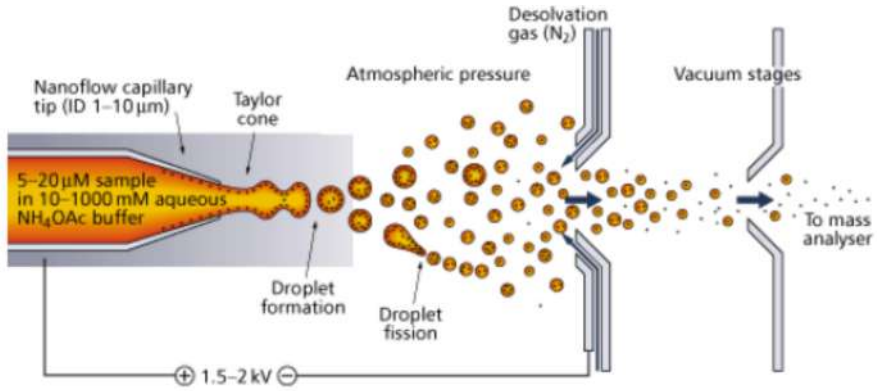


Figura 5. Esquema general del proceso de electronebulización.

El proceso de electronebulización [180] se inicia con el paso mediante un flujo bajo (300-400 µL min^{-1}) de la disolución que contiene el analito a través de un capilar. Cuando se aplica un voltaje elevado sobre el capilar (2-5 kV), de carga positiva o en función del tipo de ionización que sufra el analito, se provoca el gradiente necesario para producir la separación de cargas en la superficie del líquido. Como consecuencia de esto, el líquido sobresale de la punta del capilar formando el denominado "Taylor cone". Cuando en la disolución se alcanza el límite de Rayleigh (punto en el que la repulsión coulombica de la carga superficial es igual a la tensión superficial de la disolución), las gotas que contienen un exceso de carga positiva o negativa se separan de la punta del capilar en dirección al espectrómetro de masas generando iones gaseosos. Se han propuesto dos mecanismos: el primer mecanismo propone que al evaporarse el disolvente que forma la gota, se produce un aumento de densidad de carga, provocando que la gota se divida en gotas más pequeñas. Este proceso se repite hasta que finalmente se forman iones libres que pueden ser analizados en función de su valor m/z. El segundo mecanismo, denominado "ion evaporation", postula que el aumento de la densidad de carga provocado por la evaporación del disolvente en la gota puede dar lugar a una repulsión coulombica que supere la tensión superficial de ésta, dando lugar a una liberación de iones a partir de la superficie de la gota.

Para ayudar al proceso de electronebulización, se utiliza nitrógeno, el cual, favorece la nebulización del spray y la desolvatación de las gotas formadas. Existen dos fuentes de gas, la fuente de "gas de envoltura" (sheath gas) y la fuente auxiliar de gas. El primero fluye a través del capilar pero por fuera de la aguja, así, la muestra entra en contacto con este gas al final de la aguja.

El proceso de electronebulización puede dar lugar a iones con carga positiva o negativa. Para la elección del modo de ionización se atiende a la basicidad o acidez de los analitos de estudio. Los compuestos básicos en una solución a pH bajo dan lugar a iones protonados, usándose el modo positivo, mientras que compuestos ácidos a pH elevados dan lugar a iones desprotonados empleándose el modo negativo. El modo positivo es más general debido a que los protones se pueden unir con cierta facilidad a las moléculas a pesar de que éstas no cuenten con grupos funcionales eminentemente básicos. Sin embargo, el modo negativo es más específico ya que requiere la presencia de grupos funcionales que presenten la capacidad de perder un protón.

2.5.3.2.1.1.2. Interfase de la fuente de ionización.

La interfase de la fuente de ionización está formada por distintos componentes que se encuentran bajo condiciones de vacío (excepto en el caso de una parte del "ion sweep cone" que se encuentra a presión atmosférica). Estos componentes son: un capilar que transfiere los iones ("ion transfer capillary" o "transfer tube"), dos cartuchos calefactores "cartridge heaters", un bloque calefactor "heater block", una sonda de platino que actúa como sensor "platinum probe sensor", una bolita de prevención de tungsteno "prevent ball" que evita la eliminación del vacío al quitar el transfer tube para limpiarlo y el "ion sweep cone".

Los iones son introducidos en la región que permanece a presión atmosférica del "ion transfer capillary" y son transportados hacia la región del "skimmer" que se encuentra en condiciones de vacío. De forma habitual se aplica un potencial de 25-35 kV (positivo para iones positivos y viceversa) que facilita el movimiento de los iones hacia el "skimmer". Para mantener el vacío en la interfase de la fuente de iones, ésta se encuentra dentro de una cámara de vacío que se mantiene a una presión atmosférica de 2 mbar (1.5 Torr).

2.5.3.2.1.1.3. Ion optics.

Los "Ion Optics", u óptica del analizador de masas, tienen como función enfocar y transferir los iones producidos hasta el analizador de masas. Estos se pueden dividir en dos grupos principales según su posición: los cercanos a la fuente de iones y los más cercanos al analizador de masas.

Las Ion Optics de la fuente de iones, se componen de las lentes tubulares "tube lens", el "skimmer", lentes de radiofrecuencia "RF lens" y lentes L_0. Una vez formados los iones, pasan del "ion transfer capillary" a las "tube lens". Las "tube lens" dificultan la expansión del haz de iones al abandonar el "ion transfer capillary" y lo enfocan hacia el "skimmer". Esto permite el paso de los iones hacia el "RF lens" y actúa como deflector ya que compensa la diferencia de presión entre la "fuente de ionización" (a mayor presión) y las RF lens (a menor presión). Finalmente, las "RF lens" enfocan los iones hacia el "Ion Optic" que se mantienen bajo vacío elevado. Las lentes L_0 ayudan a la transmisión de iones y actúan como deflectoras de vacío.

Las "ion optics" del analizador de masas están compuestos por el multipolo 1, las lentes L_1, las "split lens" (lentes de división) y el multipolo 2. El multipolo 1, denominado "Flatapole", actúa como un dispositivo de transmisión, evita la transmisión de especies

neutras no deseadas hacia el detector y reduce el ruido. Las lentes L_1 actúan también como deflectoras de vacío. En las "split lens" se lleva a cabo el "Automatic Gain Control" (AGC), que controla la población de iones que entra en la C-trap en un periodo de tiempo determinado. Si entran pocos iones, la sensibilidad es baja; sin embargo, si entran demasiados iones, aumenta la densidad de carga y se producen repulsiones electrostáticas que disminuyen la exactitud de masa del análisis. El equipo dispone de 3 configuraciones de AGC: "Ultimate Mass Accuracy", que proporciona una elevada exactitud de masa y resolución, ya que mantiene una densidad iónica baja ($5*10^{-5}$ iones en la C-trap; el tiempo de inyección se calcula mediante un "prescan"); "Balanced", que ofrece mayor sensibilidad y reproducibilidad que el anterior, aunque pierde exactitud de masa debido a que permite una densidad de iones mayor ($1*10^{-6}$ iones en la C-trap; el tiempo de inyección se calcula mediante un "prescan"); y "High Dynamic Range", que ofrece una elevada precisión en la cuantificación, se emplea a resoluciones bajas aunque la exactitud de masas es más baja ya que la densidad iónica es elevada ($3*10^{-6}$ iones en la C-trap; el tiempo de inyección se calcula en el "prescan"). Tras este control, los iones pasan hacia el multipolo 2 y por último entran en la "C-trap" a través de las "Gate Lens".

2.5.3.2.1.1.4. C-trap.

Para el funcionamiento adecuado del analizador, los iones se deben introducir en el Orbitrap en grupos de forma que se pueda llevar a cabo de forma adecuada tanto la fase de "trapping" (atrapamiento) como la de "spinning" (giro). La C-trap o trampa de iones curvada, es el dispositivo encargado de introducir estos "paquetes" de iones en el analizador de masas.

La "C-trap" es un cuadrupolo de RF en forma de C que contiene N_2. Los iones que entran en la C-trap se reflejan a través de un electrodo trampa (una placa situada en el lado opuesto a la entrada de C-trap) y pierden energía progresivamente al colisionar con el N_2 presente en la C-trap, con una presión de aproximadamente 1 mTorr. Debido a la baja presión y la corta longitud de la C-trap, los iones pasan varias ocasiones a lo largo del octapolo de transferencia y la C-trap antes de ser acumulados en la C-trap donde la presión del N_2 es mayor y contiene un DC offset menor. Una vez los iones han sido atrapados por la C-trap, son expulsados disminuyendo la RF en los electrodos cuadrupolares durante unos 100-200 nseg y aplicando posteriormente pulsos de DC (1200, 1000 y 1000 V) de manera que se crea un campo en la trampa que envía grupos de iones a lo largo de líneas que convergen con la entrada del Orbitrap [181].

2.5.3.2.1.1.5. Orbitrap.

El Orbitrap está formado por dos electrodos: un electrodo central, denominado "spindle" (eje) alrededor del cual los iones se mueven en espiral, y otro electrodo externo que está dividido por la mitad de un anillo cerámico aislante.

Desde la C-trap, los paquetes o grupos de iones de cada rango de m/z viajan tangencialmente hacia el Orbitrap. Para mantener a los iones en su interior, el Orbitrap no usa radiofrecuencias o un campo magnético sino que los iones que se mueven alrededor del Orbitrap son atrapados en un campo electrostático creado por el "spindle" y por los electrodos externos. La atracción electrostática hacia el electrodo central es compensada por la fuerza centrípeta que se origina por la velocidad tangencial inicial de

los iones. De manera axial, los iones se dirigen desde las zonas externas del Orbitrap (más estrechas) hasta la zona interna (más ancha), produciéndose oscilaciones de forma axial sin la necesidad de aplicar una excitación adicional, explicándose de esta manera la forma característica del Orbitrap. La frecuencia de rotación de cada valor de m/z depende de diversos parámetros tales como la energía, la posición y el ángulo de los iones, apareciendo anillos dibujados por la rotación de los iones, de forma que los iones más ligeros son más atraídos hacia el electrodo central que los iones más pesados.

Las trayectorias de un ion combinan la mencionada rotación alrededor del electrodo central con oscilaciones armónicas a lo largo de él dependientes del componente axial (Figura 6). La frecuencia *w* de estas oscilaciones armónicas a lo largo del eje z es independiente de la energía, del ángulo y de la posición inicial del ión, por lo que depende únicamente del cociente masa/carga (m/z), del ion y de la constante instrumental *k* (ecuación 3)

$$w_z = \sqrt{\frac{k}{m/z}} \qquad (3)$$

Esta frecuencia es detectada por los electrodos externos, por lo que se provoca una transducción de la señal mediante "image current", dando lugar a una frecuencia inducida que es transformada, mediante una Transformada de Fourier rápida (FFT) en su valor de m/z correspondiente, con gran precisión y con alta resolución.

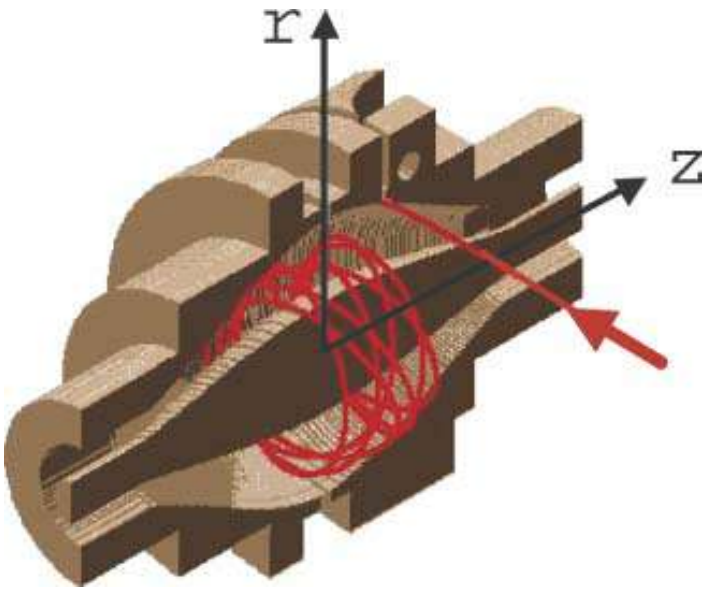


Figura 6. Trayectoria estable de los iones en una celda Orbitrap

2.5.3.2.1.2. Parámetros operacionales del Orbitrap.

2.5.3.2.1.2.1. Exactitud de masa.

La exactitud de masa es el grado de semejanza entre el valor de m/z experimental obtenido y el valor de m/z teórico (Δm). Una elevada exactitud de masa es una de las cualidades más importantes en un espectrómetro de masas ya que proporciona una gran selectividad y permite determinar la fórmula molecular de un analito ionizado de manera a partir de su masa exacta. Esta información permite tanto la identificación de compuestos prioritarios como de compuestos desconocidos. Sin embargo, normalmente, se necesita información adicional para determinar la composición de un analito, como el perfil isotópico o la generación de fragmentos iónicos. El Orbitrap puede lograr una exactitud de masa inferior a 1 ppm [182].

2.5.3.2.1.2.2. Resolución.

Los términos resolución y poder de resolución se usan de igual manera en espectrometría de masas. Así, la IUPAC [183] define la resolución como m/Δm, donde m es el valor de m/z observado y Δm es la mínima diferencia necesaria de masa para poder separar dos iones mientras que el poder de resolución es la capacidad de un determinado espectrómetro de masas de obtener un valor de resolución específico.

Además, en función de cómo se define Δm, se puede definir la resolución de otras diferentes formas:

➔ Definición del valle 10%: la resolución es igual a m/Δm donde m es el valor m/z de un analito próximo a otro analito de m/z similar que presenta misma altura de pico que el primer analito y entre ellos forman un valle de altura igual al 10% de la altura máxima de los picos. Δm es la separación entre las puntas de los picos expresado en unidades de m/z. Esta definición es utilizada principalmente en cromatografía de gases acoplada a espectrometría de masas de alta resolución (GC-HRMS).

➔ Definición de la anchura de pico: Para un solo pico compuesto por iones con una sola carga con una masa m en un espectro de masas, la resolución puede ser expresada como m/Δm donde Δm es la anchura del pico a una altura que es una fracción específica de la altura máxima del pico. Se recomienda usar estos dos valores, 50 % o 5 %.

La definición que se emplea habitualmente en LC-HRMS y la que emplea el Orbitrap es la que se refiere a la Resolución como la anchura total del pico a la mitad de su altura máxima (50 %) o “Full Width of the peak at Half its Maximum Height” (FHWM).

El valor numérico obtenido cuando se sigue la definición de FHWM es siempre mayor que el valor obtenido usando la definición del 10 % valley que mide la anchura al 5 % de la altura. De hecho, el valor numérico de la resolución FHWM necesario para conseguir una separación de masas con un 10 % valley es aproximadamente el doble del valor necesario si se sigue la definición del 10 % del valle. Por lo que una resolución de 20000 FHWM es equivalente a una resolución de 10000 (10% valley). Un instrumento que tiene una resolución de 10000 FHWM separará iones a m/z 500.0 de iones a m/z 500.1 (pero no de 1000.1 a 1000.0).

En el Orbitrap, el poder de resolución es directamente proporcional al tiempo de adquisición, así a mayor tiempo de adquisición, mayor es el poder de resolución. Sin embargo, se debe llegar a un compromiso entre el tiempo de adquisición necesario y el poder de resolución deseado [182].

Una elevada resolución es necesaria para evitar interferencias isobáricas y es particularmente importante para todo tipo de análisis de matrices complejas, como muestras biológicas o medioambientales, ya que contienen un alto número de iones que pueden interferir isobáricamente con los analitos. En estos casos, una resolución elevada permite la detección de analitos a muy baja concentración sin estar enmascarados por las interferencias isobáricas de la matriz.

2.5.3.2.1.2.3. Fragmentación.

El analizador de masas empleado tipo Orbitrap (Exactive) permite dos modos de fragmentación de las moléculas: mediante CID (collision induced dissociation) y mediante HCD (higher energy collisional dissociation).

La fragmentación mediante colisión inducida (CID) es el método de fragmentación tradicional, es rápido y de alta sensibilidad [184]. Esta técnica consiste en la disociación de los iones mediante su interacción con compuestos conocidos neutros. En el equipo, se lleva a cabo aumentando la corriente continúa del voltaje del "skimmer", las "tube lens" y el "ion transfer capillary".

En el método de fragmentación HCD, los iones son fragmentados en una celda de colisión HCD. Esta celda consiste en un multipolo recto (octapolo) situado en el interior de un tubo metálico conectado directamente con la "C-trap". Para llevar a cabo la fragmentación, se suministra un gas de colisión (generalmente N_2), a través de una interfase abierta, de manera que aumenta la presión de gas dentro de la celda. Los iones pasan a través de la C-trap hasta la celda de colisión. El voltaje existente entre la C-trap y la celda HCD permite acelerar el paso de los iones precursores (parent) hacia la celda. La presión elevada del gas dentro de la celda provoca la disminución de la movilidad de los iones, aplicándose para evitarlo un gradiente de potencial a la celda de colisión que incrementa su movilidad. La polaridad del gradiente puede cambiarse en función de si se pretende la detección en modo positivo o negativo. Los iones colisionan con el gas provocando la rotación, la extensión y la ruptura de los puentes que forman las moléculas, dando lugar a fragmentos de iones y de moléculas neutras que son eliminadas por el sistema de vacío. Una vez que los iones se han formado en la celda de colisión, se transfieren de vuelta a la C-trap para ser analizadas posteriormente por el Orbitrap.

Una de las principales ventajas del modo de fragmentación HCD con respecto al modo CID es que este primero no presenta un límite inferior de análisis de masas (low mass cut off) y además el HCD utiliza energías de disociación más elevadas que las usadas en CID, permitiendo una gran variedad de tipos de fragmentación. Sin embargo, el modo de fragmentación HCD presenta un inconveniente, ya que los tiempos de adquisición de los espectros aumentan considerablemente ya que existen más iones que necesitan ser detectados mediante una transformada de Fourier en comparación con la detección de espectros CID, de forma que se pone en riesgo la sensibilidad de detección.

2.5.3.2.2. Evolución de la espectrometría de masas de alta resolución (HRMS).

En la siguiente tabla (Tabla 5), se muestran algunos de los trabajos publicados en la última década respecto al análisis de plaguicidas empleando la espectrometría de masas de alta resolución. Cómo se puede observar en la tabla, la inmensa mayoría de métodos de análisis de plaguicidas han empleado como método de preparación la extracción mediante QuEChERS, muy habitual en el análisis de plaguicidas en alimentos. Se puede ver que la resolución de los métodos ha ido aumentando durante esta década.

La espectrometría de masas de alta resolución se ha aplicado en multitud de matrices en el análisis de plaguicidas, especialmente en matrices relacionadas principalmente con el análisis de plaguicidas en alimentos (frutas y verduras, especialmente), habiéndose aplicado también en matrices biológicas tales como orina y sangre o en el análisis de aguas. En el caso de nuestra matriz de estudio, aire, únicamente se han descrito pocos trabajos [163, 185-186] donde se ha realizado el análisis de filtros y adsorbentes empleando la espectrometría de masas de alta resolución, aunque en dos de estos [185-186] se ha empleado la cromatografía gaseosa. En nuestro conocimiento, únicamente se encuentra descrito un trabajo de análisis de plaguicidas en la atmósfera donde se emplee la cromatografía líquida acoplada a espectrometría de masas de alta resolución [163].

Tabla 5. Revisión de artículos de análisis de plaguicidas en diferentes matrices empleando HRMS

Compuestos	Matriz	Extracción/Preparación	Instrumental	Columna analítica	Resolución	Método análisis	LOD	Referencia
11 plaguicidas	Frutas y verduras	Extracción con metanol/agua (80:20)	UHPLC-QTOF-MS/MS ESI (+)	UPLC BEH C18 (1.7 µm, 50 mm x 2.1 mm)	10000	Target	0.31-12.5 pg	[187]
53 plaguicidas	Naranjas, tomates y puerro	Extracción con QuEChERS (Acetonitrilo)	LC-QTOF-MS-ESI (+)	Agilent Zorbax Eclipse XDB-C8 (5 µm, 150 mm x 4.6 mm)	15000	Target	-	[188]
97 plaguicidas	Tomates, pimientos, naranjas, puerro y calabacín	Extracción con QuEChERS (Acetonitrilo)	LC-QTOF-MS-ESI (+)/ESI (-)	XDB-C18 (1.8 µm, 50 mm x 4.6 mm)	15000	Screening	-	[189]
2 plaguicidas (clorantraniliprol y ciantraniliprol)	Lechugas y naranjas	Extracción con QuEChERS (Acetonitrilo)	LC-Orbitrap-MS-ESI (+)	C18 (5 µm, 50 mm x 2 mm)	50000	Target	10 µg/kg (LOQ)	[190]
Plaguicidas, residuos veterinarios y micotoxinas	Bollería	Extracción con QuEChERS (Acetonitrilo)	UHPLC-HRMS (Exactive)	UPLC BEH C18 (1.7 µm, 100 mm x 2.1 mm)	100000	Screening	-	[191]
240 plaguicidas	Lechugas, pimiento rojo	Extracción con acetato de etilo-1% en ácido acético	LC-Orbitrap – MS-ESI (+)	C18 (1.8 µm, 100 mm x 2.1 mm)	50000	Screening	0.5-100 µg/kg (LOQ)	[192]
Plaguicidas	Frutas y verduras	Extracción con QuEChERS	UHPLC-HRMS (Orbitrap)	Atlantis T3 (3 µm, 100 mm x 3.1 mm)	50000	Screening	10-200 µg/kg	[193]
Plaguicidas y metabolitos	Alimentos	Extracción con QuEChERS	UHPLC-QTOF-ESI (+)	XDB-C18 (1.8 µm, 50 mm x 4.6 mm)	-	Screening		[194]
Plaguicidas y otros contaminantes orgánicos	Agua potable y residual	Dilución e inyección	UHPLC-HRMS (Orbitrap)	Hypersil Gold C18 (12 µm, 20 mm x 2.1 mm)	25000	Target screening	0.01-0.38 µg/L	[195]
Plaguicidas	Filtros PM10	Extracción con MAE	UHPLC-HRMS (Orbitrap)	Hypersil Gold aQ C18 (1.9 µm, 100 mm x 2.1 mm)	50000	Target y análisis retrospectivo	6.5-75 pg m^{-3} (LOQ)	[163]
Plaguicidas	Filtro de fibra	Extracción con Soxhlet, 48	HRGC-HRMS	HP-5MS (0.25 µm,	10000	Target	0.04-0.10	[185]

organoclorados y PCBs	de cuarzo + PUF	horas con DCM. Clean-up con columna de alúmina		0.32 mm id., 30 m)	(10% valley definition)		pg m^{-3}	
Plaguicidas organoclorados y otros contaminantes persistentes	Adsorbente para fase gaseosa	Extracción con ASE (hexano/acetona). Clean-up con Florisil	VG AutoSpec-HRMS	DB-5 (0.25 µm, 0.25 mm i.d., 30 m)	> 8000	Target	11-203 pg/muestra	[186]
Metabolitos de plaguicidas	Orina	Extracción con QuEChERS	UHPLC-HRMS (Orbitrap)	Hypersil Gold aQ C18 (1.9 µm, 100 mm x 2.1 mm)	50000	Target y análisis retrospectivo	0.8-50 ng/mL (LOQ)	[196]
Productos de transformación de plaguicidas y residuos veterinarios	Alimentos y matrices relacionadas	Extracción con QuEChERS	UHPLC-HRMS (Orbitrap	Trascend 600 LC (1.7 µm, 100 mm x 2,1 mm)	10000	Análisis retrospectivo	-	[197]
Plaguicidas organoclorados	Sangre	Extracción líquido-líquido y clean-up con C18	Agilent GC 6890-Finnigan MAT 95S HRMS	ZB-Mulitresiude 2 (0.2 µm, 0.25 mm id, 30 m)	> 8000	Target	-	[198]
Plaguicidas organoclorados	Suelos, sedimentos, musgo y líquenes	Extracción con ASE (n-hexano/DCM) y clean-up con columna silica-gel	HRGC/HRMS-EI (+)	DB 5-MS (0.25 µm, 0.25 mm id, 30 m)	≥ 80000	Target	0.02-22.71 pg/g	[199]
Plaguicidas y drogas	Agua	Acidificación con HCOOH	Trascend 1250-Q Exactive	HSS T3 (1.8 µm, 150 mm × 2.1 mm)	70000 (m/z 200)	Screening	0.1 ng/L-1 µg/L	[200]
Plaguicidas	Pienso	Extracción con QuEChERS	UHPLC-HRMS (Orbitrap)	Hypersil Gold aQ C18 (1.9 µm, 100 mm x 2.1 mm)	50000	Análisis retrospectivo	-	[201]
Plaguicidas y micotoxinas	Te verde y jalea real	Extracción con QuEChERS + dilución e inyección	UHPLC-HRMS (Orbitrap)	Hypersil Gold aQ C18 (1.7 µm, 100 mm x 2.1 mm)	25000	Target	0.5-10 µg/kg	[202]
54 plaguicidas	Tomate, puerro y naranja	Extracción con QuEChERS	GC-EI-Q-Orbitrap-HRMS	TG-OCP I (0.25 µm, 0.25 mm i.d., 30 m)	60000	Target	0.5-25 µg/kg	[203]

Plaguicidas y otros contaminantes orgánicos	Comida para bebés	Extracción con QuEChERS	UHPLC-TOF-HRMS	Zorbax Rapid Resolution High Definition C_{18} (1.8 µm, 50 mm x 2.1 mm)	-	Screening	-	[204]
Residuos de plaguicidas y micotoxinas	Pimentón	Extracción con QuEChERS	UHPLC-HRMS (Orbitrap)	Kinitex C18 (2.6 µm, 100 mm X 2.1 mm)	35000	Target	0.8-3.6 µg/kg	[205]
Productos de transformación de plaguicidas y otros contaminantes ambientales	Aguas residuales	SPE	LC-ESI-QTOF-MS-ESI(+)/ESI(-)	ProntoSil C18 (5 µm, 250 mm x 4 mm)	20000	Screening	-	[206]
7 plaguicidas muy polares	Apio y lechuga	Método QuPPe-PO	UHPLC-HRMS (Orbitrap)	-	25000 (m/z 200)	Target	0.02-0.06 mg/kg	[207]
450 plaguicidas y productos de transformación	Aguas residuales	SPE	UPLC-QTOF-MS-ESI (+)	UPLC BEH C18 (1.7 µm, 100 mm x 2.1 mm)	-	Screening	-	[208]

ABREVIATURAS

A: Air/Aire.
ACN: Acetonitrilo.
AGC: Automatic Gain Control.
An: Animals/Animales.
APCI: Atmospheric Pressure Chemical Ionization.
ASE: Accelerated Solvent Extraction/Extracción acelerada con disolventes.
BW: Body Weight/ Peso corporal.
CCD: Central Composite Design.
CE: Comisión Europea.
CID: Collision Induced Dissociation.
CUPs: Currently Used Pesticides/ Plaguicidas de uso habitual.
C_{TSP}: Concentración total de las partículas suspendidas en el aire.
CV: Coeficiente de variación.
DAD: Diode array detector/ Detector de matriz de diodos.
DCM: Diclorometano.
DEE: Dietil éter.
DIE: Daily Inhalation Exposure/ Exposición Inhalatoria Diaria.
Eac: Acetato de etilo.
ECD: Detector de captura electrónica.
ED: Tiempo de exposición.
EE: Etil éter.
EFSA: European Food Safety Authority.
EIC: Cromatograma de ion extraído.
ET: Excitation Time/ Tiempo de excitación.
EV: Excitation Voltage/ Voltaje de excitación.
FFT: Transformada de Fourier rápida.
FHWM: Full-Width at Half Maximum/ Anchura de pico a media altura.
f_{OM}: Fracción de la materia orgánica.
GC: Cromatografía de gases.
GFF: Glass fiber filter/ Filtro de fibra de vidrio.
GPC: Gel Permeation Chromatography/ Cromatografía de exclusión molecular.
GW: Groundwater/ Agua subterránea.
HBRV: Health Based Reference Value/ Valor de referencia basado en salud.
HCD: Higher-energy collisional dissociation.
HI: Hazard index/ Índice de peligro.
HPLC: High Pressure Liquid Chromatography/ Cromatografía Líquida de alta presión.
HQ: Hazard Quotient/ Cociente de Peligro.
HRMS: High Resolution Mass Spectrometry/ Espectrometría de masas de alta resolución.
Hu: Humans/Humanos.
HVSs: High Volume Sampler/ Captador de alto volumen.
Hx: Hexano.
IR_{inh}: Inhalation rate/ Tasa de ventilación.
IST: Ion Source Temperature/ Temperatura de la fuente de iones.
IT: Isolation Time/ Tiempo de aislamiento.
K_{oa}: Coeficiente octanol-aire.
K_p: Coeficiente de partición entre la fase gaseosa y la fase particulada.
LC: Liquid Chromatography/ Cromatografía Líquida.

LOD: Límite de detección.
LOQ: Límite de cuantificación.
LSE: Liquid Solid Extraction/ Extracción Sólido-Líquido.
LVSs. Low Volume Sampler/ Captador de bajo volumen.
MAE: Microwave Assistent Extraction/ Extracción asistida con microondas.
MS: Mass spectrometry/ Espectrometría de masas.
MS/MS: Mass spectrometry in tandem/ Espectrometría de masas en tándem.
NI: Negative Ionization/ Ionización negativa.
N-MPA: N-(2-etil-6-metilfenil)-L-alanina.
NOAEL: No Observed Adverse Effect Values.
NPD: Nitrogenphosphorus detector/ Detector de nitrógeno-fósforo.
OCPs: Plaguicidas organoclorados.
OMS: Organización Mundial de la Salud.
PAHs: Hidrocarburos policíclicos aromáticos.
PAS: Passive aire sampler/ Muestreador pasivo.
PB: Plackett-Burman.
PBDEs: Polibromodifenil éteres.
PCNs: Naftalenos policlorados.
PCBs: Bifenilos policlorados.
PDA: Photodiode array detector/ Detector de matriz de fotodiodos.
PE: Éter de petróleo.
PF: Potency Factor/ Factor Potencial.
Pl: Plants/Plantas.
PLE: Pressurized Liquid Extracción/ Extracción con líquidos presurizados.
PM10: Material particulado de diámetro inferior a 10 micras.
PM: Particulate matter/ Material particulado.
POPs: Persistent organic pollutants/ Contaminantes orgánicos persistentes.
PUF: Polyurethane foam/ Espuma de poliuretano.
QFF: Quartz fiber filter/ Filtro de fibra de cuarzo.
QuEChERS: Quick, Easy, Cheap, Effective, Rugged, y Safe.
RF: Radiofrecuencias.
RPF: Relative Potency Factor.
RSD: Relative Standard Desviation/ Desviación relativa estándar.
S: Soil/Suelo.
SIM: Single Ion Monitoring/ Seguimiento de Ion Monitorizado.
SPE: Solid Phase Extraction/ Extracción en fase sólida.
SRM: Selected Reaction Monitoring.
SVOCs: Compuestos orgánicos semivolátiles.
THPAM: Tetrahydrophthalamic acid/ Ácido tetrahidroftalámico.
TOF: Time of Flow/ Tiempo de Vuelo.
TP: Transformation Product/ Producto de Transformación.
TR: Tiempo de Retención.
UE: Unión Europea.
USEPA: United States Environmental Protection Agency.
UV: Ultravioleta.
W: Water/Agua.

II. OBJETIVOS

3. OBJETIVOS

Los objetivos principales de esta Tesis son:

a) El desarrollo de nuevas estrategias de captación y analíticas para la determinación de plaguicidas y sus metabolitos en la atmósfera basadas en la cromatografía líquida acoplada a espectrometría de masas de alta resolución (UHPLC-HRMS).

b) La evaluación de la exposición de la población a los plaguicidas y sus metabolitos presentes en el aire ambiente.

Estos dos objetivo principales se desglosan en los siguientes objetivos específicos:

- Desarrollo de una metodología analítica mediante LC-HRMS para el análisis retrospectivo de metabolitos de plaguicidas en la atmósfera.

- Desarrollo de un nuevo método rápido de extracción de la fase particulada de los plaguicidas mediante una cafetera Espreso.

- Selección del adsorbente adecuado para la captación de la fase gaseosa de plaguicidas apolares y volátiles.

- Selección del adsorbente adecuado para la captación de la fase gaseosa de plaguicidas polares y térmicamente inestables.

- Evaluación de la exposición a plaguicidas en el aire en la población de la Comunidad Valenciana.

- Evaluación del riesgo de exposición a plaguicidas en el aire ambiente en la población de una zona rural de Francia.

III. METODOLOGÍA

4. METODOLOGÍA

En este apartado se describen todos los materiales, reactivos, patrones y equipos empleados en esta Tesis Doctoral. Además, se describe con detalle cómo y dónde se han recogido las muestras utilizadas, qué técnicas de preparación y extracción se han empleado y que métodos cromatográficos se han empleado.

También se han descrito los criterios de identificación y confirmación que se han aplicado para la detección y cuantificación de los plaguicidas y los metabolitos y la metodología utilizada para el cálculo de la exposición y evaluación del riesgo a plaguicidas.

4.1. Materiales, reactivos, patrones, equipos.

4.1.1. Material empleado.

-Cartuchos ChemComb para el captador de bajo volumen Partisol 2300.
-Cápsulas de acero inoxidable de dimensiones (26 x 25 x 23 mm de tamaño y 8.8 mL de volumen interno) de Mycoffestar GAMMA (Zurich, Suiza).
-Celdas de acero para la extracción acelerada con disolventes de Dionex (Sunnyvale, CA, EEUU).
-Filtros de borosilcato de 47 mm de Scharlau (Barcelona, España).
-Filtros de fibra de cuarzo de diámetro de 47 mm de Filtres RS (París, Francia).
-Filtros de fibra de cuarzo de diámetro 150 mm de Munktell filter AB (Falun, Sweden).
-Filtros de tamaño 0.22 µm GHP Acrodisc de Pall Life Science (Ann Arbor, MI, EEUU).
-Matraces aforados clase A de 10, 50 y 500 mL.
-Micropipetas electrónicas de volumen variable: entre 10 y 100 µL, entre 20 y 200 µL, entre 100 y 1000 µL y entre 1 y 10 mL.
-Pipetas Pasteur.
-Probetas graduadas de 50 mL y 500 mL.
-Tapones LC-Agilent Screw cap green 100/PK.
-Tapones GC- Chromacol 11 mm CRIMP CAP-PTFE silicona.
-Tubos de Teflón de 100 mL para la extracción con microondas de CEM Corporation (Mathews, NC, EEUU).
-Tubos de TurboVap de 250 mL de Zymark (Idstein, Alemania).
-Tubos de vidrio de topacio de 22 mL con tapón de rosca.
-Viales LC-Agilent High Recovery Screw Vial.
-Viales GC-Crimp IV-Vial amber con inserto de 12 x 32 mm de volumen 500 µL.

4.1.2. Reactivos utilizados.

-Acetato amónico grado HPLC (97 %) de Scharlau (Barcelona, España).
-Acetato de etilo grado HPLC de Merck (Darmstadt, Alemania).
-Acetona grado HPLC de Merck (Darmstadt, Alemania).
-Acetonitrilo grado HPLC de Scharlau (Barcelona, España).
-Ácido acético glacial de Panreac (Barcelona, España).
-Ácido fórmico suprapur (98 %) de Panreac (Barcelona, España).
-Agente dispersante Spe-ed Matrix de Applied Separations (Alletown, PA, EEUU).
-Agua grado HPLC de Merck (Darmstadt, Alemania).

-Cloruro sódico de Scharlau (Barcelona, España).
-Espumas de poliuretano (PUF) de dimensiones 150 mm x 100 mm de BSG Ingenieros (Valencia, España).
-Formiato amónico solución Ultra (100 mL, 10 M en agua) de Fluka (Steinheim, Suiza).
-Metanol grado HPLC de Scharlau (Barcelona, España).
-N-hexano grado HPLC (99 %) de Scharlau (Steinheim, Suiza).
-Nonano grado GC de Fluka (Steinheim, Suiza).
-Resina polimérica XAD-2 de Sigma Aldrich (Barcelona, España).
-Resina polimérica XAD-4 de Sigma Aldrich (Barcelona, España).
-Sulfato magnésico de Scharlau (Barcelona, España).

4.1.3. Patrones.

Los patrones de plaguicidas y metabolitos de plaguicidas empleados en los diferentes capítulos de esta Tesis doctoral son estándares comerciales de alta pureza provistos por Dr. Ehrenstorfer (Augsburgo, Alemania) y Sigma Aldrich (Barcelona, España). Para la preparación de patrones de plaguicidas y metabolitos de plaguicidas, se han pesado 10 mg de cada analito empleando una balanza analítica de precisión 0.0001 g (Metler-Toledo, Barcelona, España) disolviéndose en 50 mL de acetona. Estas disoluciones se han guardado a -20 ºC empleando viales opacos para evitar su degradación, siguiendo las directrices de la guía SANTE/11945/2015 [209].

4.1.4. Equipos.

- Balanza analítica de precisión 0.0001 g de Metler-Toledo (Bedford, MA, EEUU).
- Baño de ultrasonidos de VWR (Barcelona, España).
- Cafetera Nespresso Essenza Manual XN2003 Krups.
- Captador de alto volumen para material particulado de tamaño 10 μm (PM10) de Digitel (Madrid, España).
- Captador de bajo volumen Partisol 2300 de Thermo Fisher Scientific (Bremen, Alemania).
- Captador de bajo volumen Partisol 2000 de Thermo Electron Corporation (East Greenbush, NY, EEUU).
- Congelador a temperatura -20 ºC.
- Estufa desecadora a 130 ºC.
- Evaporador Turbo Vap LV500 de Zymark (Idstein, Alemania).
- Extractor ASE300 PLE System para la extracción acelerada con disolventes de Dionex (Sunnyvale, CA, EEUU).
- Microondas Mars System de CEM Corporation (Mathews, NC, EEUU).
- Nevera a temperatura < 10 ºC.

4.2. Toma de muestra.

4.2.1. Toma de muestra de la fase particulada.

Las muestras recogidas en la Comunidad Valenciana se realizaron empleando un captador activo de alto volumen (Digitel, Madrid) y utilizando como soporte de captación de la fase particulada filtros de fibra de cuarzo PM10 de 150 mm de diámetro (muestras de materia particulada con un diámetro inferior a 10 μm) (Figura 7). El flujo

de aire empleado fue 30 m^3 h^{-1} durante un total de 24 horas, suponiendo un volumen total de aire de aproximadamente 760 m^3.

Figura 7. Captador Digitel de Alto Volumen.

Las muestras recogidas en Francia se realizaron mediante un captador activo de bajo volumen Partisol 2000 (Thermo Electron, East Greenbush, USA) y utilizando como soporte de captación de la fase particulada filtros de fibra de cuarzo de 47 mm de diámetro (Figura 8). El flujo de aire empleado fue de 1 m^3 h^{-1} durante un total de 168 horas (1 semana), suponiendo un volumen total de aire de aproximadamente 168 m^3.

Figura 8. Captador Partisol 2000 de Bajo Volumen.

4.2.2. Toma de muestra de la fase gaseosa.

Las muestras recogidas en la Comunidad Valencia se realizaron empleando un captador de bajo volumen Partisol 2300 (Thermo Fisher Scientific, Bremen, Alemania) y utilizando como soporte de captación de la fase gaseosa una mezcla en forma de sándwich de espuma de poliuretano (PUF) y resina polimérica XAD-2 (sándwich PUF-XAD2-PUF) (Figura 9). El sándwich está formado por dos PUF de 1.4 cm de longitud y de 2.5 cm de diámetro y 5 gramos de XAD-2. El flujo de aire empleado fue de 1 m^3 h^{-1} durante un total de 168 horas (1 semana), suponiendo un volumen total de aire de aproximadamente 168 m^3.

Figura 9. Captador Partisol 2300 de Bajo Volumen.

Las muestras recogidas en Francia se realizaron mediante un captador activo de bajo volumen Partisol 2000 (Thermo Electron, East Greenbush, USA) y utilizando como soporte de captación de la fase gaseosa espumas de poliuretano (PUF) de 26 mm de diámetro y 76 mm de longitud (ver Figura 8). El flujo de aire empleado fue de 1 m^3 h^{-1} durante un total de 168 horas (1 semana), suponiendo un volumen total de aire de aproximadamente 168 m^3.

4.3. Localizaciones estudiadas.

Las muestras de aire empleadas en esta Tesis fueron recogidas en 11 zonas diferentes: diez de ellas situadas en la Comunidad Valenciana y una de ellas en una zona agrícola francesa (Oysonville). La figura 10 muestra las localizaciones de las zonas estudiadas.

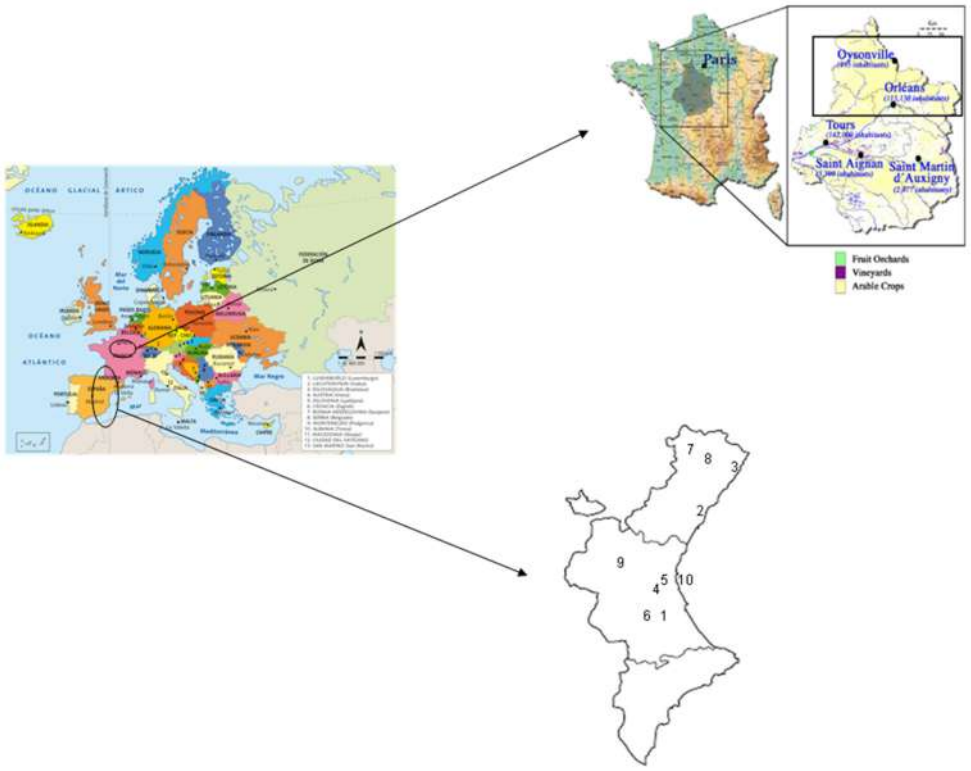


Figura 10. Localizaciones estudiadas de Francia (Oysonville) y Comunidad Valenciana (1: Alzira, 2: Burriana, 3: Benicarló, 4: Benifaió, 5: Burjassot, 6: L'Alcúdia, 7: Morella, 8: Sant Jordi, 9: Villar del Arzobispo, 10: Viveros).

La mayoría de las zonas estudiadas son zonas rurales donde es habitual la actividad agrícola, aunque también se han estudiado zonas urbanas y remotas. Además, en el capítulo 3 se recogieron muestras de aire indoor en diferentes domicilios de mujeres embarazadas de la Comunidad Valenciana. Los lugares estudiados se presentan en la siguiente tabla (Tabla 6):

Tabla 6. Localizaciones de las zonas estudiadas en esta Tesis Doctoral.

Localizaciones	Latitud	Longitud	Descripción	Número total muestras
Alzira	39º09´00″	0º27´28″	Área rural y agrícola rodeada de cítricos. Las muestras fueron recogidas a 60 m sobre el nivel del mar.	79
Burriana	39º53′52″	0º03′54″	Área rural y agrícola rodeada de cítricos. Las muestras fueron recogidas a 20 m sobre el nivel del mar.	58
Benicarló	40°25′07″	0°25′23″	Área rural y agrícola rodeada de viñedos. Las muestras fueron recogidas a 20 m sobre el nivel del mar.	25
Benifaió	39°17′07″	0°25′35″	Área rural y agrícola rodeada de cítricos. Las muestras fueron recogidas a 35 m sobre el nivel del mar.	23
Burjassot	39°30′34″	0°25'04″	Área urbana. Las muestras se recogieron a 60 metros sobre el nivel del mar.	16
L'Alcúdia	39°11′45″	0°30′26″	Área rural y rodeada de cítricos, caquis y frutales de hueso. Las muestras se recogieron a 32 metros sobre el nivel del mar.	15
Morella	40°38′14″	0°05'33″	Área remota. Las muestras fueron recogidas en la cima del Monte Mas del Aljub, situado a 1153 metros sobre el nivel del mar.	54
Oysonville (Francia)	48º23′35″	1º56'57″	Área rural y agrícola, rodeada de cultivos de trigo, cebada y colza. Las muestras se recogieron a 143 metros sobre el nivel del mar.	134
Sant Jordi	40°30′34″	0°19′55″	Área rural y agrícola rodeada de viñedos, almendros, olivos y algarrobos. Cercano a un campo de golf (Panorámica Golf) que ocupa 80 ha. Las muestras fueron recogidas a 181 metros sobre el nivel del mar.	43
Villar del Arzobispo	39°44′01″	0°49′38″	Área rural y agrícola rodeada de viñedos, cereales y olivos. Las muestras se recogieron a 520 metros sobre el nivel del mar.	9
Viveros (Valencia)	39°28′46″	0°22′10″	Área comercial y residencial. El captador se encuentra situado dentro de un parque (Viveros) con jardines. Las muestras se recogieron a 11 metros sobre el nivel del mar	48
			TOTAL	450

4.4. Preparación y extracción de las muestras.

Para la extracción de los plaguicidas tanto de la fase particulada como de la fase gaseosa de las muestras recogidas en la Comunidad Valenciana se ha empleado de forma mayoritaria la extracción con microondas (MAE) utilizando acetato de etilo como disolvente extractante. Las condiciones empleadas fueron las siguientes: temperatura de 50 ºC durante 20 minutos empleando una potencia de 1200 W y añadiendo 30 mL de acetato de etilo. Posteriormente, las muestras fueron filtradas y evaporadas empleando un concentrador de nitrógeno TurboVap LV500. Dependiendo de si las muestras se analizaban mediante UHPLC-HRMS o en el GC-MS/MS se siguió un procedimiento distinto. Para las muestras analizadas por cromatografía líquida, se redisolvieron con 1 mL de una disolución agua: metanol (70:30) y se filtraron mediante filtros de 0.22 µm pasándolos al vial correspondiente para ser analizados mediante LC-HRMS. Para las muestras analizadas por cromatografía gaseosa, se redisolvieron con 0.5 mL de hexano y se filtraron mediante filtros de 0.22 µm pasándolos al vial correspondiente para ser analizados mediante GC-MS/MS.

El método de extracción de plaguicidas por microondas ha sido comparado en uno de los trabajos de esta tesis, con un nuevo método de extracción desarrollado que emplea una cafetera Nespresso. La cafetera fue modificada ligeramente para incrementar el depósito de agua. Las cápsulas de acero inoxidables compatibles con la cafetera donde se introducen las muestras (dimensiones 26 x 25 x 23 mm de tamaño y 8.8 mL de volumen interno) fueron obtenidos de Mycoffestar GAMMA (Zurich, Suiza) y el agente dispersante Spe-ed Matrix de Applied Separations (Allentown, PA, USA). La cafetera se presenta en la figura 11:

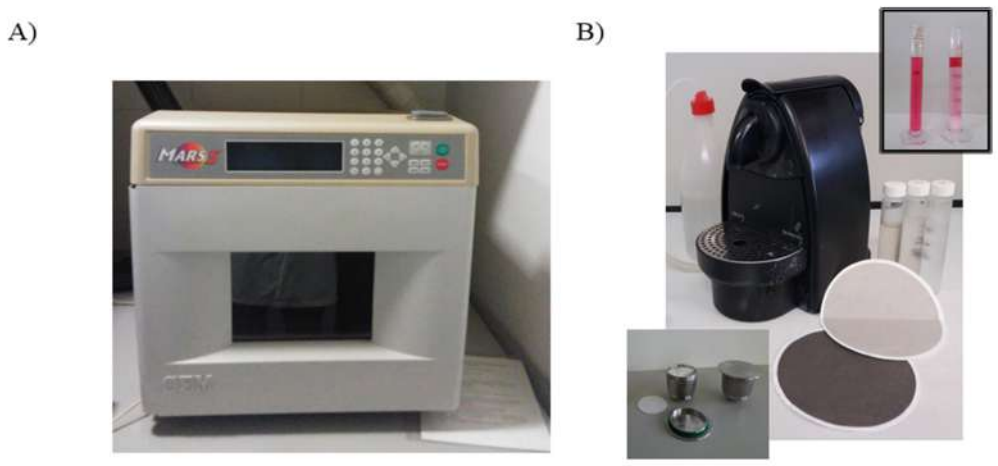


Figura 11. A) Microondas utilizado para la extracción, B) Filtros PM10, máquina Espresso y cápsulas de acero utilizadas para la extracción en el capítulo 2.

La máquina Espresso fue purgada con 200 mL 20 % en volumen de acetonitrilo: agua usando la cápsula vacía y añadiéndole 2.5 g de agente dispersante, antes de cada secuencia de extracción. Cada filtro PM10 fue plegado e introducido en la cápsula de acero llenando el volumen restante de la cápsula con agente dispersante. Además, se añadió un filtro de borosilicato en la parte superior para evitar la pérdida de material durante la extracción. Posteriormente, la cápsula fue cerrada e introducida en el

compartimento de la cafetera, extrayendo el filtro mediante 50 mL 20% en volumen de acetonitrilo: agua. Dos fracciones de 25 mL fueron recogidas en tubos de centrífuga añadiendo posteriormente para realizar el salting-out, 8 gramos de NaCl, agitados en vórtex y centrifugados durante 5 minutos a 3500 rpm. Los plaguicidas se encontraron concentrados en la capa superior, obteniéndose un volumen final de 1.1 ± 0.1 mL en cada tubo, analizándose directamente en el equipo de UHPLC-HRMS.

En el capítulo 5, las muestras de Benicarló, Benifaió, Villar del Arzobispo y Burjassot, fueron analizadas mediante cromatografía líquida acoplada a espectrometría de masas (LC-MS) y cromatografía gaseosa acoplada a espectrometría de masas (GC-MS). Para las muestras analizadas por LC-MS, fueron extraídas mediante ultrasonidos (VWR ultrasonic bath, Barcelona, España, empleando ciclos de 3 repeticiones de 15 minutos cada ciclo, usando 10 mL de acetato de etilo como disolvente extractante, ver figura 12). Posteriormente, las muestras fueron evaporadas empleando un sistema de rotavapor (50 ºC, 180 rpm, 5 min), disueltas en 500 µL de metanol y evaporadas hasta 100 mL empleando una corriente de N_2. Las muestras analizadas por GC-MS fueron extraídas empleando ultrasonidos (2 ciclos de 10 minutos con 10 mL de isooctano como disolvente extractante). A continuación, el extracto fue evaporado mediante el sistema de rotavapor (5 minutos, 40 ºC, 180 rpm), evitando la sequedad. Seguidamente, el extracto fue disuelto con 1 mL de isooctano y evaporado mediante corriente de nitrógeno. Finalmente, el extracto fue disuelto en 150 µL de 1-fenildodecano e inyectado en el sistema GC-MS.

En el capítulo 6, las muestras de filtros y espumas de poliuretano (PUF) recogidas en Oysonville (Francia) fueron extraídas de forma conjunta. Tanto los filtros como las espumas de poliuretano fueron extraídos de forma conjunta empleando la extracción con fluidos presurizados. El equipo empleado para llevar a cabo la extracción fue un ASE 300 System (Dionex, Sunnyvale, CA, USA, ver figura 12). Las condiciones de extracción empleadas fueron las siguientes: el disolvente extractante empleado fue el diclorometano, la temperatura utilizada fue de 90 ºC, con una presión de 100 bares, con un tiempo de calentamiento de 5 minutos y un tiempo estático de 5 minutos. Posteriormente, el extracto fue purificado en la celda de muestreo empleando una corriente de nitrógeno durante 150 segundos. Posteriormente, el extracto de muestra se dividió en dos fracciones diferentes. Una de las fracciones fue concentrada y redisuelta con 1 mL de hexano. Esta solución fue inyectada directamente en el cromatógrafo de gases acoplado a espectrometría de masas (GC-MS) en modo SIM (selected ion monitoring). La otra fracción fue concentrada y redisuelta con metanol. Posteriormente esta disolución fue inyectada en el cromatógrafo de líquidos acoplado a espectrometría de masas en tándem (LC-MS/MS) en modo SRM (Selected Reaction Monitoring Mode).

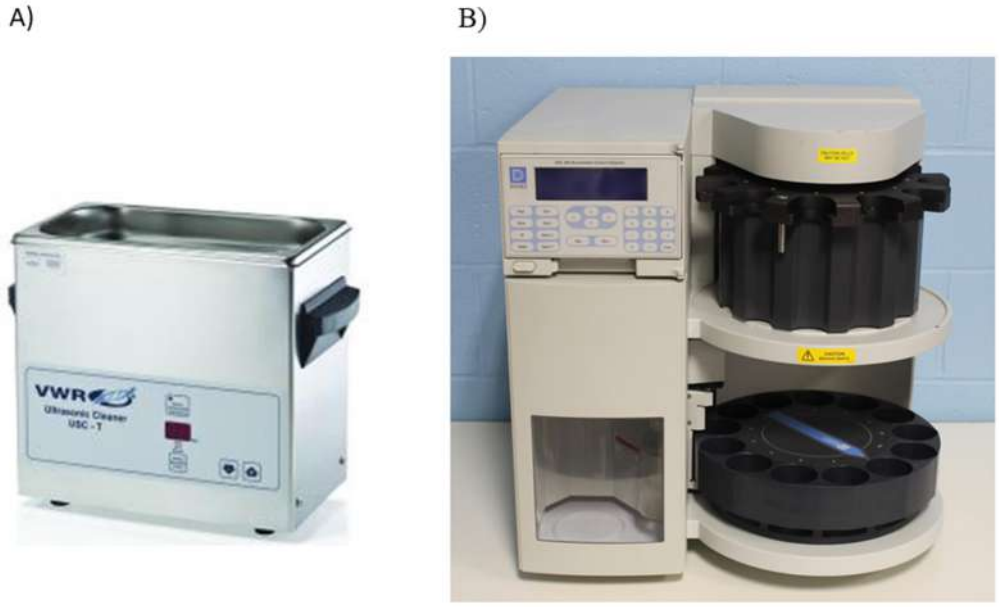


Figura 12. A) Baño de ultrasonidos empleado para la extracción en el capítulo 5, B) ASE empleado para la extracción en el capítulo 6

Los métodos de extracción empleados en cada capítulo están resumidos en la siguiente tabla (Tabla 7).

Tabla 7. Método de extracción empleado en cada capítulo

	Capítulo 1	**Capítulo 2**	**Capítulo 3**	**Capítulo 4**	**Capítulo 5**	**Capítulo 6**
Modo extracción	MAE	MAE Máquina Nespresso	MAE	MAE	Ultrasonidos	PLE
Disolvente extractante	Acetato de etilo	Acetato de etilo ACN:H_2O	Acetato de etilo	Acetato de etilo	Acetato de etilo Isooctano	Diclorometano

MAE= Extracción acelerada por microondas PLE= Extracción con fluidos presurizados.

4.5. Etapa de análisis.

La separación cromatográfica de las muestras analizadas tiene lugar mediante el uso de cromatografía líquida y cromatografía gaseosa, empleando diferentes columnas y distintos detectores. En la siguiente tabla (Tabla 8) se describe que método o métodos cromatográficos se han empleado en cada uno de los capítulos de esta Tesis:

Tabla 8. Métodos cromatográficos y sistema de detección empleados

	Capítulo 1	Capítulo 2	Capítulo 3	Capítulo 4	Capítulo 5	Capítulo 6
Compuestos estudiados	Metabolitos de plaguicidas	Plaguicidas Metabolitos de plaguicidas	Plaguicidas	Plaguicidas	Plaguicidas	Plaguicidas
Cromatografía	UHPLC	UHPLC	GC	UHPLC	UHPLC	GC
					LC	
					LC	LC
					GC	
Tipo de detector de masas	HRMS	HRMS	MS/MS	HRMS	UHPLC: HRMS	GC: MS
					LC: MS/MS	
					LC: MS	LC: MS/MS
					GC: MS	
Columna Cromatográfica	Hypersil Gold aQ	Hypersil Gold aQ	Columna capilar SGE-BPX5	Hypersil Gold aQ	UHPLC: Hypersil Gold aQ	GC: CPSIL 13CB
					LC: Luna C18	
					LC: ProtoSil 120-5 C18	LC: Gemini-NX3U C18 100A
					GC: TR-5MS	
Modo de adquisición	Full scan 50-800 Da ESI + sin/con HCD (20 eV)	Full scan 50-800 Da ESI + sin/con HCD (20 eV)	Full scan 50-650 Da Modo SRM	Full scan 50-800 Da ESI + sin/con HCD (20 eV)	UHPLC: Full scan 50-800 Da ESI + sin/con HCD (20 eV)	GC: Modo SIM
					LC: ESI+ Modo SRM	
					LC: APCI+ Modo SRM	LC: ESI+ /ESI- Modo SRM
					GC: Full scan 50-650 Da Modo SIM	
Fases eluyentes/portadoras	A) agua con 0.1 % de ácido fórmico y 5 mM de formiato amónico B) metanol con 0.1 % de ácido fórmico y 5 mM de formiato amónico.	A) agua con 0.1 % de ácido fórmico y 5 mM de formiato amónico B) metanol con 0.1 % de ácido fórmico y 5 mM de formiato amónico.	Helio (1.2 mL min^{-1})	A) agua con 0.1 % de ácido fórmico y 5 mM de formiato amónico B) metanol con 0.1 % de ácido fórmico y 5 mM de formiato amónico.	UHPLC: A) agua con 0.1 % de ácido fórmico y 5 mM de formiato amónico B) metanol con 0.1 % de ácido fórmico y 5 mM de formiato amónico.	GC: Helio (1 mL min^{-1})
					LC: A) agua con 0.1 % de ácido fórmico y 5 mM de formiato amónico B) metanol	
					LC: A) Metanol B) Agua con 0.1 % ácido acético	LC: A) Agua B) Metanol con 5 mM de formiato amónico
					GC: Helio (1 mL min^{-1})	

4.6. Criterios de identificación y confirmación.

La correcta identificación y confirmación de las sustancias analizadas es una parte de los criterios de calidad exigible a los métodos analíticos. Así, a continuación, se describen los criterios de identificación y confirmación empleados en cada uno de los equipos utilizados en esta Tesis Doctoral. Todos los criterios de identificación y confirmación están descritos siguiendo las directrices de la guía SANTE/11945/2015 [209].

4.6.1. Criterios de identificación y confirmación para los plaguicidas (UHPLC-HRMS).

Para la identificación de los analitos, se siguen los siguientes criterios:

i) Desviación de masa del ion molecular < 5 ppm respecto a la masa teórica del ion molecular.
ii) Desviación de masa del fragmento < 5 ppm respecto a la masa teórica del fragmento.
iii) Perfil isotópico similar al teórico con una tolerancia del 30%.
iv) El tiempo de retención de la muestra debe ser ± 0.1 min al tiempo de retención del patrón.

4.6.2. Criterios de identificación y confirmación para los plaguicidas (GC-MS/MS).

Para la identificación y confirmación de los plaguicidas, se siguen los siguientes criterios:

i) Detección de dos o más transiciones SRM por analito.
ii) El tiempo de retención de la muestra debe ser ± 0.1 min al tiempo de retención del patrón.
iii) La diferencia de abundancia relativa de las transiciones en las muestras no puede ser mayor al 30 % de la diferencia obtenida en los patrones.
iv) La señal/ruido (S/N) de las dos transiciones ha de ser mayor a 3.

4.6.3. Criterios de identificación y confirmación para los plaguicidas (LC-MS/MS).

Para la identificación y confirmación de los plaguicidas, se siguen los siguientes criterios:

i) Detección de dos transiciones SRM por analito.
ii) La diferencia en el tiempo de retención entre la muestra y el patrón no puede ser superior a 2.5 %.
iii) La diferencia de abundancia relativa de las transiciones en las muestras no puede ser mayor al 20 % de la diferencia obtenida en los patrones.
iv) La señal/ruido (S/N) de las dos transiciones ha de ser mayor a 3.

4.6.4. Criterios de identificación y confirmación para los plaguicidas (LC-MS y GC-MS).

Para la identificación y confirmación de los plaguicidas, se siguen los siguientes criterios:

i) Detección de 3 o más iones diagnóstico, preferiblemente incluyendo el ion molecular.
ii) El tiempo de retención de la muestra debe ser ± 0.2 min al tiempo de retención del patrón.
iii) Perfil isotópico similar al de la librería NIST.

4.6.5. Criterios de identificación y confirmación para los metabolitos de plaguicidas (UHPLC-HRMS).

El screening para la búsqueda de metabolitos se realiza mediante herramientas automatizadas, usando el programa TraceFinder. Los parámetros empleados fueron los siguientes: para el ion molecular, un umbral de 10000, con una S/N de 5, y una desviación de masa exacta inferior a 5 ppm, para los fragmentos, un umbral mínimo de 5000, para el perfil isotópico un porcentaje de fit threshold del 90 %, con una intensidad relativa permitida del 30 %, y una desviación de la exactitud de masa de 5 ppm.

Para la identificación de los metabolitos, se siguen los siguientes criterios:

i) Desviación de masa del ion molecular < 5 ppm respecto a la masa teórica del ion molecular.
ii) Desviación de masa del fragmento < 5 ppm respecto a la masa teórica del fragmento.
iii) Perfil isotópico similar al teórico con una tolerancia del 30%.
iv) Para la confirmación de los analitos, se comparan los tiempos de retención. El tiempo de retención de la muestra debe ser ± 0.1 min al tiempo de retención del patrón.

4.7. Modelo teórico de partición fase particulada-fase gaseosa.

El modelo empleado usando el coeficiente de partición octanol-aire (K_{oa}) propuesto por Harner y Bidleman en el año 1998 [87] se ha usado para la estimación de la concentración total en las muestras analizadas, donde solamente se ha recogido la fase particulada. Así, la fracción de cada plaguicida en la fase particulada se calcula a través de la siguiente fórmula:

$$Ø = (K_p C_{TSP})/ (1 + K_p C_{TSP}) \quad (1)$$

donde Ø es el porcentaje de plaguicida presente en la fase particulada, C_{TSP} es la concentración total de partículas suspendidas en el aire ($\mu g\ m^{-3}$) y K_p es el coeficiente de partición entre la fase particulada y la fase gaseosa. Este coeficiente se puede calcular a través de la siguiente ecuación:

$$\log K_p = \log K_{oa} + \log f_{OM} - 11.91 \quad (2)$$

donde K_{oa} es el coeficiente de partición y f_{OM} es la fracción de materia orgánica.

Los datos han sido calculados asumiendo un valor de f_{OM} de 0.2 y un valor de 0.55 para la C_{TSP}, que son los valores representativos en la zona estudiada [91].

4.8. Exposición crónica y evaluación del riesgo.

La inhalación de plaguicidas es una de las rutas más importantes de exposición a plaguicidas. La evaluación crónica a plaguicidas (a partir de un año) se ha evaluado para

tres grupos distintos de población: adultos, niños y bebés. Para estimar la exposición debida a la inhalación de plaguicidas, se utiliza la siguiente ecuación (ecuación 4):

$$DIE\ (mg/kg/day) = \Sigma(C \times IR_{inh} \times ED)/BW \quad (4)$$

Donde DIE es la exposición inhalatoria diaria, C es la concentración total (particulada + gaseosa) de cada plaguicida en el aire (mg m^{-3}), calculada a partir del modelo teórico de partición fase particulada-fase gaseosa, IR_{inh} es la tasa de inhalación por hora (m^3 h^{-1}), ED es el tiempo de exposición (horas) y BW es el peso corporal de cada grupo de población (kg).

Se han evaluado tres grupos de población distintos: bebés (6 meses-1.5 años), niños (1.5 años-6 años) y adultos (mayores de 12 años). Se han empleado dos escenarios diferentes en el estudio de evaluación del riesgo: en primer lugar, empleando la concentración media obtenida para cada plaguicida en cada lugar de estudio, y en segundo lugar, empleando la concentración máxima obtenida para cada plaguicida en cada área de estudio. En ambos casos se ha empleado un tiempo de exposición de 24 horas (1 día) y una frecuencia de exposición de 365 días al año. Además, se ha empleado una tasa IR_{inh} de 20 m^3 dia^{-1} para adultos, de 10 m^3 dia^{-1} para niños y de 8 m^3 dia^{-1} para bebés. El peso corporal (BW) empleado fue 70 kg para adultos, 15 kg para niños y 10 kg para bebés. La evaluación del riesgo fue estimada empleando HQ (hazard quotient, cociente de peligro), calculado de la siguiente manera (ecuación 5):

$$HQ = DIE_i/HBRV_i \quad (5)$$

donde $HBRV_i$ es el valor de referencia basado en salud para cada plaguicida. Los valores para cada HBRV fueron obtenidos a través de bases de datos de la Unión Europea (UE) y de la Agencia Norteamericana de Protección del medioambiente (USEPA, United States Environmental Protection Agency). El valor de referencia basado en salud fue definido como AOEL (acceptable operator exposure level), nivel aceptable de exposición del aplicador, que es el nivel que se aplica en el estudio de evaluación de plaguicidas y biocidas en Europa.

El nivel de riesgo se aplica a partir de valores de HQ superiores a 1, que nos indicarían que estamos expuestos a un riesgo potencial.

La exposición acumulada puede ser estimada de formas distintas. En primer lugar, empleando el índice de peligro (HI), para aquellas sustancias que presentan los mismos efectos sobre los órganos. Se calcula de la siguiente manera (ecuación 6):

$$HI = HQ_1 \text{ (plaguicida 1)} + HQ_2 \text{ (plaguicida 2)} + \ldots \quad (6)$$

En el caso de los organofosforados que presentan el mismo modo de acción, la exposición acumulada se calcula a través del RPF (Relative Potency Factor), que se calcula de la siguiente forma (ecuación 7) [210]:

$$RPFn = NOAEL_{\text{Chlorpyrifos ethyl}} / NOAEL_{\text{Organophosphate n}} \quad (7)$$

El RPF es el cociente entre el NOAEL (No Observed Adverse Effect Values) del organofosforado en concreto comparado con el de referencia (en este caso, el del etil

clorpirifos). La exposición acumulada para los organofosforados se calcula empleando la siguiente ecuación (ecuación 8):

Exposición acumulada = Σ (DIE x RPF) (mg/kg-day) (8)

El valor obtenido para los organofosforados se compara con el valor de NOAEL del etil clorpirifos (0.1 mg/kg-day). Valores de exposición acumulada obtenidos superiores a este valor, nos indicarían que existe un cierto riesgo en la población.

Para calcular el riesgo de cáncer (Cancer Risk), se emplea la siguiente ecuación (ecuación 9):

Cancer risk = DIE (mg/kg -day) x PF $(mg/kg\ -day)^{-1}$ (9)

donde PF es el factor potencial. Para aquellos plaguicidas clasificados como posibles o probables cancerígenos, el rango de PF oscila entre >0.01 y 0.1, por lo que se ha empleado 0.1 como factor potencial (tratamiento conservador).

El cálculo de la exposición puede verse afectado por numerosas fuentes de incertidumbre debido a las limitaciones de los métodos empleados. Las incertezas no cuantificables asociadas con la evaluación del riesgo, se interpretaron para la cuantificación desde un punto de vista cualitativo, siguiendo las recomendaciones del Comité Científico de la EFSA [211].

IV. RESULTADOS

5. CAPÍTULO 1. Análisis retrospectivo de metabolitos de plaguicidas en la atmósfera mediante UHPLC-HRMS.

5.1. Introducción.

Una vez emitidos a la atmósfera, los plaguicidas son susceptibles de degradaciones biológicas y químicas, produciéndose la formación de nuevas sustancias, comúnmente llamadas metabolitos (esto incluye los metabolitos biológicos, los productos de transformación y degradación y los productos de reacción). Una vez formados estos compuestos en un compartimento específico (por ej: el suelo), donde los plaguicidas experimentan una serie de transformaciones que pueden dar lugar a un amplio número de metabolitos, éstos pueden pasar a otro compartimento como puede ser la atmósfera. En algunos casos, los metabolitos presentan una toxicidad menor que los compuestos originales de los que proceden. Sin embargo, en otros casos, los metabolitos que se forman son más tóxicos o mantienen cierta actividad biológica, como por ejemplo los oxones en muestras de aire [78]. Además del producto original, los metabolitos y sus productos de transformación pueden estar presentes en la atmósfera. La presencia de metabolitos de plaguicidas en la atmósfera puede estar relacionada con la degradación química del compuesto original (parent) en el aire y de la volatilización o erosión debido al viento de los metabolitos formados en el agua y el suelo.

El espectrómetro de masas de triple cuadrupolo (QqQ) es el analizador de masas más empleado, acoplado tanto a la cromatografía líquida como la cromatografía gaseosa, para el análisis multiresiduo de plaguicidas. Esto se debe a sus bajos límites de cuantificación para un grupo de compuestos prioritarios. Sin embargo, estos instrumentos presentan una serie de limitaciones, ya que requiere la optimización de los parámetros de adquisición para cada compuesto analizado y el número de compuestos estudiado es limitado. Además no es posible realizar el análisis retrospectivo. Como alternativa a esto, el uso de la cromatografía líquida acoplada a espectrometría de masas de alta resolución (LC-HRMS) nos permite la adquisición de un número ilimitado de compuestos a través de la medida de la masa exacta (error de la exactitud de masa entre 1 y 5 ppm) combinado con elevado poder de resolución (25000-50000 FWHM) [194, 212]. La sensibilidad del LC-HRMS puede ser semejante a los métodos multirresiduos de plaguicidas empleando LC-MS/MS cuando se analizan un gran número de compuestos.

En este capítulo (artículo 1), el principal objetivo es el desarrollo de una estrategia analítica adecuada para el análisis retrospectivo de metabolitos de plaguicidas procedentes de diferentes matrices (aire, suelo, agua, plantas, animales y humanos) detectados en la atmósfera empleando la cromatografía líquida acoplada a espectrometría de masas de alta resolución (UHPLC-HRMS). Esta estrategia está basada en dos etapas diferenciadas: i) análisis post-target de metabolitos de plaguicidas a partir de la creación de una base de datos de 240 metabolitos de plaguicidas y productos de transformación; y ii) análisis non-target de compuestos desconocidos a través de la estrategia de ‘fragmentación-degradación’. La metodología analítica ha sido aplicada a 31 muestras de filtros de fibra de vidrio PM10 (materia particulada de tamaño menor de 10 μm) procedentes de dos estaciones rurales de la Comunidad Valenciana.

5.2. Resultados.

Las 31 muestras procedentes de las zonas rurales de Alzira y Burriana fueron analizadas por los métodos post-target y non-target. Para comprobar que los metabolitos detectados no se hubieran formado durante la etapa de preparación o durante la etapa de análisis, se estudiaron las recuperaciones de algunos de los plaguicidas parent de los metabolitos detectados. Estas recuperaciones oscilaron entre 80 y 120 % con RSD < 20 %.

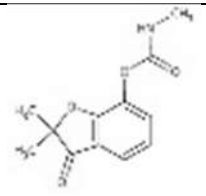
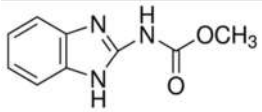
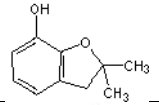
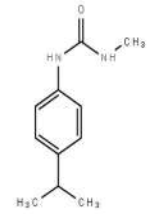
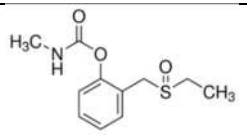
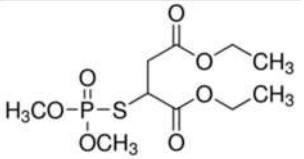
5.2.1. Creación de la base de datos de metabolitos de plaguicidas.

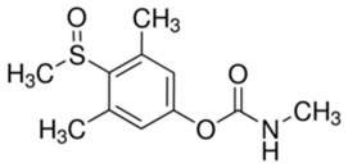
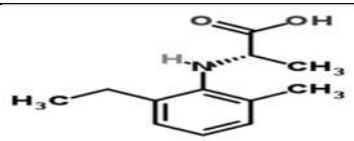
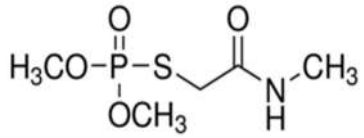
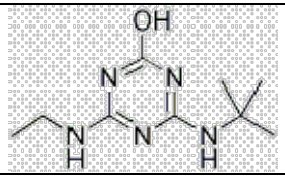
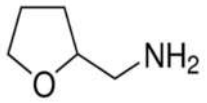
La base de datos realizada contiene 240 metabolitos de plaguicidas, presentes en diferentes matrices tales como aire, agua, suelo, plantas, animales y humanos. La tabla presenta el nombre del metabolito, el plaguicida del cual procede, el número CAS, la formula elemental y la masa del ion molecular y la formula elemental y la masa de los fragmentos. Para cada sustancia, la base de datos incluye la composición elemental (formula molecular), la masa exacta teórica del ion molecular y la información sobre la masa de los fragmentos (siempre que la información de éstos esté disponible, bien de literatura previa o bien haciendo uso de herramientas específicas como Mass Frontier) [172, 193, 197, 213]. La base de datos se presenta en la Tabla SI-1 del anexo I.

5.2.2. Análisis retrospectivo de muestras reales.

34 metabolitos de plaguicidas fueron detectados después de realizar el análisis retrospectivo a las 31 muestras, cumpliendo todos los criterios de identificación descritos anteriormente. De éstos 34 metabolitos, de 11 de ellos estaba disponible en el mercado el patrón. Los 11 fueron confirmados comparando el tiempo de retención del patrón con el de la muestra. Los 11 metabolitos confirmados se presentan en la siguiente tabla (Tabla 9), donde 9 de ellos no habían sido detectados previamente en la atmósfera.

Tabla 9. Metabolitos de plaguicidas identificados y confirmados con patrones en el análisis post-target (n=31)

Metabolito	Número CAS	Estructura	Parent	Masa monitorizada* $[M+H]^+$	Δm (ppm) $([M+H]^+)$	Δm (ppm) (FRAG. 1)	TR del patrón (min)	Rango de TR de las muestras (min)	Número de muestras detectadas	Media/Rango estimado (pg m^{-3})[1]
3-ketocarbofuran	16709-30-1		Carbofuran	236.09173	-0.62	0.83	6.37	6.39	1	98.64
Carbendazima	10605-21-7		Metil Tiofanato	192.07675	0.57-1.23	0.32-1.64	5.36	5.27-5.41	28	33.54/ 19.62-184.57
Carbofuran-7-fenol	1563-38-8		Carbofuran	165.09100	0.15-0.56	1.72-2.56	4.04	4.02-4.06	3	71.12/ 51.77-93.33
Desmetilisoproturon	34123-57-4		Isoproturon	193.13354	-0.36-0.57	0.19-1.77	7.45	7.42-7.46	2	17.01/ 12.82-21.19
Etiofencarb-sulfóxido	53380-22-6		Etiofencarb	242.08454	-1.11-(-0.53)	0.57-0.83	5.80	5.88-5.91	2	34.36/ 26.08-42.63
Malaoxon	1634-78-2		Malation	315.06618	1.67-2.24	2.14-2.34	6.90	6.89-6.95	2	39.87/ 37.63-42.11

Metiocarb-sulfóxido	2635-10-1		Metiocarb	242.08454	2.67-3	1.62-1.96	5.84	5.86-5.88	2	23.10/ 21.20-25.0
N-(2-etil-6-metilfenil)-L-alanina	82508-03-0		Metolaclor	208.13320	2.33-2.75	1.82-1.99	7.01	6.94-7.07	6	43.71/ 25.44-89.47
Ometoato	1113-02-6		Dimetoato	214.02974	-0.22-1.83	0.72-1.83	4.43	4.36-4.48	12	102.37/ 16.01-198.31
Terbutilazina-2-OH	66753-07-9		Terbutilazina	212.15059	1.73-2.87	1.58-2.56	5.98	5.97-6.08	15	36.33/ 12.77-86.45
THPAM	4795-29-3		Captan	102.09134	1.23-1.99	1.25-1.47	7.76	7.73-7.78	3	9.71/ 6.78-13.18

*Semejanza del Perfil isotópico>90 %

Los metabolitos de plaguicidas detectados con mayor frecuencia han sido la carbendazima, la terbutilazina-2-hydroxy y el ometoato, con frecuencias que oscilan entre el 90 % y el 39 %. El metabolito del metolaclor (N-MPA) y del captan (THPAM) presentan frecuencias de detección medias (entre el 10 y el 20 %). El resto de metabolitos presentan frecuencias de detección inferiores al 6 %.

Los metabolitos que presentan niveles más elevados, después del cálculo semicuantitativo de la concentración han sido el carbofuran-7-fenol, el 3-ketocarbofuran y el ometoato, con concentraciones semicuantitativas que oscilan entre 71.12 y 102.37 pg m^{-3}. Carbendazim, malaoxon, etiofencarb-sulfóxido, terbutilazina-2-hydroxy y N-MPA presentan niveles que oscilan entre los 33.51 pg m^{-3} y los 43.71 pg m^{-3}. Las concentraciones más bajas han sido obtenidas para THPAM, desmetilisoproturon y metiocarb-sulfóxido. A continuación, se presenta el ejemplo de uno de los metabolitos detectados, la terbutilazina-2-hydroxy (figura 13). El resto de los cromatogramas de los metabolitos identificados se presentan en el anexo I.

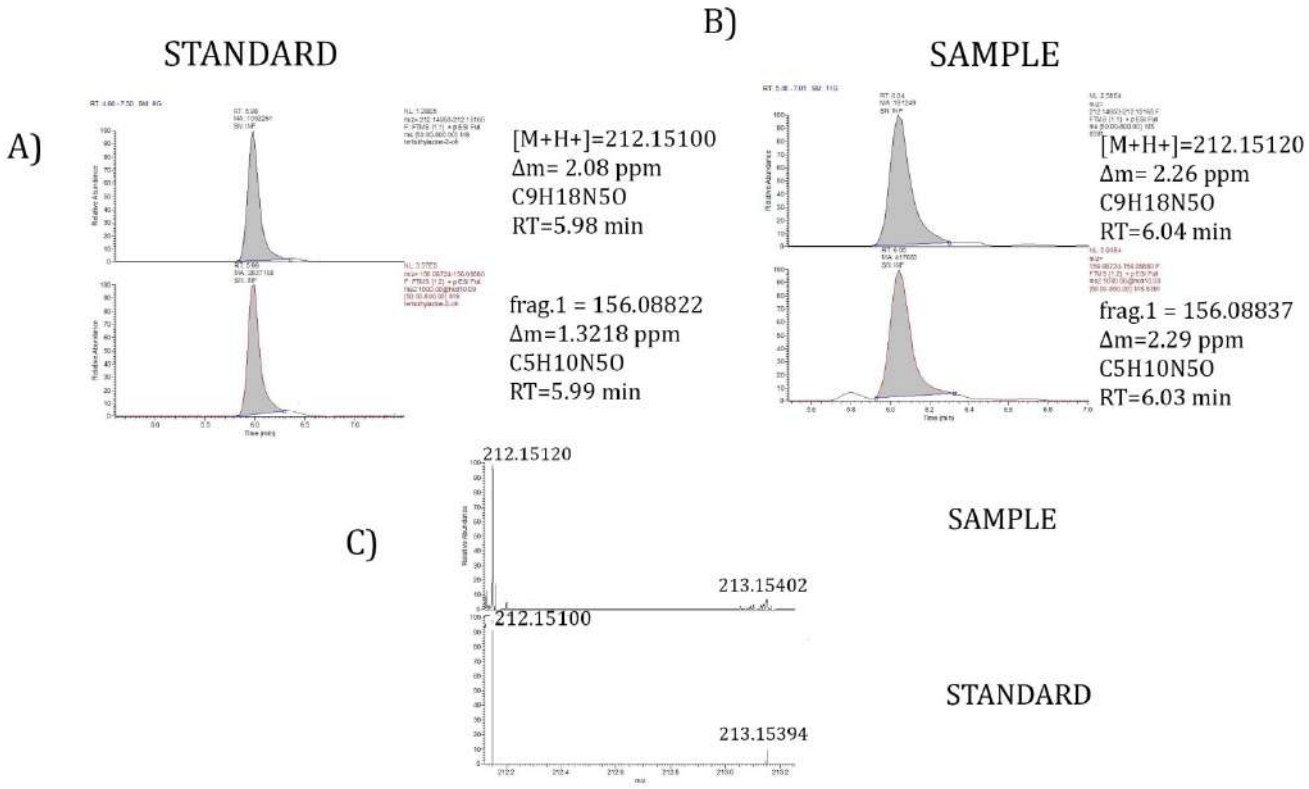


Figura 13. a) Cromatograma de ion extraído (EIC) del ion molecular y un fragmento característico del patrón de terbutilazina-2-hydroxy, b) Cromatograma de ion extraído (EIC) del ion molecular y un fragmento característico de una muestra positiva de terbutilazina-2-hydroxy, c) Perfil isotópico del patrón y de la muestra.

La terbutilazina-2-hydroxy ha sido identificada en 15 muestras. Es un metabolito de la terbutilzaina, un herbicida usado habitualmente en cítricos. La confirmación se ha realizado a través de la medida de la masa exacta ([M+H+]= 212.15120, $C_9H_{18}N_5O$), la

semejanza del perfil isotópico y la presencia de un fragmento característico (m/z 156.08837, $C_5H_{10}N_5O$). La concentración media estimada en todas las muestras está alrededor de 35 pg m^{-3}.

5.2.3. Análisis non-target de muestras reales.

La metodología empleada para la identificación de metabolitos desconocidos está basada en la estrategia de fragmentación-degradación establecida por García-Reyes et al. (2007) [174]. En esta estrategia, se establece una relación entre las fragmentaciones de los plaguicidas en el equipo (HCD, en nuestro caso) y su posible degradación de los productos en el aire. Dos posibles metabolitos han sido identificados empleando esta estrategia: un producto de transformación del malaoxon (TP-1) y un producto de transformación de la fenhexamida (TP-2).

En el caso del malaoxon ($C_{10}H_{19}O_7PS$), este metabolito ha sido identificado y confirmado en dos muestras, con una desviación de masa exacta menor de 5 ppm y dos fragmentos característicos (m/z 142.9926 y 127.01547), a un tiempo de retención de 6.86 minutos, similar al del patrón. Estos dos fragmentos (empleando HCD) también aparecen a un tiempo de retención de 4.50 minutos. Teniendo en cuenta además, que el ion de m/z 142.9925 presenta una respuesta elevada al tiempo de retención de 4.50 minutos cuando se adquiere el espectro en modo full-scan, sin emplear la fragmentación. De esta forma, el ion con m/z 142.9925 ($C_2H_8O_3PS$) puede ser al mismo tiempo un fragmento del malaoxon (tiempo de retención de 6.85 minutos) y un producto de transformación (TP-1) del malaoxon/malathion (tiempo de retención de 4.50 minutos). Este hecho se observa en la figura 14:

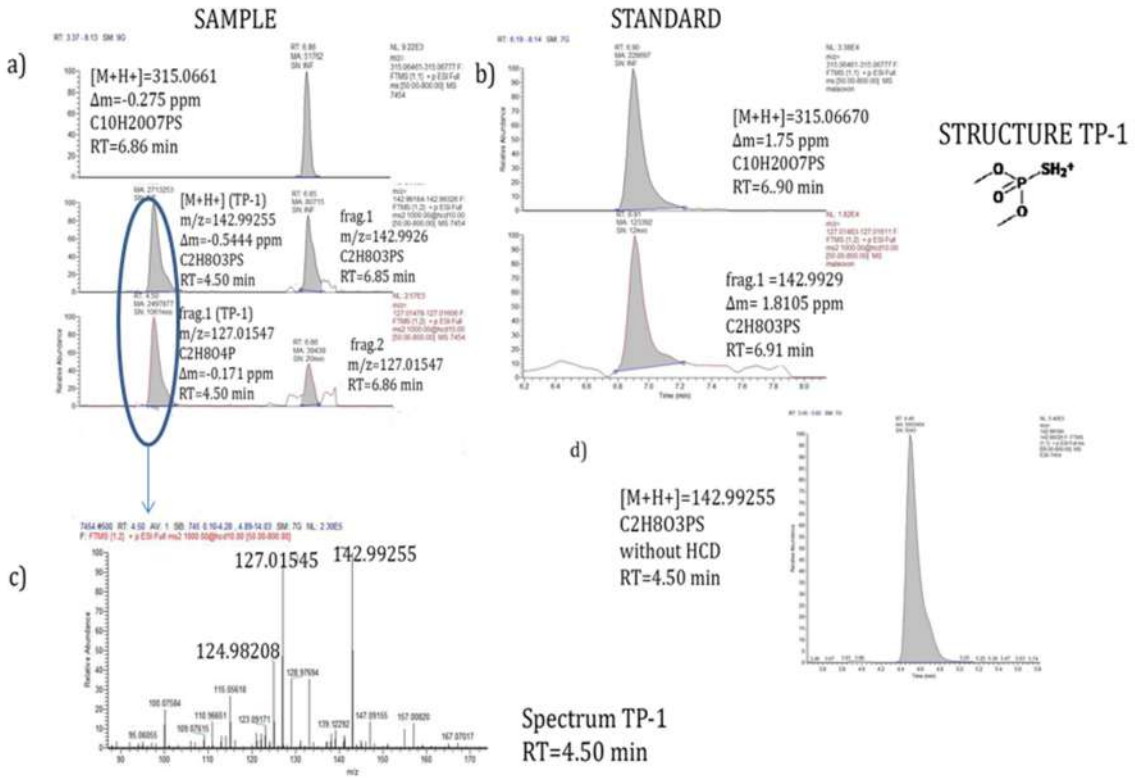


Figura 14. a) Cromatograma de ion extraído (EIC) del malaoxon (m/z=315.06601), sus fragmentos (m/z 142.9926 y m/z 127.01547) y del producto de transformación (TP-1) del malation/malaoxon (m/z 142.9926) obtenido en una muestra de aire, b) EIC del patrón del malaoxon junto a un fragmento característico, c) Espectro de masas a TR=4.50 min, que corresponde al TP-1, d) EIC del TP-1 (m/z=142.99255) sin HCD.

De igual manera se ha empleado esta estrategia para la identificación de un producto de transformación de la fenhexamida (TP-2). El desclorofenhexamid (tiempo de retención = 5.64 minutos), un metabolito del herbicida fenhexamida, fue detectado realizando el análisis retrospectivo en 9 de las muestras analizadas. Aunque el metabolito cumplía todos los criterios de identificación (exactitud de masa, perfil isotópico y presencia de dos fragmentos característicos con m/z 142.12257 y 125.09611), su confirmación no fue posible al no encontrarse en el mercado el patrón para su confirmación. Cómo se puede observar en la siguiente figura, para los fragmentos con m/z 142.12257 y 125.09611, se ha observado un pico adicional a tiempo de retención de 5.93 minutos. El espectro de masas del pico nos revela tres iones abundantes correspondientes a los dos fragmentos estudiados y a la m/z 170.11753. El origen de este ion con m/z 170.11753 se puede deber a su posible formación como producto de degradación del fenhexamid en la atmósfera o a su formación en el instrumento al emplear el modo de fragmentación HCD. Para confirmar que su presencia no se debe a su formación en el instrumento, se realiza la búsqueda de este ion en el modo full-scan (modo sin fragmentación), observándose al tiempo de retención de 5.93 minutos una señal elevada. Este hecho nos indica que su presencia se debe a su formación en la atmósfera, detectándose por tanto, un producto de transformación de la fenhexamida ($C_9H_{16}NO_2$) (ver Figura 15).

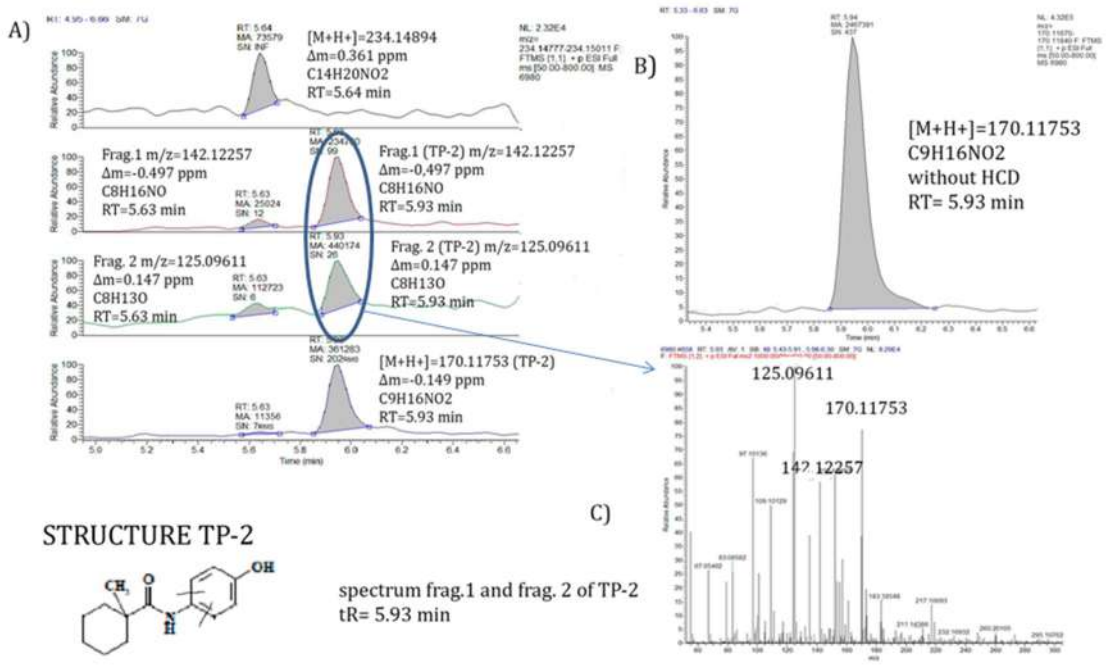


Figura 15. a) Cromatograma de ion extraído (EIC) del desclorofenhexamid (m/z=234.14894), sus fragmentos (m/z 142.12257 y m/z 125.0611) y del producto de transformación (TP-2) de la fenhexamida (m/z 170.11753, TR=5.93 min) obtenido en una muestra de aire, b) EIC del TP-1 (m/z=142.99255) sin HCD, c) Espectro de masas a TR=5.93 min, que corresponde al TP-2.

5.3. Conclusiones.

Las conclusiones a las que se han llegado después de la realización de este trabajo han sido las siguientes:

- El desarrollo y la aplicación de una estrategia exhaustiva para el análisis retrospectivo de metabolitos de plaguicidas en el aire, mediante el uso de LC-HRMS, ha resultado muy útil.

- El análisis retrospectivo se ha basado en la creación de una base de datos de aproximadamente 250 metabolitos de plaguicidas, incluyendo la masa exacta teórica y los principales fragmentos descritos bien en la literatura o bien empleando softwares específicos de fragmentación. Esta base de datos puede ser ampliada con nuevos metabolitos no sólo procedentes de la atmósfera, sino también descritos en otras matrices como agua o suelo.

- 34 metabolitos de plaguicidas han sido detectados en la atmósfera, la mayoría de ellos no descritos previamente, siendo confirmados mediante el uso de patrones un total de 11 metabolitos.

- Además, dos productos de transformación han sido identificados, uno de ellos procedente del malaoxon (metabolito del malathion) y el segundo de ellos procedente de la fenhexamida, empleando el método de fragmentación-degradación. Sin embargo, en el futuro, para la detección de compuestos desconocidos, será necesario el uso de estrategias más exhaustivas con la ayuda de estrategias computacionales para identificar nuevos metabolitos.

5.4. Artículo 1. Retrospective screening of pesticide metabolites in ambient air using liquid chromatography coupled to high-resolution mass spectrometry.

Talanta 150 (2016) 27–36

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Retrospective screening of pesticide metabolites in ambient air using liquid chromatography coupled to high-resolution mass spectrometry

Antonio López [a,c], Vicent Yusà [a,b,c,*], Maurice Millet [d], Clara Coscollà [a,c]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020 Valencia, Spain
[b] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain
[c] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain
[d] Institut de Chimie et Procedes pour l'Energie, l'Environnement et la Sante, ICPEES UMR 7515, Groupe de Physico-Chimie de l'Atmosphere, Université de Strasbourg/CNRS, France

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form
24 November 2015
Accepted 26 November 2015
Available online 1 December 2015

Keywords:
Pesticides
Metabolites
Transformation products
High resolution mass spectrometry
Ambient air
Retrospective analysis

ABSTRACT

A new methodology for the retrospective screening of pesticide metabolites in ambient air was developed, using liquid chromatography coupled to Orbitrap high-resolution mass spectrometry (UHPLC-HRMS), including two systematic workflows (i) post-run target screening (suspect screening) and (ii) non-target screening. An accurate-mass database was built and used for the post-run screening analysis. The database contained 240 pesticide metabolites found in different matrixes such as air, soil, water, plants, animals and humans. For non-target analysis, a "fragmentation-degradation" relationship strategy was selected. The proposed methodology was applied to 31 air samples (PM10) collected in the Valencian Region (Spain). In the post-target analysis 34 metabolites were identified, of which 11 (3-ketocarburan, carbofuran-7-phenol, carbendazim, desmethylisoproturon, ethiofencarb-sulfoxide, malaoxon, methiocarb-sulfoxide, N-(2-ethyl-6-methylphenyl)-L-alanine, omethoate, 2-hydroxy-terbuthylazine, and THPAM) were confirmed using analytical standards. The semiquantitative estimated concentration ranged between 6.78 and 198.31 pg m^{-3}. Likewise, two unknown degradation products of malaoxon and fenhexamid were elucidated in the non-target screening.

1. Introduction

A wide variety of pesticides can be applied in agriculture and their use depends on a range of factors including the specific pest and crop of interest. During 2010, about 208,000 tonnes of pesticide-active ingredients were used in Europe (EU-15) [1] and more than 300 active substances are nowadays authorised by the European Union for their application on various crops according to the Regulation (EC) 1107/2009 [2].

Following application, pesticides are partitioned among soil, water and the atmosphere, and a deep concern has been expressed for the possible effects of the active substances and their metabolites on human health and the environment. Once released in the environment, pesticides are susceptible of biological and chemical degradation, which may result in the formation of a range of different compounds, commonly termed "metabolites" (this includes biological metabolites, transformation and degradation products, reaction products). Once formed in a specific compartment (e.g. soil) where pesticides undergo transformations that give place to a wide pattern of metabolites, these can move to other compartments such as groundwater or air. Generally, metabolites show lower toxicity than the parent compound, however, in some instances metabolites are more toxic, or hold certain biological activity (relevant metabolites), such as oxygen analogs (oxons) in air samples [3]. Like the original molecules, metabolites and their transformation products can also be present in the atmosphere. The presence of pesticide metabolites in the atmosphere could be linked to the chemical degradation of parent compounds in air and to volatilization or wind erosion of metabolites formed in soil or water.

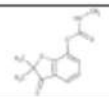
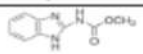
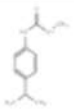
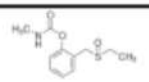
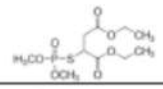
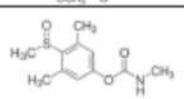
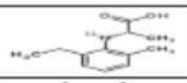
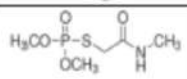
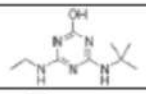
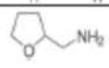
All this may add up to a large number of compounds entering the atmosphere, and it is interesting to note that unlike in water, soil and food, not many of all possible metabolites have been monitored in air, and that there is a very scarce knowledge related with their occurrence, fate and impact, showing that there is a need for more studies in these fields [4–6].

Triple quadrupole (QqQ) based mass spectrometers coupled to gas and liquid chromatographs are the most important analysers used for multiresidue pesticide analysis. This is because of their

* Corresponding author at: Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain.
E-mail address: yusa_vic@gva.es (V. Yusà).

http://dx.doi.org/10.1016/j.talanta.2015.11.068
0039-9140/

Table 1
Pesticide metabolites identified and confirmed with standards in the for post-run target screening (suspected screening) (n=31).

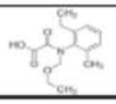
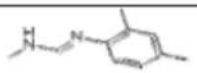
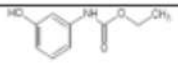
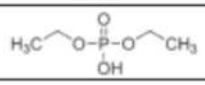
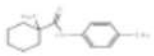
Metabolite	CAS number	Structure	Parent	Monitored Mass* $[M+H]^+$	Δm (ppm) $([M+H]^+)$	Δm (ppm) (FRAG. 1)	Retention time of standard (min)	Range of Retention time of samples (min)	Number of detected samples	Average/ Estimated range level (pg m^{-3})
3-ketocarbofuran	16709-30-1		Carbofuran	236.09173	-0.62	0.83	6.37	6.39	1	98.64
Carbendazim	10605-21-7		Methyl-Thiophanate	192.07675	0.57-1.23	0.52-1.64	5.36	5.27-5.41	28	33.54/ 19.62-184.57
Carbofuran-7-phenol	1563-38-8		Carbofuran	165.09100	0.15-0.56	1.72-2.56	4.04	4.02-4.06	3	71.12/ 51.77-93.33
Desmethylisoproturon	34123-57-4		Isoproturon	193.13354	-0.36-0.57	0.19-1.77	7.45	7.42-7.46	2	17.01/ 12.82-21.19
Ethiofencarb-sulfoxide	53380-22-6		Ethiofencarb	241.07726	-1.11 (-0.53)	0.57-0.83	5.80	5.88-5.91	2	54.36/ 26.08-42.63
Malaoxon	1634-78-2		Malathion	315.06618	1.67-2.24	2.14-2.34	6.90	6.89-6.95	2	39.87/ 37.63-42.11
Methiocarb-sulfonide	2635-10-1		Methiocarb	242.08454	2.67-3	1.62-1.96	5.84	5.86-5.88	2	23.10/ 21.20-25.0
N-(2-ethyl-6-methylphenyl)-L-alanine	82508-03-0		Metolachlor	208.13320	2.33-2.75	1.82-1.99	7.01	6.94-7.07	6	43.71/ 25.44-89.47
Omethoate	1113-02-6		Dimethoate	214.02974	-0.22-1.83	0.72-1.83	4.43	4.36-4.48	12	102.57/ 16.01-198.31
Terbuthylazine-2-OH	66753-07-9		Terbuthylazine	212.15059	1.73-2.87	1.58-2.56	5.98	5.97-6.08	15	36.33/ 12.77-86.45
THPAM	4795-20-3		Captan	102.09134	1.23-1.99	1.25-1.47	7.76	7.73-7.78	3	9.71/ 6.78-13.18

*All isotopic peaks>80 %.

excellent quantitation and identification properties for a group of target compounds. However, these instruments have certain limitations: they require acquisition parameter optimization for each analyzed compound, the number of analyzed compounds is limited, only compounds from a target list can be detected and retrospective data analysis is impossible [7]. Consequently, these techniques are "blind" to any compound present in the sample but not included in the list of monitored analytes. As an alternative, the use of liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) has an increasing use in this field. The main advantage of LC-HRMS, such as TOF and Orbitrap, is that enables the acquisition of unlimited number of species by means of accurate mass measurements (1–5 ppm) combined with high resolving power (25000–50000 FWHM) [8,9]. On the other hand, LC-HRMS could achieve similar sensibility than multi-residue methods developed for the analysis of pesticides using LC-MS/MS [10,11].

In a previous paper we worked on a LC-HRMS strategy mainly focused on the parent pesticides [11]. In this paper, we developed an analytical strategy for retrospective screening analysis of pesticide metabolites (transformation products) in ambient air, based on a comprehensive database containing about 240 metabolites (suspect screening) and in the use of the "fragmentation-

Table 2
Potential pesticide metabolites in the for post-run target screening (without analytical standards confirmation) (n = 31).

Metabolite	CAS number	Structure	Parent	Mealtored Mass[a] $[M+H]^+$	Δm (ppm) ($[M+H]^+$)	Δm (ppm) (FRAG. 1)	N[b]
[Acetic acid, [(ethoxymethyl)(2-ethyl-6-methylphenyl)-amino]oxo]			Acetochlor	266.13868	-0.49	0.41	1
N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	34256-82-1		Acetochlor	236.16450	0.24-1.14	-0.59-1.02	3
N-2,4-dimethylphenyl-N'-methylformamidine	33089-74-6		Amitraz	163.12297	-0.06-0.13	-0.12-0.23	3
THPI	85-40-5		Captan	152.07060	-0.15-0.25	0.83-1.53	3
EHPC	6641-13-0		Desmedipham	182.08117	0.21-0.93	-0.47-0.08	14
2-(1-hydroxy-methyl)-ethyl-4-methyl-6-hydroxypirimidine	28175-97-5		Diazinon	169.09715	-0.25-0.74	-0.99-0.27	3
Diethylphosphate	598-02-7		Diazinon	154.03895	-0.61-0.29	1.61-2.38	30
4-formylmorpholine	113059-82-8		Dimethomorph	116.07060	0.23-1.45	1.12-2.67	28
Deschlorofenhexamid	1333041-78-5		Fenhexamid	234.14886	-0.55-0.34	-0.64-0.87	10
3-methyl-4-nitrophenol	2581-34-2		Fenitrothion	154.04987	-0.73-0.86	-0.36-1.44	10
p-methyl-phenethylamine	3261-62-9		Iprovalicarb/Isoproturon	136.11208	0.08-0.96	0.27-1.67	27

degradation" relationship approach (non-target approach). The analytical methodology was applied to 31 PM10 samples (particulate matter with diameter $< 10\ \mu m$) collected from the monitoring network of the Regional Valencia Government (Spain).

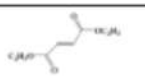
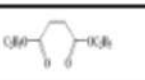
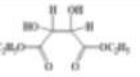
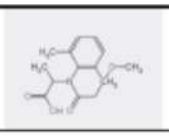
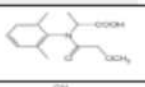
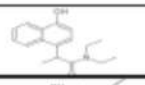
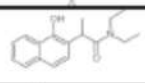
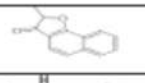
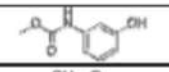
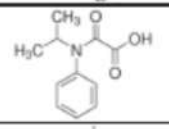
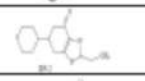
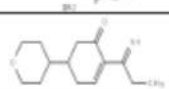
2. Experimental

2.1. Reagents and chemicals

The following reagents and chemicals were supplied by Dr. Ehrenstorfer (Augsburg, Germany): high-purity pesticide metabolite standards, carbofuran-3-keto (96.5%), phosphorothioic O,O,S-trimethyl ester (98%), dimethachlor oxalamic acid (99%), THPAM (99%), 3-phenoxybenzoic acid (99%), metolachlor oxalamic acid (98%), N-(2-Ethyl-6-methylphenyl)-L-alanine (99.4%), 3-methyl-4-nitrophenol (99%), desmethylisoproturon (99.5%), N-2,4-dimethyl-phenyl-N'methylformamidine (98.5%), carbofuran-7-phenol (99%), malaoxon (99%), methiocarb-sulfoxide (99.5%), terbuthylazine-2-OH (97.5%), ethiofencarb-sulfoxide (98.5%) , propachlor oxalamic acid (98%), omethoate (97%) and carbendazim (99%).

Individual stock standards were prepared weighing 10 mg of pure standard using a 5-decimal analytical balance and dissolving each compound in 50 mL of acetone. In addition, dilutions of 1000

Table 2 (continued)

Diethylmaleate	141-05-9		Malathion	197.08085	-0.28-0.94	0.16-1.11	22
Diethylsuccinate	123-25-1		Malathion	151.09648	-0.57-0.46	-0.63-1.88	20
Dimethyl 2,3-dihydroxy-2,3-dimethylsuccinate	15309-47-4		Malathion	207.08631	0.11-3.14	0.27-3.86	29
N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine	467430-42-8		Metalaxyl	266.13868	-0.49	1.53	1
R-2-[2,6-dimethylphenyl)-meythoxyacetyl-amino]-propionic acid	75596-99-5		Metalaxyl	266.13868	1.69	-0.70	1
N,N-diethyl-4-hydroxy-methyl-1-naphtaleneacetamide			Napropamide	272.16450	-0.86-0.57	0.48-1.11	6
N,N-diethyl-4-hydroxy-methyl-2-naphtaleneacetamide			Napropamide	272.16450	-0.47-0.23	0.66-2.22	3
α-Naphtol-2-methyl-naphtol(1,2-b)-2H-furan-3-one			Napropamide	199.07535	0.16	-0.21	1
Methyl-N-hydrophenyl-carbamate	13683-89-1		Phenmedipham	168.06551	-0.57-0.47	-0.19-0.82	10
Propachlor oxalamic acid	70628-36-3		Propachlor	208.09681	-0.16-1.68	-0.18-0.90	6
(RS)-2-ethyl-6,7-dihidro-6-perhydropyran-4yl-benzoxazol-4-(5H)-one			Tepraloxydim	250.14377	-0.22-0.14	-0.22-0.77	2
3-hydroxy-2-(1-iminopropyl)-5-perhydropyran-4-ylcyclohex-2-en-1-one			Tepraloxydim	252.15942	-0.47-1.05	0.02-0.41	2

[a] All isotopic pattern> 90%.
[b] Number of samples detected.

and 100 ng mL^{-1} were prepared and stored in capped amber vials at −21 °C [12].

Methanol and acetonitrile were of HPLC grade, supplied by Scharlau (Barcelona, Spain). Acetone, ethyl acetate and water were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Glacial acetic acid and formic acid 98% were provided by Panreac (Barcelona, Spain). Ammonium formate, solution Ultra (100 mL, 10 M in water) was provided by Fluka (Steinheim, Switzerland).

2.2. Sampling and site characterization

Valencia Region, situated on the East coast, made up 12.6 % of the total national consumption of pesticides in 2014 [13]. The main irrigated crops are citrus fruit, other fruit trees (mainly peach, apricot and plum trees), rice and garden produce (primarily watermelon, cabbage, artichoke, lettuce, cauliflower, tomatoes, potatoes and onion). The main dry crops are vineyards, olive trees and almonds [14]. Two rural sampling stations (Alzira and Burriana sites) included in the PM10 monitoring network of the Regional Valencia Government (Generalitat Valenciana), Eastern Spain, were selected. Samples were collected using a large-volume sampler from Digitel (Madrid) and quartz fiber filters of 150 nm of diameter, supplied by Munktell filter AB (Falun, Sweden). At Alzira station, samples were collected from June to July 2013, and at Burriana station, from May to June 2014. A sampling flow of 30 m^3 h^{-1} for 24 h, providing a total volume of around 760 m^3 was used.

The first station was placed in a rural area in Alzira, at approximately 1 km away from the city (0°27′28″W, 39°09′00″N). Alzira is a city (42,153 inhabitants), located at the centre of the

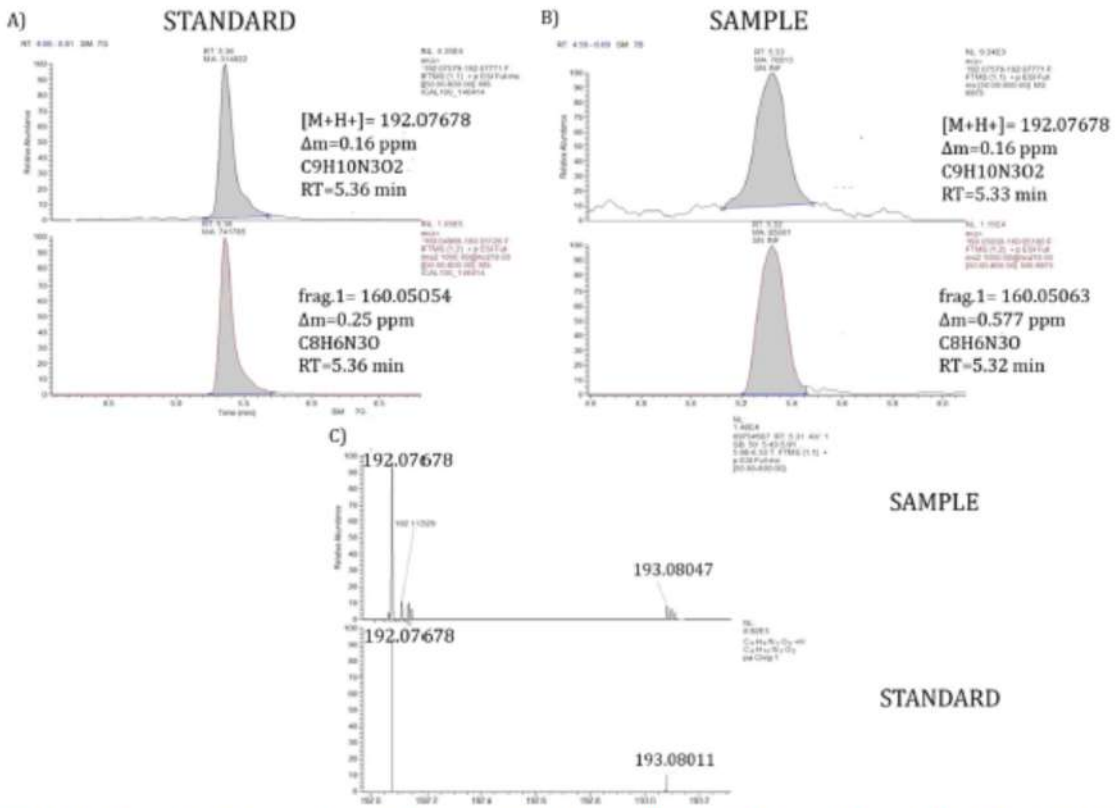


Fig. 1. (a) Accurate mass extracted ion chromatograms (XIC) of the molecular ion and a characteristic fragment for carbendazim standard; (b) Accurate mass XIC of the molecular ion and a characteristics fragment for carbendazim in air sample; (c) Isotopic patterns of the molecular ion of the sample and standard.

Valencia Region (43 km of the Valencia city), which has many citrus crops, such as orange trees, in its surroundings. A total of 19 samples were collected here.

The second station was placed in Burriana, in the north of the Valencia region (0° 05'23'' W, 39°53'41''). Burriana is a town (31,281 inhabitants) located 13 km southeast of Castellon and 63 km north of Valencia, surrounded by irrigated land. 12 samples were collected at this site. All samples were sampled at about 3 m above ground level.

In order to check potential losses or degradation during the sampling and storage period, spiked blank filters were stored and analyzed as field samples. No degradation of pesticides were found during sampling and storage.

2.3. Sample preparation

When a wide-scope analysis is applied, non selective sample preparation is preferred in order to extract the highest number of compounds. A generic extraction method developed in a previous works using microwave extraction (MAE) with ethyl acetate was employed [10,11]. In short, MAE of pesticides from PM 10 samples was carried out using a Mars system from CEM corporation (Mathews, NC, USA) equipped with Teflon® TFM 100 mL extraction vessels. The extraction conditions were as follows: a temperature of 50 °C was applied for 20 min, using a power of 1200 W, and 30 mL of ethyl acetate were added. After cooling, the reactor was opened and the extracts were filtered. After 100 µl of diethylene glycol (keeper) had been added to the extract, it was concentrated with a Turbo Vap 500 (Zymark, Idstein, Germany). The extracts were then re-dissolved with 1 mL of water: methanol (70:30) and filtered through a 0.22 µm GHP Acrodisc filter from Pall Life Science (Ann Arbor, USA) prior to the LC–HRMS determination.

A study of stability for pesticides degradation during sample preparation and final analysis was carried out. Filters were fortified with parent pesticides of the identified metabolites such as carbofuran, isoproturon and terbuthylazine.

Quality assurance protocols, transport of samples and storage conditions were carried out as described previously [11]. Each set of samples were analyzed under quality assurance protocols, including process blanks, field blank and reagent blanks

2.4. UHPLC - HRMS orbitrap analysis

LC conditions have been discussed in previous works [10,11]. Chromatographic separation was performed on an Accela liquid chromatography UHPLC system equipped with a Hypersil Gold aQ column (100 mm × 2.1 mm, 1.9 µm) both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 300 µL min^{-1} and the injection volume was 10 µL. Separations were performed using a binary gradient. The mobile phase was (A) H_2O with 0.1% formic acid and 4 mm ammonium formate and (B) methanol with 0.1% formic acid and 4 mm ammonium formate. The gradient conditions were as follows: 0–8 min, linear with 100% of A; 8–

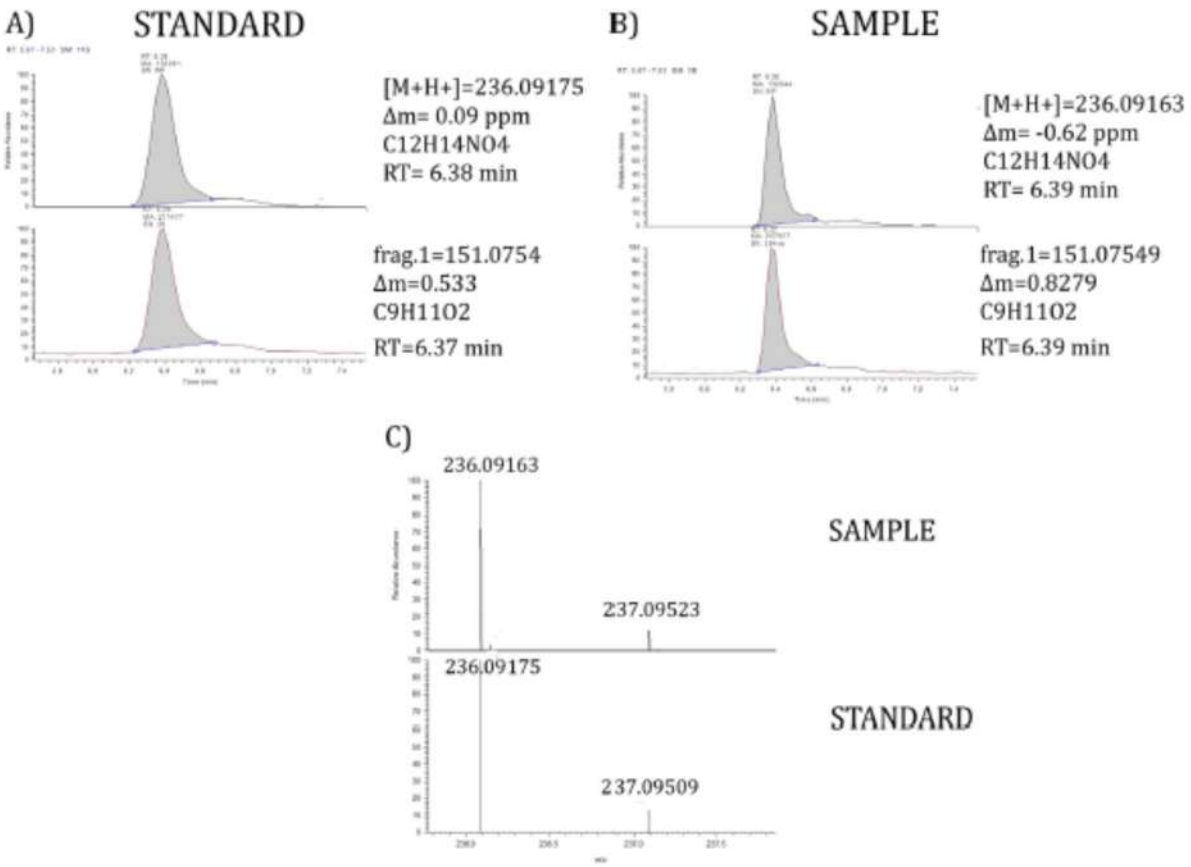


Fig. 2. (a) Accurate mass extracted ion chromatograms (XIC) of the molecular ion and a characteristic fragment for 3-ketocarbofuran standard; (b) Accurate mass extracted XIC and a characteristic fragment for 3-ketocarbofuran in air sample; (c) Isotopic patterns of the molecular ion of the sample and standard.

12 min, linear with 100% of B and 12–16 min, linear with 100% of A. The total run time lasted 16 min.

Mass spectrometric analysis was performed on a single stage Orbitrap MS (ExactiveTM, Thermofisher Scientific, Bremen, Germany). The system was equipped with a heated electrospray ionization interface (HESI-II). The detection was carried out in positive ionization mode (ESI+) using the following optimized operational parameters: spray voltage, 2.8 kV; sheath gas (N_2, >95%), 25 (adimensional); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 205 °C; and capillary temperature, 281 °C. The mass spectra was acquired using two alternating acquisition functions (i) full-scan MS without fragmentation, ESI+; mass resolving power=50,000 FWHM; scan range=80–800 Da; scan time=0.5 s (2 Hz); (ii) The same parameters but with full-scan MS all ion fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 10 eV). The automatic gain control (AGC) was set to 1×10^6 ions [11]. The external mass calibration of the spectrometer was performed using two ready-to-use calibration mixtures (Mas Cal5 (+) and Mas Cal 6 (−)) from Supelco (USA). Data acquisition and processing was performed using Thermo Scientific TraceFinderTM software version 3.1.

2.5. Identification and confirmation criteria for pesticide metabolites

For compound identification the following criteria were established [15]: (i) Mass accuracy of the molecular ion < 5 ppm; (ii) mass accuracy of the fragment ion < 5 ppm; (iii) isotopic pattern similar to the theoretical isotopic pattern (the relative intensity of the A+1 and/or A+2 isotope peaks in the real sample shall correspond to the theoretical relative intensities). For confirmation we used the reference standard solutions of those metabolites available in the market. In this case the confirmation criteria included: (iv) retention time (t_R) similar to that of the reference standard ± 0.20 min.

In post-run target screening, searching for metabolite compounds was carried out by using automated software tools. The identification and confirmation settings in the Trace Finder programme included a threshold override of 10,000, with *S/N* of 5, and a mass tolerance of 5 ppm for the molecular ion; an intensity threshold of 5000 and a mass tolerance of 5 ppm for fragments. For isotopic pattern a fit threshold of 90%, an allowed relative intensity (RI) deviation of 30%, and a mass deviation of 5 ppm were selected in the TraceFinder software.

The resulting one-point calibration (131 pg m^{-3}) on suspected peaks yielded semiquantitative concentrations. Semiquantitative approach gives only approximately concentrations, because the purpose of post-target study is basically to identify and if possible confirmate pesticide metabolites but not an accurate quantification method.

In the non-target approach, the search of pesticide metabolites was undertaken using the "fragmentation–degradation relationship" approach [16]. This methodology relies on the fact that low-molecular weight compounds often display parallel degradation and fragmentation pathways. Therefore, should a fragment ion

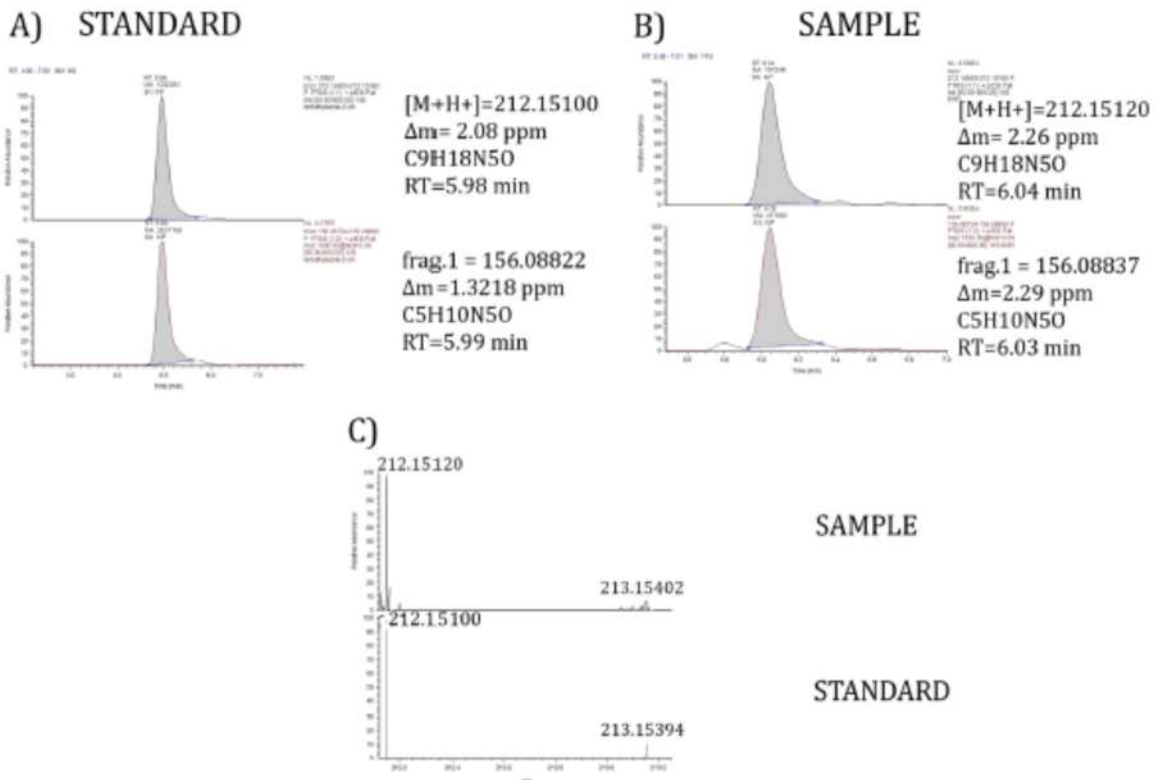


Fig. 3. (a) Accurate mass extracted ion chromatograms (XIC) of the molecular ion and a characteristics fragment for terbuthylazine-2-OH standard; (b) Accurate mass XIC of the molecular ion and a characteristic fragment for terbuthylazine-2-OH in air sample; (c) Isotopic patterns of the molecular ion of the sample and standard.

appear not only at the t_R corresponding to the parent molecule, but also at a different t_R, it may indicate the presence of a molecule with similar structure, i.e., a possible metabolite. Checking the extracted ion chromatogram (XIC) of fragment ions and their mass spectra at retention times for peaks not corresponding to the parent molecule allows the straightforward detection of novel metabolites. A final confirmation was performed comparing retention times to those obtained for reference standards (when available).

3. Results and discussion

The 31 samples from the two rural sampling sites were analyzed for post-run target and non-target screening following the developed analytical strategy developed.

The stability study to check for pesticides degradation during sample preparation and instrumental analysis do not shown any degradation during this steps. Likewise, recoveries ranging between 80% and 110%, with RSD (%) < 20%, were found for those parent pesticides whose metabolites were identified.

3.1. Development of the accurate mass database of pesticide metabolites for post-run target screening (suspect screening)

A customized theoretical database was built, containing 240 pesticide metabolites found in different matrixes such as air, soil, water, plants, animals and humans (Table SI-1).

For each substance, the screening database included the elemental composition (molecular formula) and the theoretical accurate mass of the monitored ion. Existing data from available databases [17–20] and previous literature [21] were used. This is a theoretical database where no standards were analysed to get characteristic fragments. Information about fragments were included when available in the literature [4–5,17–18, 22–25] (see Table SI-1).

3.2. Post-run target screening of real samples

34 metabolites were tentatively identified in the post-run target analysis of the chromatograms using the developed database. All these substances meet the criteria for the identification described in Section 2.5 (mass accuracies and isotopic pattern). Using the only 11 comercially available reference standard solutions, all of them (3-ketocarburan, carbofuran-7-phenol, carbendazim, desmethylisoproturon, ethiofencarb-sulfoxide, malaoxon, methiocarb-sulfoxide, N-(2-ethyl-6-methylphenyl)-L-alanine (N-MPA), omethoate, 2-hydroxy-terbuthylazine (2-HT), and THPAM) were confirmed following the confirmation criteria (mass accuracies, isotopic pattern and retention time) (Table 1). Table 2 shows the potential metabolites identified in post-run target analysis .

Carbendazim, 2-HT, and omethoate presented the highest frequency of detection ranging from 90 to 39 %. The metolachlor metabolite, N-MPA (19%) and the captan metabolite, THPAM (10%) were less frequently detected. The other identified and confirmed metabolites presented frequencies lower than 6 % (Table 1). Apart from omethoate and carbendazim [11], to our knowledge, these confirmed metabolites had not been previously described in the

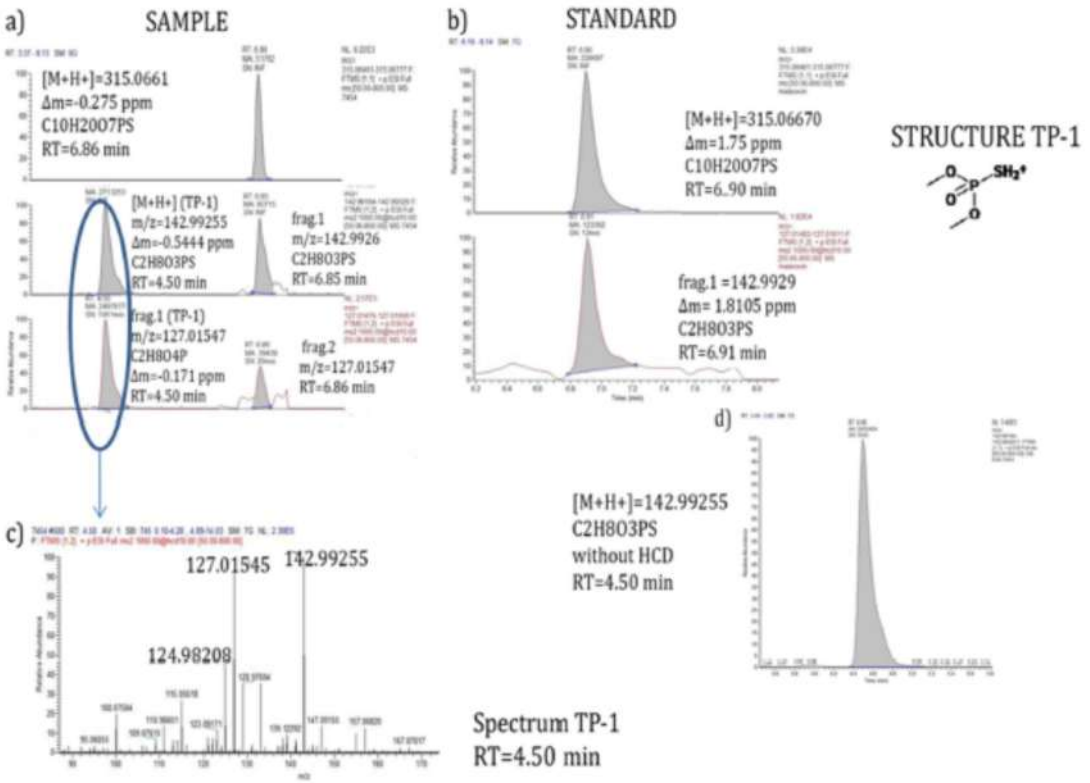


Fig. 4. (a) Extracted ion chromatograms (XIC) of malaoxon (m/z =315.0661), its fragments (m/z 142.9926; m/z 127.01547); and of a transformation product (TP-1) of malathion/malaoxon (m/z 142.99926) obtained from air sample; (b) XIC of malaoxon standard and a characteristic ion fragment; (c) Accurate mass spectrum at 4.50 min, which corresponds to malathion/malaoxon transformation product-1 (TP-1); d) XIC of PT-1 (m/z = 142.99255) without HCD.

literature in ambient air samples. Regarding the estimated concentrations (semiquantitative), the highest levels were observed for carbofuran-7-phenol, 3-ketocarbofuran and omethoate, with average concentrations ranging from 71.12 to 102.37 pg m^{-3}. Carbendazim, malaoxon, ethiofencarb-sulfoxide, 2-HT and N-MPA obtained medium concentrations ranging from 33.51 to 43.71 pg m^{-3}. The lowest concentrations were found for THPAM, desmethylisoproturon and methiocarb-sulfoxide (9.41–23.10 pg m^{-3}). As an example of the pesticide metabolites detected in post-run target screening, Fig. 1–3 show the accurate mass XICs and isotopic patterns for carbendazim, 3-ketocarbofuran and 2-HT, respectively. In Figs. 1–8 (SI) we present the XIC of other confirmed metabolites.

Carbendazim is a systemic benzimidazole fungicide used to combat a wide range of diseases, but it is currently banned in the EU. It is also the major metabolite/degradation product of thiophanate-methyl (TM) [26] and benomyl (banned in the EU). Carbendazim was identified in 28 samples (Fig. 1). Confirmation was accomplished by accurate mass measurements ($[M+H]+=$ 192.07678, $C_9H_{10}N_3O_2$), isotope pattern matching and the presence of one diagnostic fragment ion (m/z 160.05063, $C_8H_6N_3O$). This pesticide metabolite fulfil the identification criteria. The theoretical and experimental isotopic patterns for carbendazim (^{13}C, M+1) are also shown, with estimated average concentrations of around 30 pg m^{-3}.

3-ketocarbofuran (Fig. 2) and carbofuran-7-phenol are metabolites of carbofuran [27], which is currently banned in the EU. Confirmation was accomplished by accurate mass measurements ($[M+H]+=$ 236.09163, $C_{12}H_{14}NO_4$), isotope pattern matching and the presence of one diagnostic fragment ion (m/z 151.07549, $C_9H_{11}O_2$). This pesticide metabolite fulfil the identification criteria. The theoretical and experimental isotopic patterns for 3-ketocarbofuran (M+1,^{13}C) are also shown, with estimated average concentrations of around 100 pg m^{-3}.

2HT was identified in 15 samples (Fig. 3). It is a metabolite of terbuthylazine (TBZ) [28], a herbicide frequently used in citrus fruits. Confirmation was accomplished by accurate mass measurements ($[M+H]+=$212.15120, $C_9H_{18}N_5O$), isotope pattern matching and the presence of one diagnostic fragment ion (m/z 156.08837, $C_5H_{10}N_5O$). This pesticide metabolite fulfil the identification criteria. The theoretical and experimental isotopic patterns for 2-HT (M+1, ^{13}C) are also shown, with estimated average concentrations of around 35 pg m^{-3}.

Omethoate is formed during the oxidation of the organothiophosphate insecticide dimethoate. The atmospheric oxidation of dimethoate is likely to lead to the formation of the corresponding oxon (omethoate) similarly to the reaction channel of degradation of chlorpyrifos methyl and other organophosphorus insecticides [24]. Omethoate was detected in about 40% of the samples, with estimated average concentrations of around 100 pg m^{-3}.The photodegradation of ethiofencarb in solar-light is very rapid [29]. Ethiofencarb is now forbidden in the European Union but has been applied in citrus crops against several pest in the studied area.

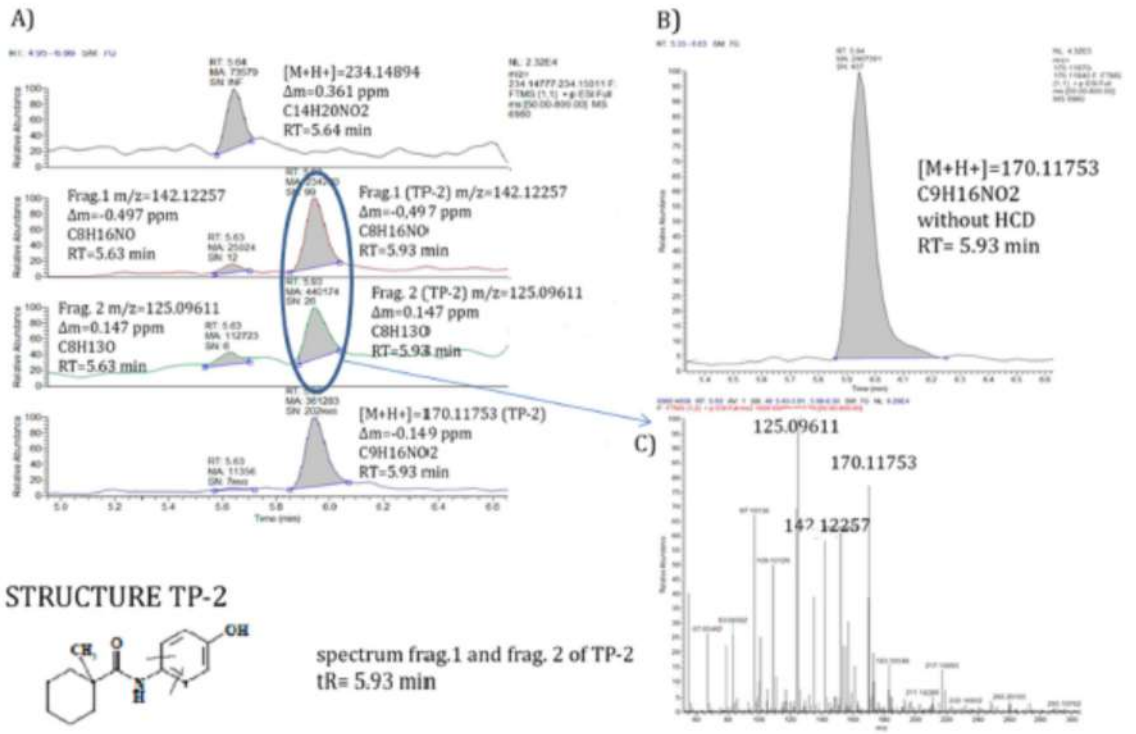


Fig. 5. (a) Extracted ion chromatograms (XIC) of deschlorofenhexamid (m/z 234.14894, Rt: 5.63), two fragments (m/z 142.12257, m/z 125.09611); and a transformation product of fenhexamid (PT-2) (m/z m /z= 170.11753; Rt: 5.93) with two fragments (m/z 142.12257, m/z 125.09611); (b) extracted ion chromatogram at m/z= 170.11753 without HCD; (c) accurate mass spectrum at 5.93 min, which corresponds to fenhexamid transformation product-2 (TP-2).

Ethiofencarb-sulfoxide was identified in two out of the 31 collected samples. Insecticide captan breaks down to form the major metabolites THPI and THPAM. Both metabolites were identified in three samples, although only THPAM was confirmed after using standards. Malaoxon and desmethylisoproturon were also detected in a few samples.

3.3. *Non-target analysis of real samples*

The procedure for the identification of non-target metabolites was based on the "fragmentation–degradation" relationship strategy established by Garcia-Reyes et al. [16]. In this approach accurate mass measurements of ions of interest are used to establish relationships between fragmentations of parent pesticides in the instrument (in our case HCD-fragmentation) and possible degradation products of these pesticides in air. Two non-target metabolites were identified following this strategy: a transformation product of malaoxon (TP-1) and a transformation product of fenhexamid (TP-2).

As commented in the post-run target section, the malaoxon metabolite ($C_{10}H_{19}O_7PS$) was identified and confirmed in two samples, using the presence of the $[M+H]^+$ and two diagnostic fragment ions (*m/z* 142.9926 and 127.01547), with t_R of 6.86 min., similar to that of the standard. These two fragments also appeared in XIC (with HCD) but at 4.50 min (Fig. 4(a). Likewise, the ion of m/z 142.9926 was present in the recorded XIC without HCD (no fragmentation) at higher response (Fig. 4(d), which means that this ion was, apart from a fragment of malaoxon (at 6.86 min), a transformation product present in the air sample (at 4.50 min). Consequently, the ion with *m/z* 142.99255 ($C_2H_8O_3PS$) could be at the same time a fragment of malaoxon (t_R: 6.85 min) and a transformation product (TP-1) of malaoxon/malathion (t_R: 4.50 min) present in ambient air.

A similar approach was used for the deschlorofenhexamid ($C_{14}H_{19}NO_2$), a metabolite of the fungicide fenhexamid which was identified in 9 samples at 5.64 min. Identification was accomplished by accurate mass measurements, isotopic pattern matching (100%) and the presence of two diagnostic fragment ions (*m/z* 142.12257 and 125.09611). Unfortunately, unequivocal confirmation could not be granted due to unavailable analytical standards. Checking the XICs for m/z m/z 142.12257 and 125.09611, an additional peak was found for each fragment ion at 5.93 min (Fig. 5a). The mass spectrum at 5.93 min revealed three abundant ions (Fig. 5c), the two previously described ions, and another one with an *m/z* 170.11753 ($C_9H_{16}NO_2$). The origin of this ion could be a fragment of the parent pesticide (fenhexamid) in the instrument (HCD fragmentation) or a possible degradation product of fenhexamid in air. The sample was also injected without applying fragmentation (without HCD) (see Fig. 5b), and higher response for *m/z* 170.11753 was detected, showing that it was a transformation product (TP-2) of fenhexamid present in the collected air sample. A tentative structural formula has been proposed both for TP-1 and TP-2 transformation products (Figs. 4 and 5), that, to our knowledge, had not been described previously.

Afterwards, the presence of the two transformation products identified in non-target analysis were searched and found using post-target strategy.

Although this approach requires manual work on the different

XICs and fragmentation spectra, consequently being time intensive, it frequently provides easy interpretations for finding new compounds and elucidating the structural formulas. There are other strategies based on peak picking on the chromatograms (peak inventories) using different mass spectral software (e.g. MzMine) and the subsequent calculation of molecular formulas and their automated search in compound databases to elucidate unknown molecules [30,31]. However, more efforts are necessary to capture and identify potentially relevant metabolites using automated computational tools.

4. Conclusions

The development and application of a comprehensive strategy for retrospective analysis of pesticide metabolites in air, using LC-HRMS, has proved to be very useful. Thirty four metabolites, the majority of which had not been previously detected in ambient air, have been identified, with eleven of them having been fully confirmed using analytical standards.

In the post-run target screening, the retrospective strategy was based on the creation of a customized database of about 250 pesticide metabolites, including the theoretical accurate mass, and their main fragments described in the literature or using specific fragmentation software. This database can eventually be extended with new metabolites and transformation products, and it appears that selecting metabolites (including transformation or degradation products) not only formed in air, but also those described in other matrices such as soil and water would provide more advantages for identifying more metabolites present in ambient air.

Two unknown transformation products, one from the malaoxon (metabolite of malathion) and the second one from fenhexamid were elucidated using a "fragmentation–degradation" relationship approach in the non-target workflow. Although this methodology provided, in some cases, good results and unknown metabolites could be elucidated, more computational efforts are necessary for the identification of unknown environmentally-relevant metabolites.

Acknowledgments

This study was financed by the Generalitat Valenciana through the DIPA Project (GV/2014/005) from the Conselleria d'Educació.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2015.11.068.

References

[1] (Association), E.E.C.P. Industry Statistics 2001–2010, 2010. Available from: (http://www.ecpa.eu/page/industry-statistics).
[2] EU. EU pesticides database. 2014 [cited 2014 June]; Available from: (http://ec.europa.eu/sanco_pesticides/public/?event=homepage).
[3] J.L. Armstrong, R.A. Fenske, M.G. Yost, K. Galvin, M. Tchong-Frenchand, J. Yu, Presence of organophosphorus pesticide oxygen analogs in air samples, Atmos. Env. 66 (2013) 145–150.
[4] A. Muñoz, T. Vera, M. Ródenas, E. Borrás, A. Mellouki, J. Treacy, H. Sidebottom, Gas-phase degradation of the herbicide ethalfluralin under atmospheric conditions, Chemosphere 95 (2014) 395–401.
[5] A. Muñoz, T. Vera, H. Sidebottom, M. Ródenas, E. Borrás, M. Vázquez, M. Raro, A. Mellouki, Studies on the atmospheric fate of propachlor (2-chloro-N-isopropylacetanilide) in the gase phase, Atmos. Environ. 49 (2012) 33–40.
[6] T.L. Reemtsma, L. Alder, U. Banasiak, Emerging pesticide metabolites in groundwater and surface water as determined by the application of a multi-method for 150 pesticide metabolites, Water Res. 47 (15) (2013) 5535–5545.
[7] L. Rajski, M. del Mar Gómez-Ramos, A.R. Fernández-Alba, Large pesticide multiresidue method by liquid chromatography – Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables, J. Chromatogr. A 1360 (2014) 119–127.
[8] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, Liquid chromatography-high resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: screening and quantitative studies, J. Chromatogr. A 1287 (2013) 24–37.
[9] L. Polgár, J.F. García-Reyes, P. Fodor, A. Gyepes, M. Dernovics, L. Ahrankó, B. Gilbert-López, A. Molina-Díaz, Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions, J. Chromatogr. A 1249 (2012) 83–91.
[10] C. Cascollà, V. Yusà, M.I. Beser, A. Pastor, Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM 2.5) by microwave-assisted extraction and liquid chromatography - tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 8817–8827.
[11] C. Cascollà, N. León, A. Pastor, V. Yusà, Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using liquid Chromatography – Orbitrap High Resolution Mass Spectrometry, J. Chromatogr. A 1368 (2014) 132–142.
[12] SANCO /2007/3131; 31/October/2007. Method validation and quality control procedures for pesticide residues analysis in food and feed, 2007.
[13] AEPLA (Asociación Empresarial para la Protección de las Plantas), Memoria AEPLA 2014, 2015. Available from: (http://www.aepla.es/tmp/images/publicaciones/P0017_Memoria_AEPLA_2014.pdf) (accessed November 2015).
[14] CAPA (Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenci- ana), Informe del sector agrario 2012, 2013. Available from: http://www.agricultura.gva.es/web/c/document_library/get_file?uuid=f0128d9b-3de3-43d7-a3f2-5bcdac0cf10d&groupId=416 (accessed November 2015).
[15] SANCO/12571/2013; 19 November 2013. Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food And Feed, 2013.
[16] J.F. García Reyes, A. Molina-Díaz, A.R. Fernandez-Alba, Identification of pesticide transformation products in food by using liquid chromatography/time-of – flight mass spectrometry via "fragmentation–degradation" relationships, Anal. Chem. 79 (2007) 307–321.
[17] T. Scientific. Pesticides Database Compound library: Environmental and Food (http://planetorbitrap.com/compoundlibrary#.VBlJ_JOMUfAM) (accessed April 2015).
[18] PPDB, pesticide property database. University of Hertfordshire. (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm) (accessed April 2015).
[19] ChemSpider software. (http://www.chemspider.com/) (accessed March 2015).
[20] PubChem compound database. (http://www.ncbi.nlm.nih.gov/pccompound) (accessed April 2015).
[21] H.G.J. Mol, P. Zomer, M. de Koning, Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry, Anal .Bioanal. Chem. 403 (2012) 2891–2908.
[22] M.L. Gómez-Pérez, R. Romero-González, P. Plaza-Bolaños, E. Génin, J. L. Martínez Vidal, A. Garrido Frenich, Wide-scope analysis of pesticide and veterinary drug residues in meat matrices by high resolution MS: detection and identification using Exactive- Orbitrap, J. Mass. Spectrom. 49 (2014) 27–36.
[23] T. Scientific. Mass Frontier 7.0. Available from: (http://www.thermoscientific.com/en/product/mass-frontier-7-0-spectral-interpretation-software.html), 2014.
[24] A. Muñoz, T. Vera, H. Sidebottom, A. Mellouki, E. Borrás, M. Ródenas, E. Clemente, M. Vázquez, Studies on the atmospheric degradation of chlorpyrifos-methyl, Environ. Sci. Technol. 45 (5) (2011) 1880–1886.
[25] A. Muñoz, A. Le Person, S. Le Calvé, A. Mellouki, E. Borrás, V. Daële, T. Vera, Studies on atmospheric degradation of diazinon in EUPHORE simulation chamber, Chemosphere 85 (2011) 724–730.
[26] Review Report EU for the Active Substance Thiophanate-methyl, 5030/VI/98-rev-8, 3 February 2005.
[27] Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance Carbofuran, EFSA Scientific Report 310, 2009, pp. 1–118.
[28] Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance Terbuthylazine, EFSA J. 9(1), 1969 (2011), pp.1–133.
[29] D. Vialaton, C. Richard, Direct photolyses of thiobencarb and ethiofencarb in aqueous phase, J. Photochem. Photobiol. A 136 (2000) 169–174.
[30] F. Hufsky, K. Scheubert, S. Böcker, Computational mass spectrometry for small-molecule fragmentation, TrAC-Trends Anal. Chem. 53 (2014) 41–48.
[31] V. Ieendert, H. Van-Langhenove, K. Demeestere, Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments, TrAC- Trends Anal. Chem. 67 (2015) 192–208.

6. CAPÍTULO 2. Análisis de plaguicidas en la atmósfera empleando extracción mediante cafetera Espresso y UHPLC-HRMS.

6.1. Introducción.

La extracción de plaguicidas presentes en la materia particulada (PM10) retenida en los filtros del captador se ha realizado habitualmente mediante la extracción con el método Soxhlet. Sin embargo, el método Soxhlet ha sido desplazado en los últimos años por nuevas metodologías analíticas de extracción como la extracción mediante líquidos presurizados (PLE) [168, 214] y la extracción asistida con microondas (MAE) [215-216], reduciendo ambos, el tiempo de extracción y el consumo de disolvente e incrementando el rendimiento de extracción para muchos de los compuestos estudiados. Sin embargo, estos métodos de extracción eficaces son muy caros, y en muchos casos, el tiempo de extracción no es todo lo rápido que se desearía. Adicionalmente, tanto el PLE como el MAE implican el calentamiento de las muestras durante un largo período de tiempo y este aspecto puede afectar a la estabilidad de los compuestos polares térmicamente poco estables [217]. Estudios previos nos han indicado que temperaturas superiores a 80 ºC empleando el MAE puede provocar la degradación de plaguicidas como carbendazima, metiocarb, flusilazol, procloraz, ciprodinil, triflumizol, tebufenpirad y buprofezin [163]. Resultados semejantes fueron observados para fungicidas como azoxystrobin, clorotalonil, ciproconazol, metconazol, miclobutanil, propioconazol, piraclostrobin, tebuconazol, tetraconazol, trifloxistrobin, que se degradaban a temperaturas mayores de 100 ºC empleando el MAE [218]. Así, la temperatura y el tiempo de extracción deben ser establecidos para evitar la degradación de los plaguicidas estudiados, observándose que una extracción rápida con condiciones suaves puede ser el tratamiento más adecuado para la extracción.

Las cafeteras espresso se han aplicado para la extracción de hidrocarburos policíclicos aromáticos y de bifenilos policlorados de suelos contaminados [219] y compuestos bioactivos procedentes de vegetales y especies [220]. En este enfoque, las muestras son introducidas en cápsulas de acero inoxidables recargables y la extracción de los compuestos estudiados se realiza en pocos segundos, empleando una mezcla de acetonitrilo: agua y etanol: agua, fijando unas condiciones de presión y temperatura moderadas: 72 ºC y 19 bares, respectivamente. Así, el uso de la máquina espresso provee una extracción fácil y rápida permitiendo múltiples aplicaciones y costes asequibles.

El objetivo de este estudio (capítulo 2) es el uso de la cafetera espresso para la extracción de 35 plaguicidas prioritarios detectados habitualmente en la atmósfera de zonas rurales. Este método está basado en una extracción con la cafetera espresso empleando como disolvente extractante una mezcla de acetonitrilo: agua seguida de una etapa de salting out y una inyección directa en un equipo de cromatografía líquida acoplada a espectrometría de masas de alta resolución (UHPLC-HRMS) de muestras de filtros PM10 recogidas procedentes de la red de control de la Comunidad Valenciana (España).

6.2. Resultados

6.2.1. Condiciones de extracción de la cafetera.

El uso de las máquinas espresso para la extracción de contaminantes orgánicas procedentes de muestras sólidas ha sido propuesto recientemente, considerando su disponibilidad, su uso y su bajo coste [219-221]. Además, las condiciones de trabajo son fáciles de configurar (72 ºC y 19 bares), siendo los principales parámetros a ajustar el disolvente empleado y el volumen de extracción. En principio, los disolventes más adecuados para emplear en esta metodología son aquellos que presentan elevadas temperaturas de ebullición, son miscibles en agua y presentan constantes dieléctricas elevadas. Así, acetonitrilo y etanol fueron empleados en estudios previos para este propósito.

En este estudio, agua y una mezcla de acetonitrilo: agua 20 % fueron los dos disolventes empleados para la extracción de plaguicidas procedentes de filtros PM10 empleando un filtro blanco cargado con 500 ng de plaguicidas para la evaluación de las condiciones iniciales. Además, tres ciclos consecutivos de extracción fueron realizados empleando 50 mL de disolvente extractante y analizados directamente por UHPLC-HRMS.

Empleando agua como disolvente extractante se obtuvieron buenas recuperaciones para 18 de los 35 plaguicidas estudiados (recuperaciones superiores al 80 %) mientras que para el resto de plaguicidas estudiados las recuperaciones obtenidas oscilaron entre el 26 % y el 76 %. Empleando 100 mL de agua se obtuvieron buenas recuperaciones para todos los plaguicidas estudiados excepto para bitertanol, difenoconazole, imazalil, tebufenpirad y tiabendazol. Con un volumen de 150 mL de agua, se obtuvieron buenas recuperaciones para difenoconazol e imazalil, pero no para el resto. Sin embargo, con el uso de una pequeña cantidad de disolvente orgánico (20 % acetonitrilo en agua) se obtuvieron recuperaciones para todos los plaguicidas estudiados entre el 77 % y el 123 %, empleando únicamente 50 mL de volumen de la mezcla agua: acetonitrilo. Así, de esta forma, las condiciones descritas anteriormente han sido las empleadas para la extracción de los plaguicidas presentes en los filtros PM10, con un tiempo de extracción de 20 segundos.

Los extractos obtenidos son compatibles con el equipo de cromatografía líquida (UHPLC-HRMS) y podrían ser inyectados directamente en el equipo. Sin embargo, se observó una sensibilidad escasa y así, se añadió una etapa de preconcentración de forma que se pudiera obtener una sensibilidad adecuada para evaluar la posible contaminación a plaguicidas en la zona estudiada.

6.2.2. Etapa de preconcentración.

Cómo se ha comentado anteriormente, los plaguicidas fueron extraídos empleando una disolución de acetonitrilo: agua. Después, se ha realizado una etapa de preconcentración posterior a la extracción mediante la cafetera para incrementar la sensibilidad del método. $MgSO_4$ y NaCl fueron empleados en la etapa de salting out mediante el método QuEChERS [222]. En este estudio, 25 mL de cada patrón de plaguicida de concentración 10 $\mu g\ L^{-1}$, preparados en disoluciones de 20, 40 y 60 % de acetonitrilo en agua y utilizando una etapa de salting out empleando 8 g de $MgSO_4$ o 8 gramos de NaCl en experimentos independientes. Las mezclas de acetonitrilo: agua fueron agitadas y

centrifugadas a 3500 rpm durante 5 minutos. El volumen de la capa superior, principalmente acetonitrilo, fue medido empleando una probeta volumétrica y analizada mediante UHPLC-HRMS. Utilizando cloruro sódico (NaCl) se obtiene un factor de preconcentración mayor que usando sulfato magnésico ($MgSO_4$), alcanzando un factor de preconcentración de 22 empleando la mezcla de 20 % acetonitrilo: agua. El uso de NaCl en la etapa de preconcentración nos permite obtener buenas recuperaciones para todos los plaguicidas estudiados (entre el 89 y el 108 %). Así, el extracto procedente de la cafetera, empleando la disolución de 20 % de acetonitrilo en agua, se puede preconcentrar directamente con 8 g de NaCl y la disolución obtenida inyectarla directamente en el sistema cromatográfico. El volumen de acetonitrilo obtenido es de 1.1 ± 0.1 mL, de forma que el factor de preconcentración obtenido fue de 22, incrementando de forma considerable la sensibilidad del sistema.

6.2.3. Parámetros analíticos del método.

El método propuesto fue validado en términos de linealidad, límites de detección (LOD), límites de cuantificación (LOQ) y de precisión, siguiendo las directrices de la guía SANTE/11945/2015 [208]. El efecto matriz (ME) fue evaluado por comparación de las pendientes de la curva con matriz y la curva preparada en metanol. La ecuación utilizada fue la siguiente (ecuación 10) [223]:

$$\text{Efecto matriz (\%)} = \left(\left(\frac{\text{pendiente en matriz}}{\text{pendiente en disolvente}}\right) - 1\right) \text{x } 100 \qquad (10)$$

Los valores de efecto matriz obtenidos oscilaron entre el -16 % y el -74 %, indicando una supresión de la señal como consecuencia de la matriz. La linealidad fue evaluada empleando calibración en matriz (entre 5 y 100 $\mu g\ L^{-1}$). Los coeficientes de linealidad (R^2) empleados oscilaron entre 0.9950 y 0.9990. El LOD y el LOQ fue calculado como, respectivamente, como 3 y 10 veces la desviación estándar de la ordenada en el origen dividida por la pendiente. Los LOD obtenidos oscilaron entre 1.3 y 3.5 pg m^{-3} y los LOQ oscilaron entre los 4.3 y 11.7 pg m^{-3}. La precisión fue evaluada como la desviación relativa (RSD) obtenida para la determinación de filtros blancos fortificados con 10 ng por triplicado. Los valores de RSD obtenidos fueron menores del 20 % en todos los casos.

La exactitud del método desarrollado fue evaluada mediante la evaluación de la recuperación a diferentes niveles (6.6-131.6 pg m^{-3}). El volumen de aire utilizado fue de 760 m^3 (flujo de 30 $m^3\ h^{-1}$ durante 24 horas). Los valores de recuperación obtenidos oscilaron entre el 77 % y el 125 %, el 70 % y el 120 % y el 74 % y el 129 % para los niveles de 10, 50 y 100 ng. El nivel de 5 ng por filtro era inferior al límite de cuantificación de muchos de los plaguicidas estudiados, obteniéndose recuperaciones para el resto de plaguicidas entre el 80 % y el 115 %.

6.2.4. Niveles de plaguicidas en la atmósfera.

10 muestras de aire fueron recogidas (filtros PM10) y divididas en dos piezas iguales. Una de ellas se analizó después de su extracción mediante cafetera y la otra de las piezas se analizó después de su extracción mediante microondas (MAE). Carbendazim y metalaxil se detectaron en todas las muestras con concentraciones que oscilaron entre los 29 y los 60 pg m^{-3} y entre los 15 y los 41 pg m^{-3}, respectivamente. En el caso de la

terbutilazina, se detectaron 8 muestras donde las concentraciones oscilaron entre los 13.5 y 30.4 pg m^{-3}, imidaclorpid se detectaron en 3 muestras que oscilaron entre 15 y 30.9 pg m^{-3}, tiabendazol se detectó en 3 de las muestras entre 10.7 y 15.8 pg m^{-3}, y tebuconazol en 2 de las muestras que oscilaron entre 13.5 y 14.5 pg m^{-3}. Acetamiprid, ciproconazol, fenbuconazol y fluazifop se detectaron en una única muestra con concentraciones de 8.5, 10.6, 8.1 y 9.2 pg m^{-3}, respectivamente. Estos resultados coinciden a los plaguicidas más empleados en la zona de acuerdo con estudios anteriores [138, 163 y capítulo 5]. La siguiente figura (Figura 16) muestra el resultado de una muestra positiva que contiene carbendazim (28.6 pg m^{-3}), metalaxil (17.2 pg m^{-3}), terbutilazina (13.8 pg m^{-3}) y tiabendazol (15.8 pg m^{-3}).

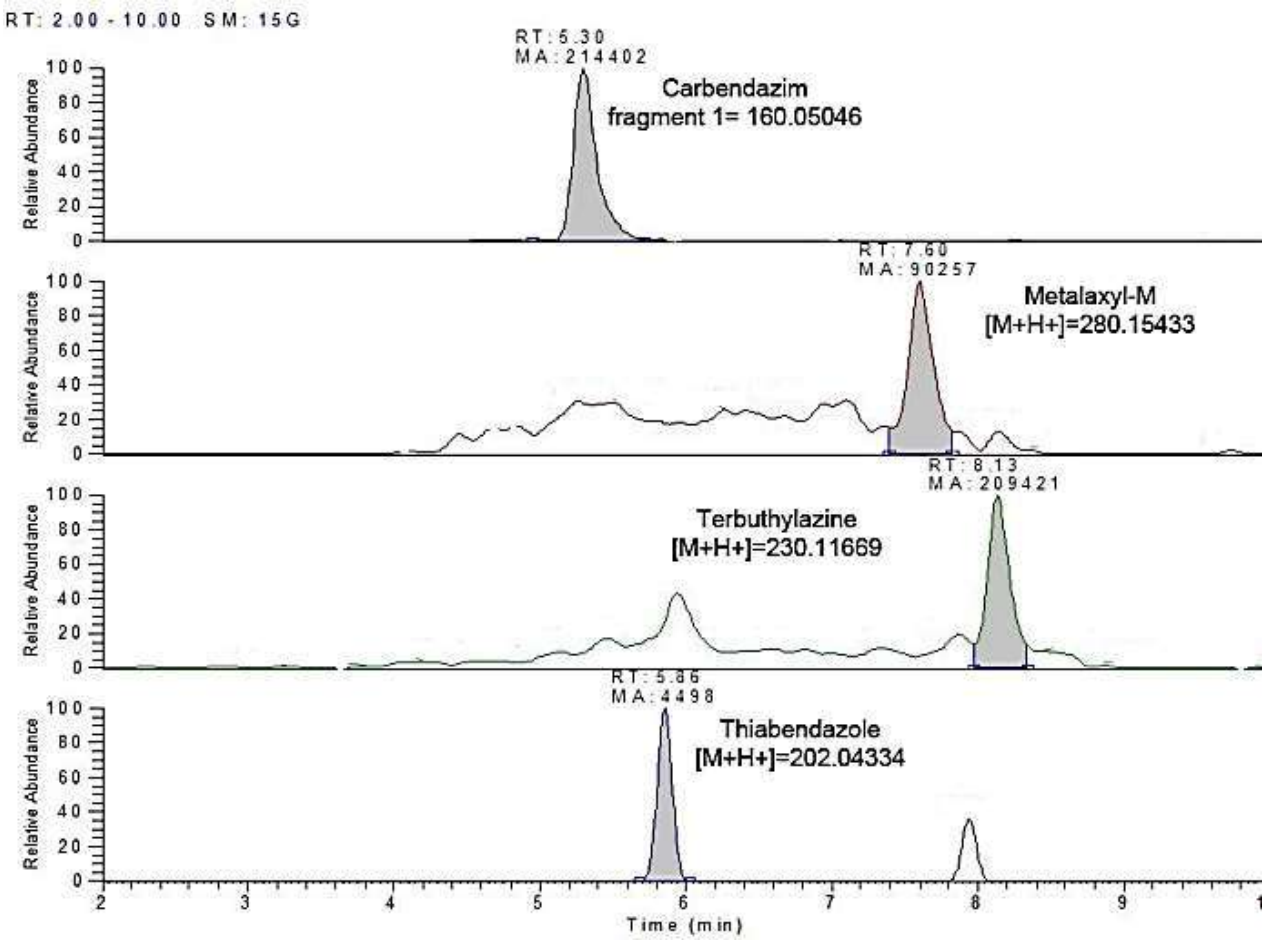


Figura 16. EIC de una muestra positiva. La muestra contiene carbendazima (28.6 pg m^{-3}), metalaxil (17.2 pg m^{-3}), terbutilazina (13.8 pg m^{-3}) y tiabendazol (15.8 pg m^{-3}).

A pesar de que el uso de la carbendazim está prohibido desde el año 2014, se ha detectado su uso en todas las muestras. Este hecho puede explicarse debido al uso extensivo realizado en el pasado y a que se trata de un metabolito del metil tiofanato, que es uno de los plaguicidas más empleados en el área de estudio para el tratamiento post-cosecha (ver capítulo 1).

Las 10 muestras fueron también analizadas mediante el método de extracción por microondas. La siguiente tabla (Tabla 10) muestra la comparación entre los dos métodos, comparándose los resultados mediante el test t-Student para muestras pareadas, sin considerar aquellas muestras que se detectaron en una única muestra.

Tabla 10. Plaguicidas detectados y concentraciones encontradas en ambos métodos de extracción

Plaguicida	Muestra	Concentración (pg m^{-3})		Diferencia	Media ± s	Test t [a]
		Cafetera Espresso	MAE			
Acetamiprid	1	8.5 ± 0.4	8.4	-0.1	- [b]	- [b]
Carbendazima	1	38.2 ± 0.2	34.6	-3.6	-3 ± 2	2.58
	2	45.3 ± 0.4	40.8	-4.5		
	3	56 ± 4	51.6	-4.7		
	4	60 ± 2	56.2	-3.7		
	5	38 ± 5	38.3	0.3		
	6	35 ± 3	36.0	0.5		
	7	42.5 ± 0.4	38.3	-4.2		
	8	28.6 ± 0.3	26.5	-2.1		
	9	29.2 ± 0.7	26.7	-2.4		
	10	34 ± 1	30.7	-3.7		
Ciproconazol	4	10.6 ± 0.3	10.6	0.0	- [b]	- [b]
Fenbuconazol	4	8.1 ± 1.1	8.5	0.4	- [b]	- [b]
Fluazifop	2	9.2 ± 0.6	8.6	-0.6	- [b]	- [b]
Imidacloprid	3	30.9 ± 1.2	30.5	-0.4	1 ± 2	0.79
	6	15.0 ± 1.3	14.9	-0.1		
	10	26.2 ± 1.2	29.5	3.3		
Metalaxil	1	25.5 ± 0.4	25.0	-0.5	0.5 ± 1.1	0.80
	2	17.7 ± 0.3	18.8	1.1		
	3	26.0 ± 1.2	26.9	0.9		
	4	29.9 ± 0.2	28.3	-1.6		
	5	24.9 ± 1.5	24.3	-0.6		
	6	41 ± 3	41.4	0.6		
	7	35.2 ± 0.5	37.3	2.0		
	8	17.2 ± 1.6	17.5	0.3		
	9	15.0 ± 0.1	16.8	1.8		
	10	21.4 ± 0.4	22.6	1.3		
Tebuconazol	3	13.5 ± 0.8	15.0	1.5	1.7 ± 0.3	9.00
	9	14.5 ± 0.1	16.4	1.9		
Terbutilazina	1	28.0 ± 0.3	26.6	-1.5	-0.4 ± 0.9	0.78
	2	24.9 ± 0.7	24.2	-0.6		
	3	30.4 ± 1.8	29.8	-0.7		
	4	24.8 ± 0.5	25.3	0.5		
	5	13.5 ± 0.9	13.5	0.0		
	6	15.0 ± 0.4	15.6	0.6		
	7	17.4 ± 0.3	17.5	0.2		
	8	13.8 ± 0.5	12.0	-1.8		
Tiabendazol	2	10.7 ± 0.4	11.7	1.0	0.8 ± 1.4	0.93
	5	12.2 ± 1.1	14.3	2.1		
	8	15.8 ± 0.1	15.0	-0.8		

[a] T-Student tabulada para 3 grados de libertad y α=0.05 = 3.18

[b] Valores de t-Student no han sido calculados para análisis individuales

Todos los análisis fueron estadísticamente significativos a un 95 %, con la excepción de dos muestras de tebuconazole que mostraron dos valores de t experimental mayores que los tabulados ($t_{n=3,\ \alpha=0.05} = 3.18$).

Los resultados obtenidos fueron globalmente comparados empleando la regresión de Deming, que consideró la desviación estándar de los valores obtenidos por ambos métodos. Los resultados obtenidos a través de la regresión de Deming se muestran en la siguiente figura (figura 17), con una pendiente de 1.032 (el intervalo con nivel de confianza del 95 % oscila entre 0.98 y 1.09) y una ordenada de -0.67 (el intervalo con un nivel de confianza del 95 % oscila entre -1.62 y 0.29).

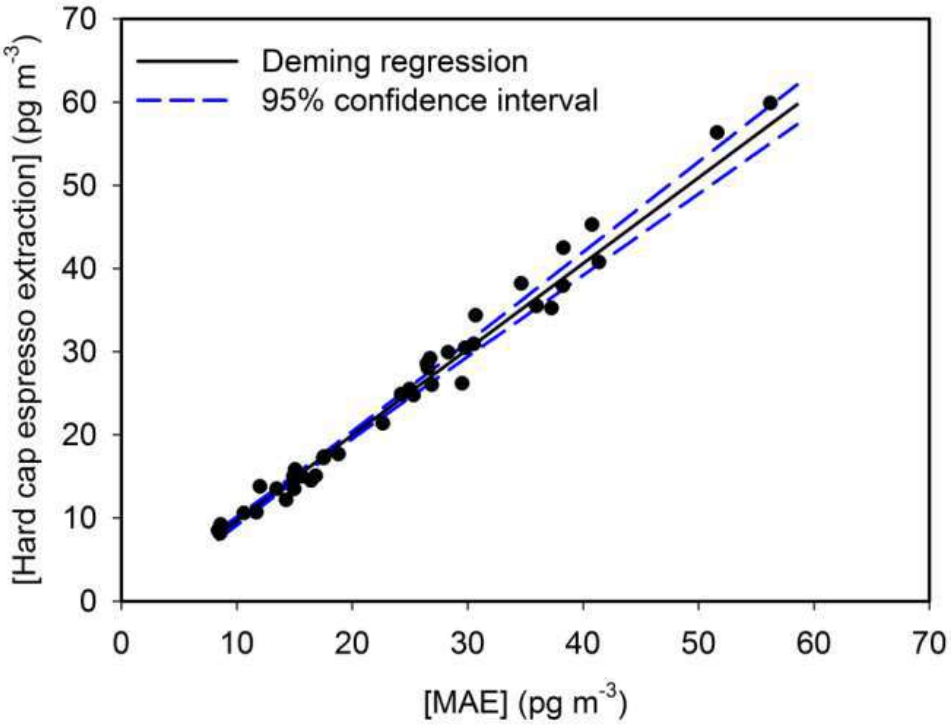


Figura 17. Regresión de Deming obtenida en la comparación de los dos métodos de extracción descritos (MAE y cafetera).

Las grandes ventajas que ofrecen la cafetera en comparación con la extracción con microondas (MAE) son las siguientes: extracción fácil y rápida, bajo nivel de contaminación cruzada, alta accesibilidad y bajo coste. Sin embargo, presenta algunos inconvenientes relacionados con las condiciones de extracción y las condiciones de seguridad. Así, por tanto, se propone la cafetera como método de extracción ya que resulta estadísticamente comparable con el método de referencia empleado mediante microondas para la extracción de plaguicidas en filtros PM10.

6.2.5. Análisis retrospectivo de las muestras.

Como alternativa a la espectrometría de masas en tándem (MS/MS), el uso de la espectrometría de masas de alta resolución (HRMS), como Orbitrap o TOF, nos permite la adquisición de espectros con una elevada exactitud de masa (1-5 ppm) combinada

con un elevado poder de resolución (25000-50000 FHWM) [194, 212], además de su capacidad de adquisición en modo full-scan, lo que permite realizar un análisis retrospectivo de las muestras analizadas. Para ello, se buscaron metabolitos de plaguicidas a partir de una base de datos ya creada con anterioridad con aproximadamente 250 metabolitos de plaguicidas (ver capítulo 1 y anexo I). Los resultados obtenidos en el análisis post-target se muestran en la siguiente tabla (Tabla 11).

Tabla 11. Metabolitos detectados en el análisis retrospectivo de muestras de filtros PM10 (n=10)

Metabolito	Formula	Parent	Masa monitorizada [a]	Δm (ppm)		Frecuencia									
				$[M+H]^+$	Fragmento	1	2	3	4	5	6	7	8	9	10
N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	$C_{14}H_{21}NO_2$	Acetoclor	236.16450	-0.2150, 0.6896	-0.7034, 0.7645		X							X	
Phosphorothioic O,O,S -trimetil ester	$C_3H_9O_3PS$	Azinfos-metil	157.00827	-0.3536, 0.1324	0.0874, 0.5147		X		X	X			X		
1,2,3,6-tetrahidroftalimida	$C_8H_9NO_2$	Captan	152.07060	0.1508	1.3882			X							
3-hydroxy-1,2,3,6-tetrahydrophthalamic acid	$C_5H_{11}NO$	Captan	102.09134	1.5606, 3.1300	3.1252, 4.7379	X		X			X	X		X	
Carbofuran-7-fenol	$C_{10}H_{12}O_2$	Carbofuran	165.09100	-0.2247, 0.0526	-0.7763, 0.1677	X		X	X	X	X		X	X	
Dietilfosfato	$C_4H_{11}O_4P$	Diazinon	155.04677	-0.8974, 0.2836	1.3003, 2.3793	X	X	X	X	X		X	X	X	X
4-formylmorfolina	$C_5H_9NO_2$	Dimetomorf	116.07060	0.5262, 1.1178	1.3567, 2.0569	X	X				X		X	X	X
Clorpirifos-oxon	$C_9H_{11}Cl_3NO_4P$	Etil clorpirifos	333.95640	-0.3897, 0.8850	-1.2648, 1.3720				X	X		X		X	X
Desclorofenhexamid	$C_{14}H_{19}NO_2$	Fenhexamida	234.14886	-0.1101	0.2007					X					
Dietilmaleato	$C_8H_{12}O_4$	Malation	173.08083	-0.2583, 0.5351	-0.1727, 1.1487	X		X	X	X	X	X	X	X	X
Malaoxon	$C_{10}H_{19}O_7PS$	Malation	315.06618	-0.9603	0.2098		X								
N-(2-etil-6-metilfenil)-L-alanina	$C_{12}H_{17}NO_2$	Metolaclor	208.13320	0.2895	0.5178	X									
Ácido propacloroxanilico	$C_{11}H_{13}NO_3$	Propacloro	208.09682	-0.1610	-0.1714	X									
Terbutilazina-2-OH	$C_9H_{17}N_5O$	Terbutilazina	212.15059	-1.084, -0.0767	-0.3517, -0.3401						X	X			

[a] Semejanza Perfil Isotópico > 90 %

Para ambos métodos se han obtenido resultados similares. La frecuencia de detección oscila entre el 90 % del dietilmaleato y el dietilfosfato y el 10 % del N-(2-etil-6-metilfenil)-L-alanina, ácido propacloroxanilico, desclorofenhexamid, malaoxon y 1,2,3,6-tetrahidroftalimida. En el anexo II, se muestran cromatogramas de algunas de las muestras detectadas en el análisis retrospectivo.

6.3. Conclusiones

-El método desarrollado para la determinación de plaguicidas en la atmósfera, basados en el muestreo de materia particular de tamaño hasta 10 micras (PM10), una posterior extracción mediante la cafetera y una etapa de preconcentración mediante el salting out hacen posible la determinación cuantitativa de 35 plaguicidas.

-Los LOQ obtenidos son 6.5 pg m^{-3} para la mayoría de los plaguicidas estudiados, obteniéndose recuperaciones entre 70 % y 129 % en los niveles fortificados para su estudio (entre 5 y 100 ng).

-El uso del análisis retrospectivo nos permite la evaluación de la posible presencia de metabolitos en las muestras recogidas en las áreas agrícolas estudiadas.

-El uso de la cafetera para la etapa de extracción y de una etapa posterior de preconcentración con salting-out mejora de forma significativa el tiempo de extracción (la extracción se realiza en 20 segundos) y el consumo de reactivos (50 mL de una disolución del 20 % de acetonitrilo en agua y 8 g de NaCl por muestra) de forma que se obtiene un método rápido, de bajo coste y medioambientalmente sostenible comparándolo con los métodos habituales de extracción (PLE y MAE).

6.4. Artículo 2: Comprehensive analysis of airborne pesticides using hard cap espresso extraction-liquid chromatography-high-resolution mass spectrometry.

Journal of Chromatography A, 1506 (2017) 27–36

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comprehensive analysis of airborne pesticides using hard cap espresso extraction-liquid chromatography-high-resolution mass spectrometry

Antonio López[a], Clara Coscollà[a], Vicent Yusà[a,b], Sergio Armenta[b], Miguel de la Guardia[b], Francesc A. Esteve-Turrillas[b,*]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Cataluña, 46020 Valencia, Spain
[b] Department of Analytical Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form 15 May 2017
Accepted 16 May 2017
Available online 18 May 2017

Keywords:
Nespresso
Hard cap espresso machine
High resolution mass spectrometry
Pesticides
Metabolites
Ambient air

ABSTRACT

A hard cap espresso extraction procedure has been developed to recover airborne pesticides in particulate matter trapped in filters. This extraction step was made for 20 s at 72 °C and 19 bar using 50 mL of 20% (v/v) acetonitrile in water. After that, based on NaCl salting out, extracts were concentrated 22 times and analysed by ultra-high performance liquid chromatography – high resolution mass spectrometry. 35 pesticides were evaluated, as a proof of concept, being validated the whole methodology and compared the extraction method with that based on microwave assisted extraction for 20 min. In short, the method avoids cross-contamination of samples, it is relatively fast and consumes only 10 mL acetonitrile and 8 g NaCl per sample; thus, offering a low cost and green alternatively to available methods based on pressurized solvent extraction or microwave-assisted treatment.

1. Introduction

The presence of pesticides in the atmosphere of agricultural areas is one of the main consequences of their massive use during the application periods, the volatilization from soil, sediments and plants after application, and the wind erosion of soil particles [1]. Pesticides can be found in both, gas and particulate phases, with a partition ratio that depends on their physico-chemical properties and environmental factors, [2]. Particulate matter (PM10) is a complex mixture of suspended particles mainly inorganic compounds, trace metals, elemental carbon, carbonaceous organic matter, crustal compounds and water [3,4]. PM10 is currently employed as an air pollution indicator, taking into account that the respirable fraction of PM is the most dangerous for human health and the environment.

Pesticide extraction from airborne PM10 filters has been traditionally carried out with organic solvents by using Soxhlet extraction [5,6]. However, Soxhlet applications have been replaced in the last decade by novel extraction methodologies like pressurized liquid extraction (PLE) [7,8] and microwave assisted extraction (MAE) [9,10], reducing both, extraction time and solvent consumption, and increasing the extraction yield of many compounds. However, these high efficacy extraction methods are quite expensive and, in some cases, the extraction time is not as fast as desired. Additionally, PLE and MAE involve heating of samples for long periods of time and it may affect the stability of thermally labile polar compounds, especially in the case of modern agricultural pesticides like *N*-methylcarbamates, sulphonyl urea, and chlorophenoxy acid herbicides [11]. Previous studies indicated that MAE at temperatures higher than 80 °C may degrade compounds like carbendazim, methiocarb, flusilazole, prochloraz, cyprodinil, triflumizole, tebufenpyrad, and buprofezin [12]. Similar results were observed for fungicides like azoxystrobin, chlorothalonil, cyproconazole, metconazole, myclobutanil, propiconazole, pyraclostrobin, tebuconazole, tetraconazole, and trifloxystrobin that provided thermal degradations at temperatures higher than 100 °C using MAE [13]. So, extraction temperature and time must be accurately established to avoid any target compound degradation, being observed that a quick extraction using mild conditions can be the most adequate treatment.

Conventional hard cap espresso machines have been applied for the extraction of polycyclic aromatic hydrocarbons [14] and poly-

* Corresponding author.
E-mail address: francesc.a.esteve@uv.es (F.A. Esteve-Turrillas).

http://dx.doi.org/10.1016/j.chroma.2017.05.040
0021-9673/

chlorinated biphenyls [15] from contaminated soils, and bioactive compounds from vegetables and spices [16]. In this approach, samples are introduced in refillable stainless steel capsules and target analytes are extracted in few seconds using acetonitrile:water or ethanol:water mixtures at a fixed and moderate temperature and pressure conditions of 72 °C and 19 bar, respectively. So, the use of hard cap espresso machines provides an easy, fast and reliable extraction method with multiple potential applications and affordable costs.

The aim of this study is to enhance the use of hard cap espresso machine for a novel application related to the extraction of 35 commonly use pesticides from airborne PM10 retained in filters employed for air active sampling monitoring campaigns in urban and agricultural areas. The proposed method is based on the use of a hard cap espresso extraction with acetonitrile:water mixtures followed by a salting out step and direct injection into an ultra-high performance liquid chromatograph with a high resolution mass spectrometer detector (UHPLC-HRMS) as a proof of concept of the tremendous possibilities of the use of this simple and low cost extraction system for accurate sample preparation. The method has been also applied to the analysis of PM10 filters collected from the monitoring network of the Regional Government of Valencia (Spain).

2. Material and methods

2.1. Chemicals and reagents

High purity standard pesticides, thiamethoxam (98.5 m/m), carbendazim (99% m/m), pirimicarb-desmethyl (99.5% m/m), thiabendazole (98.5% m/m), imidacloprid (99% m/m), dimethoate (98% m/m), acetamiprid (99% m/m), pirimicarb (98.7% m/m), carbofuran (99% m/m), imazalil (97.5% m/m), diuron (98% m/m), metalaxyl (99.5% m/m), pyrimethanil (99% m/m), fluazifop (96% m/m), azoxystrobin (99.5% m/m), methidathion (98.5% m/m), terbuthylazine (99.5% m/m), fluquinconazole (99% m/m), cyproconazole (99% m/m), fenbuconazole (98.7% m/m), myclobutanil (99% m/m), fenoxycarb (99.5% m/m), fenhexamid (99.5% m/m), iprovalicarb (97.5% m/m), flusilazole (99.5% m/m), benalaxyl (99.5% m/m), tebuconazole (98.5% m/m), difenoconazole (98.7% m/m), cyprodinil (97.5% m/m), bitertanol (98% m/m), prochloraz (99.5% m/m), triflumizole (98.5% m/m), tebufenpyrad (98% m/m), pyriproxifen (98% m/m) and buprofezin (99% m/m), were supplied by Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standards were prepared in acetone and stored in capped amber vials at −21 °C. Methanol and acetonitrile were HPLC grade supplied by Scharlau (Barcelona, Spain). HPLC grade acetone, ethyl acetate and water were purchased from Merck (Darmstadt, Germany). Glacial acetic acid (Reag. Ph. Eur.) and formic acid 98% were provided by Panreac (Barcelona, Spain). Ammonium acetate for HPLC (97%) and sodium chloride were purchased from Scharlau. Ammonium formate, solution Ultra (100 mL, 10 M in water) was provided by Fluka (Steinheim, Switzerland).

2.2. Sampling and site characterization

Valencia Region, situated on the East coast of Spain, made up 12.6% of the total national consumption of pesticides in 2014 [17]. The main irrigated crops are citrus fruit, other fruit trees (mainly peach, apricot and plum trees), rice and garden produce (primarily watermelon, cabbage, artichoke, lettuce, cauliflower, tomatoes, potatoes and onion). The main dry crops are vineyards, olive trees and almonds [18].

Samples were collected in Burriana, in the north of the Valencia region (39°53′22″N 0°05′33″W). Burriana is a town (31281 inhabitants) located at 13 km southeast of Castellon and 63 km north of Valencia, surrounded by irrigated land. A total of 10 samples were collected from May to July in 2016. Air was sampled at 3 m above ground level.

Fig. 1. PM10 filters, hard cap espresso machine, and stainless steel reusable caps employed in this study. Inset: Detail of the salting-out effect using a colouring agent for identification purposes.

PM10 samples were collected using a large-volume sampler from Digitel (Madrid, Spain) and quartz fiber filters of 150 mm diameter, supplied by Munktell filter AB (Falun, Sweden). A sampling flow of 30 m^3 h^{-1} for 24 h, which provided a total volume of air around 760 m^3, was used through this study.

PM10 filters were also used for air sampling of non-agricultural sites, in order to have blank filters, containing dust and organic matter, to be employed for the preparation of spiked filters. Filters were baking during 24 h at 300 °C to eliminate possible pesticides and other organic compounds as in previous studies [9].

2.3. Hard-cap espresso extraction

A Nespresso© Essenza Manual XN2003 Krups coffee machine was obtained from a local appliance store. The employed coffee machine was slightly modified to increase water reservoir as in previous studies [14–16]. Nespresso© compatible stainless steel refillable capsules (26 × 25 × 23 mm size, 8.8 mL internal volume) were obtained from Mycoffestar GAMMA (Zurich, Switzerland), filled with samples and Spe-ed Matrix dispersing agent from Applied Separations (Allentown, PA, USA) and covered inside with 47 mm borosilicate filters obtained from Scharlau (Barcelona, Spain) as shown in Fig. 1.

Espresso machine was purged with 200 mL 20% (v/v) acetonitrile in water using an empty capsule, filled with approximately 2.5 g dispersing agent, before each batch of extractions to clean and pre-heat the system. PM10 filter was folded and introduced in the stainless steel capsule filling the residual volume of the capsule with dispersing agent. A borosilicate filter was placed in the top to avoid losses of solid materials. The capsule was closed and introduced in the extraction compartment of the espresso machine

Table 1
UHPLC-HRMS parameters for the analysis of the pesticides extracted from airborne PM10 filters.

Compound	Formula	RT (min)[a]	Ion (m/z)	
			Monitored mass [M−H]⁻	Fragment
Acetamiprid	$C_{10}H_{11}ClN_4$	6.30	223.07450	126.01057
Azoxystrobin	$C_{22}H_{17}N_3O_5$	8.19	404.12410	372.09764
Benalaxyl	$C_{20}H_{23}NO_3$	9.02	326.17507	148.11204
Bitertanol	$C_{20}H_{23}N_3O_2$	9.14	338.18630	99.08064
Buprofezin	$C_{16}H_{23}N_3OS$	9.52	306.16346	201.10581
Carbendazim	$C_9H_9N_3O_2$	5.30	192.07675	160.05046
Carbofuran	$C_{12}H_{15}NO_3$	7.29	222.11247	165.09105
Cyproconazole	$C_{15}H_{18}ClN_3O$	8.63	292.12112	70.04033
Cyprodinil	$C_{14}H_{15}N_3$	9.08	226.13387	108.08090
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	9.34	406.07197	251.00255
Dimethoate	$C_5H_{12}NO_3PS_2$	6.18	230.00690	198.96466
Diuron	$C_9H_{10}Cl_2N_2O$	8.13	233.02429	72.04479
Fenbuconazole	$C_{19}H_{17}ClN_4$	8.78	337.12145	125.01532
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	8.63	302.07091	97.10144
Fenoxycarb	$C_{17}H_{19}NO_4$	8.87	302.13868	116.07074
Fluazifop	$C_{15}H_{12}F_3NO_4$	8.28	328.07912	282.07350
Fluquinconazole	$C_{16}H_8Cl_2FN_5O$	8.66	376.01627	349.00491
Flusilazole	$C_{16}H_{15}F_2N_3Si$	8.84	316.10761	247.07464
Imazalil	$C_{14}H_{14}Cl_2N_2O$	7.77	297.05560	159.03072
Imidacloprid	$C_9H_{10}ClN_5O_2$	5.94	256.05958	175.09787
Iprovalicarb	$C_{18}H_{28}N_2O_3$	8.57	321.21727	119.08568
Metalaxyl	$C_{15}H_{21}NO_4$	7.60	280.15433	220.13327
Methidathion	$C_6H_{11}N_2O_4PS_3$	8.08	302.96913	85.03985
Myclobutanil	$C_{15}H_{17}ClN_4$	8.54	289.12145	125.01526
Pirimicarb	$C_{11}H_{18}N_4O_2$	6.81	239.15025	72.04482
Pirimicarb-desmethyl	$C_{10}H_{16}N_4O_2$	5.76	225.13460	168.11317
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	9.20	376.03809	307.99991
Pyrimethanil	$C_{12}H_{13}N_3$	8.40	200.11822	107.06054
Pyriproxifen	$C_{20}H_{19}NO_3$	9.70	322.14377	279.26530
Tebuconazole	$C_{16}H_{22}ClN_3O$	9.02	308.15242	70.04040
Tebufenpyrad	$C_{18}H_{24}ClN_3O$	9.55	334.16807	117.02147
Terbuthylazine	$C_9H_{14}ClN_5$	8.13	230.11670	174.05400
Thiabendazole	$C_{10}H_7N_3S$	5.86	202.04334	175.09788
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	5.46	292.02656	211.06483
Triflumizole	$C_{15}H_{15}ClF_3N_3O$	9.43	346.09285	278.05508

[a] Retention time using the conditions mentioned in the text.

and extracted using 50 mL 20% (v/v) acetonitrile in water, which were measured using a glass graduated cylinder. Two 25 mL fractions were aliquoted in centrifuge tubes and they were salted–out by adding 8 g NaCl, shaken in a vortex and centrifuged for 5 min at 3500 rpm. Pesticides were concentrated in the upper acetonitrile layer with a final volume of 1.1 ± 0.1 mL in each tube, which were directly analysed by UHPLC-HRMS.

A purge with 50 mL 20% (v/v) acetonitrile in water was carried out between samples to clean the system and to avoid cross contaminations. After the extraction of samples, acetonitrile residues were removed to the system purging with 200 mL deionized water to avoid long-term contact of solvent with the internal parts of the espresso machine.

Spiked filters were prepared as follow: a blank PM10 filter was folded, introduced inside the capsule with dispersing agent, and directly spiked with 250 µL of pesticide standard solutions prepared in acetone at different concentration levels. The cap was fully filled with dispersing agent, and a borosilicate filter was placed at the top. The capsule was closed and stored in the fridge at 4 °C overnight until analysis.

2.4. Reference extraction procedure

A MAE method was employed as reference method for the extraction of pesticide sampled in PM10 filters. A Mars system from CEM Corporation (Mathews, NC, USA) equipped with Teflon® TFM 100 mL extraction vessels was employed. The extraction conditions were as follows: a temperature of 50 °C was applied for 20 min, using a power of 1200 W, and 30 mL of ethyl acetate were added [12]. Then, 100 µL of diethylene glycol was added as keeper to the extract and, it was concentrated using a Turbo Vap 500 (Zymark, Idstein, Germany). Extracts were re-dissolved with 1 mL of water:methanol (70:30, v/v) and filtered through a 0.22 µm GHP Acrodisc filter from Pall Life Science (Ann Arbor, MI, USA) prior to the UHPLC–HRMS determination.

Calibration solutions were prepared by adding variable volumes of concentrated pesticide solutions directly to the PM10 blank filter extracts.

2.5. UHPLC-HRMS analysis

Chromatography separation of pesticides was performed on an Accela UHPLC system equipped with an Hypersil Gold a Q column (100 mm × 2.1 mm, 1.9 µm) both from Thermo Fisher Scientific (Bremen, Germany). The flow rate used was 300 µL min^{-1} and the injection volume was 10 µL. Separations were performed using a binary gradient. The mobile phase was a gradient of water with 0.1% (v/v) formic acid and 4 mM ammonium formate (A) and methanol with 0.1% (v/v) formic acid and 4 mM ammonium formate (B) and the gradient conditions were as follows: 0–8 min, linear with 100% of A; 8–12 min, linear with 100% of B and 12–16 min, linear with 100% of A. The total run time was 16 min. Mass spectrometry analysis was performed on a single stage Orbitrap MS (Exactive™ from Thermofisher Scientific). The system was equipped with a heated electrospray ionization interface (HESI-II). The detection was carried out in positive ionization mode (ESI+) using the following optimised operational parameters: spray voltage, 2.8 kV; sheath gas (N_2, >95%); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 205 °C; and capillary temperature, 281 °C. The mass spectra were acquired using two alternating acquisition func-

tions (i) full scan MS without fragmentation, ESI+; mass resolving power = 50000 FWHM; scan range = 50–800 Da; scan time = 0.5 s (2 Hz); (ii) The same parameters but with full scan MS all ion fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 10 eV). The automatic gain control was set to 1×10^6 ions. The external mass calibration of the spectrometer was performed using two ready-to-use calibration mixtures (MasCal5 (+) and MasCal 6 (−)) from Supelco (Bellefonte, PA, USA). Data acquisition and processing was performed using Thermo Scientific TraceFinder software version 3.2.

2.5.1. Identification and confirmation criteria for pesticides

Extracted ion chromatograms for individual compounds were reconstructed from the full-scan data with a mass tolerance of 5 ppm. Retention times and the employed MS detection parameters for each evaluated pesticide are shown in Table 1.

Identification of analytes in the target quantitative method were assessed using the following criteria: (i) mass accuracy of the molecular ion lower than 5 ppm; (ii) mass accuracy of the fragment ion (HCD) lower than 5 ppm and/or isotope pattern similar to the theoretical one; (iii) ion ratio similar to the standards with a relative tolerance of ± 30%, and (iv) retention time similar to that of the calibration standard ± 0.1 min [19].

2.5.2. Identification criteria for pesticide metabolites

In post-run target screening, searching for metabolite compounds was carried out by using automated software tools. The identification and confirmation settings in the Trace Finder programme included a threshold override of 10000, with S/N of 5, and a mass tolerance of 5 ppm for the molecular ion; an intensity threshold of 5000 and a mass tolerance of 5 ppm for fragments. For isotopic pattern a fit threshold of 90%, an allowed relative intensity (RI) deviation of 30%, and a mass deviation of 5 ppm were selected in the TraceFinder software.

For compound identification the following criteria were established [19]: i) mass accuracy of the parent ion lower than 5 ppm; ii) mass accuracy of the fragment ion with error lower than 5 ppm; and iii) isotopic pattern similar to the theoretical isotopic pattern (the relative intensity of the A + 1 and/or A + 2 isotope peaks in the real sample shall correspond to the theoretical relative intensities).

2.6. Database for post-run target screening analysis

A customized theoretical database was built in a previous study [20], containing 263 pesticide metabolites. For each substance, the screening database included the elemental composition (molecular formula) and the theoretical accurate mass of the monitored ion. Existing data from available databases [21–24] and previous literature [25] were used. This is a theoretical database where no standards were analysed to get characteristic fragments. Information about fragments was included when available in the literature, mainly from HRMS (exact mass) and QqQ (nominal mass) studies [26–31].

3. Results and discussion

3.1. Hard cap espresso extraction conditions

The use of hard cap espresso machine to extract organic pollutants from solid samples has been recently proposed, considering its availability, ease of use, and low cost acquisition [14–16]. Furthermore, working conditions are easy to be set-up as 72 °C and 19 bar, being the main parameters to be adjusted the type of solvent employed and the extraction volume. In principle, the most appropriate solvents to be used with this setup are those with high boiling point, water miscibility, and high dielectric constant. Thus, acetonitrile and ethanol were employed in previous studies for this purpose.

In this study, water and 20% (v/v) acetonitrile in water were employed for the extraction of pesticides from PM10 filters using a blank filter spiked with 500 ng target compounds to evaluate the starting conditions. Then, three consecutive extractions of the spiked filters were carried out using 50 mL extractant and they were directly analysed by UHPLC-HRMS. Fig. 2 shows the extraction recovery for the evaluated pesticides using from 1 to 3 successive extractions with pure water (Fig. 2A) and 20% v/v acetonitrile (Fig. 2B). As it can be seen, water was able to extract the pesticides from the filter with recoveries higher than 80% for 18 of 35 pesticides and ranging from 26 to 76% for the rest. The extraction with 100 mL water provided a quantitative recovery for all pesticides, except for bitertanol, buprofezin, difenoconazole, imazalil, tebufenpyrad and thiabendazole. The use of 150 mL water as extractant made quantitative the recovery for difenoconazole and imazalil, but not for the others. However, the use of a slight amount of organic solvent in the extractant, such as 20% (v/v) acetonitrile in water, provided a quantitative recovery for all the evaluated pesticides with values ranging from 77 to 123% using only 50 mL volume (see the inset of Fig. 2B). Thus, the aforementioned conditions were proposed for the extraction of pesticides from PM10 filters in a really short extraction time of 20 s.

The obtained extracts are compatible with UHPLC-HRMS and they can be directly analysed. However, a scarce sensitivity was observed due to the high dilution of the original sample and, thus, a preconcentration step must be added to the procedure in order to obtain the required sensitivity to provide an effective tool to assess the contamination of agricultural air by pesticides.

3.2. Salting out preconcentration of extracts

Pesticide extracts were acetonitrile:water mixtures. A salting out clean-up and preconcentration step was assayed after the hard cap espresso extraction to increase the sensitivity of the procedure. $MgSO_4$ and NaCl salts were employed in the salting out step of QuEChERS procedures [32]. In this study, 25 mL of a 10 $\mu g\,L^{-1}$ pesticide standards, prepared in 20, 40 and 60% (v/v) acetonitrile in water, were extracted using a salting out procedure with 8 g of $MgSO_4$ or 8 g of NaCl in independent experiments. The mixtures of water:acetonitrile were shaken in a vortex and then centrifuged at 3500 rpm for 5 min. The volume of the upper layer, mainly acetonitrile, was measured using a volumetric cylinder and analysed by UHPLC-HRMS in order to check the sensitivity obtained for the target pesticides. Table 2, shows the obtained volume, the preconcentration factor and the average recovery obtained for the target pesticides in each aforementioned experiment. The use of NaCl provided a higher preconcentration factor than $MgSO_4$, reaching a maximum value of 22 from a 20% (v/v) acetonitrile in water solution. NaCl salting out also provided quantitative recoveries of the evaluated pesticides, with values ranging from 89 to 108%. Thus, the obtained hard cap espresso extracts of PM10 samples, using 20% (v/v) acetonitrile in water, could be directly preconcentrated by salting out with 8 g NaCl and the obtained acetonitrile solution analysed by UHPLC-HRMS. The obtained acetonitrile volume was 1.1 ± 0.1 mL; so, a preconcentration factor of 22 was obtained that considerably increased the sensitivity of the method.

3.3. Analytical features of the method

The proposed method was validated in terms of linearity, limits of detection (LOD) and quantification (LOQ), and precision, following SANTE/11945/2015 guidelines [19]. Matrix effect (ME) was evaluated by comparison of the slopes of matrix-matched

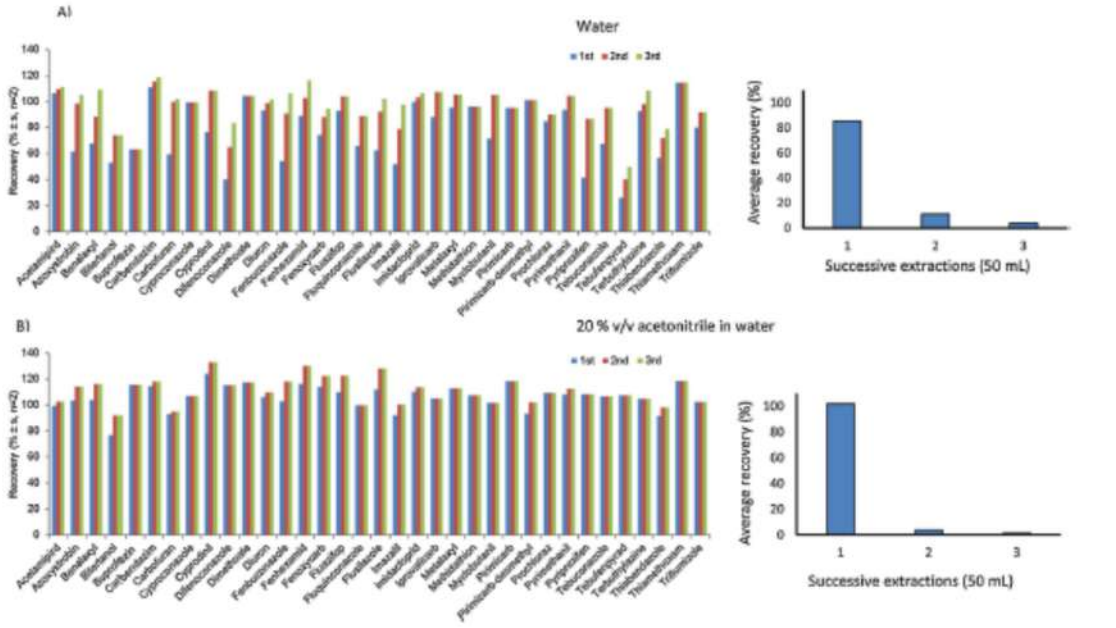


Fig. 2. Recoveries obtained for the extraction of the evaluated pesticides from PM10 filters using a hard cap espresso machine with pure water (A) and 20% (v/v) acetonitrile in water (B) as extraction solvents. Insets: Average recovery for the extraction of the selected pesticides as a function of the extractant volume.

Table 2
Salting out effect of $MgSO_4$ and NaCl on the preconcentration of 25 mL 10 $\mu g\,L^{-1}$ pesticide standards prepared in different acetonitrile:water mixtures (n = 3).

Salting out	[Acetonitrile] (%, v/v)	Volume (mL)	Preconcentration	Recovery (% ± s)[a]
$MgSO_4$	20	6.7 ± 0.3	3.7	68 ± 12
	40	18.1 ± 0.9	1.4	91 ± 37
	60	18.8 ± 1.8	1.3	100 ± 20
NaCl	20	1.1 ± 0.2	22.2	108 ± 30
	40	6.3 ± 0.9	3.6	89 ± 15
	60	13.8 ± 0.8	1.9	95 ± 15

[a] Recovery was determined as the average of values found for the 35 studied pesticides.

calibration curves with those obtained for standards prepared in methanol, using Eq. (1) [33].

$$Matrix\ effect\ (\%) = \left(\left(\frac{slope\ in\ matrix}{slope\ in\ methanol}\right) - 1\right) \times 100 \quad (1)$$

The obtained ME values ranged from −16 to −74%, indicating a suppression of the signal as consequence of the matrix. Linearity was evaluated using matrix-matched calibration standards at six concentration levels from 5 to 100 $\mu g\,L^{-1}$. The obtained determination coefficients (R^2) ranged from 0.9950 to 0.9990. LOD and LOQ were calculated as 3 and 10 times, respectively, the standard deviation of the intercept of the calibration curve divided by the slope. The obtained LOD values ranged from 1.3 to 3.5 pg m^{-3} and LOQ ranged from 4.3 to 11.7 pg m^{-3}. Precision was evaluated as the relative standard deviation (RSD) obtained for the determination of blank PM10 filters spiked at 10 ng in triplicate. The obtained RSD values were lower than 20% in all cases (see Table 3).

Trueness of the developed procedure was evaluated by pesticide recoveries using blank PM10 filters spiked at 5, 10, 50 and 100 ng per filter. These concentration levels correlated with pesticide air concentrations from 6.6 to 131.6 pg m^{-3} for a sampling volume of 760 m^3 (24 h sampling at a 30 $m^3\,h^{-1}$ flow). The obtained recoveries can be seen in Table 3 and they ranged from 77 to 125%, from 70 to 120%, and from 74 to 129%, for 10, 50 and 100 ng spiked levels, respectively. PM10 filter spiked at 6.6 pg m^{-3} were lower than the LOQ for some pesticides and provided recoveries from 80 to 115% for the other ones.

3.4. Levels of studied pesticides in ambient air

Ten air samples were collected using PM10 filters, divided in two equal pieces. One of them was analysed after using the proposed hard cap espresso extraction method and the second half after MAE extraction. Table 4 shows the pesticides found with their concentration levels. Carbendazim and metalaxyl were found in all analysed samples with concentrations ranging from 29 to 60 pg m^{-3} and from 15 to 41 pg m^{-3}, respectively. The pesticide occurrence and concentration range found for the other evaluated pesticides were in 8 samples for terbuthylazine ranging from 13.5 to 30.4 pg m^{-3}, 3 samples for imidacloprid ranging from 15 to 30.9 pg m^{-3} and thiabendazole from 10.7 to 15.8 pg m^{-3}, and 2 samples for tebuconazole ranging from 13.5 to 14.5 pg m^{-3}. Acetamiprid, cyproconazole, fenbuconazole and fluazifop were found separately in a single sample with concentration values of 8.5, 10.6, 8.1, and 9.2 pg m^{-3}, respectively. These results corresponded to the most employed pesticides in the sampled area and are in accordance with previous studies [12,34,35]. Fig. 3 shows

Table 3
Main features validation of the procedure for UHPLC–MS determination of pesticides retained in PM10 filters by using hard cap espresso extraction and NaCl salting out preconcentration.

Pesticide	Calibration curve	R^2 [a]	LOD [b] ($pg\,m^{-3}$)	LOQ [b] ($pg\,m^{-3}$)	RSDr (%)	ME [c] (%)	ME [d] (%)	Recovery (% ± s, n = 2) [e]			
								6.6 $pg\,m^{-3}$ (5 ng)	13.2 $pg\,m^{-3}$ (10 ng)	65.8 $pg\,m^{-3}$ (50 ng)	131.6 $pg\,m^{-3}$ (100 ng)
Acetamiprid	3.81e5X+1.43e4	0.9985	1.3	4.5	10	−45	55	99 ± 6	114 ± 1	107 ± 5	109 ± 5
Azoxystrobin	1.09e5X – 3.18e5	0.9982	1.3	4.3	12	−32	68	80 ± 5	81 ± 1	70 ± 3	79 ± 4
Benalaxyl	2.21e5X – 2.85e5	0.9968	2.3	7.7	8	−19	81	[f]	81 ± 1	72 ± 3	79 ± 3
Bitertanol	1.26e4X – 3.75e4	0.9957	3.2	10.5	15	−17	83	[f]	96 ± 1	81 ± 4	93 ± 4
Buprofezin	2.63e4X – 2.44e4	0.9986	1.6	5.2	12	−44	56	91 ± 4	90 ± 1	79 ± 3	86 ± 4
Carbendazim	1.06e5X+3.63e4	0.9969	1.9	6.5	18	−69	31	115 ± 2	114 ± 3	114 ± 7	129 ± 7
Carbofuran	1.85e5X+1.84e5	0.9951	3.1	10.3	10	−42	58	[f]	113 ± 1	106 ± 5	107 ± 5
Cyproconazole	1.88e5X – 1.74e5	0.999	2.0	6.6	4	−27	73	114 ± 3	104 ± 1	101 ± 5	100 ± 4
Cyprodinil	1.96e6X+1.81e5	0.9955	1.7	5.8	9	−48	52	109 ± 5	95 ± 1	92 ± 4	92 ± 4
Difenoconazole	2.70e5X – 8.62e4	0.996	2.6	8.5	10	−28	72	[f]	107 ± 1	108 ± 5	102 ± 5
Dimethoate	7.61e4X+1.22e4	0.995	2.0	6.6	13	−60	40	109 ± 5	124 ± 1	120 ± 6	121 ± 5
Diuron	2.26e5X – 1.54e5	0.9965	2.0	6.6	8	−53	47	80 ± 1	94 ± 1	90 ± 4	90 ± 4
Fenbuconazole	1.26e5X – 1.83e5	0.9976	1.8	6.0	11	−27	73	83 ± 1	90 ± 1	91 ± 4	85 ± 4
Fenhexamid	2.24e5X – 2.08e4	0.9955	2.7	9.0	8	−52	48	[f]	89 ± 1	99 ± 5	85 ± 4
Fenoxycarb	1.05e5X – 1.29e5	0.9966	2.1	7.1	10	−54	46	[f]	87 ± 1	81 ± 4	84 ± 4
Fluazifop	1.94e5X – 2.45e5	0.9965	1.4	4.8	8	−17	83	93 ± 3	98 ± 1	98 ± 5	94 ± 4
Fluquinconazole	1.28e4X – 2.06e4	0.9958	2.1	6.9	14	−35	65	[f]	105 ± 1	104 ± 5	102 ± 4
Flusilazole	4.33e5X – 5.59e5	0.9956	2.5	8.5	13	−42	58	[f]	96 ± 1	94 ± 4	93 ± 4
Imazalil	2.98e5X – 4.03e5	0.9976	2.2	7.5	13	−29	71	[f]	77 ± 1	79 ± 4	74 ± 3
Imidacloprid	1.09e5X – 6.38e4	0.9956	2.9	9.8	10	−45	55	[f]	105 ± 1	111 ± 5	102 ± 5
Iprovalicarb	7.87e4X – 1.91e5	0.9959	2.1	6.9	12	−16	84	[f]	79 ± 1	77 ± 3	76 ± 3
Metalaxyl	2.05e5X – 3.56e5	0.9955	3.5	11.7	10	−32	68	[f]	89 ± 2	86 ± 4	86 ± 4
Methidathion	1.57e4X – 3.51e4	0.9963	2.0	6.5	10	−50	50	92 ± 5	94 ± 1	90 ± 4	91 ± 4
Myclobutanil	1.01e5X – 8.92e4	0.9955	2.0	6.6	8	−29	71	95 ± 3	92 ± 3	90 ± 4	87 ± 4
Pirimicarb	1.82e5X – 6.45e4	0.9953	2.1	7.0	12	−42	58	[f]	122 ± 3	114 ± 5	116 ± 5
Pirimicarb-desmethyl	1.87e5X – 2.57e5	0.9962	3.1	10.3	12	−47	53	[f]	125 ± 2	112 ± 5	121 ± 5
Prochloraz	7.94e3X – 2.81e4	0.9989	1.9	6.5	6	−39	61	110 ± 5	107 ± 1	101 ± 5	103 ± 5
Pyrimethanil	1.05e6X – 7.11e5	0.9966	2.0	6.6	9	−45	55	87 ± 4	95 ± 1	94 ± 4	92 ± 4
Pyriproxifen	6.57e4X – 7.10e4	0.9979	1.8	6.0	13	−48	52	92 ± 2	106 ± 1	102 ± 4	102 ± 5
Tebuconazole	3.06e5X – 2.04e5	0.9978	2.1	7.0	6	−28	72	[f]	96 ± 2	96 ± 4	92 ± 4
Tebufenpyrad	7.24e5X – 4.48e5	0.9952	2.6	8.5	11	−64	36	[f]	109 ± 1	100 ± 4	105 ± 5
Terbuthylazine	2.43e5X – 3.01e5	0.9973	2.2	7.4	5	−43	57	[f]	98 ± 1	98 ± 4	94 ± 4
Thiabendazole	1.41e6X+1.88e5	0.9967	2.9	9.8	8	−68	32	[f]	121 ± 1	115 ± 5	116 ± 5
Thiamethoxam	7.26e4X – 6.30e4	0.9979	1.8	5.9	2	−74	26	99 ± 3	114 ± 1	116 ± 5	110 ± 5
Triflumizole	6.25e3X – 2.11e4	0.9958	2.8	9.5	10	−71	29	[f]	109 ± 1	103 ± 4	105 ± 5

[a] Determination coefficient (R^2).
[b] Limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times, respectively, the standard deviation of the intercept of the calibration line divided by the slope and multiplied by the dilution factor.
[c] Relative standard deviation of a blank PM10 filter spiked at 10 ng (n = 3).
[d] Air pesticide concentrations were calculated for a 24 h sampling at a 30 $m^3\,h^{-1}$ flow, that corresponds to a sampling volume of 760 m^3. Data indicated in parenthesis correspond to ng of pesticide mixtures spiked on blank PM10 filters from which recovery studies were made.
[e] Matrix effect was calculated as ((slope in the matrix/slope in solvent)-1)*100.
[f] Lower than limit of quantification.

Table 5
Results of retrospective analysis of the occurrence of pesticide metabolites in ten analysed PM10 samples.

Found metabolite	Formula	Parent	Monitored mass [a]	Δm (ppm)		Occurrence									
				[M+H]+	Fragment	1	2	3	4	5	6	7	8	9	10
Diethylmaleate	$C_8H_{12}O_4$	Malathion	173.08083	−0.2583, 0.5351	−0.1727, 1.1487	X		X	X	X	X	X	X	X	X
Diethylphosphate	$C_4H_{11}O_4P$	Diazinon	155.04677	−0.8974, 0.2836	1.3003, 2.3793	X	X	X	X	X		X	X	X	X
Carbofuran-7-phenol	$C_{10}H_{12}O_2$	Carbofuran	165.09100	−0.2247, 0.0526	−0.7763, 0.1677	X		X	X	X	X		X	X	
4-formylmorpholine	$C_5H_9NO_2$	Dimethomorph	116.07060	0.5262, 1.1178	1.3567, 2.0569	X	X				X		X	X	X
3-hydroxy-1,2,3,6-tetrahydrophthalamic acid	$C_8H_{11}NO$	Captan	102.09134	1.5606, 3.1300	3.1252, 4.7379	X		X			X	X		X	
Chlorpyrifos-oxon	$C_9H_{11}Cl_3NO_4P$	Chlorpyrifos-ethyl	333.95640	−0.1202, 0.8850	−1.2648, 1.3720				X	X		X		X	X
Phosphorothioic *O,O,S* –trimethyl ester	$C_3H_9O_3PS$	Acimphos-methyl	157.00827	−0.3536, 0.1324	0.0874, 0.5147		X		X	X			X		
N-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	$C_{14}H_{21}NO_2$	Acetochlor	236.16450	−0.2150, 0.6896	−0.7034, 0.7645		X							X	
Terbuthylazine-2-OH	$C_9H_{17}N_5O$	Terbuthylazine	212.15059	−0.5802, −0.0767	−0.3517, −0.3401						X	X			
N-(2-ethyl-6-methylphenyl)-L-alanine	$C_{12}H_{17}NO_2$	Metolachlor	208.13320	0.2895	0.5178			X							
Propachlor oxanilic acid	$C_{11}H_{13}NO_3$	Propachlor	208.09682	−0.1610	−0.1714	X									
Deschlorofenexamid	$C_{14}H_{19}NO_2$	Fenhexamid	234.14886	−0.1101	0.2007					X					
Malaoxon	$C_{10}H_{19}O_7PS$	Malathion	315.06618	−0.9603	0.2098			X							
1,2,3,6-tetrahydrophthalimide	$C_8H_9NO_2$	Captan	152.07060	0.1508	1.3882				X						

[a] All isotopic pattern >90%.

of about 250 pesticide metabolites, including the theoretical accurate mass and their main fragments described in the literature or using specific fragmentation software [20]. Table 5 shows a summary of the metabolites tentatively identified in the measured samples applying hard cap expresso and MAE extractions by using retrospective analysis. Similar results were obtained in both extraction techniques. The pesticide metabolite occurrence in the analysed samples was 90% diethylmaleate and diethylphosphate, 70% carbofuran-7-phenol, 60% 4-formylmorpholine, 50% 3-hydroxy-1,2,3,6-tetrahydrophthalamic acid and chlorpyrifos-oxon, 40% phosphorothioic*O,O,S*-trimethyl ester, 20% *N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamideand terbuthylazine-2-OH, and 10% *N*-(2-ethyl-6-methylphenyl)-L-alanine, propachloroxanilic acid, deschlorofenexamid, malaoxon and 1,2,3,6-tetrahydrophthalimide. Table 5 also shows information about the respective precursor pesticide and molecular structure for each identified compound. Supplementary data file shows the accurate mass extracted ion chromatograms of the molecular ion and characteristic fragments of metabolites found in this study. So, it can be seen that UHPLC-HRMS offers a powerful tool for the confirmation of the presence of detected pesticides in airborne particulated materials retained in PM10 filters.

4. Conclusions

The method developed for pesticide determination in air, based on PM10 sampling, hard cap espresso extraction and salting out preconcentration made possible the quantitative determination of 35 pesticides by UHPLC-HRMS at pg m^{-3} concentration levels with LOQ of 6.5 pg m^{-3} for most of the studied pesticides, being obtained recovery percentages from 70 to 129% at spiked levels on PM10 filters from 5 till 100 ng of mixtures of the studied pesticides. The use of retrospective analysis permitted to evaluate the pesticide metabolite occurrence in samples taken from the atmosphere of agronomical areas.

In short, it has been evidenced that hard cap espresso extraction and salting out preconcentration can drastically improve sample preparation methods allowing a reduction of time (extraction step involved 20 s) and reagents required (50 mL of 20% (v/v) acetonitrile in water and 8 g NaCl per sample) providing a fast, low cost and green alternative to PLE or MAE of pesticides from filters deployed in open air.

Acknowledgments

Authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (Projects CTQ 2014-52841-P and CTQ 2016-78053-R), Generalitat Valenciana (Project PROMETEO-II 2014-077) and Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (AIRPEST project UGP-15-250).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.05.040.

References

[1] C. Coscollà, V. Yusà, Pesticides and agricultural air quality, in: M. de la guardia, S. Armenta (Eds.), Comprehensive Analytical Chemistry, vol. 73, Elsevier, 2016, pp. 423–490.
[2] T.F. Bidleman, Atmospheric processes, Environ. Sci. Technol. 22 (1988) 361–367.

[3] W.E. Wilson, J.C. Chow, C. Claiborn, W. Fusheng, J. Engelbrecht, J.G. Watson, Monitoring of particulate matter outdoors, Chemosphere 49 (2002) 1009–1043.
[4] Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe, Off. J. Eur. Union (2008) 1–44 (L152/1, 11.06.2008).
[5] H. Alegria, T.F. Bidleman, M.S. Figueroa, Organochlorine pesticides in the ambient air of Chiapas, Mexico, Environ. Pollut. 140 (2006) 483.
[6] D.T. Waite, A.J. Cessna, R. Grover, L.A. Kerr, A.D. Snihura, Environmental concentrations of agricultural herbicides: 2,4-d and triallate, J. Environ. Qual. 31 (2002) 129–144.
[7] Y. Yang, D. Li, D. Mu, Levels, seasonal variations and sources of organochlorine pesticides in ambient air of Guangzhou China, Atmos. Environ. 42 (2008) 677–687.
[8] C. Coscollà, V. Yusà, P. Martí, A. Pastor, Analysis of currently used pesticides in fine airborne particulate matter (PM 2. 5) by pressurized liquid extraction and liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1200 (2008) 100–107.
[9] C. Coscollà, V. Yusà, M.I. Beser, A. Pastor, Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM 2. 5) by microwave-assisted extraction and liquid chromatography?tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 8817–8827.
[10] J. He, R. Balasubramanian, S. Karthikeyan, U.M. Joshi, Determination of semi-volatile organochlorine compounds in the atmosphere of Singapore using accelerated solvent extraction, Chemosphere 75 (2009) 640–648.
[11] J. Cheng, M. Liu, X. Zhang, L. Ding, Y. Yu, X. Wang, H. Jin, H. Zhang, Determination of triazine herbicides in sheep liver by microwave-assisted extraction and high performance liquid chromatography, Anal. Chim. Acta 590 (2007) 34–39.
[12] C. Coscollà, N. León, A. Pastor, V. Yusà, Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using liquid chromatography–orbitrap high resolution mass spectrometry, J. Chromatogr. A 1368 (2014) 132–142.
[13] K.L. Smalling, K.M. Kuivila, Multi-residue method for the analysis of 85 current-use and legacy pesticides in bed and suspended sediments, J. Chromatogr. A 1210 (2008) 8–18.
[14] S. Armenta, M. de la Guardia, F.A. Esteve-Turrillas, Hard cap espresso machines in analytical chemistry: what else? Anal. Chem. 88 (2016) 6570–6576.
[15] D. Gallart-Mateu, A. Pastor, M. de la Guardia, S. Armenta, F.A. Esteve-Turrillas, Hard cap espresso extraction–stir bar preconcentration of polychlorinated biphenyls in soil and sediments, Anal. Chim. Acta 952 (2017) 41–49.
[16] M.T. Martinez-Sena, M. de la Guardia, F.A. Esteve-Turrillas, S. Armenta, Hard cap espresso extraction and liquid chromatography determination of bioactive compounds in vegetables and spices, Food Chem. (2017), http://dx.doi.org/10.1016/j.foodchem.2017.05.101.
[17] AEPLA (Asociación Empresarial para la Protección de las Plantas), 2015. Memoria AEPLA 2014. Available from: http://www.aepla.es/tmp/images/publicaciones/P0017_Memoria_AEPLA_2014.pdf (Accessed April 2017).
[18] CAPA (Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenciana), 2013. Informe del sector agrario 2012. Available from: http://www.agricultura.gva.es/web/c/document_library/get_file?uuid%4f0128d9b-3de3-43d7-a3f2-5bcdac0cf10d&groupId%416 (Accessed April 2017).
[19] SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.
[20] A. López, V. Yusà, M. Millet, C. Coscollà, Retrospective screening of pesticide metabolites in ambient air using liquid chromatography coupled to high-resolution mass spectrometry, Talanta 150 (2016) 27–36.
[21] Thermo Scientific.Pesticides Database Compound Library: Environmental and Food http://planetorbitrap.com/compoundlibrary#.VIlI_KMUfAM (Accessed April 2017).
[22] PPDB, pesticide property database, University of Hertfordshire. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (Accessed April 2017).
[23] ChemSpider software http://www.chemspider.com/ (Accessed April 2017).
[24] PubChem compound database http://www.ncbi.nlm.nih.gov/pccompound (Accessed April 2017).
[25] H.G.J. Mol, P. Zomer, M. de Koning, Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry, Anal. Bioanal. Chem. 403 (2012) 2891–2908.
[26] A. Muñoz, T. Vera, M. Ródenas, E. Borrás, A. Mellouki, J. Treacy, H. Sidebottom, Gas-phase degradation of the herbicide ethafluralin under atmospheric conditions, Chemosphere 95 (2014) 395–401.
[27] A. Muñoz, T. Vera, H. Sidebottom, M. Ródenas, E. Borrás, M. Vázquez, M. Raro, A. Mellouki, Studies on the atmospheric fate of propachlor (2-chloro-N-isopropylacetanilide) in the gas-phase, Atmos. Environ. 49 (2012) 33–40.
[28] M.L. Gómez-Pérez, R. Romero-González, P. Plaza-Bolaños, E. Génin, J.L. Martínez Vidal, A. GarridoFrenich, Wide-scope analysis of pesticide and veterinary drug residues in meat matrices by high resolution MS: detection and identification using Exactive- Orbitrap, J. Mass Spectrom. 49 (2014) 27–36.
[29] Thermo Scientific.Mass Frontier 7.0. 2014. Available from: http://www.thermoscientific.com/en/product/mass-frontier-7-0-spectral-interpretation-software.html (Accessed April 2017).
[30] A. Muñoz, T. Vera, H. Sidebottom, A. Mellouki, E. Borrás, M. Ródenas, E. Clemente, M. Vázquez, Studies on the atmospheric degradation of chlorpyrifos-methyl, Environ. Sci. Technol. 45 (5) (2011) 1880–1886.
[31] A. Muñoz, A. Le Person, S. Le Calvé, A. Mellouki, E. Borrás, V. Daële, T. Vera, Studies on atmospheric degradation of diazinon in EUPHORE simulation chamber, Chemosphere 85 (2011) 724–730.
[32] S.J. Lehotay, Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study, J. AOAC Int. 90 (2007) 485–520.
[33] A. Lozano, I. Rajski, N. Belmonte-Valles, A. Uclés, S. Uclés, M. Mezcua, A.R. Fernández-Alba, Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: validation and pilot survey in real samples, J. Chromatogr. A 1268 (2012) 109–122.
[34] C. Coscollà, E. Hart, A. Pastor, V. Yusà, LC-MS characterization of contemporary pesticides in PM10 of valencia region Spain, Atmos. Environ. 77 (2013) 394–403.
[35] A. López, V. Yusà, A. Muñoz, T. Vera, E. Borràs, M. Ródenas, C. Coscollà, Risk assessment of airborne pesticides in a Mediterranean region of Spain, Sci. Total Environ. 574 (2017) 724–734.
[36] I. Rajski, M.M. Gómez-Ramos, A.R. Fernández-Alba, Large pesticide multiresidue method by liquid chromatography–Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables, J. Chromatogr. A 1360 (2014) 119–127.
[37] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, Liquid chromatography-high resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: screening and quantitative studies, J. Chromatogr. A 1287 (2013) 24–37.
[38] I. Polgár, J.F. García-Reyes, P. Fodor, A. Gyepes, M. Dernovics, L. Abrankó, B. Gilbert-López, A. Molina-Díaz, Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions, J. Chromatogr. A 1249 (2012) 83–91.

7. CAPÍTULO 3. Selección del adsorbente adecuado y optimización del método de GC-MS/MS para el análisis de plaguicidas en la atmósfera.

7.1. Introducción.

Una fracción de los plaguicidas aplicados en prácticas agrícolas puede ser emitida a la atmósfera durante la pulverización [62]. Varios días o semanas después de la aplicación, los plaguicidas pueden ser transferidos a la atmósfera mediante procesos de volatilización desde el suelo y las plantas [65]. Los plaguicidas están presentes de forma simultánea tanto en la fase particulada como en la fase gaseosa. Su distribución entre las dos fases depende de las propiedades fisicoquímicas del plaguicida empleado, como la presión de vapor o su solubilidad en el agua [86-88, 224].

Por otra parte, la contaminación del aire en ambientes interiores y sus posibles efectos toxicológicos propicia cierta preocupación en la población, debido a que un gran porcentaje de nuestro tiempo (aproximadamente un 80 %) lo pasamos en sitios cerrados, estando potencialmente expuestos a una gran cantidad de contaminantes, incluyendo los plaguicidas [225-226]. Además, en los últimos tiempos, el incremento del uso de plaguicidas en los hogares ha provocado un aumento de la vigilancia y del posible riesgo a la exposición a éstos en ambientes interiores [91, 227]. Los insecticidas, son, en general, los plaguicidas más empleados en ambientes interiores (piretroides y organofosforados, sobretodo).

Para la determinación de la fase gaseosa de los plaguicidas presentes en la atmósfera, se siguen tres etapas principales: a) muestreo; b) extracción de la muestra; c) análisis de los plaguicidas.

Para la captación de la fase gaseosa de los plaguicidas, varios adsorbentes (tanto individuales como mezclas) se han empleado de forma habitual para el muestreo: PUF [142, 145]; XAD-2 [136, 143, 228-232]; XAD-4 [141]; sándwich PUF-XAD2 [229]; Tenax-GC [230] y Carbopack [231]. Sin embargo, en la gran mayoría de casos, los adsorbentes empleados no habían sido validados previamente para el análisis de los plaguicidas prioritarios. Por tanto, sin la validación del adsorbente adecuado, no es seguro que se hubiera realizado una retención adecuada y la concentración final obtenida no fuera la correcta.

Para la selección del adsorbente adecuado, es necesario conocer su capacidad de retención y el breakthrough (colmatación) para los plaguicidas estudiados. La capacidad de retención permite identificar la cantidad de plaguicida perdida durante la captación de la muestra. Por otra parte, el breakthrough nos permite conocer la cantidad máxima de plaguicida que puede ser retenida en el adsorbente específico [228-231] en condiciones de muestreo (tiempo y flujo adecuado).

La etapa de extracción de la muestra tiene como objetivo extraer la mayor cantidad de plaguicidas posibles del adsorbente elegido. Las metodologías empleadas habitualmente que incluyen la extracción líquido-líquido (principalmente Soxhlet) han sido sustituidas por metodologías que reducen el tiempo de extracción y el consumo de disolventes como la extracción con fluidos presurizados (PLE) o la extracción asistida por microondas (MAE) [133, 134, 139, 142].

Para los plaguicidas apolares y volátiles, la cromatografía gaseosa es la técnica más adecuada debido a su gran eficiencia para la separación de los analitos y la disponibilidad de un amplio rango de detectores [90, 136, 141, 230, 233, 233].

Este capítulo 3 de la Tesis tiene tres objetivos principales: i) la selección del adsorbente adecuado para el muestreo de un amplio rango de plaguicidas tanto en la atmósfera como en ambientes interiores; ii) la optimización de los parámetros del GC-MS/MS y iii) la validación del método analítico empleando MAE y GC-MS/MS para la aplicación en muestras reales.

7.2. Resultados.

7.2.1. Optimización de los parámetros del MS-MS.

Para la optimización de los parámetros del MS/MS, primero fue necesario obtener un espectro en modo barrido full-scan para seleccionar los iones más abundante y selectivo para cada plaguicida, seleccionando los dos más abundantes para cada compuesto. La Tabla 12 muestra las dos transiciones seleccionadas para cada plaguicida, la más intensa (en negrita) para la cuantificación y la segunda, para la confirmación.

Tabla 12. Parámetros experimentales seleccionados para cada plaguicida

Analito	TR (min)	Transiciones (m/z)
Etoprofos	11.26	157.91>129.81/ **157.91>113.82**
Trifluralina	11.31	**306.16 > 264.10**/ 306.16 > 159.95
Difenilamina	11.36	**169.06>168.05**/ 169.06>167.04
Clorprofam	11.59	213.03 > 126.96/ **213.03 > 170.98**
Diazinon	12.86	**179.13 > 137.04**/ 179.13 > 164.06
Lindano	12.98	**181>144.92**/ 219>182.99
Pirimetanil	13.43	**199.18 > 198.16**/ 198.11 > 117.95
Metil clorpirifos	14.33	**286.06 > 271.03**/ 286.06 > 240.98
Vinclozolina	14.38	212.04 > 144.91/ **212.04 > 171.96**
Tolclofos-metil	14.54	**265.04 > 250.05**/ 265.04 > 220.03
Fenitrotion	15.30	**277.19 > 260.09**/ 277.19 > 108.98
Malation	15.42	173.08 > 126.96/ **173.08 > 98.89**
Etil clorpirifos	15.59	**314.07 > 257.99**/ 198.89 > 170.92
Aldrin	15.73	**263>227.96/** 263>192.87
Triadimefon	15.99	**208.09 > 181.02**/ 208.09 > 110.88
Fipronil	16.94	367.02 > 255.02/ **367.08 > 213.03**
Penconazol	16.96	**248.13 > 156.97**/ 248.13 > 192.04
Folpet	17.76	**260.03 > 129.92**/ 260.03 > 232.07
Alfa-endosulfan	18.19	195>158.91/ **241>205.69**
Dieldrin	19.10	277>240.90/ **279>242.94**
Kresoxim-metil	19.15	206.05 > 131.03/ **206.05 > 116.00**
Fludioxonil	19.54	248.14 > 153.98/ **248.14 > 126.96**
Beta-endosulfan	20.38	195>158.92/ **241>205.69**
Quinoxifen	21.53	272.13 > 237.16/ **237.04 > 208.11**
Endosulfan-sulfato	21.55	**272>236.76**/ 273>238.93
Propargita	22.18	**135.05 > 107.02**/ 135.05 > 76.99
Bifentrina	22.85	**181.05 > 165.01**/ 181.05 > 166.04
Iprodiona	23.20	**314.08 > 245.02**/ 314.08 > 271.16
Dicofol	23.65	250.99 > 110.94/ **250.99 > 138.94**

Lambda-cialotrina	25.75	196.99>141.02/ **181.02>151.97**
Permetrina	26.83	**183.07 > 168.01**/ 183.07 > 153.02
Ciflutrina	28.18	206.02>151/ **206.02>177.09**
Cipermetrina	30.42	**181.07>152.20/** 163.01>126.98
Deltametrina	32.43	252.88>93.18/ **180.98>151.92**
HCH Gamma D6 (Patrón Interno)	12.90	**223.92>187.24**

Cuatro factores fueron los factores seleccionados para la optimización del espectrómetro de masas: voltaje de excitación (EV, excitation voltage), tiempo de excitación (ET, excitation time), temperatura de la fuente (IST, ion source temperature), tiempo de aislamiento (IT, isolation time). Durante el tiempo en que los iones están atrapados en la trampa (IT), el potencial es alterado para seleccionar un único ion o un grupo pequeño de iones, eliminando el resto de iones. Posteriormente, se aplica un potencial (EV) para la excitación de los iones seleccionados, fragmentando el ion precursor y formándose iones productos que pueden ser estudiados (ET). IST es la temperatura de la fuente donde se forman los iones antes de ser atrapados en el analizador de masas.

El efecto de los cuatros factores en la respuesta de cada plaguicida se ha estudiado mediante un Plackett-Burman (PB), estudiando su efecto al 95 % de nivel de confianza ($p < 0.05$) (ver Tabla SI-3, anexo III). EV presentó un efecto significativo para un gran número de los plaguicidas estudiados; ET e IT presentó efecto significativo en dos de los plaguicidas e IST únicamente en uno (folpet). Así, por tanto, EV, ET e IT fueron los parámetros seleccionados para la optimización completa mediante el uso de un CCD (Central Composite Design), mientras que la IST fue fijada a 250 ºC. Para cada plaguicida fue posible una optimización individual de cada uno de los tres parámetros estudiados (ver Tabla SI-4, Anexo III). La figura 18 muestra, como ejemplo, varias superficies de respuesta obtenidas para algunos de los plaguicidas estudiados (diazinon, quinoxifen y trifluralina).

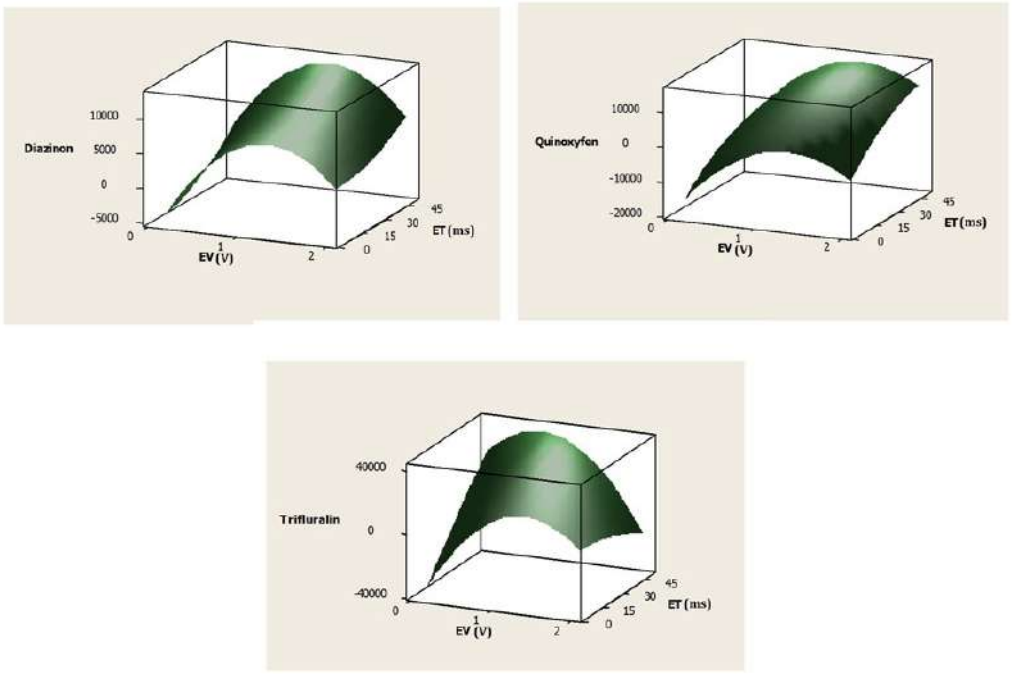


Figura 18. Superficie de respuesta obtenida para diazinon, quinoxifen y trifluralina (Se muestra el efecto de dos variables independientes, EV y ET, dejando constante la otra variable, IT).

7.2.2. Estudio del efecto matriz.

Como se puede observar en la Tabla SI-5 del Anexo III, todos los plaguicidas presentan amplificación de la señal en presencia de las matrices estudiadas. Los valores oscilan entre un 20 % (difenilamina) y un 100 % (ciflutrina). Consecuentemente, la calibración se realiza sobre la matriz, empleándose además un patrón interno para la cuantificación de los plaguicidas detectados.

7.2.3. Estudio de los adsorbentes.

Antes del estudio de la capacidad de retención, es necesario conocer la recuperación obtenida para cada adsorbente siguiendo el método analítico. Así, empleando PUF-XAD2-PUF, se ha obtenido una recuperación entre el 75 % y el 110 % para 22 de los 28 plaguicidas que presentan una capacidad de retención adecuada en los tres niveles estudiados (2.5, 10 y 50 ng). Fenitrotion, malation y fipronil presentan buenas recuperaciones en los dos niveles más altos estudiados mientras que etil clorpirifos, clorprofam y endosulfan sulfato sólo presentan buena recuperación en el nivel más alto estudiado. Este hecho se observa en la siguiente tabla (Tabla 13):

Tabla 13. Parámetros analíticos del método empleando sándwich PUF-XAD2-PUF como adsorbente

Plaguicida	Recuperación (%) ± SD			LOQ* (pg m^{-3})	Rango (pg m^{-3})
	2.5 ng (equivalente a 16.1 pg m^{-3})	10 ng (equivalente a 64.5 pg m^{-3})	50 ng (equivalente a 322.6 pg m^{-3})		
Etoprofos	82±1	95±10	100±5	16.1	16.1-1613
Trifluralina	95±6	105±3	107±3	16.1	16.1-1613
Difenilamina	100±10	110±10	110±10	16.1	16.1-1613
Clorprofam	47±4	54±2	77.3±0.9	322.6	322.6-1613
Diazinon	85±10	95±10	105±5	16.1	16.1-1613
Lindano	105±5	110 ±10	99± 4	16.1	16.1-1613
Pirimetanil	101±6	110±20	99 ± 6	16.1	16.1-1613
Metil clorpirifos	100±3	94±7	98±2	16.1	16.1-1613
Vinclozolina	103.1±0.7	110±5	108±3	16.1	16.1-1613
Tolclofos-metil	86±7	98±7	102±7	16.1	16.1-1613
Fenitrotion	62±3	90±10	96±4	64.5	64.5-1613
Malation	44±8	110±10	93±4	64.5	64.5-1613
Etil clorpirifos	51±4	67±7	90±10	322.6	322.6-1613
Aldrin	101±5	100±4	90±10	16.1	16.1-1613
Triadimefon	107±5	100±10	91±9	16.1	16.1-1613
Fipronil	52±4	82±5	83±2	64.5	64.5-1613
Penconazol	107±3	96±4	99±6	16.1	16.1-1613
Alfa-endosulfan	104±1	110±6	105±2	16.1	16.1-1613
Dieldrin	109±6	101±8	110±10	16.1	16.1-1613
Beta-endosulfan	95±3	90±10	102± 10	16.1	16.1-1613
Quinoxifen	102±6	94±6	90±10	16.1	16.1-1613
Endosulfan-sulfato	52±6	65±4	99 ± 8	322.6	322.6-1613
Bifentrina	95±4	110±10	110±10	16.1	16.1-1613
Iprodiona	106±4	94±7	90±10	16.1	16.1-1613
Lambda-cialotrina	110±8	90±10	107 ± 10	16.1	16.1-1613
Permetrina	98.0±0.7	108±2	110±10	16.1	16.1-1613
Cipermetrina	107±4	98±7	95±5	16.1	16.1-1613
Deltametrina	102±3	95±5	90±10	16.1	16.1-1613

* Usando PUF-XAD2-PUF como adsorbente y empleando un captador de bajo volumen (Volumen Total= 155 m^3).

Para el XAD-2, 10 de los 22 plaguicidas estudiados con una adecuada capacidad de retención presentan buenas recuperaciones en los tres niveles estudiados (pirimetanil, malation, etil clorpirifos, triadimefon, penconazol, folpet, alfa-endosulfan, dieldrin, propargite y ciflutrin). Los otros 12 plaguicidas únicamente presentan buenas recuperaciones al nivel de fortificación más elevado (ver Tabla SI-6, Anexo III).

Para el XAD-4, sólo 6 de los plaguicidas con buena capacidad de retención presentan una recuperación adecuada para los tres niveles estudiados (etoprofos, diazinon, metil clorpirfos, tolclofos-metil, clorprofam y lindano). Pirimetanil, fenitrotion, etil clorpirifos, triadimefon, beta-endosulfan, iprodiona, lambda cihalotrina y cipermetrina presentan buenas recuperaciones en los dos niveles más altos, mientras que el resto sólo presentan recuperaciones adecuadas al nivel más alto (ver Tabla SI-7, Anexo III).

7.2.4. Capacidad de retención y evaluación del breakthrough.

Se ha evaluado la capacidad de retención para los tres adsorbentes empleados para seleccionar el más adecuado para la captación de la fase gaseosa. La fórmula empleada para el cálculo de la capacidad de retención ha sido la siguiente (ecuación 11):

Capacidad de retención (%)= [plaguicida obtenido] / [plaguicida fortificado] * 100 (11)

La siguiente Tabla (Tabla 14) muestra los resultados obtenidos de capacidad de retención para los tres adsorbentes estudiados:

Tabla 14. Capacidad de retención para los adsorbentes empleados (n=3) (nivel de fortificación alto=50 ng)

Plaguicida	PUF-XAD2-PUF Capacidad de retención (%)± SD	XAD-2 Capacidad de retención (%)± SD	XAD-4 Capacidad de retención (%)± SD
Etoprofos	92±5	-	82±2
Trifluralina	96±5	-	79±4
Difenilamina	99±2	87±5	-
Clorprofam	90±10	91±4	77±3
Diazinon	85±10	76±3	78±4
Lindano	88±4	-	81±5
Pirimetanil	81±3	82±6	84±3
Metil clorpirifos	85±2	83±2	81±5
Vinclozolina	99±3	-	-
Tolclofos-metil	85±5	79±5	85±5
Fenitrotion	100±8	-	84±2
Malation	92±2	86±4	-
Etil clorpirifos	105±4	76±3	79±5
Aldrin	83±4	-	75±4
Triadimefon	92±7	84±2	83±6
Fipronil	83±9	-	77±2
Penconazol	76±2	84±7	-
Folpet	-	-	-
Alfa-endosulfan	91±6	75±6	71±4
Dieldrin	84±4	85±3	-
Kresoxim-metil	-	88±4	71±2
Fludioxonil	-	-	-
Beta-endosulfan	87±6	-	77±2
Quinoxifen	86±4	-	-
Endosulfan-sulfato	77±3	79±3	-
Propargita	-	75±4	-
Bifentrina	89±2	77±5	78±1
Iprodiona	95±5	101±3	77±3
Dicofol	-	-	-
Lambda-cialotrina	80±2	82±3	77±4
Permetrina	95±1	79±3	-
Ciflutrina	-	78±4	-
Cipermetrina	85±3	86±2	80±5
Deltametrina	86±2	75±2	-

Cómo se puede observar, empleando el sándwich PUF-XAD2-PUF, 28 de los 34 plaguicidas estudiados presentaron una capacidad de retención que osciló entre el 75 % y el 100 %. Únicamente folpet, kresoxim-metil, fludioxonil, propargita, dicofol y ciflutrina presentaron valores por debajo del 70 %. En el caso del XAD-2, 22 de los plaguicidas estudiados presentaron una capacidad de retención adecuada y 20 plaguicidas presentaron una capacidad de retención adecuada empleando como adsorbente XAD-4.

El breakthrough fue evaluado por separado, analizando la sección inferior del adsorbente, para comprobar si se ha producido un traspaso desde la sección superior hasta sección inferior. Para la evaluación del breakthrough se ha seguido el criterio descritos por Tsirapoulos et al. (2006) [234]: se determinará que existe breakthrough cuando los niveles detectados en la sección inferior sean superiores al 1 % del nivel detectado en la sección superior. Este ensayo se realizó por triplicado, empleando

también un blanco analítico. Únicamente se detectaron concentraciones en la parte inferior para trifluralina, malation y etil clorpirifos en los tres adsorbentes empleados, siendo inferiores al límite de cuantificación para cada uno de ellos. En el resto de plaguicidas estudiados, no se detectó nada en la parte inferior.

Así, por tanto, teniendo en cuenta la capacidad de retención y el breakthrough, el adsorbente seleccionado fue el sándwich PUF-XAD2-PUF.

7.2.5. Parámetros analíticos del método.

La validación del método fue realizado empleando como adsorbente el sándwich PUF-XAD2-PUF. Se observó una linealidad adecuada (R^2> 0.99) para la curva de calibrado realizada en matriz entre 5 y 500 ng mL^{-1}. La exactitud y la precisión se evaluaron mediante la recuperación a tres niveles (2, 10 y 50 ng, equivalentes a 16.1, 64.5 y 322.6 pg m^{-3}) (ver Tabla 13), obteniéndose recuperaciones adecuadas (70-120 %) para la gran mayoría de plaguicidas con CV < 30 %.

El límite de cuantificación (ver Tabla 13), se ha determinado como la concentración más baja en la cual se ha obtenido una recuperación y precisión adecuada. Así, los niveles de LOQ oscilan entre 16.1 y 322.6 pg m^{-3}, al emplear un volumen de aire de 155 m^3. Si comparamos los LOQ obtenidos con los obtenidos en estudios previos tanto en la atmósfera [235] como en ambientes interiores [236-240] son valores similares, lo que nos permite afirmar que nuestra metodología es adecuada para la captación de la fase gaseosa de los plaguicidas tanto en la atmósfera como en ambientes interiores.

7.2.6. Aplicación a muestras reales.

Para el estudio de la aplicabilidad de la metodología desarrollada, se analizaron 10 muestras de aire recogidas en viviendas desde el mes de Julio al mes de Diciembre de 2015 en la Comunidad Valenciana, empleando las condiciones descritas en el apartado 4 de metodología. Los resultados obtenidos se muestran en la Tabla 15.

Se han detectado un total de 6 plaguicidas en al menos una muestra. Las frecuencias de detección oscilan entre un 20 % y el 100 % (porcentaje de las muestras por encima del LOD), detectándose cipermetrina y lambda cihalotrina en todas las muestras. Las concentraciones de los plaguicidas detectados oscilan entre 1.46 ng m^{-3} (bifentrina) y 22.02 ng m^{-3} (lambda cihalotrina).

Tabla 15. Concentración de plaguicidas en fase gaseosa en 10 viviendas de la Comunidad Valenciana (España) (ng m^{-3})

Plaguicidas	**Muestras**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Bifentrina	6.72	-	<LOQ	1.46	-	-	-	-	-	-
Cipermetrina	16.26	15.32	17.59	15.58	9.73	8.07	3.09	20.03	17.83	21.46
Difenilamina	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lambda cihalotrina	14.86	14.76	16.45	16.25	9.50	10.60	<LOQ	22.02	14.72	17.54
Permetrina	16.23	11.55	2.31	<LOQ	<LOQ	2.97	<LOQ	14.72	10.83	15.10
Pirimetanil	17.47	<LOQ	5.06	<LOQ	<LOQ	<LOQ	<LOQ	11.65	<LOQ	2.70

La siguiente figura (Figura 19), muestra el cromatograma de una muestra positiva:

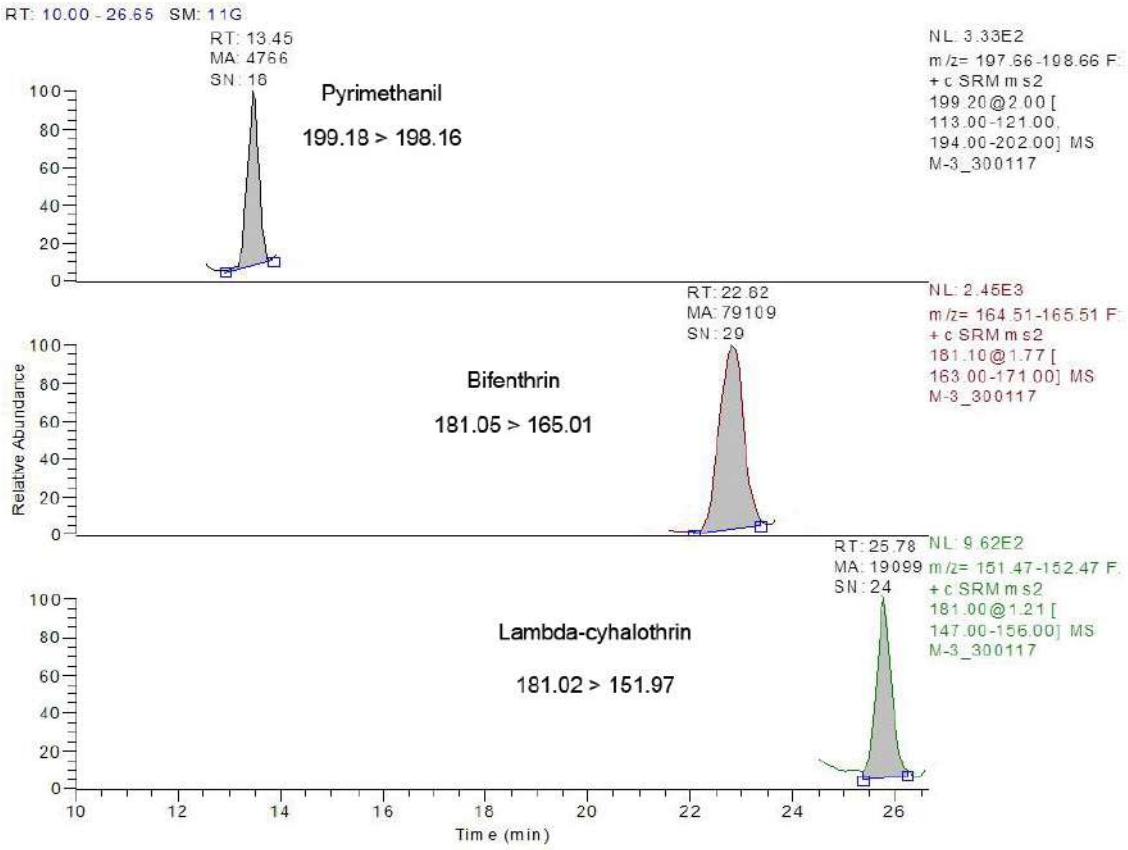


Figura 19. Cromatograma de una muestra positiva que contiene: pirimetanil: 17.47 ng m^{-3}, bifentrina: 6.72 ng m^{-3} y lambda-cyhalothrin: 14.86 ng m^{-3}.

Los niveles detectados de bifentrina en este estudio han sido similares a los detectados por Bradman et al., 2007 [240] en EEUU (concentración máxima de bifentrina detectada fue de 3.1 ng m^{-3}) y mayores que los niveles detectados en Tailandia por Pentamwa et al., (2008) [241] (niveles que oscilaron entre 0.02 y 0.59 ng m^{-3}).

En el caso de la cipermetrina, los niveles detectados fueron similares a los detectados en EEUU por Lu et al., (2013) [239] (concentración máxima de 5.5 ng m^{-3}) y mayores que los niveles detectados en Francia por Laborie et al., (2006) [237] (concentración media de 4.7 ng m^{-3}).

Los valores de lambda cihalotrina obtenidos fueron superiores a los detectados por Lu et al., (2013) (concentración media de 0.52 ng m^{-3}). Los niveles de permetrina obtenidos fueron superiores a los detectados en Tailandia [241] (concentración máxima de 0.34 ng m^{-3}) y similares a los obtenidos en EEUU [240].

7.3. Conclusiones.

Las conclusiones a las que se han llegado después de la realización de este trabajo son las siguientes:

- El sándwich PUF-XAD2-PUF fue seleccionado como el adsorbente más adecuado para el muestreo de la fase gaseosa de 34 plaguicidas (incluyendo persistentes y de uso habitual en la agricultura). La estrategia analítica ha incluido una extracción mediante MAE y una posterior determinación empleando GC-MS/MS, con LOQ que variaban desde 16.1 hasta 322.6 pg m^{-3}.

- El método desarrollado puede ser empleado tanto para ambientes exteriores como para ambientes interiores. Empleando la metodología desarrollada, se analizaron 10 muestras procedentes de viviendas de la Comunidad Valenciana. Seis plaguicidas fueron detectados en al menos una muestra, con concentraciones entre 1.46 y 22.02 ng m^{-3}. En un futuro próximo, esta metodología podrá ser aplicada para el control de los niveles de plaguicidas tanto en ambientes interiores como en la atmósfera.

7.4. Artículo 3: Selection of sampling adsorbents and optimisation and validation of a GC-MS/MS method for airborne pesticides.

INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY, 2017
VOL. 97, NO. 10, 949–964
https://doi.org/10.1080/03067319.2017.1373771

ARTICLE

Selection of sampling adsorbents and optimisation and validation of a GC-MS/MS method for airborne pesticides

Antonio López[a,b,c], Clara Coscollà[a,c] and Vicent Yusà[a,b,c]

[a]Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Valencia, Spain; [b]Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Burjassot, Spain; [c]Public Health Laboratory of Valencia, Valencia, Spain

ABSTRACT

A methodology for the sampling and determination of airborne pesticides has been developed. The trapping efficiency of three adsorbents, namely XAD-2,XAD-4 and a sandwich sorbent (PUF-XAD2-PUF), was tested for 34 pesticides and the latter was selected because it presented the highest retention capacity without breakthrough. Pesticides were determined by gas chromatography coupled to an ion trap mass spectrometer in tandem. The method showed recoveries ranging from 70% to 120% with limits of quantification in the range of 16.1–322.6 pg m^{-3} when 155 m^3 were sampled. This analytical strategy was applied to 10 indoor air samples collected in dwellings from the Valencian Region. Six pesticides, namely diphenylamine, pyrimethanil, bifenthrin, lambda-cyhalothrin, permethrin and cypermethrin were detected in indoor samples with concentrations ranging from 1.46 to 22.02 ng m^{-3}.

ARTICLE HISTORY
Received 21 June 2017
Accepted 22 August 2017

KEYWORDS
Pesticides; GC-MS/MS; adsorbents; gaseous phase; air samples

1. Introduction

A broad range of pesticides can be applied both outdoors (in agriculture) and indoors. In agricultural practices, the active substance used depends on a range of factors including the specific pest and crop of interest. During 2014, about 400,000 tonnes of pesticide active ingredients were used in Europe (EU-15) [1,2] and around 500 active substances are nowadays authorised by the European Union for their application on various crops according to the Regulation (EC) 1107/2009 [3].

A major fraction of the applied pesticides can be emitted to the atmosphere during spraying in agricultural practices [4]. Within days or weeks after application, pesticides can be transferred to the air through the volatilisation process from soil and plants [5]. Airborne pesticides are simultaneously present in both particle and gaseous phases. The distribution among these phases depends on the physicochemical properties of the compound considered, such as vapour pressure and water solubility. Different models have been proposed to describe this gaseous/particle phases partitioning [6–9]. On the other hand, indoor air pollution and its toxicological effects have become a growing concern, since humans spend more than 80% of their time in indoor environments, where they are potentially exposed to a wide variety of pollutants including pesticides [10,11]. Moreover, the increased use of

CONTACT Vicent Yusà yusa_vic@gva.es

pesticides for pest control in homes has claimed extra surveillance to the risk of indoor exposure to these substances [12,13]. In general, insecticides are commonly used in indoors ambients (synthetic-pyrethroid and organophosphate pesticide classes, mainly). Nowadays, most persistent pesticides have been banned or strongly restricted in many countries but they are still detected in the environment due to the intensive use in the past and in indoor ambients due to the indoor–outdoor exchange [14].

The pesticide determination in the gaseous phase of the ambient air includes three main steps: (a) sampling; (b) sample extraction and (c) the analytical determination of the pesticides.

For trapping airborne pesticides, many adsorbents including polyurethane foam (PUF) [15,16], XAD-2 [17–23], XAD-4 [24], PUF-XAD2 sandwich [18], Tenax-GC [19] and Carbopack B [19] have been described and used in different studies. However, in many studies, the selected adsorbent had not been previously validated for the target pesticides. Consequently, in these studies and monitoring programmes it is not sure that the adsorbent employed had an appropriate retention capacity for the target pesticides and consequently the final concentration could be misleading.

For choosing an appropriate adsorbent, it is necessary to know its retention capacity and its breakthrough for the target pesticides. The retention capacity allows identifying the pesticide levels loss at trapping during air sampling. Breakthrough evaluation enables to know the maximum amount of pesticide that can be retained on a specific mass of adsorbent [17,20] in the sampling conditions given (time, flow).

The extraction step aims to extract the largest amount of pesticide from the sampling material. Methodologies that involve liquid–solid extraction of pesticides, mainly Soxhlet extraction, have been used usually for the pesticide extraction [16,25–27]. Accelerated solvent extraction and microwave-assisted extraction (MAE) are alternatives procedures for the pesticide extraction from the sampling materials. MAE allows reduction of both extraction time and organic solvent consumption compared to other extraction methodologies such as Soxhlet.

For apolar and volatile pesticides, gas chromatography (GC) is the most frequently employed technique due to its excellent separation efficiency and the availability of a wide range of sensitive and specific detectors [19,21,23,24,28,29].

We have carried out a study in the field of airborne pesticides in order to achieve three main goals: (a) to select the appropriate solid sorbent for sampling a wide range of current and persistent pesticides in ambient air and indoor air; (b) to optimise the gas chromatography coupled to an ion trap mass spectrometer in tandem (GC-MS/MS) parameters; and (c) to validate the analytical method using MAE and GC-MS/MS for application to real samples.

In a previous work, we have developed a method for the determination of pesticides in airborne particulate matter [30]. Here we are focused on the gaseous phase.

2. Experimental

2.1. *Reagents and chemicals*

The following high-purity standard pesticides were supplied by Dr. Ehrenstorfer (Augsburg, Germany): aldrin (99%), bifenthrin (99.5 %), chlorpropham (99.5 %),

chlorothalonil (99.5%), cypermethrin (99%), cyfluthrin (99.5%), deltamethrin (99.5%), diazinon (96%), dieldrin (99%), dicofol (99%), diphenylamine (99%), α-endosulfan (97%), β-endosulfan (98%), endosulfan-sulphate (97%), ethyl-chlorpyrifos (99.5%), ethoprophos (93%), fenitrothion (97.5%), fipronil (98%), fludioxonil (99%), folpet (99%), HCH gamma D6 (97.5%), iprodione (99.5%), kresoxim-methyl (98%), lambda-cyhalothrin (98%), lindane (99%), malathion (99%), methyl-chlorpyrifos (98.5%), penconazole (99%), permethrin (99%), pyrimethanil (97.5%), propargite (94.5%), quinoxyfen (99%), tolclofos-methyl (98.3%), triadimefon (99.5%), trifluralin (99.5%) and vinclozolin (99%).

Individual stock standards were prepared weighting 10 mg of pure standard using a five-decimal analytical balance and dissolving each compound in 50 mL of acetone. They were stored in capped amber vials at −21°C [31]. Mix working solutions at 1 and 10 µg mL^{-1} were prepared with acetone. Calibration solutions (5, 10, 20, 50, 100, 250, 500 and 1000 ng mL^{-1}) were prepared adding variable volumes of the mix working solutions in matrix (matrix matched standards).

Ethyl acetate (UV-IR-HPLC preparative) and acetone for GC was purchased from Merck (Darmstadt, Germany). *N*-Hexane 99% HPLC grade was supplied by Scharlau (Sentmenat, Spain). Nonane puriss p.a. standard for GC was supplied by Fluka (Steinheim, Switzerland).

The following three solid sorbents were employed: XAD-2 (Sigma-Aldrich, Barcelona, Spain), PUF (BSG Ingenieros, Valencia, Spain) and XAD-4 (Sigma-Aldrich). Amberlite XAD-2 polymeric adsorbent is a hydrophobic cross-linked polystyrene copolymer resin. The resin is widely employed to adsorb soluble organic compounds from aqueous streams and organic solvents, generally in cyclic columnar operations. PUF is white and turns yellow upon exposure to light. PUFs are good for trapping volatile compounds and they are commonly used for the sampling of gaseous persistent organic pollutants like polychlorinated biphenyls and organochlorine pesticides. XAD-4 is a polymeric adsorbent supplied as white insoluble beads. It is a non-ionic cross-linked polymer which has adsorptive properties due to its macroreticular structure (containing both a continuous polymer phase and a continuous pore phase), its high surface area and the aromatic nature of its surface.

A Partisol 2300 low-volume sampler (Thermo Fisher Scientific, Bremen, Germany) was employed coupled to ChemComb cartridge (Thermo Fisher Scientific).

2.2. *Sample collection and extraction*

For testing the sorbents, we worked at field conditions: sampling around 160 m^3 of air in total, using a flow air of 1 m^3 h^{-1} during 1 week. The tested solid sorbents presented two sections: the upper section was used for evaluating the retention efficiency during sampling and the lower section was employed for evaluating the breakthrough. In total, 5 g of XAD-2 or XAD-4 were weighed, individually. Sandwich PUF-XAD2-PUF was composed of 5 g of XAD-2 between two PUFs of 1.4 cm long.

A generic extraction method developed using MAE with ethyl acetate was employed to recover pesticides from the three studied sorbents. Microwave extraction of pesticides was carried out using a MARS System from CEM Corporation (Mathews, NC, USA) equipped with Teflon® TFM 100-mL extraction vessels. The corresponding solid sorbent was extracted at 50°C for 20 min, using a power of 1200 W and 30 mL of ethyl acetate [31]. After cooling, the reactor was opened and the extracts were filtered. Then, 20 µL of nonane (keeper) were added to the extract and concentrated with Turbo Vap 500

(Zymark, Idstein, Germany). The extracts were redissolved with 0.5 mL of *n*-hexane and filtered through a 0.22-µm GHP Acrodisc filter from Pall Life Sciences (Ann Arbor, MI, USA) prior to injection in the GC-MS/MS.

2.3. *Optimisation of GC-ion trap mass spectrometer (ITMS)*

Analyses were performed on a Finnigan ITMS Polaris Q (Austin, TX, USA). The mass spectrometer was connected by a heated transfer line to a Thermoquest Trace GC 2000 (Waltham, MA, USA) gas chromatograph equipped with a Combi Pal Autosampler from CTC Analytics AG (Zwingen, Switzerland). The analyses were carried out with a 30 m × 0.25 mm i.d., 0.25-µm film thickness SGE-BPX5 capillary column (Trajan, Austin, TX, USA). The carrier gas was helium (constant flow, 1.2 mL min^{-1}). A PTV Silcosteel liner of 1 × 2.75 × 120 mm was installed in the split/splitless injector and the temperature was set at 250°C. A high-pressure Microseal septum was purchased from Supelco (Bellefonte, PA, USA). The GC temperature programme was as follows: initial 90°C, hold 5 min; rate 25°C min^{-1}, to 180°C; rate 5°C min^{-1}, to 280°C, hold 3 min; rate 10°C min^{-1}, to 300°C, hold 3 min. At last, the exposition column is heated at 310°C during 3 min for removing possible contaminated interferences. The transfer line was set at 250°C. The electron impact ionisation was selected to work with an electron energy (EE) of 70 eV. The ionisation source temperature was set at 250°C. XCalibur 1.2 was used for data acquisition.

According to the literature [32–37], five main factors affect the Ion Trap MS/MS: excitation voltage (EV), excitation time (ET), ion source temperature (IST), isolation time (IT) and EE. However, the EE should not be changed since library fragments are obtained at 70 eV. Consequently, EV, ET, IST and IT were optimised.

A Plackett–Burman (P-B) design was selected as a screening method to evaluate the four selected factors that could have an influence on the analytical response [38]. The P-B design assumes that the interactions can be completely ignored and so the main effects are calculated with a reduced number of experiments (12 plus a triplicate central point) (Table SI-1). The estimated effects of the factors and their statistical significance at 95% confidence level ($p < 0.05$) were studied. In order to have generous degrees of freedom for testing the statistical significance of the estimated effects, 12 runs plus a triplicate centre point were used. The minimum, maximum and centre point values used in the P-B were as follows: EV (0.1–1.05–2 V), ET (5–27.5–50 ms), IST (150°C–200°C–250°C), IT (4–19–34 ms).

Then, after the selection of the factors that potentially affect the MS/MS, a central composite design (CCD) was employed and preferred to one-factor-at-a-time to optimise analytical methods [39]. The CCD design allows a more accurate optimisation of the three significant parameters (EV, ET and IT). This design consists of a full factorial design (eight cube points, six centre points in cube, six axial points). The 20 runs were randomised to provide protection against the effect of hidden variables. The values corresponding to every factor in each experiment and the responses for each compound are shown in Table SI-2. This experimental design allows to obtain the response surface and the factor settings or operating conditions to maximise pesticide response. These factor settings (EV, IT and ET) were individually studied to maximise the response for each of the 34 compounds using the response optimiser in the Minitab programme. The response optimiser parameters were as follows:

'low': the minimum response for each pesticide obtained in the CCD experiment, 'goal': maximise and 'target': the maximum response for each compound obtained in the CCD.

Numerical analysis of data resulting from the experimental design was carried out using statistical package MINITAB for Windows, Release 14, Minitab INC (State College, PA, USA).

2.4. Retention capacity and breakthrough

Retention capacity was assessed by spiking the sorbents (PUF-XAD2-PUF, XAD-2 and XAD-4) with 50 ng (equivalent to 322 pg m^{-3} at the sampling condition) and by pulling air through using a constant air flow (1 m^3 h^{-1}) during 1 week using Partisol 2300 (low volume sampler). This test was performed in triplicate. The recovery study of pesticides in each solid sorbent allowed us to quantify the pesticide loss during sampling. Retention capacity was calculated using the following equation:

$$\text{Retention capacity}(\%) = [\text{pesticide obtained}]/[\text{pesticide spiked}] * 100$$

Moreover, breakthrough was evaluated by separately analysing the lower section of the sorbent for the eventual passage of the analytes from the first to the second section. For testing the breakthrough, sampling conditions were as follows: a flow air of 1 m^3 h^{-1} during 1 week was employed (around 160 m^3). The criterion used to evaluate the breakthrough was the one described by Tsiropoulos et al. [40]: when the pesticide level detected in the lower section of the cartridge was greater than 1% of that found in the upper section. These assays were carried out in triplicate together with an analytical blank.

2.5. Matrix effect

Matrix effect was evaluated by comparing the response of standard prepared in pure solvent with the response of matrix-matched standard. The following equation was employed: $ME(\%) = B/A * 100$ (A: standard in pure solvent and B: spiked solid sorbent). Both A and B sets had concentrations of 322.6 pg m^{-3}.

2.6. Validation study and quality control (QC) protocol

There is no available reference material of pesticides in airborne gaseous phase, so the method validation was carried out using spiked solid sorbents samples at three levels (2, 10 and 50 ng equivalents to 16.1, 64.5 and 322.6 pg m^{-3}) with three replicates at each spiked level. The analytical characteristics evaluated were accuracy (measured as mean recovery), precision (expressed at repeatability), sensitivity and specificity. Linearity was studied using matrix-matched standards, analysing each of them in triplicate at eight concentrations between 5 and 500 ng mL^{-1} in vial. The limit of quantification (LOQ) was established checking the signal to noise (S/N) of the quantification ion at decreasing concentrations of a spiked matrix solution, establishing it at S/N > 10 and checking good pesticide recoveries at that level. The S/N for the confirmation ion was in all cases above 3. Each set of solid sorbents were analysed under quality assurance protocols, including process blanks, spiked blank samples, blank sample and reagent blanks. Two process blanks were used, consisting of a sealed envelope containing solid sorbent, and were used as QC samples during deployment, retrieval

and transportation of the field filters. These field blanks were processed and analysed in the same way as the analytical samples. The analysis of solid sorbents was performed immediately after sampling or after a storage period at −20°C (3 months maximum).

In order to check potential losses during the storage period and assess recoveries during sample preparation, spiked blank samples were stored and analysed as field samples. Blank samples were baked 24 h at 130°C in the oven to eliminate possible pollutants in the matrix.

2.7. *Identification and confirmation criteria*

For a positive identification of the substances (confirmation criteria) in accordance with the SANTE guidelines [30], the following rules were applied: (a) monitoring of two or more SRM transitions per compound, (b) the GC relative retention time of the analyte in the sample must be within 0.1 min of the retention time of the standard, (c) the relative abundance of the SRM transition signals must be within 30% of the ratio obtained for the standards, and (d) the S/N of the two diagnostic ions must be >3.

3. Results and discussion

3.1. *Optimisation of MS-MS parameters*

An optimisation of MS/MS parameters is required in order to maximise the signal for each pesticide in multiresidue methods. The first step of the MS/MS optimisation was to obtain a full-scan spectra of each pesticide in order to select the most selective and abundant ion (as the precursor ion). Precursor ions were isolated in the ion trap and fragmented by collision-induced dissociation, selecting the two most abundant product ions for each compound. This MS/MS experiment was carried out with the default parameters provided by ITMS system. Based on the elution profile of the pesticides, a time-scheduled acquisition method was built, comprising eight time windows, between 6 and 12 transitions, each. Table 1 shows the two monitored transitions for each pesticide, the most intense one (in bold) for quantification and the second one, for confirmation.

Four factors are usually selected such as EV, ET, IST and IT. During the time in which the ions are maintained in the trap (IT), voltages are altered to isolate a single ion or ion window, thus retaining only the ion of interest and purging everything else. Then, voltages are applied to excite (EV) and fragment the precursor ion into product ions all of which can then be scanned out (ET). The IST is the temperature of the source where the ions are formed before being trapped by the mass analyser.

The estimated effects in the P-B of the four factors and their statistical significance at 95% confidence level ($p < 0.05$) are shown in Table SI-3. As can be seen in Table SI-3, EV had a significant effect on the analytical response for most of the pesticides studied. Both ET and IT had a significant effect in two pesticides and IST had a significant effect only in one pesticide (folpet). Consequently, EV, ET and IT were selected for further optimisation, and IST was set at 250°C. To optimise these parameters, the variation of the response (peak area) at different values of ET, EV and IT was studied using a CCD (Table SI-2) [38].

Table 1. Selected GC-MS/MS experimental parameters for each pesticide.

Analyte	RT (min)	Transitions (m/z)
Ethoprophos	11.26	157.91 > 129.81/**157.91 > 113.82**
Trifluralin	11.31	**306.16 > 264.10**/306.16 > 159.95
Diphenylamine	11.36	**169.06 > 168.05**/169.06 > 167.04
Chlorpropham	11.59	213.03 > 126.96/**213.03 > 170.98**
Diazinon	12.86	**179.13 > 137.04**/179.13 > 164.06
Lindane	12.98	**181 > 144.92**/219 > 182.99
Pyrimethanil	13.43	**199.18 > 198.16**/198.11 > 117.95
Chlorpyrifos-methyl	14.33	**286.06 > 271.03**/286.06 > 240.98
Vinclozolin	14.38	212.04 > 144.91/**212.04 > 171.96**
Toldofos-methyl	14.54	**265.04 > 250.05**/265.04 > 220.03
Fenitrothion	15.30	**277.19 > 260.09**/277.19 > 108.98
Malathion	15.42	173.08 > 126.96/**173.08 > 98.89**
Chlorpyrifos-ethyl	15.59	**314.07 > 257.99**/198.89 > 170.92
Aldrin	15.73	**263 > 227.96**/263 > 192.87
Triadimefon	15.99	**208.09 > 181.02**/208.09 > 110.88
Fipronil	16.94	367.02 > 255.02/**367.08 > 213.03**
Penconazole	16.96	**248.13 > 156.97**/248.13 > 192.04
Folpet	17.76	**260.03 > 129.92**/260.03 > 232.07
Alfa-endosulfan	18.19	195 > 158.91/**241 > 205.69**
Dieldrin	19.10	277 > 240.90/**279 > 242.94**
Kresoxim-methyl	19.15	206.05 > 131.03/**206.05 > 116.00**
Fludioxonil	19.54	248.14 > 153.98/**248.14 > 126.96**
Beta-endosulfan	20.38	195 > 158.92/**241 > 205.69**
Quinoxyfen	21.53	272.13 > 237.16/**237.04 > 208.11**
Endosulfan-sulphate	21.55	**272 > 236.76**/273 > 238.93
Propargite	22.18	**135.05 > 107.02**/135.05 > 76.99
Bifenthrin	22.85	**181.05 > 165.01**/181.05 > 166.04
Iprodione	23.20	**314.08 > 245.02**/314.08 > 271.16
Dicofol	23.65	250.99 > 110.94/**250.99 > 138.94**
Lambda-cyhalothrin	25.75	196.99 > 141.02/**181.02 > 151.97**
Permethrin	26.83	**183.07 > 168.01**/183.07 > 153.02
Cyfluthrin	28.18	206.02 > 151/**206.02 > 177.09**
Cypermethrin	30.42	**181.07 > 152.20**/163.01 > 126.98
Deltamethrin	32.43	252.88 > 93.18/**180.98 > 151.92**
HCH Gamma D6 (Internal Standard)	12.90	**223.92 > 187.24**

Figure 1 shows, as an example, some response surfaces obtained by using the three dimensional response surfaces for diazinon, quinoxyfen and trifluralin. The three-dimensional response surfaces show the effect of two independent variables (EV and ET) on a given response, at a constant value of the other independent variable (IT).

For each compound, an individual optimisation of each studied parameter is possible. Table SI-4 shows the optimised value for EV, ET and IT for each pesticide studied.

3.2. *Matrix effect study*

All pesticides presented a high-signal enhancement in presence of all matrices ranging from 20% (diphenylamine) to 100% (cyfluthrin) (see Table SI-5). Consequently, a matrix-matched calibration method and an internal standard were used for an accurate quantification.

3.3. *Recovery study*

Before studying the retention capacity, we checked sorbent recovery when using the analytical method. Table 2 illustrates the recoveries at three levels of pesticides (only pesticides with good retention capacity) using PUF-XAD2-PUF as solid sorbent. As can

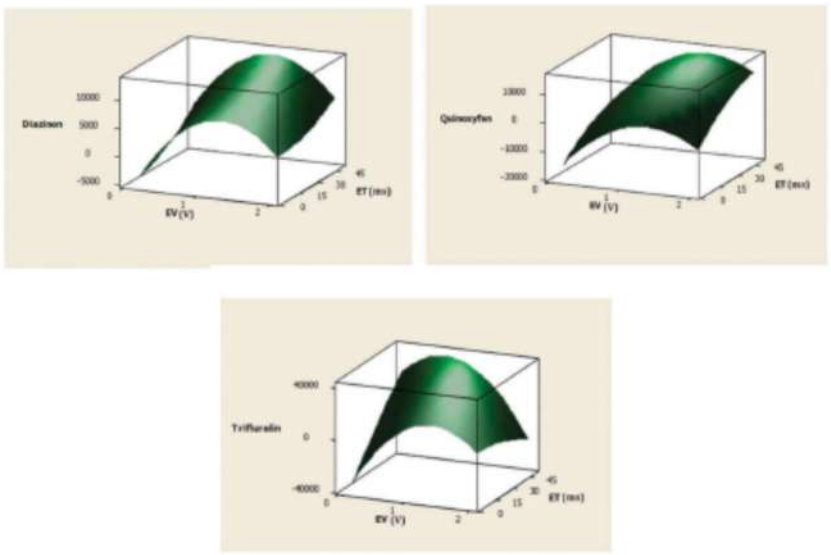


Figure 1. Response surface for diazinon, quinoxyfen and trifluralin.

be seen, using PUF-XAD2-PUF, 22 out of the 28 currently used pesticides (CUPs) studied with good retention capacities presented recoveries between 75% and 110% at the three levels studied. Fenitrothion, malathion and fipronil presented good recoveries at two higher levels studied and chlorpyrifos-ethyl, chlorpropham and endosulfan-sulphate only presented good recoveries at the highest level studied (50 ng).

For XAD-2, 10 out of the 22 CUPs studied with good retention capacities presented good recoveries (pyrimethanil, malathion, chlorpyrifos-ethyl, triadimefon, penconazole, folpet, alfa-endosulfan, dieldrin, propargite and cyfluthrin) at the three levels studied. The other 12 CUPs with good retention capacity presented good recoveries only at the highest spiked level (50 ng) (see Table SI-6). Only 6 out of the 20 CUPs with good retention capacities presented good recoveries using XAD-4 as solid sorbent (ethoprophos, diazinon, chlorpyrifos-methyl, tolclofos-methyl, chlorpropham and lindane). Pyrimethanil, fenitrothion, chlorpyrifos-ethyl, triadimefon, beta-endosulfan, iprodione, lambda-cyhalothrin and cypermethrin presented good recoveries at two high spiked levels (10 and 50 ng) and the other CUPs presented good recoveries only at the highest level (50 ng) (see Table SI-7).

3.4. *Retention capacity and breakthrough evaluation*

We evaluated the retention capacity of the three sorbents in order to choose the most appropriate for sampling the target pesticides. Table 3 shows the retention capacity of the sorbents for each of the 34 pesticides. As can be seen, using sandwich PUF-XAD2-PUF, 28 of the studied pesticides presented retention capacities ranging from 75% to

Table 2. Analytical performance of the method using sandwich PUF-XAD2-PUF as solid sorbent.

	Recovery (%) ± SD				
Pesticide	2.5 ng (equivalent to 16.1 pg m^{-3})	10 ng (equivalent to 64.5 pg m^{-3})	50 ng (equivalent to 322.6 pg m^{-3})	LOQ[a] (pg m^{-3})	Levels range (pg m^{-3})
Ethoprophos	82 ± 1	95 ± 10	100 ± 5	16.1	16.1–1613
Trifluralin	95 ± 6	105 ± 3	107 ± 3	16.1	16.1–1613
Diphenylamine	100 ± 10	110 ± 10	110 ± 10	16.1	16.1–1613
Chlorpropham	47 ± 4	54 ± 2	77.3 ± 0.9	322.6	322.6–1613
Diazinon	85 ± 10	95 ± 10	105 ± 5	16.1	16.1–1613
Lindane	105 ± 5	110 ± 10	99 ± 4	16.1	16.1–1613
Pyrimethanil	101 ± 6	110 ± 20	99 ± 6	16.1	16.1–1613
Chlorpyrifos-m	100 ± 3	94 ± 7	98 ± 2	16.1	16.1–1613
Vinclozolin	103.1 ± 0.7	110 ± 5	108 ± 3	16.1	16.1–1613
Toldofos-methyl	86 ± 7	98 ± 7	102 ± 7	16.1	16.1–1613
Fenitrothion	62 ± 3	90 ± 10	96 ± 4	64.5	64.5–1613
Malathion	44 ± 8	110 ± 10	93 ± 4	64.5	64.5–1613
Chlorpyrifos-ethyl	51 ± 4	67 ± 7	90 ± 10	322.6	322.6–1613
Aldrin	101 ± 5	100 ± 4	90 ± 10	16.1	16.1–1613
Triadimefon	107 ± 5	100 ± 10	91 ± 9	16.1	16.1–1613
Fipronil	52 ± 4	82 ± 5	83 ± 2	64.5	64.5–1613
Penconazole	107 ± 3	96 ± 4	99 ± 6	16.1	16.1–1613
Alfa-endosulfan	104 ± 1	110 ± 6	105 ± 2	16.1	16.1–1613
Dieldrin	109 ± 6	101 ± 8	110 ± 10	16.1	16.1–1613
Beta-endosulfan	95 ± 3	90 ± 10	102 ± 10	16.1	16.1–1613
Quinoxyfen	102 ± 6	94 ± 6	90 ± 10	16.1	16.1–1613
Endosulfan-sulphate	52 ± 6	65 ± 4	99 ± 8	322.6	322.6–1613
Bifenthrin	95 ± 4	110 ± 10	110 ± 10	16.1	16.1–1613
Iprodione	106 ± 4	94 ± 7	90 ± 10	16.1	16.1–1613
Lambda-cyhalothrin	110 ± 8	90 ± 10	107 ± 10	16.1	16.1–1613
Permethrin	98.0 ± 0.7	108 ± 2	110 ± 10	16.1	16.1–1613
Cypermethrin	107 ± 4	98 ± 7	95 ± 5	16.1	16.1–1613
Deltamethrin	102 ± 3	95 ± 5	90 ± 10	16.1	16.1–1613

[a]Using PUF-XAD2-PUF as solid sorbent and using a low-volume sampler (total volume = 155 m^3).

Table 3. Retention capacity of pesticides from spiked solid sorbents (n = 3) (higher fortification level = 50 ng).

Pesticide	PUF-XAD2-PUF Retention capacity (%) ± SD	XAD-2 Retention capacity (%) ± SD	XAD-4 Retention capacity (%) ± SD
Ethoprophos	92 ± 5	–	82 ± 2
Trifluralin	96 ± 5	–	79 ± 4
Diphenylamine	99 ± 2	87 ± 5	–
Chlorpropham	90 ± 10	91 ± 4	77 ± 3
Diazinon	85 ± 10	76 ± 3	78 ± 4
Lindane	88 ± 4	–	81 ± 5
Pyrimethanil	81 ± 3	82 ± 6	84 ± 3
Chlorpyrifos-m	85 ± 2	83 ± 2	81 ± 5
Vinclozolin	99 ± 3	–	–
Tolclofos-methyl	85 ± 5	79 ± 5	85 ± 5
Fenitrothion	100 ± 8	–	84 ± 2
Malathion	92 ± 2	86 ± 4	–
Chlorpyrifos-e	105 ± 4	76 ± 3	79 ± 5
Aldrin	83 ± 4	–	75 ± 4
Triadimefon	92 ± 7	84 ± 2	83 ± 6
Fipronil	83 ± 9	–	77 ± 2
Penconazole	76 ± 2	84 ± 7	–
Folpet	–	–	–
Alfa-endosulfan	91 ± 6	75 ± 6	71 ± 4
Dieldrin	84 ± 4	85 ± 3	–
Kresoxim-methyl	–	88 ± 4	71 ± 2
Fludioxonil	–	–	–
Beta-endosulfan	87 ± 6	–	77 ± 2
Quinoxyfen	86 ± 4	–	–
Endosulfan-sulphate	77 ± 3	79 ± 3	–
Propargite	–	75 ± 4	–
Bifenthrin	89 ± 2	77 ± 5	78 ± 1
Iprodione	95 ± 5	101 ± 3	77 ± 3
Dicofol	–	–	–
Lambda-cyhalothrin	80 ± 2	82 ± 3	77 ± 4
Permethrin	95 ± 1	79 ± 3	–
Cyfluthrin	–	78 ± 4	–
Cypermethrin	85 ± 3	86 ± 2	80 ± 5
Deltamethrin	86 ± 2	75 ± 2	–

Retention capacity was calculated using the following equation: Retention capacity(%) = [pesticide obtained]/[pesticide spiked] * 100.

100%. Folpet, kresoxim-methyl, fludioxonil, propargite, dicofol and cyfluthrin presented retention capacities lower than 70%. For XAD-2 and XAD-4, 22 and 15 pesticides presented retention capacities from 75% to 100%, respectively.

The occurrence of breakthrough implies unsatisfactory sampling efficiency. Breakthrough levels observed in trifluralin, malathion and chlorpyrifos-ethyl using the three solid sorbents were lower than the LOQ, after analysing the back section of the cartridge for all the target pesticides at different concentrations. Breakthrough was not observed in any of the other pesticides studied.

Taking into account the retention capacity study, the sandwich PUF-XAD2-PUF was selected as the sampling adsorbent for the target airborne pesticides.

3.5. *Analytical performance of the method*

The validation of the method was performed using sandwich PUF-XAD2-PUF as sorbent. Matrix-matched calibration plots showed good linearity with correlation coefficients

($R^2 > 0.99$) between 5 and 500 ng mL^{-1} for the solid sorbents studied. The specificity of the method was tested by analysing blank samples.

There is no reference material of pesticides in airborne particulate matter, so the accuracy (recovery) of the method was carried out using spiked solid sorbents samples. Accuracy and precision were estimated by means of recovery experiments ($n = 3$) at three concentration levels (2, 10 and 50 ng equivalents to 16.1, 64.5 and 322.6 pg m^{-3}). As it can be seen in Table 2, most pesticides presented suitable recoveries with values between 70% and 120% at three spiked levels and CV< 30%.

The quantification limit was determined as the lowest concentration giving good recoveries and precision for each pesticide. The LOQ ranged from 16.1 to 322.6 pg m^{-3}, when air volumes of 155 m^3 were collected (see Table 2). LOQs obtained using PUF-XAD2-PUF are satisfactory because outdoor levels of pesticides in previous studies [41] ranged from 8.7 pg m^{-3} to 59.1 ng m^{-3} and indoor levels ranged from 0.03 to 380 ng m^{-3} [14,42–45] for the pesticides studied.

Table 4 compares the methodology described in this work with other similar methodologies [15,17,18,42,44–47] described for trapping pesticides in the gaseous phase in outdoor and indoor environments. Table 4 shows that our methodology presents adequate characteristics to sampling pesticides in indoor and outdoor air.

3.6. *Application to real air samples*

In order to study the applicability of the methodology developed, 10 indoor air samples were collected in dwellings from July to December of 2015 in the Valencian Region (Spain) using the Partisol 2300 and the conditions described earlier. The results obtained are shown in Table 5.

In indoor air samples, six pesticides were detected in at least one sample, which highlights both the applicability of the analytical method and the use of pesticides in this area. The frequencies of detection (percentage of samples above the limit of detection) ranged from 20% to 100%, with cypermethrin and lambda-cyhalothrin present in all samples. The concentrations of the detected pesticides ranged from 1.46 ng m^{-3} (bifenthrin) to 22.02 ng m^{-3} (lambda-cyhalothrin). Figure 2 shows a chromatogram of a positive indoor air sample.

In this study, the obtained concentration for bifenthrin was similar to levels obtained in the USA by Bradman et al. (maximum bifenthrin level was 3.1 ng m^{-3}) [45] and higher than levels obtained in Thailand by Pentamwa and Ann [46] (levels ranging from 0.02 to 0.59 ng m^{-3}). Cypermethrin levels obtained were similar to levels in USA and higher than levels obtained in France by Laborie et al. [42] (average of 4.7 ng m^{-3}) and the USA by Lu et al. [44] (maximum cypermethrin level was 5.5 ng m^{-3}). Lambda-cyhalothrin results were higher than levels obtained by Lu et al. [44] (average of 0.52 ng m^{-3}). The levels obtained for permethrin were higher than levels in Thailand [46] (maximum permethrin level was 0.34 ng m^{-3}) and similar to levels in USA [45] (maximum permethrin level was 11 ng m^{-3}).

4. Conclusions

The sandwich PUF-XAD2-PUF was selected as the appropriate adsorbent for sampling 34 persistent and CUPs from the gaseous phase of air. The analytical strategy included a MAE and a GC-MS/MS determination and present LOQ ranging from 16.1 to 322.6 pg m^{-3}.

Table 4. Sampling comparison methodology.

Number of pesticides studied	Sorbent employed	Air flow (m^3 h^{-1})	Sampling time (days)	Total sampling volume (m^3)	LOQ	Outdoor/indoor ambient	Reference
34	PUF-XAD2-PUF	1 (LVS)	7	155	16.1–322.6 pg m^{-3}	Indoor/outdoor	Our methodology
27	PUF	25 (HVS)	7	4310	0.05–20 ng mL^{-1}	Outdoor	[15]
11	XAD-2	0.12 (LVS)	0.33	1	30.4–334 ng m^{-3}	Outdoor	[17]
8	XAD-2	10–15 (HVS)	2	480–720	2.5–1250 pg m^{-3}	Outdoor	[18]
2	XAD-2	0.7 (LVS)	15	252	0.07–0.28 pg m^{-3}	Indoor	[42]
8	PUF	4.2 (HVS)	1	101	5.2–624 ng g^{-1}	Indoor	[44]
24	PUF	0.15 (LVS)	1	3.6	2–100 ng g^{-1}	Indoor	[45]
32	PUF	2.4 (LVS)	1	58	0.066–0.20 ng m^{-3}	Indoor	[46]
6	XAD-2	0.09 (LVS)	0.73	1.6	0.33–133 ng m^{-3}	Outdoor	[47]

LVS: low-volume sampler; HVS: high-volume sampler.

Table 5. Concentration of pesticides gaseous phase in 10 indoor air samples in Valencia Region, Spain (ng m^{-3}).

Pesticides	Samples									
	1	2	3	4	5	6	7	8	9	10
Bifenthrin	6.72	–	<LOQ	1.46	–	–	–	–	–	–
Cypermethrin	16.26	15.32	17.59	15.58	9.73	8.07	3.09	20.03	17.83	21.46
Diphenylamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lambda-cyhalothrin	14.86	14.76	16.45	16.25	9.50	10.60	<LOQ	22.02	14.72	17.54
Permethrin	16.23	11.55	2.31	<LOQ	<LOQ	2.97	<LOQ	14.72	10.83	15.10
Pyrimethanil	17.47	<LOQ	5.06	<LOQ	<LOQ	<LOQ	<LOQ	11.65	<LOQ	2.70

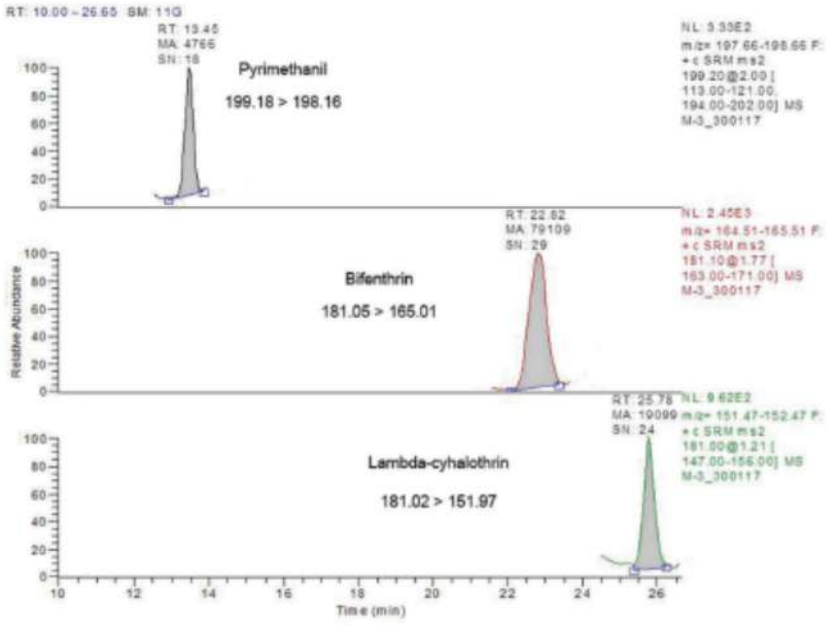


Figure 2. Sample ion chromatogram with concentrations of pyrimethanil: 17.47 ng m^{-3}, bifenthrin: 6.72 ng m^{-3} and lambda-cyhalothrin: 14.86 ng m^{-3}.

The developed method could be used for both indoor and outdoor ambients. Using the optimised method, pesticide occurrence was investigated in 10 indoor air samples. In indoor air, six pesticides were detected in at least one sample, with concentrations ranging from 1.46 to 22.03 ng m^{-3}. In the near future, this method could be applied to monitor the levels of CUPs in indoor and outdoor ambients.

Supplemental data for this article can be accessed here.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work has been funded by the LIFE-IRRILIFE project (LIFE 14/ENV/ES/000119) and the AIRPEST project (UGP–15–120).

References

[1] Eurostat, *Eurostat Statistical Books: Agriculture, Forestry and Fishery Statistics* (Publications Office of the European Union, Luxembourg, 2016 Edition).

[2] ECPA (European Crop Protection Association), (2010). Industry Statistics 2001–2010. http://www.ecpa.eu/page/industry-statistics

[3] EU pesticides database, (2017). http://ec.europa.eu/sanco_pesticides/public/?event=homepage [accessed May 2017].
[4] F. Van den Berg, R. Kubiak, W.G. Benjey, M.S. Majewski, S.R. Yates, G.L. Reeves, J.H. Smelt and A.M.A. Van Der Linden, Water, Air, Soil Pollut. **115**, 195 (1999). doi:10.1023/A:1005234329622.
[5] C. Bedos, M.-F. Rousseau-Djabri, D. Flura, S. Masson, E. Barriusoa and P. Cellier, Atmos. Environ. **36**, 5917 (2002). doi:10.1016/S1352-2310(02)00775-6.
[6] J.F. Pankow, Atmos. Environ. **28**, 185 (1994). doi:10.1016/1352-2310(94)90093-0.
[7] A. Finizio, D. Mackay, T. Bidleman and T. Harner, Atmos. Environ. **31,** 2289 (1997). doi:10.1016/S1352-2310(97)00013-7.
[8] T. Harner and T.F. Bidleman, Environ. Sci. Technol. **32,** 1494 (1998). doi:10.1021/es970890r.
[9] A. Sofuoglu, E. Cetin, S.S. Bozacioglu, G.D. Sener and M. Odabasi, Atmos. Environ. **38,** 4483 (2004). doi:10.1016/j.atmosenv.2004.05.036.
[10] W. Hadnagy, G. Leng, D. Sugiri, U. Ranft and H. Idel, Int. J. Hyg. Environ. Health **206**, 93 (2003). doi:10.1078/1438-4639-00201.
[11] Y. Zhang, J. Mo and C.J. Weschler, Environ. Health Perspect. **121,** 751 (2013). doi:10.1289/ehp.1205983.
[12] V. Yusà, C. Coscollà and M. Millet, Atmos. Environ. **96,** 322 (2014). doi:10.1016/j.atmosenv.2014.07.047.
[13] D. Zhang, X. Zhan, X. Wu, X. Yang, G. Liang, Z. Zheng, Z. Li, Y. Wu and X. Zheng, Parasitol. Res. **113,** 399 (2014). doi:10.1007/s00436-013-3668-9.
[14] P. Pentamwa, N. Kanaratanadilok and N.T.K. Oanh, Environ. Monit. Assess. **181,** 363 (2011). doi:10.1007/s10661-010-1834-4.
[15] C. Degrendale, K. Okonski, L. Melymuk, L. Landlova, P. Kukučka, O. Audy, J. Kohoutek, P. Cupr and J. Klánova, Atmos. Chem. Phys. Discuss. **15,** 23651 (2015). doi:10.5194/acpd-15-23651-2015.
[16] H. Li, M. Hongzhu, M.J. Lidy and J. You, Chemosphere **95,** 363 (2014). doi:10.1016/j.chemosphere.2013.09.046.
[17] L.G. Dos Santos, L. Lourencetti, A.A. Pinto, W.A. Pignati, E.F.G.C. Dores and J. Environ, Sci. Heal. B **46,** 150 (2011). doi:10.1080/03601234.2011.535381.
[18] R. Dobson, A. Scheyer, A.L. Rizet, P. Mirabel and M. Millet, Anal. Bioanal. Chem. **386,** 1781 (2006). doi:10.1007/s00216-006-0737-2.
[19] P. Avino, G. Cinelli, I. Notardonato and M.V. Russo, Aerosol Air Qual. Res. **11,** 783 (2011).
[20] J.L. Freixo, E.F.G.C. Dores and R.D. Villa, Anal. Methods **7**, 8255 (2015). doi:10.1039/C5AY01691F.
[21] M.V. Russo, P. Avino, G. Cinelli and I. Notardonato, Anal. Bional. Chem. **404,** 1517 (2012). doi:10.1007/s00216-012-6205-2.
[22] C. Raeppel, M. Fabritius, M. Nief, B.M. Appenzeller and M. Millet, Talanta **121,** 24 (2014). doi:10.1016/j.talanta.2013.12.040.
[23] S.Y. Baek, J. Jung and Y.S. Chang, Atmos. Environ. **64,** 40 (2013). doi:10.1016/j.atmosenv.2012.09.015.
[24] M. Hengel and P. Lee, Environ. Monit. Assess. **186,** 1343 (2014). doi:10.1007/s10661-013-3395-9.
[25] S.S. Cindoruk, Chemosphere **82,** 78 (2011). doi:10.1016/j.chemosphere.2010.10.003.
[26] J.S. Park, S.K. Shin, W.I. Kim and B.H. Kim, Atmos. Environ. **45,** 7496 (2011). doi:10.1016/j.atmosenv.2010.10.030.
[27] G.Z. Jin, S.M. Kim, S.Y. Lee, J.S. Park, D.H. Kim, M.J. Lee, K.T. Sim, H.G. Kang, I.G. Kim, S.K. Shin, S.Y. Lee, K.S. Seok and S.R. Hwang, Atmos. Environ. **68,** 333 (2013). doi:10.1016/j.atmosenv.2012.10.036.
[28] Q. Li, X. Wang, J. Song, H. Sui, L. Huang and L. Li, Atmos. Environ. **61**, 620 (2012). doi:10.1016/j.atmosenv.2012.07.068.
[29] C. Schummer, L. Tuduri, O. Briand, B.M. Appenzeller and M. Millet, Environ. Pollut. **170,** 88 (2012). doi:10.1016/j.envpol.2012.05.025.
[30] C. Coscollà, M. Castillo, A. Pastor and V. Yusà, Anal. Chim. Acta **693**, 72 (2011). doi:10.1016/j.aca.2011.03.017.

[31] European Comission, Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. SANTE/11945/2015, (Directorate General Health and Consumer Protection, Brussels, 2016)
[32] V.C. Fernandes, J.L. Vera, V.F. Domingues, L.M.S. Silva, N. Mateus and C. Delerue-Matos, J. Am. Soc. Mass. Spectrom. **23**, 2187 (2012). doi:10.1007/s13361-012-0488-6.
[33] V. Yusà, O. Pardo, A. Pastor and M. De La Guardia, Anal. Chim. Acta **557**, 304 (2006). doi:10.1016/j.aca.2005.10.041.
[34] C. Coscollà, S. Navarro-Olivares, P. Martí and V. Yusà, Talanta **119,** 544 (2014). doi:10.1016/j.talanta.2013.11.052.
[35] C. Naert, S. De Saeger and C. Van Peteghem, Rapid Commun. Mass Spectrom. **18,** 2317 (2004). doi:10.1002/rcm.1629.
[36] M. Mandalakis, M. Tsapakis and E.G. Stephanou, J. Chromatogr. **925,** 183 (2001). doi:10.1016/S0021-9673(01)01033-0.
[37] S.L. Blanco and J.M. Vieites, Anal. Chim. Acta **672,** 137 (2010). doi:10.1016/j.aca.2010.04.008.
[38] D. Massart, C. Vandeginste, B.G.M. Buydens, S. De Jong, P. Lewi and J. Smeyers-Vrbeke, *Handbook of Chemometrics and Qualimetrics Part A, Volume 20A in Data Handling in Science and Technology* (Elsevier, Amsterdam, 1997).
[39] V. Flotron, J. Houessou, A. Bosio, C. Delteil and A. Bermond, J. Chromatogr. **999,** 175 (2003). doi:10.1016/S0021-9673(03)00424-2.
[40] N.G. Tsiropoulos, E.B. Bakeas, V. Raptis and S.S. Batistatou, Anal Chim. Acta. **573–574**, 209 (2006). doi:10.1016/j.aca.2006.03.091.
[41] C. Coscollà and V. Yusà, Pesticides and Agricultural Air Quality in *The Quality of Air*, edited by M. de la Guardia and S. Armenta (Elsevier, Amsterdam, 2016).
[42] S. Laborie, E. Moreau-Guigon, F. Alliot, A. Desportes, L. Oziol and M. Chevrauil, Talanta **147,** 132 (2016). doi:10.1016/j.talanta.2015.09.028.
[43] H. Li, M.J. Lidy and J. You, Chemosphere **144,** 2427 (2016). doi:10.1016/j.chemosphere.2015.11.025.
[44] C. Lu, G. Adamkiewicz, K.R. Attfield, M. Kapp, J.D. Spengler, L. Tao and S.H. Xie, Environ. Sci. Technol. **47,** 2018 (2013). doi:10.1021/es303912n.
[45] A. Bradman, D. Whitaker, L. Quirós, R. Castorina, B.C. Henn, M. Nishioka, J. Morgan, D.B. Barr, M. Harnly, J.A. Brisbin, L.S. Sheldon, T.E. Mckone, B. Eskenazi and J. Expo, Sci. Environ. Epidemiol. **17,** 331 (2007). doi:10.1038/sj.jes.7500507.
[46] P. Pentamwa and N.T. Ann, N Y Acad. Sci. **1140,** 91 (2008). doi:10.1196/annals.1454.005.
[47] P. Avino, G. Cinelli, I. Notardonato and M.V. Russo, Anal. Bioanal. Chem. **400,** 3561 (2011). doi:10.1007/s00216-011-5039-7.

8. CAPÍTULO 4. Evaluación de diferentes adsorbents y validación de un método mediante LC-HRMS para la determinación de 28 plaguicidas en la atmósfera.

8.1. Introducción.

El control de los plaguicidas en la atmósfera es importante desde el punto de vista de la calidad del aire y su consecuente repercusión en la salud pública, debido a la exposición por inhalación [235]. Además, los niveles de plaguicidas en el aire ambiente es un indicador apropiado para las políticas de uso sostenible de plaguicidas (Regulación 1107/2009 de la Comisión Europea) [9]. Una fracción de los plaguicidas aplicados puede ser emitida a la atmósfera durante su aplicación en las distintas prácticas agrícolas [62]. Pasados unos días o incluso semanas de la aplicación, los plaguicidas, en una segunda fase, pueden ser transferidos a la atmósfera mediante procesos de volatilización desde el suelo o las plantas [65]. El efecto deriva es relevante únicamente a una distancia limitada desde el lugar de aplicación. Para el estudio del desplazamiento de los plaguicidas a largas distancias, la volatilización es el principal proceso de emisión junto con la erosión de las partículas de plaguicidas debido al viento. Además, habitualmente, los plaguicidas en la atmósfera están presentes tanto en la fase particulada como en la fase gaseosa, distribuyéndose en ambas fases según sus propiedades fisicoquímicas [87, 88, 89, 224].

Durante muchos años, el análisis de plaguicidas se ha centrado en la determinación de contaminantes orgánicos persistentes (POPs), como pueden ser los organclorados. Estos compuestos son volátiles y térmicamente estables. En los últimos años, el uso de plaguicidas más polares, no volátiles y menos estables térmicamente (carbamatos, triazinas o fenoxiácidos) ha provocado el incremento del uso de la cromatografía líquida [170]. Se han realizado pocos estudios de análisis de la fase gaseosa de los plaguicidas empleando esta técnica [101, 123, 130, 140, 141, 145], estudiándose únicamente un plaguicida o un grupo pequeño de plaguicidas. En cambio, nuestra metodología descrita en este Capítulo, nos permite el estudio de 33 CUPs polares empleados en la agricultura empleando la cromatografía líquida acoplada a espectrometría de masas de alta resolución (HRMS). Por otro lado, el uso de HRMS nos permite la adquisición mediante un alto poder de resolución (25000-50000), obteniéndose un método con elevada sensibilidad y selectividad.

Este Capítulo 4 de esta Tesis tiene tres objetivos principales: en primer lugar, la selección del adsorbente adecuado para el muestreo de los plaguicidas polares; en segundo lugar, la validación del método analítico empleando MAE y UHPLC-HRMS, y, por último, su aplicación en muestras reales.

8.2. Resultados.

8.2.1. Estudio del efecto matriz.

19 de los plaguicidas estudiados en este capítulo (azoxiestrobina, benalaxil, carbofuran, ciproconazol, ciprodinil, difenoconazol, diuron, fenbuconazol, fenhexamida, fluazifop, flusilazol, imazalil, iprovalicarb, miclobutanilo, pirimetanil, tebuconazol, tebufenpirad, terbutilazina y tiametoxam) presentaron una elevada supresión de la señal (porcentajes entre el 20 % y el 60 %) en presencia de la matriz oscilando los valores entre el 1 %

(azoxistrobin, benalaxil, ciprodinil y difenoconazol) y el 41 %, correspondiente al tiametoxam (ver Tabla SI-1, anexo IV). Siete de los plaguicidas estudiados (acetamiprid, dimetoato, imidacloprid, metalaxil, ometoato, pirimicarb y tiabendazol) presentaron moderada supresión de la señal (porcentajes entre el 60 % y el 80 %). Únicamente la carbendazima presentó una baja supresión de la señal (83 %) y sólo el pirimicarb- desmetil no presentó efecto matriz (94 %). Por tanto, la calibración se realiza sobre la matriz para una correcta cuantificación de los plaguicidas.

8.2.2. Estudio de los adsorbentes.

Previo al estudio de la capacidad de retención, es necesario conocer la recuperación obtenida para cada uno de los adsorbentes estudiados empleando el método analítico desarrollado. Así, para el sándwich PUF-XAD2-PUF, se ha obtenido una recuperación entre el 75 % y el 110 % para 22 de los 28 plaguicidas que presentan una capacidad de retención adecuada en todos los niveles estudiados (2.5, 10 y 50 ng). Este hecho se observa en la Tabla 16:

Tabla 16. Parámetros analíticos del método empleando sándwich PUF-XAD2-PUF como adsorbente

Plaguicida	Recuperación (%) ± SD			LOQ (pg m^{-3})	Rango (pg m^{-3})
	5 ng (equivalente a 32.2 pg m^{-3})	20 ng (equivalente a 129.0 pg m^{-3})	100 ng (equivalente a 645.2 pg m^{-3})		
Acetamipird	93±2	94±2	107±2	32.2	32.2-1290.4
Azoxiestrobina	63±4	83±3	80±7	129.0	129.0-1290.4
Benalaxil	84±1	92±1	87±1	32.2	32.2-1290.4
Carbendazima	91±2	97±1	112±1	32.2	32.2-1290.4
Carbofurano	98±2	101±1	110±1	32.2	32.2-1290.4
Ciproconazol	61±3	88±2	104±3	129.0	129.0-1290.4
Ciprodinil	87±1	93±1	105±3	32.2	32.2-1290.4
Difenoconazol	57±4	78±2	81±1	129.0	129.0-1290.4
Dimetoato	93±2	93±2	117±4	32.2	32.2-1290.4
Diuron	92±1	95±2	91±3	32.2	32.2-1290.4
Fenbuconazol	88±2	93±2	83±2	32.2	32.2-1290.4
Fenhexamida	60±2	83±1	91±2	129.0	129.0-1290.4
Fluazifop	87±2	76±1	82±2	32.2	32.2-1290.4
Flusilazol	58±3	92±1	105±5	129.0	129.0-1290.4
Imazalil	91±1	92±1	106±2	32.2	32.2-1290.4
Imidacloprid	88±2	100±2	107±2	32.2	32.2-1290.4
Iprovalicarb	90±2	92±1	112±1	32.2	32.2-1290.4
Metalaxil	97±1	97±1	115±4	32.2	32.2-1290.4
Miclobutanilo	92±1	96±1	104±4	32.2	32.2-1290.4
Ometoato	91.1±0.5	97±2	116±4	32.2	32.2-1290.4
Pirimicarb	89±1	92±1	110±3	32.2	32.2-1290.4
Pirimicarb-desmetil	89±1	93±1	102±7	32.2	32.2-1290.4
Pirimetanil	91±2	93±2	90±1	32.2	32.2-1290.4
Tebuconazol	88±1	90±2	94±2	32.2	32.2-1290.4
Tebufenpirad	64±2	86±1	90±1	129.0	129.0-1290.4
Terbutilazina	86±2	98±1	99±4	32.2	32.2-1290.4
Tiabendazol	90±1	96±1	99±4	32.2	32.2-1290.4
Tiametoxam	92.8±0.5	95±1	92±0.2	32.2	32.2-1290.4

Para el XAD-2, 15 de los plaguicidas estudiados con una adecuada capacidad de retención presentan buenas recuperaciones en los tres niveles estudiados (acetamiprid, azoxiestrobina, benalaxil, carbendazima, carbofuran, ciprodinil, diuron, imazalil, iprovalicarb, metalaxil, pirimicarb-desmetil, pirimetanil, terbutilazina, tiabendazol y tiametoxam). Ciproconazol, dimetoato, flusilazol, imidacloprid, miclobutanilo, ometoato, pirimicarb y tebuconazol presentan beunas recuperaciones a los dos niveles de fortificación más elevados. Difenoconazol y fenhexamid presentan buenas recuperaciones únicamente al nivel más alto (ver Tabla SI-2, Anexo IV).

Para el XAD-4, 13 de los plaguicidas con buena capacidad de retención presentan una recuperación adecuada para los tres niveles estudiados (acetamiprid, buprofezin, carbendazima, carbofuran, ciproconazol, dimetoato, imazalil, imidacloprid, iprovalicarb, metalaxil, tebuconazol, terbutilazina y tiabendazol). Azoxiestrobina, flusilazol y ometoato presentan buenas recuperaciones en los dos niveles más altos de fortificación (129.0 y 645.2 pg m^{-3}). Bitertanol, difenoconazol, fenbuconazol, fluquinconazol, metidation y piriproxifen sólo presentan buenas recuperaciones al nivel de fortificación más alto (ver Tabla SI-3, Anexo IV).

8.2.3. Capacidad de retención y evaluación del breakthrough.

Se ha evaluado la capacidad de retención para los tres adsorbentes estudiados para seleccionar el más adecuado para la captación de los plaguicidas. Para el cálculo de la capacidad de retención se ha empleado la ecuación (11) descrita en el capítulo 3. La tabla 17 muestra los resultados de capacidad de retención obtenidos para los adsorbentes estudiados:

Tabla 17. Capacidad de retención para los adsorbentes empleados (n=3) (nivel de fortificación alto=100 ng)

Plaguicida	PUF-XAD2-PUF Capacidad de retención (%)± SD	XAD-2 Capacidad de retención (%)± SD	XAD-4 Capacidad de retención (%)± SD
Acetamiprid	98±4	90±1	92±3
Azoxiestrobina	90±2	91±1	78±1
Benalaxil	87±1	79±2	-
Bitertanol	-	-	91±3
Buprofezina	-	-	88±2
Carbendazima	84.6±0.3	90±2	87±3
Carbofurano	85±2	91±2	94±2
Ciproconazol	82±2	85±2	80±4
Ciprodinil	100±1	90±3	-
Difenoconazol	80±1	91±4	80±3
Dimetoato	92±3	86±3	93±2
Diuron	85±2	88±3	-
Fenbuconazol	76±1	-	82±3
Fenhexamida	95±1	77±2	-
Fluazifop	81±1	-	-
Fluquinconazol	-	-	88±2
Flusilazol	91±3	88±2	83±3
Imazalil	92±4	92±1	84±4
Imidacloprid	92±1	93±2	85±3
Iprovalicarb	91.8±0.4	91±3	84±2
Metalaxil	87±5	90±3	82±5
Metidation	-	-	89±3
Miclobutanilo	96±4	85±2	-
Ometoato	98±2	86±2	88±3
Pirimicarb	95.9±0.7	86.4±0.8	-
Pirimicarb-desmetil	74±2	84±3	-
Pirimetanil	94±2	85±3	-
Piriproxifen	-	-	92±3
Tebuconazol	95±2	82±2	85±3
Tebufenpirad	82±2	-	-
Terbutilazina	93.7±0.4	84±3	88±3
Tiabendazol	76±2	78±2	80±2
Tiametoxam	97 ±1	80±2	-

Cómo se puede observar, empleando el sándwich PUF-XAD2-PUF, 28 de los 33 plaguicidas estudiados presentaron una capacidad de retención que osciló entre el 75 % y el 100 %. Únicamente bitertanol, buprofezina, fenoxicarb, fluquinconazol, metidatión, piriproxifen y triflumizol presentaron valores por debajo del 70 %.

En el caso del XAD-2, 25 de los plaguicidas estudiados presentaron una capacidad de retención adecuada (entre el 75 % y el 100 %) y 22 plaguicidas presentaron una capacidad de retención adecuada empleando como adsorbente XAD-4.

El breakthrough fue evaluado por separado, analizando la sección inferior del adsorbente, para comprobar si se ha producido un traspaso desde la sección superior hasta sección inferior. Para la evaluación del breakthrough se ha seguido el criterio descritos por Tsirapoulos et al. (2006) [234]: se determinará que existe breakthrough cuando los niveles detectados en la sección inferior sean superiores al 1 % del nivel

detectado en la sección superior. Este ensayo se realizó por triplicado, empleando también un blanco analítico. En ningún caso de los estudiados se ha observado breakthrough.

Así, por tanto, teniendo en cuenta la capacidad de retención, donde se obtuvieron mejores capacidades de retención empleando la mezcla PUF-XAD2-PUF y el breakthrough, donde en ninguno de los ensayos se detectó una colmatación de los plaguicidas, el adsorbente seleccionado para la captación de los plaguicidas fue el sándwich PUF-XAD2-PUF.

8.2.4. Parámetros analíticos del método.

La validación del método fue realizado empleando como adsorbente el sándwich PUF-XAD2-PUF. Se observó una linealidad adecuada (R^2> 0.99) para la curva de calibrado realizada en matriz entre 5 y 200 ng mL^{-1}. La exactitud y la precisión se evaluaron a través de la recuperación a tres niveles (5, 20 y 100 ng, equivalentes a 32.2, 129.0 y 645.2 pg m^{-3}) (ver Tabla 16), obteniéndose recuperaciones adecuadas (70-120 %) para la gran mayoría de plaguicidas con CV < 30 %.

El límite de cuantificación (ver Tabla 16), se ha determinado como la concentración más baja en la cual se ha obtenido una recuperación y precisión adecuada. Así, los niveles de LOQ oscilan entre 32.2 y 129.0 pg m^{-3}, al emplear un volumen de aire de 155 m^3. Si comparamos los LOQ obtenidos con los obtenidos en estudios previos, presenta límites de cuantificación más bajos que los obtenidos para aquellas metodologías similares que emplearon muestradores activos de bajo volumen [101, 130, 140, 141], principalmente debido a la sensibilidad de la espectrometría de masas de alta resolución (HRMS) y al volumen total muestreado. Por otro lado, los límites obtenidos en nuestro estudio son ligeramente superiores a los obtenidos mediante el empleo de muestreadores de alto volumen [123, 145]. En la tabla SI-4 del anexo VI se muestran los límites de cuantificación individuales obtenida para cada plaguicida y comparados con otros estudios similares.

En el caso de la carbendazima, el límite de cuantificación obtenido (32.2 pg m^{-3}) es ligeramente superior al obtenido en la República Checa [145] (2.8 pg m^{-3}). La diferencia que se observa es debido al diferente sistema de muestreo (muestreador de bajo volumen frente a muestreador de alto volumen). Para el dimetoato, nuestro LOQ es superior a los obtenidos en aquellos estudios que emplean muestreadores de alto volumen [123, 145] pero es inferior a aquellos estudios que han empleado también muestreador de bajo volumen [141]. Para el diuron, el límite de cuantificación obtenido es ligeramente superior al obtenido por Degrendele et al. [145] (14 pg m^{-3}) pero inferior al obtenido en EEUU (5140 pg m^{-3}).

8.2.5. Aplicación a muestras reales.

Para estudiar la aplicabilidad de nuestra metodología descrita en este Capítulo, 15 muestras de aire ambiente fueron recogidas en un área rural de la Comunidad Valenciana (L'Alcúdia) empleando las condiciones descritas en el apartado 4 de metodología. Los resultados se presentan en la siguiente Tabla (Tabla 18):

Tabla 18. Concentración de plaguicidas en fase gaseosa en 15 muestras rurales de la Comunidad Valenciana (España) (pg m^{-3})

Plaguicidas	Muestras														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acetamiprid					1618.87										
Carbendazima							<LOQ								
Carbofuran	937.78														
Imidacloprid		1105.84							411.16						542.33
Iprovalicarb	1223.13														
Metalaxil											4385.22			472.54	
Miclobutanil	5514.59														
Pirimicarb		1516.64			848.63	776.26									
Pirimetanil	11011.45												2585.68	2668.52	
Terbutilazina													2391.88		

La siguiente figura (Figura 20), muestra el cromatograma de una muestra positiva:

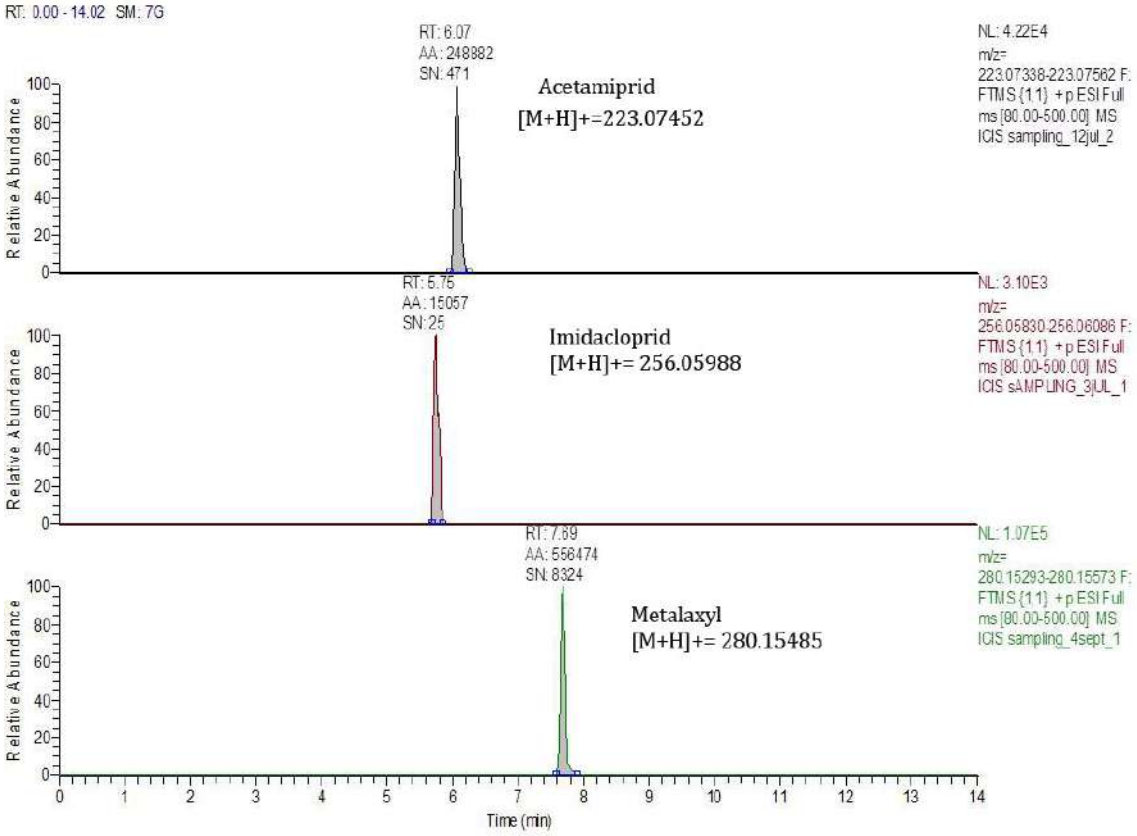


Figura 20. Cromatograma que contiene acetamiprid (1618.87 pg m^{-3}), imidacloprid (1105.84 pg m^{-3}) y metalaxyl (4385. 22 pg m^{-3}).

Se han detectado un total de 10 plaguicidas en al menos una muestra. Las frecuencias de detección oscilaron entre el 6.70 % (acetamiprid, carbendazima, carbofuran, iprovalicarb, miclobutanil y terbutilazina) y el 20.10 % (imidacloprid, pirimicarb y pirimetanil). Las concentraciones de los plaguicidas detectados oscilan entre 411.46 pg m^{-3} (correspondiente al imidacloprid) y 11011.45 pg m^{-3} (correspondiente al pirimetanil).

Sólo se detectó carbendazima en una de las muestras. El nivel obtenido es similar al obtenido en Alemania [242] y República Checa [145]. El insecticida carbofuran se ha detectado en una muestra con una concentración de 937.78 pg m^{-3}. El nivel obtenido es inferior al obtenido en Francia [97] (rango entre 1430 pg m^{-3} y 28970 pg m^{-3}) y en Taiwan (4.3 E10 pg m^{-3}) [243] y más alto que los valores detctados en Canadá [120] (rango entre 38 y 787 pg m^{-3}).

El fungicida metalaxil se ha detectado en dos muestras con concentraciones que oscilaron entre 472.54 pg m^{-3} y 4385.22 pg m^{-3}. Los niveles obtenidos son inferiores que los obtenidos en Canadá (150-26700 pg m^{-3}) [113, 114]. Miclobutanil es otro fungicida que sólo se ha detectado en una de las muestras con una concentración de

5514.59 pg m^{-3}. Los niveles obtenidos son superiores a los obtenidos en Francia [90] (40-3090 pg m^{-3}).

El insecticida pirimicarb fue detectado en 3 muestras de aire, obteniéndose concentraciones entre 776.26 pg m^{-3} y 1516.64 pg m^{-3}. Los niveles obtenidos son inferiores a los detectados en Canadá [113] (40-5100 pg m^{-3}).

El herbicida terbutilazina fue detectado en una de las muestras, con una concentración de 2391.88 pg m^{-3}. La concentración obtenida es superior a la obtenida en República Checa [145] (0.04 pg m^{-3}-33.82 pg m^{-3}) y en Alemania [242] (0.7 pg m^{-3}-156.1 pg m^{-3}).

8.3. Conclusiones.

Las conclusiones a las que se han llegado después de la realización de este trabajo son las siguientes:

- El control y la vigilancia de los plaguicidas en la atmósfera ha adquirido un interés creciente desde el punto de vista de la protección de la salud. Para poder realizar esta actividad de forma adecuada, es necesario crear una adecuada metodología para muestrear y analizar el mayor número de plaguicidas posibles. En este capítulo, se ha querido demostrar la eficiacia del sándwich PUF-XAD2-PUF como un adsorbente adecuado para el muestreo y el análisis de plaguicidas polares y poco volátiles. En el capítulo 3, ya se ha demostrado la eficicacia de este adsorbente para el muestreo y el análisis de plaguicidas volátiles y térmicamente estables. La estrategia analítica ha incluido una extracción mediante MAE y una posterior determinación empleando UHPLC-HRMS, con límites de cuantificación que oscilaron desde 32.2 hasta 129.0 pg m^{-3}.

- Por otra parte, el empleo de la espectrometría de masas de alta resolución (HRMS) permite a posteriori un análisis retrospectivo de las muestras, tal y cómo se ha detallado en el Capítulo 1.

8.4. Artículo 4: Evaluation of sampling adsorbents and validation of a LC-HRMS method for determination of 28 airborne pesticides.

*Manuscript
Click here to view linked References

Evaluation of sampling adsorbents and validation of a LC-HRMS method for determination of 28 airborne pesticides.

Antonio López [a, b, c], Clara Coscollà [a, c] and Vicent Yusà [a, b, c]*

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020, Valencia, Spain.
[b] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain.
[c] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain.

Abstract

A new strategy for sampling and determination of liquid chromatography-amenable (LC-amenable) airborne pesticides has been developed. The trapping efficiency of three adsorbents (sandwich PUF-XAD2-PUF; XAD-2 and XAD-4) was tested for 33 currently used pesticides and the first adsorbent (PUF-XAD2-PUF) was selected because it presented the highest retention capacity without breakthrough. A validation of the analytical methodology that includes microwave extraction with ethyl acetate, and determination by liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) was performed. The method showed recoveries ranging from 75 % to 120 % with quantification limits in the range of 32.2-129.0 pg m^{-3} when 155 m^3 were sampled.

This analytical strategy was applied to 15 air samples collected in a rural area of Valencia Region (Spain). 10 pesticides, namely acetamiprid, carbendazim, carbofuran, imidacloprid, iprovalicarb, metalaxyl, myclobutanil, pirimicarb, pyrimethanil and terbuthylazine were detected in air samples with concentrations ranging from 411.16 pg m^{-3} (imidacloprid) to 11011.45 pg m^{-3} (pyrimethanil).

Keywords: Pesticides, UHPLC-HRMS, adsorbents, gaseous phase, air samples.

*Corresponding author. Tel.: +34 961925865; fax: +34 961925704.
E-mail addresses: yusa_vic@gva.es (V. Yusà)

1. Introduction

The control of pesticides commonly used in agriculture is relevant from the point of view of air quality and its impact on human health (inhalation exposure) [1]. Moreover, pesticide control could be an appropriate indicator for the sustainable use of pesticides (Regulation EC 1107/2009) [2]. Pesticide selection in agriculture depends on multiple factors, including the crop to be protected and the target pest. Last years, pesticide use has increased in Europe. In 2014, about 400,000 tonnes of pesticide active ingredients were used [3, 4] and around 500 active substances are authorised by the European Union nowadays for their application on various crops [2].

Pesticides are emitted to atmosphere during application and after application (post-application time). On the one hand, a fraction of the applied pesticide during emission is deposited in soil and plants and other fraction (20-30 %) is emitted to the atmosphere (spray drift) [5]. On the other hand, several days or weeks after, pesticides can be transferred to the air through the volatilization process from plants and soil [6]. Spray drift is only relevant in a limited distance from the application site. For long distances,

volatilization is the predominant emission process. Moreover, wind erosion of soil particles containing pesticide particles (soil tillage operations) could be another primary source of airborne pesticides.

Airborne pesticides are usually present in both gaseous and particulate phase. Physicochemical properties such as vapour pressure or water solubility are the main factors contributing to this phase distribution. Different theoretical models have been proposed to describe gaseous-particulate partitioning [7-10].

For a long time, pesticide control was focused on the persistent ones, such as organochlorines pesticides (OCPs). These compounds are volatile and thermally stable, and are determined using gas chromatography. The increase in the use of polar, less volatile, and thermolabile pesticides (carbamates, triazines, phenoxyacids) has promoted the use of liquid chromatography for their determination [11]. Few works in the literature describe methods for the determination of polar pesticides in the gas phase by liquid chromatography coupled to MS or other detectors [12-17]. Many of them study only one or a few number of pesticides.

In a previous study we worked on pesticide determination in airborne particulate matter [18]. The methodology presented in this work are focused on the gaseous phase, and enables the multiresidue study of 33 currently used pesticides using liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS). The use of HRMS allows to the acquisition of polar pesticides with high sensitivity and high resolving power (25000-50000 FWHM). The present study has three main goals: i) select the appropriate solid sorbent for sampling polar currently used pesticides in the gas phase of the air, ii) to validate the analytical method using microwave accelerated extraction (MAE) and UHPLC-HRMS and iii) application of the methodology to real samples.

2. Experimental

2.1. Reagents and chemicals

The following high-purity standard pesticides were supplied by Dr. Ehrenstorfer (Augsburg, Germany): acetamiprid (99 %), azoxystrobin (99.5 %), benalaxyl (99.5 %), bitertanol (98 %), buprofezin (99 %), carbendazim (99 %), carbofuran (99 %), cyproconazole (99 %), cyprodinil (97.5 %), difenoconazole (98.7 %), dimethoate (98 %), diuron (98 %), fenbuconazole (98.7 %), fenhexamid (99.5 %), fluazifop (96 %), fluquinconazole (99 %), flusilazole (99.5 %), imazalil (97.5 %), imidacloprid (99 %), iprovalicarb (97.5 %), metalaxyl (99.5 %), methidathion (98.5 %), myclobutanil (99 %), omethoate (97 %), pirimicarb (98.7 %), pirimicarb-desmethyl (99.5 %), pyrimethanil (99 %), tebuconazole (98.5 %), terbuthylazine (99.5 %), thiabendazole (98.5 %) and thiamethoxam (98.5 %).

Individual stock standards were prepared weighting 10 mg of pure standard using a 5-decimal analytical balance and dissolving each compound in 50 mL of acetone. They were stored in capped amber vials at −21 ºC [19]. Mix working solutions at 10 and 1 mg L^{-1} were prepared with methanol. Calibration solutions (5, 10, 20, 50, 100 and 200 ng

mL^{-1}) were prepared by adding variable volumes of the mix working solutions in matrix.

Methanol and acetonitrile were HPLC grade supplied by Scharlau (Barcelona, Spain). Acetone, ethyl acetate and water were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Glacial acetic acid (Reag. Ph. Eur.) and formic acid 98 % were provided by Panreac (Barcelona, Spain). Ammonium acetate for HPLC (97 %) was purchased from Scharlau (Barcelona, Spain). Ammonium formate, solution Ultra (100 ml, 10 M in water) was provided by Fluka (Steinheim, Switzerland).

The following three solid sorbents: PUF (BSG Ingenieros, Valencia, Spain), XAD-2 (Sigma Aldrich, Barcelona, Spain) and XAD-4 (Sigma Aldrich, Barcelona, Spain) were employed. PUF is white and turns yellow upon exposure to light. PUFs are good for trapping volatile compounds and they are commonly used for the sampling of gaseous persistent organic pollutants like PCBs and organochlorine pesticides. Amberlite XAD-2 polymeric adsorbent is a hydrophobic crosslinked polystyrene copolymer resin. The resin is widely employed to adsorb soluble organic compounds from aqueous streams and organic solvents, generally in cyclic columnar operations. XAD-4 is a polymeric adsorbent supplied as white insoluble beads. It is a non-ionic crosslinked polymer which has adsorptive properties due to its macroreticular structure (containing both a continuous polymer phase and a continuous pore phase), its high surface area, and the aromatic nature of its surface.

A Partisol 2300 low-volume sampler (Thermo Fisher Scientific, Bremen, Germany) was employed coupled to ChemComb cartridge (Thermo Fisher Scientific, Bremen, Germany).

2.2. Sample collection and extraction

For testing the sorbents we worked at field conditions: sampling around 160 m^3 of air in total, using a flow air of 1 $m^3\ h^{-1}$ during 1 week. The tested solid sorbents presented two sections: the upper section was used for evaluating the retention efficiency during sampling and the lower section was employed for evaluating the breakthrough. In total, 5 g of XAD-2 or XAD-4 were weighed, individually. Sandwich PUF-XAD2-PUF was composed of 5 g of XAD-2 between two polyurethane foams of 1.4 cm long.

A generic extraction method developed using MAE with ethyl acetate was employed to recover pesticides from the three studied sorbents. Microwave extraction of pesticides was carried out using a Mars System from CEM Corporation (Mathews, NC, USA) equipped with Teflon® TFM 100 mL extraction vessels. The corresponding solid sorbent were extracted at 50 ºC for 20 min, using a power of 1200 W, and 30 mL of ethyl acetate [18]. After cooling, the reactor was opened and the extracts were filtered. After, 100 µL of diethylene glycol (keeper) were added to the extract and concentrated with Turbo Vap 500 (Zymark, Idstein, Germany). The extracts were re-dissolved with 1 mL of water: methanol (70:30) and filtered through a 0.22 µm GHP Acrodisc filter from Pall Life Science (Ann Arbor, USA) prior to inject in the LC-HRMS.

2.3. Site characterization

Valencia Region, situated on the East coast, made up 12.6 % of the total national consumption of pesticides in 2014 [20]. The main irrigated crops are citrus fruit, other fruit trees (mainly peach, apricot and plum trees), rice and garden produce (primarily watermelon, cabbage, artichoke, lettuce, cauliflower, tomatoes, potatoes and onion). The main dry crops are vineyards, olive trees and almonds [21]. Samples were collected in L'Alcudia, a rural zone of Valencia Region from April to September 2017. L'Alcudia (39°11'45"N, 0°30'26"W) is a city (11820 inhabitants) located at the centre of the Valencia Region (35 km of the Valencia city), surrounded by citrus crops, fruit orchards and persimmon crops. A total of 15 samples were collected about 30 m above ground level.

2.4. UHPLC-HRMS

LC conditions have been discussed in previous works [18, 22]. Chromatographic separation was performed on an Accela liquid chromatography UHPLC system equipped with a Hypersil Gold aQ column (100 mm x 2.1 mm, 1.9 µm) both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 300 µL min^{-1} and the injection volume was 10 µL. Separations were performed using a binary gradient. The mobile phase was (A) H_2O with 0.1% formic acid and 4 mM ammonium formate and (B) methanol with 0.1 % formic acid and 4 mM ammonium formate. The gradient conditions were as follows: 0-8 min, linear with 100 % of A; 8-12 min, linear with 100% of B and 12-16 min, linear with 100 % of A. The total run time lasted 16 min.

Mass spectrometric analysis was performed on a single stage Orbitrap MS (Exactive ™, ThermoFisher Scientific, Bremen, Germany). The system was equipped with a heated electrospray ionization interface (HESI-II). The detection was carried out in positive ionization mode (ESI+) using the following optimized operational parameters: spray voltage, 2.8 kV; sheath gas (N2, >95 %), 25 (adimensional); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 205 ∘C; and capillary temperature, 281 ∘C. The mass spectra was acquired using two alternating acquisition functions (i) full-scan MS without fragmentation, ESI+; mass resolving power = 50000 FWHM; scan range = 50–800 Da; scan time = 0.5 s (2Hz); (ii) The same parameters but with full-scan MS all ion fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 10 eV). The automatic gain control (AGC) was set to 1×10^6 ions [18]. The external mass calibration of the spectrometer was performed using two ready-to-use calibration mixtures (Mass Cal5 (+) and Mass Cal 6 (−)) from Supelco (USA). Data acquisition and processing was performed using Thermo Scientific TraceFinder ™ software version 3.2. Table 1 shows the mass data for each pesticide studied.

2.5. Retention capacity and breakthrough

With slight modifications, we have used the same procedure described previously by López et al. 2017 [23]. Retention capacity was assessed by spiking the sorbents (PUF-XAD2-PUF, XAD-2 and XAD-4) with 100 ng (equivalent to 645 pg m^{-3} at the sampling condition) and by pulling air through using a constant air flow (1 m^3 h^{-1}) during 1 week using Partisol 2300 (low-volume sampler). This test was performed in triplicate. The pesticide recovery in each solid sorbent allowed us to quantify the pesticide loss during sampling. Retention capacity was calculated using the following equation:

Retention capacity (%) = [pesticide obtained] / [pesticide spiked]*100

Moreover, breakthrough was evaluated by separately analysing the lower section of the sorbent for the eventual passage of the analytes from the upper to the lower section. For testing the breakthrough, sampling conditions were as follow: a flow air of 1 m^3 h^{-1} during one week was employed (total volume of around 155 m^3). The criteria used to evaluate the breakthrough was the one reported by Tsiropoulos et al. (2006) [24]: when the pesticide detected in the lower section of the cartridge was greater than 1% of that found in the upper section. These assays were carried out in triplicate together with an analytical blank.

2.6. Matrix effect

Matrix effect was evaluated by comparing the response of standard prepared in pure solvent with the response of matrix-matched standard. The following equation was employed: ME (%) = B/A *100 (A: standard in pure solvent and B: extract solid sorbent). Both A and B sets had concentrations of 645.2 pg m^{-3} (equivalent to 100 ng).

Values obtained between 20 - 60 % indicate high-ion suppression. Levels between 60-80 % indicate moderate ion suppression. Results obtained between 80-90 % indicate low ion suppression. No matrix effect was observed when matrix effects levels oscillate between 90 % and 110 %. Levels higher than 110 % indicate enhancement of the matrix signal.

2.7. Validation study and quality control protocol

According to our knowledge, there are no available pesticide reference materials in airborne gaseous phase. The method validation was performed at three levels by triplicate (5, 20 and 100 ng equivalents to 32.2, 129.0 and 645.2 pg m^{-3}) using spiked solid sorbents samples. We evaluated the following analytical characteristics: accuracy (expressed as mean recovery), sensitivity, specificity and precision (expressed at repeatability). Moreover, linearity was studied using matrix-matched standards, analysing six concentrations between 5-200 ng mL^{-1} in vial (by triplicate). Quantification limits (LOQ) was established studying the signal to noise (S/N) of the quantification ion at decreasing concentrations of a spiked matrix solution, establishing it at S/N>10 and checking good pesticide recoveries at that level. The signal to noise for the confirmation ion was in all cases above 3. In each batch, we include quality assurance protocols, including process blanks, spiked blank samples, blank sample and reagent blanks. Two process blanks were used: consisting of a sealed envelope containing solid sorbent, and were used as quality control (QC) samples during deployment, retrieval and transportation of the field filters. These field blanks were processed and analysed in the same way as the analytical samples. The analysis of solid sorbents was performed immediately after sampling or after storage period at -20 °C (3 months maximum).

In order to check potential losses during the storage period and assess recoveries during sample preparation, spiked blank samples were stored and analysed as field samples. Blank samples were baked 24 h at 130 °C in the oven to eliminate possible pollutants in the matrix.

2.8. Identification and confirmation criteria

Using the EU guidelines [19], the following steps has used to identify a positive compound: a) Mass accuracy of the molecular ion ($M+H^+$) were lower than 5 ppm; b) mass accuracy of the fragment ion were lower than 5 ppm; c) isotopic pattern similar to the theoretical isotopic pattern (the relative intensity of the A+1 and/or A+2 isotope peaks in the real sample shall correspond to the theoretical relative intensities). Moreover, for confirmation: d) the retention time (RT) of the sample is similar to that of the reference standard (± 0.1 min).

3. Results and discussion

3.1. Matrix effect study

19 of the studied pesticides (azoxystrobin, benalaxyl, carbofuran, cyproconazole, cyprodinil, difenoconazole, diuron, fenbuconazole, fenhexamid, fluazifop, flusilazole, imazalil, iprovalicarb, myclobutanil, pyrimethanil, tebuconazole, tebufenpyrad, terbuthylazine and thiamethoxam) presented high ion suppression in presence of the matrix ranging from 1 % (azoxystrobin, benalaxyl, cyprodinil and difenoconazole) to 41 % (pirimicarb-desmethyl). 7 of the studied pesticides (acetamiprid, dimethoate, imidacloprid, metalaxyl, omethoate, pirimicarb and thiabendazole) presented moderate ion suppression in presence of the matrix ranging from 60 % (omethoate) to 79 % (thiabendazole). Only one pesticide presented low ion suppression (carbendazim, 83 %) and only one pesticide (pirimicarb-desmethyl, 94 %) not presented matrix effect (see Table SI-1). Consequently, a matrix-matched calibration method was used for an accurate quantification.

3.2. Study of solid sorbents

In a previous work we have developed a method for pesticide determination in airborne particulate matter [18]. Now, we are focused on gaseous phase.

3.2.1. Recovery study

Before studying the retention capacity, we checked for sorbent recoveries when using the analytical method for each sorbent. Table 2 illustrates the recoveries at three levels (only pesticides with adequate retention capacity) using PUF-XAD2-PUF as solid sorbent. As can be seen, using PUF-XAD2-PUF, 22 out of the 28 CUPs studied with good retention capacities presented recoveries between 75 % and 120 % at the three levels studied. Azoxystrobin, cyproconazole, difenoconazole, fenhexamid, flusilazole and tebufenpyrad presented good recoveries only at the two higher levels (20 and 100 ng).

For XAD-2, 15 out of the 25 CUPs presented good recoveries at the three levels studied. Cyproconazole, dimethoate, flusilazole, imidacloprid, myclobutanil, omethoate, pirimicarb and tebuconazole presented good recoveries at two higher levels (20 and 100 ng). Difenoconazole and fenhexamid presented good recoveries only at the highest level (100 ng) (see Table SI-2).

13 CUPs presented good recoveries at the three levels studies using XAD-4 as solid sorbent (see Table SI-3). Azoxystrobin, flusilazole and omethoate presented good

recoveries at two higher levels (129.0 and 645.2 pg m^{-3}). Bitertanol, difenoconazole, fenbuconazole, fluquinconazole, methidathion and pyriproxifen presented good recoveries only at the highest level (645.2 pg m^{-3}).

3.2.2. Retention capacity and Breakthrough evaluation

We evaluated the retention capacity of the three sorbents in order to choose the most appropriate for sampling the target pesticides (Table 3). As can be seen, using sandwich PUF-XAD2-PUF, 28 out of the studied pesticides presented retention capacities ranging from 75 % to 100 %. Bitertanol, buprofezin, fluquinconazole, methidathion and pyriproxyfen presented retention capacities lower than 70 %.

For XAD-2, 25 pesticides presented good retention capacities. Bitertanol, buprofezin, fenbuconazole, fluazifop, fluquinconazole, methidathion, pyriproxyfen and tebufenpyrad presented retention capacities lower than 70 %. For XAD-4, 22 pesticides presented good retention capacities. Benalaxyl, cyprodinil, diuron, fenhexamid, fluazifop, myclobutanil, pirimicarb, pirimicarb-desmethyl, pyrimethanil, tebufenpyrad and thiamethoxam presented retention capacities lower than 70 %.

The occurrence of the breakthrough implies unsatisfactory sampling efficiency. Breakthrough was not observed in any of the three sorbents, after analysing the back section of the cartridge for all target pesticides at different levels.

Taking into account the retention capacity study, the sandwich PUF-XAD2-PUF was selected as the sampling adsorbent for the target airborne pesticides. Likewise, for this sorbent, the extraction procedure presented appropriate recoveries for 22 out of the studied pesticides (see section 3.2.1).

3.2.3. Analytical performance of the method

Method validation was carried out using sandwich PUF-XAD2-PUF as solid sorbent. Matrix-matched calibration plots showed good linearity with correlation coefficients (R^2>0.99) between 5 and 200 ng mL^{-1} for the sandwich PUF-XAD2-PUF. Method specificity was tested by analysing blank samples.

The accuracy (recoveries) of the analytical method was performed using spiked soil sorbent because there is not available reference material of pesticides in airborne gaseous phase. Accuracy and precision were estimated by means of recovery experiments (by triplicate, n=3) at three concentrations levels (5, 20 and 100 ng equivalents to 32.2, 129.0 and 645.2 pg m^{-3}). As it can be seen in Table 2, most pesticides presented suitable recoveries with values between 75 and 120 % at three spiked levels and RSD < 30%. Figure 1 shows a chromatogram of a spiked sample at 129.0 pg m^{-3}.

The quantification limit was determined as the lowest concentration giving good recoveries and precision for each pesticide. The LOQ ranged from 32.2 to 129.0 pg m^{-3}, when air volumes of 155 m^3 were collected (see Table 2). Quantification limits obtained using PUF-XAD2-PUF are adequate because outdoor levels of pesticides in previous studies [1] were ranged from 0.2 pg m^{-3} to 33.3 ng m^{-3} for the pesticides studied.

Table 4 compares the methodology describe in this work with other similar methodologies [12-17] described for trapping the gaseous phase of pesticides and their analytical determination by liquid chromatography.

Sandwich PUF-XAD2-PUF has been selected for sampling the gaseous phase of pesticides as opposite as other studies [12, 13, 14, 17] that used PUF as solid sorbent. PUF is an appropriate sorbent for trapping volatile compounds, especially persistent organic pollutants such as PCBs and OCPs. However, only PUF is not an appropriate solid sorbent for sampling LC-amenable pesticides.

Our methodology present quantification limits lower than other methodologies that use low volume samplers [12, 14, 15, 16], mainly due to the sensitivity of the HRMS and the total volume sampled. Moreover, quantification limits obtained in this study were slightly higher than LOQ obtained by Degrendele et al. 2016 [17] but they used high-volume sampler instead of low-volume sampler. Raina et al. [13] obtained detection limits ranged from 0.15 pg m^{-3} to 28.8 pg m^{-3}, using a high-volume sampler (total volume = 2700 m^3). Table SI-4 shows the detection/quantification limits obtained for each pesticide studied comparing its levels with the limits obtained in similar studies [12-17].

Quantification limit obtained for carbendazim in the present study (32.2 pg m^{-3}) is slightly higher than LOQ obtained in Czech Republic [17] (2.8 pg m^{-3}). Differences obtained are due to different sampling system (low volume sampler instead of high-volume sampler). Quantification limit obtained for dimethoate (32.2 pg m^{-3}) is higher than levels obtained in the studies [13, 17] that use high-volume sampler (0.18 pg m^{-3} and 1.67 pg m^{-3}, respectively). However, LOQ of dimethoate is lower than quantification level obtained in USA (2320 pg m^{-3}) [16] using a low-volume sampler too (total sampling volume is higher in our study). LOQ obtained for diuron is higher than quantification limit obtained in Czech Republic [17] (14 pg m^{-3}) but lower than quantification limit obtained by Hengel et al. 2014 [16] (5140 pg m^{-3}) that used a low-volume sampler.

3.3. Application to real air samples

In order to study the applicability of the methodology developed, 15 outdoor air samples were collected in a rural area (L'Alcudia) of Valencia Region (Spain) from April to September of 2017 using the Partisol 2300 and the conditions described earlier. The obtained results are shown in Table 5.

Ten pesticides were detected in at least one sample, which highlights both the applicability of the analytical method and the use of pesticides in this area. The frequencies of detection (percentage of samples above the limit of detection, LOD) were ranged from 6.70 % (acetamiprid, carbendazim, carbofuran, iprovalicarb, myclobutanil and terbuthylazine) to 20.10 % (imidacloprid, pirimicarb and pyrimethanil). The concentrations of the detected pesticides ranged from 411.16 pg m^{-3} (imidacloprid) to 11011.45 pg m^{-3} (pyrimethanil). Figure 2 shows a chromatogram of several detected pesticides.

Carbendazim is a fungicide used in beans, cereals and chickpeas to control a range of diseases including Septoria, Fusarium and Scletoria. Carbendazim has only been

detected in one of the 15 samples with concentration below LOQ. The obtained concentration was similar than levels obtained in Czech Republic (concentration in the gaseous phase) [17] and in Germany (gaseous phase + particulate phase) [25].

Carbofuran is an insecticide and nematicide used for soil treatments to control soil and foliar pests in citrus trees, potatoes and corn. Carbofuran has been detected in one sample with concentration of 937.78 pg m^{-3}. The obtained level was lower than levels obtained in France [26] (gaseous phase + particulate phase levels ranging from 1430 to 28970 pg m^{-3}), Taiwan (4.3 E10 pg m^{-3}) [27] and higher than Canada (using gas chromatography, gaseous phase + particulate phase levels ranging from 38-787 pg m^{-3}) [28].

Metalaxyl is a fungicide used in vegetables to control diseases caused by air- and soil-borne pathogens. Metalaxyl has been detected in two samples with levels of 472.54 pg m^{-3} and 4385.22 pg m^{-3}, respectively. Levels obtained in Valencia were lower than levels obtained in Canada (using gas chromatography, gaseous phase + particulate phase levels ranging from 150-26700 pg m^{-3}) [29, 30].

Myclobutanil is a fungicide employed in fruit trees to control ascomycetes, fungi imperfecti and basidiomycetes. Myclobutanil has only been detected in one sample with level of 5514.59 pg m^{-3}. Concentrations obtained in Valencia were higher than levels obtained in France (using gas chromatography, gaseous phase + particulate phase levels ranging from 40-3090 pg m^{-3}) [31].

Pirimicarb is an insecticide employed in citrus trees, vegetables and potatoes for aphid control. Pirimicarb has been detected in 3 samples, with levels ranging from 776.26 pg m^{-3} to 1516.64 pg m^{-3}. Levels obtained in this study were lower than levels obtained in Canada [30] (using gas chromatography, gaseous phase + particulate phase levels ranging from 40-5100 pg m^{-3}).

Terbuthylazine is an herbicide employed in citrus trees, apple trees and maize to control grass and broad-leaved weeds in a variety of situations including forestry. Terbuthylazine was detected in one sample, with concentration of 2391.88 pg m^{-3}. The obtained level was higher than levels obtained in Czech Republic (levels ranging from 0.04 pg m^{-3} to 33.821 pg m^{-3}) [17] and in Germany (levels ranging from 0.7 pg m^{-3} to 156.1 pg m^{-3}, using gaseous phase and particulate phase) [25].

4. Conclusions

Control and surveillance of currently used pesticides (CUPs) in air has a growing interest from the point of view of health protection. In order to carry out this activity, an appropriate methodology for sampling and analysis is necessary due to the wide range of pesticides used. Previously López et al. 2017 [23] demonstrated the usefulness of sandwich PUF-XAD2-PUF for volatile and apolar pesticides (GC-amenable pesticides). In this study, we demonstrate that the sandwich is available for sampling and analysis non-volatile and polar pesticides (LC-amenable airborne pesticides).

The methodology developed presents LOQ ranging from 32.2 pg m^{-3} to 129.0 pg m^{-3}. These values are adequate for its application in surveillance and control programmes.

At the same time, UHPLC-HRMS use can enable us to perform a retrospective analysis of samples analysed.

Acknowledgements

This work has been funded by the LIFE-IRRILIFE project (LIFE 14/ENV/ES/000119) and the AIRPEST project (UGP-15-120).

References

[1] C. Coscollà, V. Yusà, Chapter 17: Pesticides and agricultural air quality in: M.de la Guardia, S. Armenta (Eds.), The Quality of Air, Elsevier, Amsterdam, 2016, pp. 423–490.

[2] EU pesticides database, 2017. Available from: http://ec.europa.eu/sanco_pesticides/public/?event=homepage [accessed June 2017].

[3] Eurostat, 2016. Eurostat Statistical Books. Agriculture, forestry and fishery statistics, 2016 Edition, ISSN 2363-2488.

[4] ECPA (European Crop Protection Association), 2010. Industry Statistics 2001-2010.Available from: http://www.ecpa.eu/page/industry-statistics.

[5] F. Van der Berg, W.G. Kubiak, M.S. Benjey, S.R. Majewski, S.R. Yates, G.L. Reeves, Emission of pesticides into the air, Water, Air, Soil Pollut. 115 (1999) 195-218.

[6] C. Bedos, M.F. Rousseau-Djabri, D. Flura, S. Masson, E. Barriuso, P. Cellier, Rate of pesticide volatilization from soil: an experimental approach with a wind tunnel system applied to trifluralin, Atmos. Environ. 36 (2002) 5917-5925.

[7] J. F. Pankow, An absorption model of gas/particle partitioning in the atmosphere, Atmos. Environ. 28 (1994) 185-188.

[8] A. Finizio, D. Mackay, T. Bidleman, T. Harner, Octanol-air partition coefficient as a predictor of partitioning of semi-volatile organic chemicals to aerosols, Atmos. Environ. 31 (15) (1997) 2289-2296.

[9] T. Harner, T. F. Bidleman, Octanol-air partition coefficient for describing particle/gas partitioning of aromatic compounds in urban air, Environ. Sci. Technol. 32 (1998) 1494-1502.

[10] A. Sofuoglu, E. Cetin, S. S. Bozacioglu, G. D. Sener, M. Odabasi, Short-term variation in ambient concentrations and gas/particle partitioning of organochlorine pesticides in Izmir, Turkey, Atmos. Environ. 38 (2004) 4483-4493.

[11] G. Bouvier, O. Blanchard, I. Momas, N. Seta, Pesticide exposure of non-occupationally exposed subjects compared to some occupational exposure: A French pilot study, *Sci. Total Environ.* 366 (2006) 74-91.

[12] L. Baraud, D. Tessier, J.J. Aaron, J.P. Quisefit, J. Pinart, A multi-residue method for characterization and determination of atmospheric pesticides measured at two French urban and rural sampling sites, Anal. Bional. Chem 377 (2003) 1148-1152.

[13] R. Raina, L. Sun, Trace level determination of selected organophosphorus pesticides and their degradation products in environmental air samples by liquid chromatography-positive ion electrospray tandem mass spectrometry, J. Environ. Sci. Health B 43 (4) (2008) 323-332.

[14] M.M. Morshed, D. Omar, R. Mohamad, S. Wahed, M.A. Rahman, Airborne paraquat measurement and its exposure to spray operators in treated field environment, Int. J Agric Biol. 12(5) (2010) 679-684.

[15] J.L. Armstrong, R.L. Dills, J. Yu, M.G. Yost, R.A. Fenske, A sensitive LC-MS/MS method for measurement of organophosphorus pesticides and their oxygen analogs in air sampling matrices, J. Environ. Sci. Health B 49 (2014) 102-108.

[16] M. Hengel, P. Lee, Community air monitoring for pesticides-part 2: multiresidue determination of pesticides in air by gas chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry, Environ. Monit. Assess 186 (2014) 1343-1353.

[17] C. Degrendele, K. Okonski, L. Melymuk, L. Landlová, P. Kukucka, O. Audy, J. Kohoutek, P. Cupr, J. Klánová, Pesticides in the atmosphere: a comparison of gas-particle partitioning and particle size distribution of legacy and current-use pesticides, Atmos. Chem. Phys. 16 (2016) 1531-1544.

[18] C. Coscollà, N. León, A. Pastor, V. Yusà, Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using Liquid Chromatography – Orbitrap High Resolution Mass Spectrometry, J. Chromatogr. A 1368 (2014) 132-142.

[19] SANTE, 2015. SANTE/ 11945/2015; 1 January 2016, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

[20] AEPLA (Asociación Empresarial para la Protección de las Plantas), 2015. Memoria AEPLA 2015. Disponible en: http://www.aepla.es/tmp/images/publicaciones/P0017_Memoria_AEPLA_2014.pdf

[21] CAPA (Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenciana), 2013. Informe del sector agrario 2012.Available from: http://www.agricultura.gva.es/web/c/document_library/get_file?uuid¼f0128d9b-3de3-43d7-a3f2-5bcdac0cf10d&groupId¼16 (accessed November 2015).

[22] A. López, V. Yusà, M. Millet, C. Coscollà, Retrospective screening of pesticide metabolites in ambient air using liquid chromatography coupled to high-resolution mass spectrometry, Talanta 150 (2016) 27-36.

[23] A. López, C. Coscollà, V. Yusà, Selection of sampling adsorbents and optimization and validation of a GC-MS/MS method for airborne pesticides, Int. J. Environ. Anal. Chem. 97 (2017) 949-964.

[24] N. G. Tsiropoulos, E. B. Bakeas, V. Raptis, S. S. Batistatou, Evaluation of solid sorbents for the determination of fenhexamid, metalaxyl-M, pyrimethanil, malathion and myclobutanil residues in air samples: Application to monitoring malathion and fenhexamid dissipation in greenhouse air using C-18 or Supelpak-2 for sampling. Anal. Chim. Acta. 573-574 (2006) 209-215.

[25] C. Mai, N. Theobald, G. Lammel, H. Hühnerfuss, Spatial, seasonal and vertical distributions of currently-used pesticides in the marine boundary layer of the North Sea, Atmos. Environ. 75 (2013) 92-102.

[26] A. Sanusi, M. Millet, P. Mirabel, H. Wortham, Comparison of atmospheric pesticide concentrations measured at three sampling sites: local, regional and long-range transport, Sci. Total. Environ. 263 (2000) 263-277.

[27] H. P. Li, J. H. Li, G. C. Li, J. F. Jen, Simultaneous determination of airborne carbamates in workplace by high performance liquid chromatography with fluorescence detection, Talanta 63 (2004) 547-553.

[28] F. Aulagnier, L. Poissant, D. Brunet, C. Beauvais, M. Pilote, C. Deblois, N. Dassylva, Pesticide measured in air and precipitation in the Yamaska Basin (Québec): Occurrence and concentrations in 2004, Sci. Total. Environ. 394 (2008) 338-348.

[29] Y. Yao, L. Tuduri, T. Harner, P. Blanchard, D. Waite, L. Poissant, C. Murphy, W. Belzer, F. Aulagnier, Y. F. Li, E. Sverko, Spatial and temporal distribution of pesticide air concentrations in Canadian agricultural regions. Atmos. Environ. 40 (2006) 4339-4351.

[30] L. M. White, W. R. Ernst, G. Julien, C. Garron, M. Leger, Ambient air concentrations of pesticides used in potato cultivation in Prince Edward Island, Canada, Pest. Manag. Sci. 62 (2006) 126-136.

[31] C. Schummer, E. Mothiron, B. M. R. Appenzeller, A. L. Rizet, R. Wenning, M. Millet, Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France, Environ. Pollut. 158 (2010) 576-584.

Table 1

Table 1. Mass list, elemental composition, accurate mass data of the compounds analysed, retention time and limit of quantification of the pesticides analysed

Compound name	Elemental Composition	Diagnostic ion	Accurate mass, m/z ion (Da)	Fragment, m/z ion (Da)	RT (min)
Acetamiprid	$C_{10}H_{11}ClN_4$	$[M+H]^+$	223.07451	126.01057	6.14
Azoxystrobin	$C_{22}H_{17}N_3O_5$	$[M+H]^+$	404.12410	372.09764	8.19
Benalaxyl	$C_{20}H_{23}NO_3$	$[M+H]^+$	326.17507	148.11204	9.02
Bitertanol	$C_{20}H_{23}N_3O_2$	$[M+H]^+$	338.18630	99.08064	9.14
Buprofezin	$C_{16}H_{23}N_3OS$	$[M+H]^+$	306.16346	201.10581	9.52
Carbendazim	$C_9H_9N_3O_2$	$[M+H]^+$	192.07675	160.05046	5.23
Carbofuran	$C_{12}H_{15}NO_3$	$[M+H]^+$	222.11247	165.09105	7.29
Cyproconazole	$C_{15}H_{18}ClN_3O$	$[M+H]^+$	292.12112	70.04033	8.63
Cyprodinil	$C_{14}H_{15}N_3$	$[M+H]^+$	226.13387	108.0809	9.08
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$	$[M+H]^+$	406.07197	251.00255	9.34
Dimethoate	$C_5H_{12}NO_3PS_2$	$[M+H]^+$	230.00689	198.96466	6.18
Diuron	$C_9H_{10}Cl_2N_2O$	$[M+H]^+$	233.02429	72.04479	8.13
Fenbuconazole	$C_{19}H_{17}ClN_4$	$[M+H]^+$	337.12145	125.01532	8.78
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	$[M+H]^+$	302.07091	97.10144	8.63
Fluazifop	$C_{15}H_{12}F_3NO_4$	$[M+H]^+$	328.07912	282.0735	8.28
Fluquinconazole	$C_{16}H_8Cl_2FN_5O$	$[M+H]^+$	376.01627	349.00491	8.66
Flusilazole	$C_{16}H_{15}F_2N_3Si$	$[M+H]^+$	316.10760	247.07464	8.84
Imazalil	$C_{14}H_{14}Cl_2N_2O$	$[M+H]^+$	297.05559	159.03072	7.77
Imidacloprid	$C_9H_{10}ClN_5O_2$	$[M+H]^+$	256.05957	175.09787	5.78
Iprovalicarb	$C_{18}H_{28}N_2O_3$	$[M+H]^+$	321.21726	119.08568	8.57
Metalaxyl	$C_{15}H_{21}NO_4$	$[M+H]^+$	280.15433	220.13327	7.71
Methidathion	$C_6H_{11}N_2O_4PS_3$	$[M+H]^+$	302.96913	85.03985	8.08
Myclobutanil	$C_{15}H_{17}ClN_4$	$[M+H]^+$	289.12145	125.01526	8.54
Omethoate	$C_5H_{12}NO_4PS$	$[M+H]^+$	214.02974	182.98751	4.20
Pirimicarb	$C_{11}H_{18}N_4O_2$	$[M+H]^+$	239.15025	72.04482	6.81
Pirimicarb-desmethyl	$C_{10}H_{16}N_4O_2$	$[M+H]^+$	225.13460	168.11317	5.56

Pyrimethanil	$C_{12}H_{13}N_3$	$[M+H]^+$	200.11822	107.06054	8.40
Tebuconazole	$C_{16}H_{22}ClN_3O$	$[M+H]^+$	308.15242	70.04040	9.02
Terbuthylazine	$C_9H_{16}ClN_5$	$[M+H]^+$	230.11669	174.05400	8.46
Thiabendazole	$C_{10}H_7N_3S$	$[M+H]^+$	202.04334	175.09788	5.97
Thiamethoxam	$C_8H_{11}ClN_4O_3S$	$[M+H]^+$	292.02656	211.06483	5.46

Table 2

Table 2. Analytical performance of the method using sandwich PUF-XAD2-PUF as solid sorbent

Pesticide**	Recovery (%) ± SD			LOQ* ($pg\ m^{-3}$)	Levels range ($pg\ m^{-3}$)
	5 ng (equivalent to 32.2 $pg\ m^{-3}$)	20 ng (equivalent to 129.0 $pg\ m^{-3}$)	100 ng (equivalent to 645.2 $pg\ m^{-3}$)		
Acetamipird	93±2	94±2	107±2	32.2	32.2-1290.4
Azoxystrobin	63±4	83±3	80±7	129.0	129.0-1290.4
Benalaxyl	84±1	92±1	87±1	32.2	32.2-1290.4
Carbendazim	91±2	97±1	112±1	32.2	32.2-1290.4
Carbofuran	98±2	101±1	110±1	32.2	32.2-1290.4
Cyproconazole	61±3	88±2	104±3	129.0	129.0-1290.4
Cyprodinil	87±1	93±1	105±3	32.2	32.2-1290.4
Difenoconazole	57±4	78±2	81±1	129.0	129.0-1290.4
Dimethoate	93±2	93±2	117±4	32.2	32.2-1290.4
Diuron	92±1	95±2	91±3	32.2	32.2-1290.4
Fenbuconazole	88±2	93±2	83±2	32.2	32.2-1290.4
Fenhexamid	60±2	83±1	91±2	129.0	129.0-1290.4
Fluazifop	87±2	76±1	82±2	32.2	32.2-1290.4
Flusilazole	58±3	92±1	105±5	129.0	129.0-1290.4
Imazalil	91±1	92±1	106±2	32.2	32.2-1290.4
Imidacloprid	88±2	100±2	107±2	32.2	32.2-1290.4
Iprovalicarb	90±2	92±1	112±1	32.2	32.2-1290.4
Metalaxyl	97±1	97±1	115±4	32.2	32.2-1290.4
Myclobutanil	92±1	96±1	104±4	32.2	32.2-1290.4
Omethoate	91.1±0.5	97±2	116±4	32.2	32.2-1290.4
Pirimicarb	89±1	92±1	110±3	32.2	32.2-1290.4
Pirimicarb-desmethyl	89±1	93±1	102±7	32.2	32.2-1290.4
Pyrimethanil	91±2	93±2	90±1	32.2	32.2-1290.4
Tebuconazole	88±1	90±2	94±2	32.2	32.2-1290.4
Tebufenpyrad	64±2	86±1	90±1	129.0	129.0-1290.4
Terbuthylazine	86±2	98±1	99±4	32.2	32.2-1290.4
Thiabendazole	90±1	96±1	99±4	32.2	32.2-1290.4
Thiamethoxam	92.8±0.5	95±1	92±0.2	32.2	32.2-1290.4

* Using PUF-XAD2-PUF as solid sorbent and using a low-volume sampler (Total Volume= 155 m^3).
** Pesticides with adequate retention capacity.

Table 3

Table 3. Retention capacity of pesticides from spiked solid sorbents (n=3) (higher fortification level=100 ng)

Pesticide	PUF-XAD2-PUF Retention capacity (%)± SD	XAD-2 Retention capacity (%)± SD	XAD-4 Retention capacity (%)± SD
Acetamipird	98±4	90±1	92±3
Azoxystrobin	90±2	91±1	78±1
Benalaxyl	87±1	79±2	-
Bitertanol	-	-	91±3
Buprofezin	-	-	88±2
Carbendazim	84.6±0.3	90±2	87±3
Carbofuran	85±2	91±2	94±2
Cyproconazole	82±2	85±2	80±4
Cyprodinil	100±1	90±3	-
Difenoconazole	80±1	91±4	80±3
Dimethoate	92±3	86±3	93±2
Diuron	85±2	88±3	-
Fenbuconazole	76±1	-	82±3
Fenhexamid	95±1	77±2	-
Fluazifop	81±1	-	-
Fluquinconazole	-	-	88±2
Flusilazole	91±3	88±2	83±3
Imazalil	92±4	92±1	84±4
Imidacloprid	92±1	93±2	85±3
Iprovalicarb	91.8±0.4	91±3	84±2
Metalaxyl	87±5	90±3	82±5
Methidathion	-	-	89±3
Myclobutanil	96±4	85±2	-
Omethoate	98±2	86±2	88±3
Pirimicarb	95.9±0.7	86.4±0.8	-
Pirimicarb-desmethyl	74±2	84±3	-
Pyrimethanil	94±2	85±3	-
Pyriproxifen	-	-	92±3
Tebuconazole	95±2	82±2	85±3
Tebufenpyrad	82±2	-	-
Terbuthylazine	93.7±0.4	84±3	88±3
Thiabendazole	76±2	78±2	80±2
Thiamethoxam	97 ±1	80±2	-

Retention capacity was calculated using the following equation: Retention capacity=[pesticide obtained]/[pesticide spiked]*100

Table 4

Table 4. Sampling comparison between methodologies that used liquid chromatography for trapping the gaseous phase of pesticide

Number of pesticides studied	Sorbent employed	Chromatographic system	Air Flow ($m^3 h^{-1}$)	Sampling Time (days)	Total Sampling Volume (m^3)	LOD/LOQ	Reference
28	PUF-XAD2-PUF	UHPLC-HRMS	1 (LVS)	7	155	LOQ: 32.2-129.0 pg m^{-3}	Present study
15	PUF	HPLC-UV	12.5/2.3 (HVS/LVS)	1/2	300/110.4	LOD: 0.7-13.8 ng m^{-3}	[12]
10	PUF	LC-MS/MS	16 (HVS)	7	2700	LOD: 0.15-28.8 pg m^{-3}	[13]
1	PUF	HPLC-PDA	0.06 (LVS)	0.5	0.72	LOQ: 33.3 ng m^{-3}	[14]
4	PUF/XAD-2	LC-MS/MS	0.12 (LVS)	1	2.88	LOD: 0.7 ng m^{-3}	[15]
19	XAD-4	LC-MS	0.9 (LVS)	1	21.6	LOD: 1160-7600 pg m^{-3}	[16]
37	PUF	HPLC-MS/MS	25 (HVS)	7	4310	LOQ: 1.67-1110 pg m^{-3}	[17]

LVS= Low -volume sampler; HVS=High-volume sampler

Table 5

Table 5. Concentration of pesticides gaseous phase in 15 samples in L'Alcúdia (Valencia Region, Spain) (pg m^{-3})

Pesticides	Samples														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acetamiprid					1618.87										
Carbendazim							<LOQ								
Carbofuran	937.78														
Imidacloprid		1105.84							411.16						542.33
Iprovalicarb	1223.13														
Metalaxyl											4385.22			472.54	
Myclobutanil	5514.59														
Pirimicarb		1516.64			848.63	776.26									
Pyrimethanil	11011.45												2585.68	2668.52	
Terbuthylazine													2391.88		

Figure 1
Click here to download high resolution image

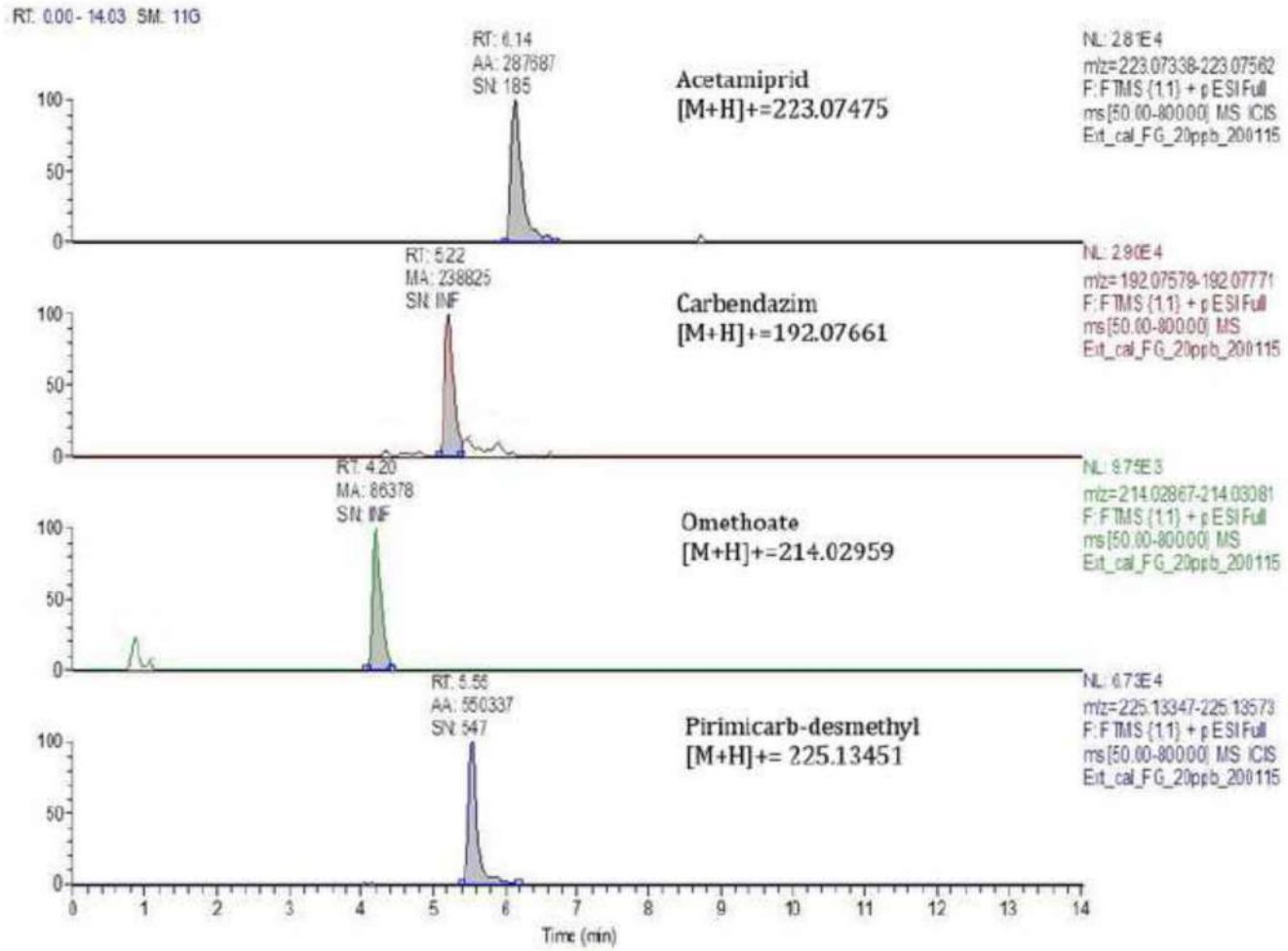
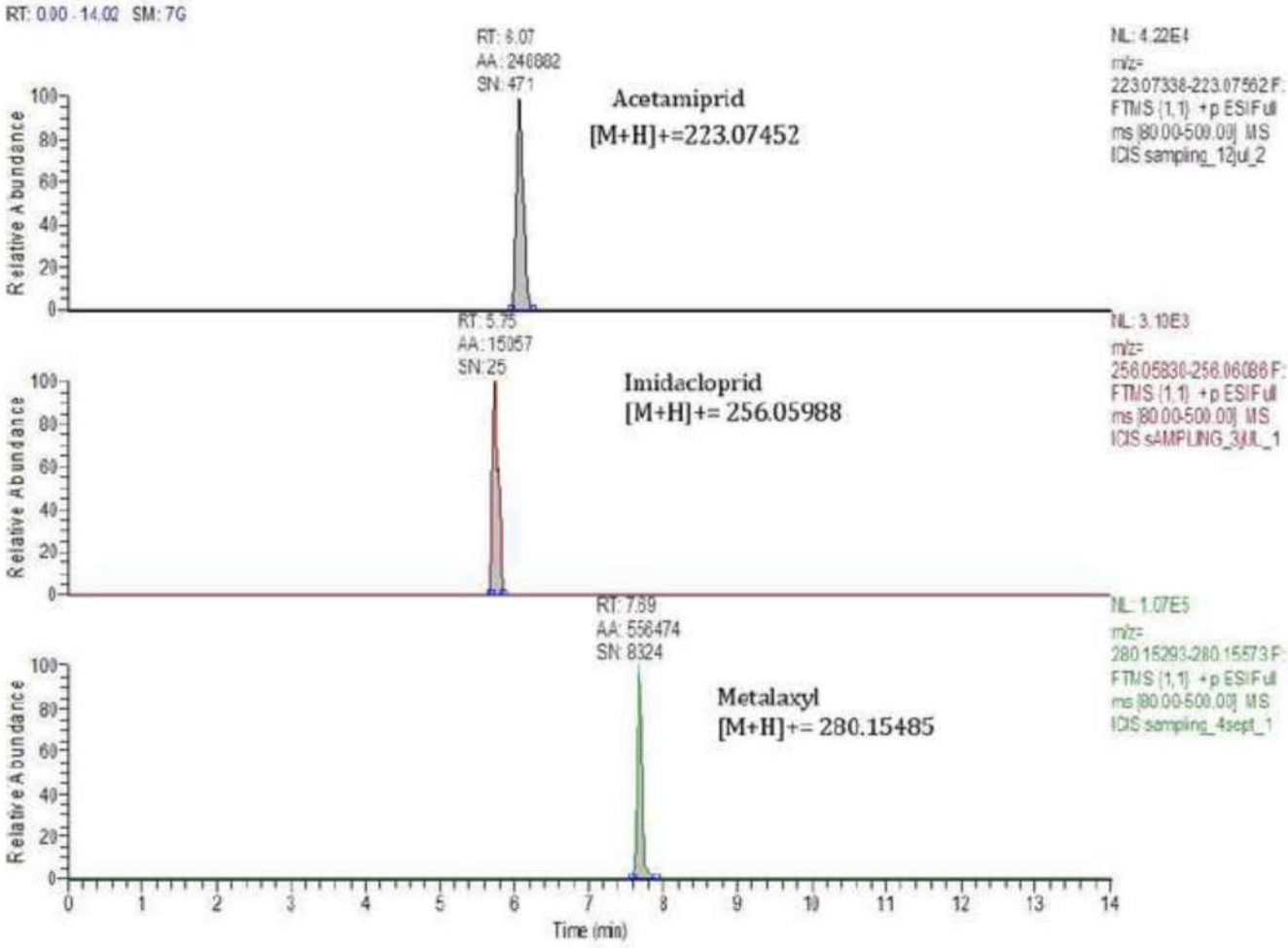


Figure 2
Click here to download high resolution image



9. CAPÍTULO 5. Evaluación del riesgo a la inhalación de plaguicidas en una región Mediterránea de España.

9.1. Introducción.

La exposición continuada a plaguicidas por parte de la población general, y en especial, en los grupos más sensibles de la población (bebés, niños, mujeres embarazadas) puede causar cáncer, problemas reproductivos, neurológicos, inmunológicos y otros efectos negativos en la salud [10-11].

La exposición a plaguicidas proviene principalmente de 3 vías principales: ingestión (alimentos, agua), vía dérmica (a través de la piel) o mediante la inhalación (aire ambiente, aire interior). Para poder estimar la exposición y el riesgo a los plaguicidas de uso habitual en agricultura (CUPs) presentes en el aire, debemos conocer su concentración, tanto en la fase particulada como en la fase gaseosa. Para ello, será necesario el uso de muestreadores capaces de recoger de forma simultánea la fase particulada y la fase gaseosa. Actualmente no existe una armonización en la etapa de recogida de muestras de aire para la captación de la fase gaseosa, debido al gran abanico de propiedades fisicoquímicas que presentan las sustancias activas que se emplean de forma habitual. En cambio, los métodos empleados para captar la fase particulada son mucho más homogéneos, ya que en general, los plaguicidas son captados mediante filtros de fibra de vidrio (GFF) o de fibra de cuarzo (QFF) [79].

Los plaguicidas autorizados para su aplicación en los diferentes cultivos no deberían causar efectos perjudiciales para los seres humanos. La EFSA publicó en el año 2014 una guía respecto a la exposición a plaguicidas y su riesgo para los aplicadores, trabajadores agrícolas, paseantes y residentes [244]. Sin embargo, los estudios realizados son escasos y no existe una metodología estandarizada para calcular el riesgo en la población general. El riesgo existente para la población ha sido estudiado tanto en zonas rurales como en zonas urbanas de todo el mundo. En España, en el año 2014, se propuso una metodología para el cálculo de exposición por inhalación a plaguicidas, partiendo de las concentraciones de plaguicidas en la fase particulada (PM10) y empleando modelos teóricos para calcular la concentración total de plaguicidas [91]. Además, en otros lugares se han realizado estudios similares para estudiar la exposición a plaguicidas organofosforados en zonas urbanas del sur de China [142] y la exposición a plaguicidas organofosforados en zonas rurales y urbanas de California [245].

En este capítulo (capítulo 5), el objetivo principal es el estudio del riesgo de la inhalación de plaguicidas en la población de la Comunidad Valenciana, a partir de la concentración de plaguicidas encontrada en la fase particulada (PM10) a través de los métodos cuantitativos (target analysis) y semicuantitativos (retrospective screening). Esta estrategia ha sido aplicada en seis estaciones rurales, dos estaciones urbanas y una estación remota situadas en diferentes lugares de la Comunidad Valenciana. El riesgo por inhalación ha sido estudiado en tres grupos de población diferentes (adultos, niños y bebés).

9.2. Resultados.

9.2.1. Concentraciones totales.

345 muestras de aire fueron analizadas en este estudio, detectándose un total de 40 plaguicidas. Las concentraciones medias oscilaron entre 8 pg m^{-3} (pirimetanil) y 30000 pg m^{-3} (abamectina). De los 40 plaguicidas detectados, 18 de ellos eran insecticidas, donde destaca la concentración media presente en el ometoato (4000 pg m^{-3}), donde los niveles encontrados son superiores a los obtenidos en la misma zona de estudio (6-2726 pg m^{-3}) [136] y en Canadá (0.9-2.7 pg m^{-3}) [123]. 16 fungicidas fueron detectados en las diferentes áreas estudiadas. Dentro de los fungicidas, los niveles de tebuconazole detectados (930 pg m^{-3}) fueron superiores a los detectados en la misma zona en otros estudios anteriores [131, 138]. Se han detectado 4 herbicidas, tres de ellos por primera vez (clorprofam, endotal y propanil). El propanil presentó la concentración media más elevada (340 pg m^{-3}), ya que a pesar de que actualmente está prohibido su uso por la UE, se permitió su uso excepcionalmente durante el periodo de estudio para el tratamiento de los arrozales. Además, la terbutilazina presentó el nivel máximo más elevado (34000 pg m^{-3}), mayor que el obtenido en anteriores estudios en la misma zona (máxima concentración de 946 pg m^{-3}) [138]. Sólo 2 nematicidas fueron detectados (carbofurano y etoprofos) con concentraciones medias que oscilaron entre 18 pg m^{-3} y 100 pg m^{-3}. El empleo del carbofurano está prohibido en la UE, así que la detección de este nematicida nos indica un posible uso ilegal de esta sustancia. La siguiente Tabla (Tabla 19) presenta un resumen de los resultados globales obtenidos en este estudio.

Tabla 19. Concentración global de los plaguicidas detectados en los filtros PM10 en todas las estaciones de muestreo (N=345)

Plaguicida	Frecuencia de detección (%) [a]	Media (pg m^{-3}) [b,c]	Rango (pg m^{-3})[c]	LOQ (pg m^{-3})[c]
Abamectin (M)	3	30000	<LQ-45000	13000 [3]
Acetamiprid (I)	3	16	12-31	7[1]/7[2]
Azoxiestrobina (F)	4	160	<LQ-800	7[1]/7[2]/3.2[3]
Bendiocarb* (I)	1	20	16-24	-
Bifentrina (I, A)	23	18	<LQ-80	7[1]/7[2]
Bitertanol (F)	4	200	<LQ-800	13[1]/13[2]/26[3]
Buprofezin (I)	17	330	<LQ-3800	13[1]/13[2]/100 [3]/ 0.5[4]
Carbendazima (F)	47	140	<LQ-2000	7[1]/7[2]/19[3]
Carbofuran (I,A,N)	5	18	<LQ-46	7[1]/7[2]
Etil Clorpirifos (I)	29	70	<LQ-210	33[1]/0.6[4]
Metil Clorpirifos (I, A)	6	10	<LQ-10	7[1]
Clorotalonilo (F)	10	13	<LQ-30	7[1]/7[2]
Clorprofam (H)	5	9	<LQ-10	7[1]
Diazinon (I, A)	32	20	<LQ-220	7[1]
Diclorvos (I, A)	5	380	120-910	7[1]
Difenilamina (F)	9	420	<LQ-1900	7[1]
Dimetoato (I, A)	11	50	<LQ-230	7[1]/7[2]
Dioxacarb* (I)	1	30	13-41	-
Endotal* (H)	1	30	26-26	-
Etoprofos (I, N)	11	100	<LQ-1200	7[1]
Fludioxonil (F)	7	19	<LQ-39	7[1]
Folpet (F)	16	38	<LQ-160	7[1]
Hexythiazox (A)	14	350	8-6000	7[3]
Imazalil (F)	4	140	<LQ-340	14[1]/7[2]/19 [3]
Imidacloprid (I)	13	100	<LQ-900	7[1]/7[2]/3 [3]
Iprodiona (F)	5	33	<LQ-36	7[1]
Kresoxim metil (F)	6	10	<LQ-14	7[1]
Malation (I, A)	14	12	<LQ-90	7[1]/0.5[4]
Metalaxyl (F)	45	250	<LQ-1000	7[1]/7[2]/250 [3]
Ometoato (I, A)	56	4000	<LQ-17000	7[1]/2.60[2]/3300 [3]
o-fenilfenol* (F)	1	22	11-33	-
Prohexadiona* (P)	3	56	13-90	-
Propanil (H)	3	340	24-900	5[3]
Pirimetanil (H)	5	8	<LQ-8	7[1]/7[2]
Piriproxifen (I)	8	300	<LQ-3800	7[1]/9[3]/0.4 [4]
Spinosad (I)	2	510	190-800	-
Tebuconazol (F)	19	930	<LQ-7000	7[1]/7[2]/330 [3]/0.6 [4]
Terbutilazina (H)	53	100	<LQ-34000	7[1]/7[2]
Tiabendazol (F)	5	340	<LQ-900	7[1]/75[2]/50[3]
Triciclazol* (F)	1	24	15-29	-

[a] La frecuencia de detección ha sido calculada para concentraciones superiores al LOD.
[b] La media ha sido calculada para aquellas muestras con concentraciones superiores al LOQ.
[c] Los resultados han sido redondeados considerando la variabilidad del método analítico (20 %).
* Concentraciones semicuantitativas
[1]= LC-MS/MS; [2]=UHPLC-HRMS; [3]=LC-MS, [4]=GC-MS
I= Insecticida; F= Fungicida; H= Herbicida; N= Nematicida; M=Derivados de microorganismos; P= Regulador del crecimiento de las plantas

9.2.2. Distribución espacial y temporal de los plaguicidas

En general, los plaguicidas detectados se encontraban en las zonas rurales estudiadas, cercanas a cultivos de cítricos, viñedos y otros cultivos habituales de la zona. Sólo buprofezin y carbendazim fueron detectados en todas las estaciones a distintas concentraciones. Así, por tanto, cada área de estudio presenta un perfil característico. En el Anexo V (Tablas SI-2 y SI-3) se muestran las concentraciones medias, frecuencias de detección y rangos para cada plaguicida en cada estación de muestreo.

En las zonas rurales de Alzira, Burriana, Benifaió, rodeadas de cítricos principalmente, los plaguicidas detectados en mayor frecuencia y concentración son el ometoato y la carbendazima. El ometoato, a pesar de estar prohibido desde el año 2003 por la UE, es muy habitual su detección debido a que la oxidación del dimetoato (plaguicida permitido para su uso) provoca la formación del ometoato (el correspondiente oxon), de forma similar a lo que ocurre con otros organotiofosfatos como el diazinon o el metil clorpirifos. Concentraciones elevadas de ometoato fueron detectadas en Burriana (1600 pg m^{-3}) y Benifaió (8000 pg m^{-3}). La carbendazima actualmente está prohibida por la UE, pero estaba permitida en el periodo de estudio. Los niveles de carbendazima obtenidos son inferiores a los detectados en la misma zona en estudios anteriores [138].

Las zonas rurales de Benicarló, Villar del Arzobispo y Sant Jordi están rodeadas por cultivos de secano como son los viñedos, cereales y olivares, donde es habitual la detección del ometoato. Elevadas concentraciones de ometoato fueron detectadas en Benicarló (7000 pg m^{-3}) y Sant Jordi (17000 pg m^{-3}). Los niveles detectados fueron superiores a los encontrados en una zona rural de Canadá (2.7 pg m^{-3}) [123] pero inferiores a los encontrados en una zona vinícola de Italia (30000 pg m^{-3}) [136].

La zona remota estudiada (Morella) está localizada lejos de toda zona agrícola, de forma que la frecuencia de detección de plaguicidas y la concentración detectada fue baja. Sin embargo, se encontraron niveles elevados de tiabendazol en una de las muestras (600 pg m^{-3}), concentración muy superior a la encontrada en un estudio anterior (13-80 pg m^{-3}) [138], debido seguramente al transporte de partículas desde las zonas rurales.

En las zonas urbanas estudiadas, destaca la frecuencia de uso del metalaxil en Viveros, debido seguramente a su uso en jardines, el uso de diclorvos y ometoato en Burjassot y las elevadas concentraciones encontradas de difenilamina y tebuconazol (1200 y 7000 pg m^{-3}, respectivamente) en la estación de Burjassot.

En alguna de las estaciones rurales estudiadas (Alzira, Burriana, Benicarló y Benifaió) se ha observado una variación temporal a lo largo de los años de estudio para algunos de los plaguicidas estudiados. Así, para el ometoato, la terbutilazina, el metalaxil y la carbendazim se han observado valores elevados en los primeros años (2008-2009) y se ha observado una reducción de los niveles detectados en los siguientes años de estudio (ver Figura SI-1 del anexo V).

9.2.3. Evaluación del riesgo.

Los posibles efectos adversos que presenta la exposición a plaguicidas en la población general, y específicamente en los grupos de población más susceptibles, son un problema de concienciación pública [10-11]. Así, en este estudio, se ha evaluado la

exposición diaria a plaguicidas mediante inhalación y su riesgo derivado, empleando la ecuación 4 descrita en el apartado 4.8 de esta Tesis. Los valores máximos obtenidos para la exposición diaria fueron similares en todas las estaciones estudiadas excepto en la zona rural de Alzira, donde se obtuvo un valor máximo de 4.09 E-03 correspondiente a la prohexadiona. Los valores obtenidos para la exposición diaria obtenidos en nuestra zona de estudio fueron inferiores a los obtenidos en California para clorpirifos, malation y dimetoato.

Además, como se ha mencionado en el apartado de metodología, se ha calculado también el índice de peligro (HQ) para cada plaguicida, empleando la ecuación 5 descrita en el apartado 4.8. Los valores máximos obtenidos de HQ para la población más vulnerable (bebés) en las zonas rurales de Burriana, Benicarló y Villar del Arzobispo, corresponden a diazinon (0.41), hexythiazox (0.497) y ometoato (0.503), respectivamente. Cómo todos los valores obtenidos están por debajo del valor de riesgo (HQ<1), se puede considerar que los niveles obtenidos en las zonas rurales estudiadas no presentan riesgos para la salud [246]. La siguiente Tabla (Tabla 20) muestra los valores globales de DIE y HQ para los plaguicidas detectados en las distintas estaciones de muestreo estudiadas. La Tabla SI-4 del anexo V muestra los valores de DIE y HQ en cada una de las estaciones de muestreo.

Tabla 20. Valores máximos de Exposición Inhalatoria diaria (mg kg^{-1} dia^{-1}) y cocientes de riesgo (HQ_{AOEL}) para los plaguicidas detectados

Plaguicida	Concentración máxima (pg m^{-3})*	Adultos (>12 años)		Niños (1-6 años)		Bebés (>6-1.5 años)	
		DIE [a]	HQ_{AOEL}	DIE [b]	HQ_{AOEL}	DIE [c]	HQ_{AOEL}
Abamectina	45000	1.80E-05	7.19E-03	4.24E-05	1.70E-02	5.00E-05	2.00E-02
Acetamiprid	31	9.19E-09	1.31E-07	2.17E-08	3.10E-07	2.56E-08	3.66E-07
Azoxiestrobina	800	2.21E-07	1.10E-06	5.21E-07	2.61E-06	6.14E-07	3.07E-06
Bendiocarb	24	2.06E-06	3.17E-04	4.86E-06	7.48E-04	5.73E-06	8.81E-04
Bifentrina	80	2.37E-08	3.16E-06	5.60E-08	7.47E-06	6.60E-08	8.80E-06
Bitertanol	800	2.26E-07	1.13E-06	5.34E-07	2.67E-06	6.30E-07	3.15E-06
Buprofezin	3800	2.42E-06	6.05E-05	5.72E-06	1.43E-04	6.74E-06	1.68E-04
Carbendazima	2000	2.35E-04	1.18E-02	5.56E-04	2.78E-02	6.55E-04	3.28E-02
Carbofuran	46	5.21E-08	1.74E-04	1.23E-07	4.10E-04	1.45E-07	4.84E-04
Etil Clorpirifos	210	2.21E-06	2.21E-03	5.22E-06	5.22E-03	6.15E-06	6.15E-03
Metil Clorpirifos	10	2.65E-07	2.65E-05	6.25E-07	6.25E-05	7.37E-07	7.37E-05
Clorotalonil	30	8.70E-07	9.66E-05	2.05E-06	2.28E-04	2.42E-06	2.69E-04
Clorprofam	10	8.54E-08	1.71E-06	2.02E-07	4.03E-06	2.38E-07	4.75E-06
Diazinon	220	2.95E-06	1.47E-02	6.96E-06	3.48E-02	8.20E-06	4.10E-02
Diclorvos	910	3.18E-05	6.36E-02	7.52E-05	7.50E-02	8.86E-05	1.77E-01
Difenilamina	1900	1.02E-04	1.02E-03	2.41E-04	2.41E-03	2.84E-04	2.84E-03
Dimetoato	230	1.34E-07	1.34E-04	3.15E-07	3.15E-04	3.72E-07	3.72E-04
Etoprofos	1200	3.46E-07	3.46E-04	8.17E-07	8.17E-04	9.62E-07	9.62E-04
Fludioxonil	39	1.12E-08	1.90E-08	2.64E-08	4.48E-08	3.12E-08	5.28E-08
Folpet	160	1.37E-06	1.37E-05	3.24E-06	3.24E-05	3.82E-06	3.82E-05
Hexythiazox	6000	1.61E-04	1.79E-02	3.79E-04	4.22E-02	4.47E-04	4.97E-02
Imazalil	340	2.05E-07	4.10E-06	4.84E-07	9.67E-06	5.70E-07	1.14E-05
Imidacloprid	900	2.54E-07	3.17E-06	5.99E-07	7.48E-06	7.06E-07	8.82E-06
Iprodiona	36	1.05E-08	3.49E-08	2.47E-08	8.23E-08	2.91E-08	9.70E-08
Kresoxim-metil	14	6.00E-09	6.67E-09	1.42E-08	1.57E-08	1.67E-08	1.86E-08
Malation	90	2.07E-07	6.90E-06	4.89E-07	1.63E-05	5.76E-07	1.92E-05
Metalaxil	1000	7.31E-05	9.14E-04	1.73E-04	2.16E-03	2.04E-04	2.54E-03
Ometoato	17000	5.43E-06	1.81E-02	1.28E-05	4.27E-02	1.51E-05	5.03E-02
o-fenilfenol	33	2.49E-06	6.23E-06	5.89E-06	1.47E-05	6.94E-06	1.74E-05
Prohexadiona	90	1.47E-03	4.20E-03	3.47E-03	9.92E-03	4.09E-03	1.17E-02
Propanil	900	2.41E-06	1.21E-04	5.70E-06	2.85E-04	6.72E-06	3.36E-04
Pirimetanil	8	4.93E-08	4.11E-07	1.16E-07	9.69E-07	1.37E-07	1.14E-06
Piriproxifen	3800	1.28E-06	3.21E-05	3.03E-06	7.58E-05	3.57E-06	8.93E-05
Spinosad	800	2.26E-07	5.66E-06	5.35E-07	1.34E-05	6.30E-07	1.58E-05
Tebuconazol	7000	1.99E-06	1.99E-03	4.69E-06	4.69E-03	5.53E-06	5.53E-03
Terbutilazina	34000	4.32E-05	1.35E-02	1.02E-04	3.19E-02	1.20E-04	3.75E-02

Tiabendazol	900	2.58E-07	2.58E-06	6.10E-07	6.10E-06	7.19E-07	7.19E-06

[a] USEPA 1989, USEPA 2004, USEPA 1991 (DIE: Exposición Inhalatoria Diaria; peso corporal=70 kg, Tasa de inhalación=20 m^3 $día^{-1}$).
[b] USEPA 2004, USEPA 1991 (DIE: Exposición Inhalatoria Diaria; peso corporal=15 kg, Tasa de inhalación=10 m^3 $día^{-1}$)
[c] (DIE: Exposición Inhalatoria Diaria; peso corporal=10 kg, Tasa de inhalación=8 m^3 $día^{-1}$)
***** Los resultados han sido redondeados considerando la variabilidad del método analítico (20 %)

Además, empleando la ecuación 6 (apartado 4.8), se ha podido calcular la exposición por acumulación para aquellos plaguicidas que presentan un modo semejante de acción (neonicotinoides, benzimidazoles, carbamatos, triazoles, organofosforados). Todos los valores calculados fueron inferiores a 1 (HI<1), de forma que se considera que no existe riesgo debido a la inhalación por acumulación. Los resultados obtenidos para los índices de riesgo acumulados fueron semejantes a los obtenidos en anteriores estudios en la zona [91] y a estudios realizados en California para organofosforados en el año 2011 [247].

El riesgo de cáncer se ha calculado para aquellos plaguicidas clasificados como posibles cancerígenos para el ser humano siguiendo la clasificación realizada por la EPA [248] en el año 2013 y empleando la ecuación 9 (apartado 4.8). Así, valores de riesgo de cáncer obtenidos mayores a 1 E-06, indicarían preocupación respecto a los niveles obtenidos para ese plaguicida concreto. Para carbendazima y hexitiazox se han obtenidos para bebés valores inferiores a 1 E-06 en cuatro de las estaciones rurales (Alzira, Burriana, Benicarló y Benifaió) y en una de las estaciones urbanas (Burjassot). Estos valores obtenidos presentan mayor riesgo que los valores obtenidos en un estudio previo [91]. En la siguiente Tabla (Tabla 21), se presentan los valores de riesgo de cáncer en cada una de las estaciones estudiadas:

Tabla 21. Riesgo de cáncer para los plaguicidas potencialmente cancerígenos

Alzira (N=79)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Bifentrina	2.37E-09	5.60E-09	6.60E-09
Carbendazima	4.38E-06	1.04E-05	1.22E-05
Clorotalonil	8.70E-08	2.05E-07	2.42E-07
Dimetoato	4.64E-09	1.10E-08	1.29E-08
Folpet	7.04E-08	1.66E-07	1.96E-07
Malation	2.07E-08	4.89E-08	5.76E-08
Tebuconazol	3.88E-10	9.17E-10	1.08E-09
Burriana (N=48)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Bifentrina	5.66E-10	1.34E-09	1.57E-09
Carbendazima	3.80E-06	8.98E-06	1.06E-05
Clorotalonil	3.38E-08	7.99E-08	9.42E-08
Dimetoato	4.85E-09	1.14E-08	1.35E-08
Etoprofos	2.25E-07	5.31E-07	6.26E-07
Folpet	3.77E-08	8.90E-08	1.05E-07
Imazalil	1.30E-08	3.06E-08	3.61E-08
Iprodiona	1.05E-09	2.47E-09	2.91E-09
Kresoxim-m	3.59E-10	8.47E-10	9.99E-10
Malation	2.90E-09	6.84E-09	8.06E-09
Pirimetanil	3.41E-09	8.05E-09	9.49E-09
Tebuconazol	3.15E-10	7.45E-10	8.78E-10
Benicarló (N=25)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Carbendazima	2.35E-05	5.56E-05	6.55E-05
Hexythiazox	1.61E-05	3.79E-05	4.47E-05
Imazalil	2.05E-08	4.84E-08	5.70E-08
Tebuconazol	3.76E-09	8.88E-09	1.05E-08

Benifaió (N=23)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Carbendazima	6.12E-06	1.44E-05	1.70E-05
Hexythiazox	3.01E-06	7.10E-06	8.37E-06
Imazalil	1.65E-08	3.90E-08	4.59E-08
Tebuconazol	1.84E-08	4.34E-08	5.11E-08
Villar del Arzobispo (N=9)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Carbendazim	4.37E-07	1.03E-06	1.22E-06
Hexythiazox	1.08E-06	2.54E-06	3.00E-06
Sant Jordi (N=43)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Bifentrina	6.90E-10	1.63E-09	1.92E-09
Carbendazima	2.39E-06	5.65E-06	6.66E-06
Clorotalonil	1.88E-08	4.44E-08	5.23E-08
Dimetoato	1.34E-08	3.15E-08	3.72E-08
Etoprofos	9.60E-08	2.27E-07	2.67E-07
Folpet	1.37E-07	3.24E-07	3.82E-07
Iprodione	8.78E-10	2.07E-09	2.44E-09
Kresoxim-m	6.00E-10	1.42E-09	1.67E-09
Malation	1.01E-08	2.39E-08	2.82E-08
Tebuconazol	2.71E-08	6.41E-08	7.55E-08
Morella (N=54)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Carbendazima	1.13E-06	2.66E-06	3.13E-06
Clorotalonil	6.16E-08	1.46E-07	1.71E-07
Folpet	4.65E-08	1.10E-07	1.29E-07
Hexythiazox	5.55E-07	1.31E-06	1.54E-06
Kresoxim-m	1.86E-10	4.39E-10	5.17E-10
Tebuconazol	2.80E-10	6.61E-10	7.79E-10
Viveros (Valencia) (N=48)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Bifentrina	8.81E-10	2.08E-09	2.45E-09
Carbendazima	9.31E-07	2.20E-06	2.59E-06
Clorotalonil	3.27E-08	7.72E-08	9.10E-08
Dimetoato	1.21E-08	2.86E-08	3.38E-08
Etoprofos	3.46E-08	8.17E-08	9.62E-08
Folpet	5.66E-08	1.34E-07	1.58E-07
Malation	1.47E-09	3.48E-09	4.10E-09
Pirimetanil	4.93E-09	1.16E-08	1.37E-08
Tebuconazol	2.37E-10	5.60E-10	6.60E-10
Burjassot (N=16)			
Plaguicida	**Adultos**	**Niños**	**Bebés**
Carbendazima	1.01E-05	2.39E-05	2.82E-05
Diclorvos	3.18E-06	7.52E-06	8.86E-06
Hexythiazox	2.63E-06	6.21E-06	7.32E-06
Tebuconazol	1.99E-07	4.69E-07	5.53E-07

9.3. Conclusiones.

-En este trabajo se han detectado un total de 40 plaguicidas (la mayoría insecticidas y fungicidas) en la atmósfera de diferentes zonas rurales, urbanas y remotas de la Comunidad Valenciana. Sólo dos plaguicidas fueron detectados en todas las estaciones (buprofezin y carbendazim). Las zonas rurales y urbanas presentan diferentes perfiles característicos según su localización (prácticas agrícolas, tiempo de aplicación y clima).

- La exposición debido a la inhalación (DIE) de plaguicidas oscila entre 3.88 E-09 mg kg^{-1} dia $^{-1}$ a 4.09 E-09 mg kg^{-1} dia^{-1}. El índice de peligro (HQ) fue evaluado para diferentes grupos de población (bebés, niños y adultos). Los valores máximos de HQ obtenidos en adultos (6.36 E-02), niños (7.50 E-02) y bebés (1.77 E-01), no representan un riesgo significativo para la salud en las diferentes zonas de estudio.

-Los índices de peligro acumulados (HI) calculados fueron menores de 1 en todos los grupos de población, considerando estos valores como apropiados para la salud de la población de la zona estudiada.

-Los valores de riesgo de cáncer para bebés calculados fueron menor que 10^{-6} (valor indicativo a partir del cual existe cierto riesgo) excepto para la carbendazima y el hexitiazox en varias de las estaciones empleadas.

-En un futuro próximo, será necesario establecer valores de referencia en salud para los niveles de plaguicidas en la atmósfera.

9.4. Artículo 5: Risk assessment of airborne pesticides in a Mediterranean region of Spain.

Science of the Total Environment 574 (2017) 734–734

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Risk assessment of airborne pesticides in a Mediterranean region of Spain

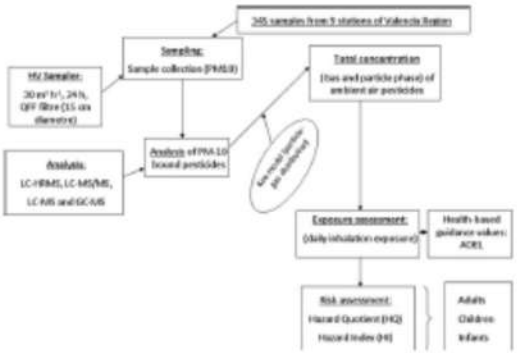
Antonio López [a,b,c,e], Vicent Yusà [a,b,c,e], Amalia Muñoz [d,e], Teresa Vera [d,e], Esther Borràs [d,e], Milagros Ródenas [d,e], Clara Coscollà [a,c,e,*]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020, Valencia, Spain
[b] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain
[c] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020, Valencia, Spain
[d] Instituto Universitario UMH-CEAM, C/Charles Darwin, 14, Parque Tecnológico, 46980 Paterna, Valencia, Spain
[e] Atmospheric Pesticide Research Group of Valencia, Spain

HIGHLIGHTS

- Levels of airborne pesticides ranged from 8 to 30,000 pg m^{-3}.
- Inhalation exposure was estimated for 40 airborne pesticides.
- Hazard Quotient was <1 for all pesticides detected in the three groups of population.
- Hazard Index was less than 1 for the three groups of population.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form 10 August 2016
Accepted 20 August 2016
Available online xxxx

Editor: Marco Trevisan

Keywords:
Pesticides
Ambient air
Risk assessment
Gas/particle partitioning

ABSTRACT

A risk assessment strategy based on the quantitative target analysis and semi-quantitative retrospective screening determination of pesticides in PM10 has been developed. The proposed methodology was applied to 345 real samples from nine stations of a Mediterranean area in Spain, and the risk was assessed for adult, children and infants. Forty pesticides were detected with average concentrations ranging from 8 to 30,000 pg m^{-3}. Each station showed its specific pesticide profile, which is linked to the different types of crops around each station. For adults, children and infants the estimated chronic inhalation risk, expressed as Hazard Quotient (HQ), was <1 for all pesticides. The cumulative exposure for organophosphates, neonicotinoids, benzimidazoles, carbamates, microorganism and triazoles pesticides (HI, Hazard Index) were <1 for the three groups of populations assessed. For infants, the cancer risk estimated for the detected pesticides classified as possible and potential carcinogens were lower than 1.0 E-06, except for carbendazim and hexythiazox.

* Corresponding author at: Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020, Valencia, Spain.
E-mail address: coscolla_cla@gva.es (C. Coscollà).

http://dx.doi.org/10.1016/j.scitotenv.2016.08.149
0048-9697/

1. Introduction

A wide variety of pesticides can be applied in agriculture and their identity depends on a range of factors including the specific pest and crop of interest. During 2013, about 300,000 t of pesticide active ingredients were used in Europe (EU-15) (Eurostat, 2013; ECPA, 2010) and around 500 active substances are nowadays authorised by the European Union for their application on various crops according to the Regulation (EC) 1107/2009 (EU Pesticide Database, 2016). The potentially adverse effects of exposure to pesticides on the general population, and specifically on the more susceptible groups such as infants and children, are a public health concern (Marks et al., 2010; London et al., 2012).

Apart from ingestion of food and drinking water, inhalation of ambient air could be a relevant pathway of exposure to pesticides. To perform an inhalation risk assessment of exposures to atmospheric CUPs (currently used pesticides), it is necessary to know the total concentration (gas + particulate phases) of these compounds in the ambient air. This requires that the air samplers collect both the particle phase and the gas phase (using appropriate adsorbents). At present, there is a lack of consistency in sampling methodologies. Standardization of sampling methods for pesticides in the gas phase is the most difficult part, owing to the wide range of physicochemical properties of the active substance currently in use (Yusà et al., 2009). On the contrary, the methods for collection of pesticides bound to the particulate matter seem to be more homogenous, and in general, pesticides are trapped in glass fibre filters (GFF) or quartz fibre filters (QFFs) (Yusà et al., 2009).

The pesticides applied to crops must have no harmful effects on humans. Although international guidelines on pesticide exposure and risk assessment for operators, workers, bystanders and residents have recently been developed (EFSA, 2014), the methodologies focused on the exposure and risk assessment of airborne pesticides on the general population are scarce. Risk assessment to inhalated pesticides have been studied in rural and urban areas worldwide. In Spain, Yusà et al. (2014) proposed a screening approach for inhalation chronic risk assessment of CUPs present in rural ambient air, based on the concentration of these compounds in the inhalable particulate matter (PM10). In that study, total pesticide concentration was estimated applying theoretical partitioning models. In addition, Li et al. (2014) studied the inhalation exposure of organophosphate pesticides in an urban community of South China and Lee et al. (2002) in rural and urban stations in California region (USA).

In this paper, we have assessed the inhalation risk of pesticides in Valencia Region population based on the quantitative (target) and semi-quantitative (retrospective screening) determination of pesticides in PM10. The strategy was applied to six rural stations, two urban stations and one remote station placed in Valencia Region, and the risk was assessed for three populations (adults, children and infants).

2. Experimental

2.1. Reagents and chemicals

Certified commercial standards were of high purity and purchase from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma Aldrich (Barcelona, Spain). Individual stock standards were prepared weighing 10 mg of pure standard using a 5 decimal analytical balance and dissolving each compound in 50 mL of acetone. They were stored in capped amber vials at −21 °C (SANCO, 2007). Mix working solutions at 10 and 1 mg L^{-1} were prepared with methanol. Calibration solutions were prepared by adding variable volumes of the mix working solutions in the PM10 blank filters.

Methanol and acetonitrile were HPLC-grade supplied by Scharlau (Barcelona, Spain). Acetone, ethyl acetate and water were of HPLC-grade and were purchased from Merck (Darmstadt, Germany). Glacial acetic acid and formic acid 98% were provided by Panreac (Barcelona, Spain). Ammonium formate, solution Ultra (100 mL, 10 M in water) was provided by Fluka (Steinheim, Switzerland).

2.2. Sampling and site characterization

Valencia Region is a Mediterranean area and is situated on the East coast of Spain. This region made up 12.6% of the total national consumption of pesticides in 2014 (AEPLA, 2014). The main irrigated crops are citrus fruit, other fruit trees (mainly peach, apricot and plum trees), rice and garden produce (primarily watermelon, cabbage, artichoke, lettuce, cauliflower, tomatoes, potatoes and onion). The main dry crops are vineyards, olive trees and almonds (CAPA, 2013).

Samples were collected using a high-volume sampler from Digitel (Madrid) and quartz fibre filters of 150 mm of diameter, supplied by Munktell filter AB (Falun, Sweden). A sampling flow of 30 $m^3\ h^{-1}$ for 24 h that provides a volume of around 720 m^3 was used. A total of 345 samples were collected from 2008 to 2014, following pesticide applications in the Valencia Region between February to November. PM10 samples were collected from six rural sampling stations (Alzira, Burriana, Sant Jordi, Benicarló, Villar del Arzobispo and Benifaió), two urban stations (Viveros and Burjassot) and one remote station (Morella) (Table 1). Rural stations are close to many citrus crops (orange trees), vineyards and cereals.

Prior to exposition, filters were baked for 24 h at 300 °C to eliminate organics. To determine the weight of particulate matter accumulated during 24-h exposition of filters, a Metler Toledo MX5 microbalance (from Bedford, MA, USA) was used. Filters were previously conditioned according to European standard sampling at a temperature of 20 ± 1 °C and at relative humidity of 50 ± 5 °C for at least 48 h, and then weighted.

2.3. Sample preparation

For Alzira, Burriana, Morella, Sant Jordi and Viveros stations, air samples were analyzed by LC-MS/MS (Liquid Chromatography coupled to mass spectrometry in tandem), except for Alzira samples in 2013 and Burriana samples in 2014. In this period and stations, air samples were analyzed by UHPLC-HRMS (Liquid Chromatography coupled to high resolution mass spectrometry). A generic extraction method developed in a previous work using microwave extraction (MAE) with ethyl acetate was employed (Coscollà et al., 2014). MAE of pesticides from PM10 samples was carried out using a Mars system from CEM corporation (Mathews, NC, USA) equipped with Teflon® TFM 100 mL extraction vessels. The extraction conditions were as follows: a temperature of 50 °C was applied for 20 min, using a power of 1200 W, and 30 mL of ethyl acetate were added. After cooling, the reactor was opened and the extracts were filtered. After 100 μl of diethylene glycol (keeper) were added to the extract, it was concentrated with Turbo Vap 500 (Zymark, Idstein, Germany). The extracts were re-dissolved with 1 mL of water: methanol (70:30) and filtered through a 0.22 μm GHP Acrodisc filter from Pall Life Science (Ann Arbor, USA) prior to the LC-MS/MS or LC-HRMS determination.

In the case of Benicarló, Benifaió, Villar del Arzobispo and Burjassot sampling sites, air samples were analyzed by LC-MS (Liquid chromatography mass spectrometry detection) and GC–MS (Gas chromatography mass spectrometry detection). For LC-MS, air samples were ultrasonically extracted (VWR ultrasonic bath, Barcelona, Spain) three times with 10 mL of ethylacetate 15 min each time. The extract was concentrated in a rotavapor system (50 °C, 180 rpm) for 5 min, to total dryness. Then, the extract was dissolved in 500 μL of methanol and reduced to 100 μL under a gentle stream of N_2. Finally, the extract was injected (5 μL) in the LC-MS. In GC–MS, air samples were ultrasonically extracted (VWR ultrasonic bath, Barcelona, Spain) two times with 10 mL of isooctane for 10 min each time. The extract was concentrated in a rotavapor system (40 °C, 180 rpm) for 5 min, avoiding total dryness. Then, the extract was dissolved in 1 mL of isooctane and dried under a gentle stream

Table 1
Description of sampling sites.

Sampling site	Latitude	Longitude	Description	Total sample number
Alzira	39°09′ 00″	0°27′28″	Rural and agricultural area surrounded by citrus groves (orange trees). Samples collected about 60 m above sea level.	79
Burriana	39°53′ 52″	0°03′54″	Rural and agricultural area surrounded by citrus groves (orange trees). Samples collected about 20 m above sea level.	48
Benicarló	40°25′ 07″	0°25′23″	Rural and agricultural area surrounded by vineyards. Samples collected about 20 m above sea level.	25
Benifaió	39°17′ 07″	0°25′35″	Rural and agricultural area surrounded by citrus groves (orange trees). Samples collected about 35 m above sea level.	23
Villar del Arzobispo	39°44′ 01″	0°49′38″	Rural and agricultural area surrounded by vineyards, cereals and olive trees. Samples collected about 520 m above sea level.	9
Sant Jordi	40°30′ 34″	0°19′55″	Rural and agricultural area (vineyards, almond, olive and carob trees). Next to a golf course (Panorámica Golf), which occupies 80 ha. Samples collected at 181 m above sea level.	43
Morella	40°38′ 14″	0°05′33″	Remote area. Samples collected at the peak of Monte Mas del Aljub, 1153 m above sea level.	54
Viveros (Valencia)	39°28′ 46″	0°22′10″	Commercial and residential area and inside a park (Viveros) with gardens. Samples collected at 11 m above sea level.	48
Burjassot	39°30′ 34″	0°25′04″	Urban area. Samples collected about 60 m above sea level.	16
				345

of N_2. Finally, the extract was dissolved in 150 μL of 1-phenyldodecane, 5 mg L^{-1} in isooctane, to inject 1 μL in the GC–MS.

2.4. Analytical determination

2.4.1. LC-MS/MS

The LC-MS/MS systems consists on a Finnigan Surveyor Autosampler, a Finnigan Surveyor LC quaternary Pump and a Finnigan TSQ Quantum Ultra detector (San José, CA, USA). Chromatographic separation was performed on a Luna C18 (2) column (150 mm × 2.00 mm I.D., 5 μm particle size) from Phenomenex (Madrid, Spain). The mobile phase was a gradient of H_2O with 0.1% formic acid and 5 mM ammonium formate (A) and methanol (B) and the gradient conditions were as follows: 0–8 min, linear from 30 to 35% B; 8–12 min, linear from 35 to 90% B; 12–22 min, isocratic 90% B; 22–23 min, linear from 90% to 30%; 23–25 min, isocratic 30%. The flow rate was 200 μL min^{-1}. The autosampler and column temperatures were set at 20 °C and a 10 μL volume injection was used. Mass spectrometry parameters were optimized in a previous study (Coscollà et al., 2009).

2.4.2. UHPLC-Orbitrap-HRMS

Chromatographic separation was performed on an Accela liquid chromatography UHPLC system equipped with a Hypersil Gold aQ column (100 mm × 2.1 mm, 1.9 μm) both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 300 μL min^{-1} and the injection volume was 10 μL. Separations were performed using a binary gradient. The mobile phase was a gradient of H_2O with 0.1% formic acid and 4 mM ammonium formate (A) and methanol with 0.1% formic acid and 4 mM ammonium formate (B) and the gradient conditions were as follow: 0–8 min, linear with 100% of A; 8–12 min, linear with 100% of B and 12–16 min, linear with 100% of A. The total run time was 16 min. The UHPLC system was coupled to a single stage Orbitrap mass spectrometer (Exactive ™, Thermo Fisher Scientific, Bremen, Germany). Coscollà et al. (2014) published the High Resolution Mass Spectrometry (HRMS) parameters.

2.4.3. LC-Ms

Surveyor LC-LCQ Advantage MS was used (Thermo Fisher Scientific Co., Waltham, MA, USA) with a ProntoSil 120–5 C18 column (150 × 2 mm; 5 μm) (supplied by Scharlab, Spain). For the chromatographic separation, the mobile phases were A: methanol and B: water at 0.1% acetic acid. The flow rate was selected at 200 μL min^{-1} and the gradient profile design was at 0 min 50% A, from 0 to 35 min linear gradient to 90% A, and at 90% A until the end of the chromatogram (50 min.). The detector is a simple quadrupole. All the pesticides were detected using APCI + (Atmospheric-Pressure chemical ionization) mode with nitrogen as the sheath gas and helium as the auxiliary gas. The operating conditions were: vaporizer temperature 400 °C; sheath gas flow rate 37 arbitrary units (arb); auxiliary gas flow rate 20 arb; discharge current 5 μA; capillary temperature 185 °C; capillary voltage 46 V and tube lens offset 20 V. Full-scan mode was selected for all the samples in the mass range 50–900 *m/z*. In addition, the Selected Reaction Monitoring mode (SRM) was used to detect and quantify all the pesticides with the exception of abamectin and tebuconazole (only in full-scan mode). Collision energy was optimized for each pesticide in syringe infusion mode.

2.4.4. GC–MS

TRACE-DSQ II GC–MS was employed (Thermo Fisher Scientific Co., Waltham, MA, USA) with a TR-5MS column of 30 m × 0.25 mm I.D × 0.25 μm film thickness was supplied by Thermo Fisher Scientific. The chromatograph was programmed at 60 °C for 1 min, then ramped at a rate of 5 °C min^{-1} to 170 °C, 1 °C min^{-1} to 200 °C, 15 °C min^{-1} to 280 °C and held at 280 °C for 5 min. The injection port was held at 280 °C and the transfer line from GC to MS was held at 300 °C. An automatic injector apparatus introduced the samples in splitless mode (t = 0.75 s), using an on-column helium carrier gas flow of 1 mL min^{-1}. Mass spectrometric conditions were previously optimized (Borrás et al., 2011).

2.5. Analytical performance parameters and identification criteria

In target analysis, the method performance acceptability criteria were those described in the guideline SANCO/12571/2013, namely recoveries between 70 and 120%, with an associated repeatability RSD (precision) ≤ 20%. The limits of quantification (LOQ) in the different systems employed were shown in Table 2.

In LC-MS/MS, for a positive identification (confirmation criteria) in accordance with the EU guidelines (SANCO, 2013), the following rules has been applied: (i) monitoring of two SRM transitions per compound, (ii) the LC retention time of the analyte in the sample must be within 2.5% of retention time in the standard, (iii) the relative abundance of the SRM transitions signals must be within 20% of the ratio obtained for the standards, and (iv) the S/N of the two diagnostic ions must be > 3.

In HRMS methodology, target and post-target analysis were carried out. In target quantitative method, identification and confirmation of analytes accomplished in the target quantitative method the followed criteria (SANCO, 2013): i) Mass accuracy of the molecular ion < 5 ppm; ii) mass accuracy of the fragment ion (HCD) < 5 ppm; iii) isotopic pattern similar to the theoretical (the relative intensity of the A + 1 and/or A + 2 isotope peaks in the real sample shall correspond to the theorical relative intensities); iv) retention time similar to that of the calibration standard ± 0.20 min. For identification and confirmation in the retrospective screening analysis the criteria were the same, with the exception of iv) retention time similar to that of the standard ± 0.20 min, when standard was available.

In the retrospective screening, searching for pesticide compounds was carried out using a home-made database (López et al., 2016) and using an automated software tool (Trace Finder, Thermo). To construct the database, both experimental and theoretical data available from databases (T. Scientific, 2014a; University of Hertfordshire, 2007; Royal Society of Chemistry, 2011; National Center for Biotechnology Information, 2004) and published literature (Mol et al., 2012) were used. A customized theoretical database was built, containing 720 substances including authorised and banished pesticides. For each substance, the screening database included the elemental composition (molecular formula) and the theoretical accurate mass of the monitored molecular (quasi)ion. This is a theoretical database where no standards were used to get characteristic fragments. Information about fragments were included when available in the literature, mainly from HRMS (exact mass) and QqQ (nominal mass) studies (T. Scientific, 2014a; University of Hertfordshire, 2007; Mol et al., 2012; T. Scientific, 2014b). The identification and confirmation settings in the Trace Finder programme included a threshold override of 10,000, with S/N of 5, and a mass tolerance of 5 ppm for the molecular ion; an intensity threshold of 5000 and a mass tolerance of 5 ppm for fragments. For isotopic pattern a fit threshold of 90%, an allowed relative intensity (RI) deviation of 30%, and a mass deviation of 5 ppm were selected in the TraceFinder software. The resulting one-point calibration (100 ng mL^{-1} ≈ 131 pg m^{-3}) on confirmed peaks yielded semiquantitative concentrations.

In LC-MS and GC–MS, identification and confirmation of analytes accomplished in the target quantitative method the followed criteria (SANCO, 2013): i) ≥ 3 o more diagnostic ions, preferably including the (quasi) molecular ion; ii) retention time similar to that of the calibration standard ± 0.20 min; iii) isotopic pattern similar using NIST library.

Table 2
Overall concentration in PM10 of detected pesticides in all sampling sites (N = 345).

Pesticide	Frequency of detection (%)[a]	Average (pg m^{-3})[b,c]	Range (pg m^{-3})[c]	LOQ (pg m^{-3})[c]
Abamectin (M)	3	30,000	<LQ-45,000	13,000 [3]
Acetamiprid (I)	3	16	12–31	7[1]/7[2]
Azoxystrobin (F)	4	160	<LQ-800	7[1]/7[2]/3.2[3]
Bendiocarb[d] (I)	1	20	16–24	–
Bifenthrin (I,A)	23	18	<LQ-80	7[1]/7[2]
Bitertanol (F)	4	200	<LQ-800	13[1]/13[2]/26[3]
Buprofezin (I)	17	330	<LQ-3800	13[1]/13[2]/100 [3]/0.5[4]
Carbendazim (F)	47	140	<LQ-2000	7[1]/7[2]/19[3]
Carbofuran (I,A,N)	5	18	<LQ-46	7[1]/7[2]
Chlorpyrifos (I)	29	70	<LQ-210	33[1]/0.6[4]
Chlorpyriphos-methyl (I, A)	6	10	<LQ-10	7[1]
Chlorothalonil (F)	10	13	<LQ-30	7[1]/7[2]
Chlorpropham (H)	5	9	<LQ-10	7[1]
Diazinon (I,A)	32	20	<LQ-220	7[1]
Dichlorvos (I,A)	5	380	120–910	7[1]
Diphenylamine (F)	9	420	<LQ-1900	7[1]
Dimethoate (I,A)	11	50	<LQ-230	7[1]/7[2]
Dioxacarb[d] (I)	1	30	13–41	–
Endothal[d] (H)	1	30	26–26	–
Ethoprophos (I,N)	11	100	<LQ-1200	7[1]
Fludioxonil (F)	7	19	<LQ-39	7[1]
Folpet (F)	16	38	<LQ-160	7[1]
Hexythiazox (A)	14	350	8–6000	7[3]
Imazalil (F)	4	140	<LQ-340	14[1]/7[2]/19 [3]
Imidacloprid (I)	13	100	<LQ-900	7[1]/7[2]/3 [3]
Iprodione (F)	5	33	<LQ-36	7[1]
Kresoxim methyl (F)	6	10	<LQ-14	7[1]
Malathion (I,A)	14	12	<LQ-90	7[1]/0.5[4]
Metalaxyl (F)	45	250	<LQ-1000	7[1]/7[2]/250 [3]
Omethoate (I,A)	56	4000	<LQ-17,000	7[1]/260[2]/3300 [3]
o-phenylphenol[d] (F)	1	22	11–33	–
Prohexadione[d] (P)	3	56	13–90	–
Propanil (H)	3	340	24–900	5[3]
Pyrimethanil (H)	5	8	<LQ-8	7[1]/7[2]
Pyriproxyfen (I)	8	300	<LQ-3800	7[1]/9[2]/0.4[4]
Spinosad (I)	2	510	190–800	–
Tebuconazole (F)	19	930	<LQ-7000	7[1]/7[2]/330 [3]/0.6[4]
Terbuthylazine (H)	53	100	<LQ-34,000	7[1]/7[2]
Thiabendazole (F)	5	340	<LQ-900	7[1]/75[2]/50[3]
Tricyclazole[d] (F)	1	24	15–29	–

[1] = LC-MS/MS; [2] = UHPLC-HRMS; [3] = LC-MS; [4] = GC-MS.
I = insecticide; F = fungicide; H = herbicide; N = nematicide; M = micro-organism derived; P = plant-growth regulator.
[a] Frequency of detection was calculated based on samples with concentrations above the limit of detection (LOD).
[b] The average was calculated from the arithmetic mean from samples with concentrations higher than LOQ.
[c] The results are rounded considering the variability of the analytical method (20%).
[d] Semiquantitative concentrations.

2.6. Quality control protocol

Each set of samples were analyzed under quality assurance protocols, including process blanks, field blank and reagent blanks. In order to determine pollutant backgrounds in the analytical process, a process and a reagent blank was employed as a control and was treated in the same way as the samples. Two field blanks were used; each consisting of a sealed envelop containing a filter, and were used as quality control (QC) samples during deployment, retrieval and transportation of the field filters. These field blanks were processed and analyzed in the same way as the analytical samples. The analysis of filters was performed immediately after sampling or after storage period at − 20 °C (3 months maximum). In order to check potential losses during the storage period, spiked blank filters were stored and analyzed as field samples.

2.7. Theoretical gaseous-particle partitioning model

Koa model proposed by Harner and Bidleman (1998) has been used in order to estimate total concentrations in all samples, where only pesticide particle phase was collected (Yusà et al., 2014). In this model the potential distribution of pesticides in the particulate phase follows the equation:

$$\emptyset = (K_p C_{TSP})/(1 + K_p C_{TSP})$$

where ø is the particulate percentage (fraction of the compound in the particle phase), C_{TSP} is the concentration of total suspended particles in the air (μg m^{-3}), and K_p is the gas/particle partition coefficient.

The K_p could be calculated using the equation:

$$\log K_p = \log K_{oa} + \log f_{OM} - 11.91$$

where log K_{oa} is the octanol-air partitioning coefficient, and log f_{OM} is the fraction of organic matter. The data was calculated assuming a f_{OM} of 0.2 and a C_{TSP} of 55 mg m^{-3} given these are representative values in the studied area (Yusà et al., 2014).

2.8. Chronic exposure and risk assessment

Inhalation of atmospheric pesticides is an important route for pesticide exposure. Chronic (>1 year) inhalation exposures were assessed for adults, children and infants. To estimate the inhalation exposure from the atmospheric pesticides, the following equation was used (USEPA, 1997b; WHO, 1999):

$$\mathrm{DIE\ (mg/kg/day)} = \Sigma(C \times IR_{inh} \times ED)/BW \quad (1)$$

where DIE is the Daily Inhalation Exposure; C is the total (particle + gas phases) concentration of each pesticide in the air (mg m^{-3}), calculated from the theoretical gaseous-particle partitioning model; IR_{inh} is the inhalation rate per hour ($m^3\ h^{-1}$); ED is the exposure duration (h) to air and BW is the body weight of the subject (kg).

Three groups of population were included in the risk assessment: infants (6 months-1.5 years), children (1–6 years) and adults (>12 years). Two conservative exposure scenarios were considered for the chronic exposure assessment i) using the average total concentration of the detected pesticides during the sampling period, and ii) using the maximum concentration for each pesticide during the sampling period. In both scenarios a conservative ED of 24 h was considered. Likewise, a very conservative assumption was adopted related with the fraction of a year over which the exposure occurs (exposure frequency). We have considered an exposure frequency of 1 which means an exposure over 12 months per year.

IRinh applied was 20 $m^3\ day^{-1}$ for adults, 10 $m^3\ day^{-1}$ for children and 8 $m^3\ day^{-1}$ for infants. BW was 70 kg for adults, 15 kg for children and 10 kg for infants (USEPA, 1989, 1991, 2004).

The risk assessment was estimated using the Hazard Quotients (HQ) as a risk descriptor, which where calculated as follows:

$$HQ = DIE_i/HBRV_i \quad (2)$$

where $HBRV_i$ is Health Based Reference Values. The values for each HBRV were retrieved from data bases of the European Union (EU) (EU Pesticides database) and USEPA (United States Environmental Protection Agency) (EPA, 2012; USEPA, 1995, 1997a, 2000, 2012). HBRV was defined as AOEL, Acceptable Operator Exposure Level, and it is applied in the assessment and review of pesticides and biocides within Europe.

The HQ level of concern was set to 1.0, thus an HQ > 1 indicated that a potential risk may be present.

The cumulative exposure was estimated using a Hazard Index (HI) approach for pesticides that have a common mode of action, applying the following formula:

$$HI = HQ_1(\mathrm{pesticide\ 1}) + HQ_2(\mathrm{pesticide\ 2}) + HQ_3(\mathrm{pesticide\ 3}) + \ldots\ (\mathrm{and\ so\ forth}) \quad (3)$$

These risks (HQ, HI) expressly apply to the populations in the vicinity of air monitoring stations.

For Cancer Risk the following equation has been used:

$$\mathrm{Cancer\ risk} = \mathrm{DIE\ (mg/kg{-}day)} \times \mathrm{PF\ (mg/kg{-}day)^{-1}} \quad (4)$$

where PF is the Potency Factor. For Possible or Likely carcinogens the potency factor ranges between >0.01 and 0.1 (Gunier et al., 2001; Lee et al., 2002), so we have used 0.1 for all these pesticides.

Exposure calculation can be affected by numerous sources of uncertainty due to limitations of scientific knowledge including factors related to both the availability of data and the methodology used. Unquantifiable uncertainties associated with exposure assessment were considered for the interpretation of the results from a qualitative point of view, following the recommendations of the Scientific Committee of EFSA (EFSA, 2006). Table SI-1 shows and briefly explains the factors or sources of uncertainty considered. While it is desirable to provide risk managers of the uncertainties associated with the exposure assessment, it is difficult in some ways to express the impact of uncertainties.

3. Results and discussion

3.1. Overall pesticide concentrations (PM10)

Spain was the European country with the highest consumption of pesticides (19.5%) in 2013 (Eurostat, 2015). Valencia region is a Mediterranean area located at the East coast of Spain. In this region the use of pesticides is very intensive in agricultural practices. In the present study, 345 real samples were analyzed for airborne pesticides. Overall, 40 pesticides were detected in the atmosphere in PM10, mainly of them were insecticides (37%) and fungicides (33%) (see Table 2).

Regarding to insecticides, a total of 18 (acetamiprid, bendiocarb, bifenthrin, buprofezin, carbofuran, chlorpyrifos, chlorpyrifos-methyl, diazinon, dichlorvos, dimethoate, dioxacarb, ethoprophos, imidacloprid, malathion, omethoate, pyriproxifen and spinosad) were detected, at frequencies from 1% to 56%. Of these, omethoate had the highest frequency of detection (56%) and the highest average concentration (4000 pg m^{-3}). Levels obtained for omethoate in this study were higher than the levels obtained in previous studies in the same region in Spain ranging from 6 to 2726 pg m^{-3} (Coscollà et al., 2013) and in Canada from 0.9 to 2.7 pg m^{-3} (Raina and Sun, 2008). However, Russo et al. (2012) found higher concentrations of omethoate in Italy than our study, ranging from 10,000 to 30,000 pg m^{-3} (Russo et al., 2012).

In the case of fungicides, 16 compounds (azoxystrobin, bitertanol, carbendazim, chlorothalonil, diphenylamine, fludioxonil, folpet, imazalil, iprodione, kresoxim-methyl, metalaxyl, o-phenylphenol, pyrimethanil, tebuconazole, thiabendazole and tricyclazole) were detected in the air at frequencies from 0.5% to 46%. Of these, carbendazim had the highest frequency of detection (47%) and tebuconazole had the highest average concentration (930 pg m^{-3}) and the highest maximum concentration (7000 pg m^{-3}). Carbendazim is a benzimidazole fungicide banned in EU now, but their use was permitted during the studied sampling period. It has been detected in the present study (average concentration: 140 pg m^{-3}) and in a previous study in the particle phase in the same area (Coscollà et al., 2013). Tebuconazole is a triazole fungicide which can be used to treat pathogenic diseases. Many triazole fungicides have been described in ambient air in the literature such as cyproconazole, difenoconazole, epoxyconazole, flusilazole, myclobutanil, penconazole, tebuconazole, tetraconazole, triadimefon (Coscollà and Yusà, 2016). From these, we only have detected tebuconazole with average concentrations of 930 pg m^{-3}. Levels of tebuconazole were higher than obtained in the same region in other studies in particle phase (Coscollà et al., 2013; Borràs et al., 2011) but lower than levels obtained in a urban region of France (Schummer et al., 2010). It is important to mention that Schummer et al. (2010) measured both particle and gaseous phase presenting higher average concentrations of 1490 pg m^{-3}.

In the literature, different herbicide families have been detected in air such as aryloxyalkanoic acid (2,4-D, MCPA, MCPB, mecoprop), chloroacetamides (acetochlor, alachlor, butachlor, dimethachlor, metazachlor, metolachlor, propachlor), triazines (ametryn, atrazine, propazine, simazine, terbuthylazine), thiocarbamate (butylate, cycloate, molinate), carbamates (desmedipham) and ureas (isoproturon, linuron, metobromuron). More than half of the detected herbicides, mainly chloracetamides, triazines and thiocarbamates, are currently forbidden under the EU Regulations (Coscollà and Yusà, 2016). We have detected terbuthylazine and three herbicides for the first time in the literature (chlorpropham, endothal and propanil). Frequencies of detection ranged from 1% (endothal) to 53% (terbuthylazine). Propanil had the highest average concentration (340 pg m^{-3}) and terbuthylazine the highest maximum concentration (34,000 pg m^{-3}). Terbuthylazine has been previously detected in the same are but with lower maximum concentrations (946 pg m^{-3}) (Coscollà et al., 2013). Although propanil is

currently banned in the European Union, its exceptional use was allowed in this region to the treatment of rice crops during the study period (except for 2013). On contrary, Borrás et al. (2011) did not found this pesticide in the same region.

Only two nematicide pesticides (carbofuran and ethoprophos) were observed with low frequency detection (<12%) and average concentrations ranged between 18 pg m^{-3} (carbofuran) to 100 pg m^{-3} (ethoprophos). Carbofuran is banned in EU, suggesting possible illegal use of these pesticides. Carbofuran levels were very similar than obtained in the same region in 2009 (Coscollà et al., 2013). In contrast, taking into account particle and gaseous phases higher average concentrations of carbofuran were described in France and Canada in remote, rural and urban stations (Coscollà et al., 2013; Aulagnier et al., 2008; Sanusi et al., 2000). Average concentration of ethoprophos in our study (100 pg m^{-3}) was lower than in a rural site in USA (350 pg m^{-3}), where only gaseous phase was measured (Peck and Hornbuckle, 2005).

3.2. Spatial distribution and temporal trends of pesticides

In general, most of the pesticides were highly detected in rural areas. Pesticides found in these zones are related to their use in the citrus groves, vineyards and the other crops that define these sampling regions. Only buprofezin and carbendazim were detected in all stations at different concentrations. Each station had its specific profile regarding average pesticide concentrations (Hart et al., 2012; Coscollà et al., 2013) (Tables SI-2 and SI-3).

Except for remote station, omethoate insecticide has been detected in all stations (rural and urban stations). In the case of omethoate, it is important to note that it has been banned in the European Union since 2003. These levels are not likely related with an illegal use, but with the fact that omethoate is a transformation product from dimethoate (Avino et al., 2011). Dimethoate usage is permitted by European Union. The atmospheric oxidation of dimethoate is likely to lead to the formation of the corresponding oxon (omethoate) similarly to the reaction channel of other organothiophosphate insecticides such as diazinon, chlorpyrifos methyl or chlorpyrifos (Muñoz et al., 2011a, 2011b, 2014).

Dimethoate is used on citrus fruit plagued with aphids (*Aphis sp., Toxoptera aurantii*) in rural stations in Mediterranean areas. Average concentration (50 pg m^{-3}) of dimethoate obtained in this work was similar than a previous one (Coscollà et al., 2013). Higher concentrations of dimethoate are described in gaseous phase in rural sites in California (1200 pg m^{-3}) (CEPA, 2014) and Italy (20,000–70,000 pg m^{-3}) (Russo et al., 2012). However, in rural stations of Canada where gaseous and particle phases were measured, lower range of concentrations were showed (1.3–19 pg m^{-3}) (Baraud et al., 2003).

Abamectin is an insecticide and acaricide pesticide which has been detected in rural stations (Benicarló, Benifaió and Villar del Arzobispo sampling sites). This compound presented the maximum concentration between all the studied substances, achieving 45,000 pg m^{-3} in Villar del Arzobispo station. Abamectin is used for the treatment of *Tetranychus urticae* and *Eriophyes sheldoni* in citrics, for *Phyllocnista citrella* in fruit trees (apple and peach trees) and for mites and *Frankliniella* in vineyards. Benicarló and Benifaió sampling sites are surrounded of citric crops, and Villar del Arzobispo has fruit trees and vineyards. Consequently, it seems logical to detected high levels of this insecticide-acaricide in these rural stations because it has been applied in agricultural practices.

Alzira, Burriana and Benifaió are rural stations and mainly surrounded by citrus groves, which are the most commonly Mediterranean crops. The pesticides detected most frequently and in the highest concentrations were omethoate and carbendazim in these rural stations. In Alzira sampling site, carbendazim presented the highest average concentration (100 pg m^{-3}). Levels of carbendazim were lower than obtained before in this Mediterranean region applied to the treatment of the crops in the same rural station (367 pg m^{-3}) (Coscollà et al., 2013). High omethoate concentrations were presented in Burriana (1600 pg m^{-3}) and Benifaió (8000 pg m^{-3}) stations. In addition, Benicarló, Villar del Arzobispo and Sant Jordi are rural stations surrounded by dry crops such as vineyards, cereals and olive trees which presented frequently omethoate detections. Dimethoate is widely used for *Batrocera Oleae* in olive trees in the region. Average concentration in Benicarló (7000 pg m^{-3}), in Villar del Arzobispo (17,000 pg m^{-3}), and in Sant Jordi (330 pg m^{-3}) were found. Omethoate levels were lower in a rural station located in Canada (maximum concentration: 2.7 pg m^{-3}) (Raina and Sun, 2008) but higher values were obtained in Italy (maximum concentration: 30,000 pg m^{-3}) (Russo et al., 2012).

The remote station of Morella is located away from any agricultural land use, low frequencies of detection and low levels were detected. Nevertheless, highest concentrations of thiabendazole were detected in one sample (600 pg m^{-3}), highest levels than obtained in the same region before (13–80 pg m^{-3}), probably due to long range transport from the rural areas (Coscollà et al., 2013).

Samples were also collected in two urban sites. Metalaxyl was detected in most samples in Valencia city probably due to their use in gardering practices. Metalaxyl has been previoulsy described in urban sites in the literature (Hart et al., 2012). Burjassot town is the another urban station. Higher concentrations than Valencia city were found, especially in diphenylamine and tebuconazole (1200 pg m^{-3} and 7000 pg m^{-3}, respectively). Dichlorvos and omethoate were detected in all samples in Burjassot station. Tebuconazole has been also detected in urban sampling sites in France (Schummer et al., 2010) and Spain (Coscollà et al., 2013). In contrast, dichlorvos has been only described in rural sites in USA (Peck and Hornbuckle, 2005). Diphenylamine has been described for the first time in the literature.

In rural stations (Alzira, Burriana, Benicarló and Benifaió sampling sites), temporal trends were observed for some pesticides. As an example, omethoate was highly detected in the period 2008–2009 (7000–8000 pg m^{-3}). In 2010, this insecticide presented also high concentrations in ambient air (210 pg m^{-3}). In contrast, lower levels were found in the following years (2013–2014). Terbuthylazine herbicide was not searched from 2008 to 2009. However, in the period 2010–2014, the highest levels were described in 2010 (110 pg m^{-3}). Lower concentrations were presented in the following years (2013: 26 pg m^{-3} and 2014: 15 pg m^{-3}). Consequently, omethoate and terbuthylazine were detected in higher concentration in the first years of the study and decreasing levels were observed in the following years. Similar trends are observed for other studied pesticides such as carbendazim and metalaxyl (see Fig. SI-1).

3.3. Risk assessment

The potentially adverse effects of exposure to pesticides on the general population, and specifically on the more susceptible groups such as infants and children, are a public health concern (Marks et al., 2010; London et al., 2012). Apart from ingestion of foods and drinking water, inhalation of ambient air could be a relevant pathway of exposure to pesticides. Although Guidance Document on pesticide exposure and risk assessment of pesticides in air for operators, workers, bystanders and residents has been developed (EFSA, 2014), the methodologies focused on risk assessment of air pesticides on the general population are scarce.

In this study, pesticide inhalation exposure was calculated through the DIE (daily inhalation exposure). The maximum DIE for each pesticide was calculated from the application of Eq. (1) for adults, children and infants (see Tables 3 and SI-4). Total pesticide concentration (particle + gas phase) considered in DIE was estimated as previously mentioned applying the Koa model (Yusà et al., 2014). Fig. 1 compare maximum DIE levels for infants obtained in all studied stations. DIE levels obtained in all stations were very similar except for Alzira sampling site. Alzira is a rural station which obtained maximum DIE of

4.09 E-03 mg kg^{-1} day^{-1} in infants for prohexadione. Yusà et al. (2014) obtained similar estimated maximum DIE for chlorothalonil (1.26E-05 mg kg^{-1} day) in children in the same sampling station in 2010.

Another study about risk assessment in California was carried out by Luo and Zhang (Luo and Zhang, 2009). They estimated exposure to organophosphates among the children population through multiple exposure pathways. Pesticide concentration in air was calculated using models of simulating environmental fate and chemical concentration in environmental media. Estimated children-inhalation exposure to chlorpyrifos, dimethoate and malathion was on average 8.8E-04, 8.6E-04 and 5.05E-05 mg kg^{-1} day, respectively. They concluded that intakes from inhalation were significantly lower than those from ingestion (by two orders or more). This exposure to inhalated pesticides was higher than in our study. We have calculated on average a DIE of 5.22E-06 for chlorpyrifos, of 3.15E-07 for dimethoate and 4.89E-07 for malathion in children population.

As mentioned, in our study the risk assessment was calculated as HQ for the AOEL health-based reference values (HQ_{AOEL}) (Eq. (2)). Tables 3 and SI-4 also show the HQs obtained in the studied populations. Fig. 2 compares maximum Hazard Quotients for infants obtained in all stations. Hazard Quotients obtained in rural regions were higher than HQ obtained in remote and urban stations except for dichlorvos in Burjassot station due to their low AOEL. Maximum HQs were obtained in rural stations for diazinon (4.10 E-02), hexythiazox (4.97 E-02) and omethoate (5.03 E-02) in Burriana, Benicarló and Villar del Arzobispo, respectively. All Hazard Quotients obtained in the present study were lower than 1 in infants, which is generally considered to be health protective (Cangialosi et al., 2008). Similar results were described in a previous study in the same region, in which taking into account the worst scenario (maximum exposures), the HQ_{AOEL} were always lower than 1.90E-03, 1.55E-03 and 1.40E-02 in adults, children and infants, respectively (Yusà et al., 2014). However, HQs obtained in these works were slightly higher than levels obtained in an urban area in China for some CUPs (Li et al., 2014). In a study from airborne pesticides in the USA, (Lee et al., 2002) assessed inhalation risks to California communities from airborne agricultural pesticides by probability distribution analysis using ambient air data provided by the California Air Resources Board and the California Department of Pesticide Regulation. Risks were estimated for the median and 75th and 95th percentiles of probability (50%, 25% and 5% of the exposed populations). They found child chronic HQs (50th) of 0.3 and 0.02 for chlorpyrifos and diazinon, respectively. Using the maximum concentration levels, the authors also calculated the child acute HQs for chlorpyrifos and diazinon, with values of 4.0 and 0.8, respectively.

Figs. SI-2 to SI-10 show the seasonal trends of maximum HQs obtained in the studied stations from february to november (2008–2014). Four stations presented maximum HQ (Burriana, Benifaió, Morella and Burjassot) in summer season, three stations (Alzira, Benicarló, and Sant Jordi) in spring seasons and two stations (Villar del Arzobispo and Viveros) in autumn season. Consequently, summer and spring

Table 3
Overall maximum total (particulate and gaseous) concentrations (pg m^{-3}), overall maximum Daily inhalation exposure (mg kg^{-1} day^{-1}) and overall maximum Hazard Quotient (HQ_{AOEL}) for the detected pesticides.

		Adults (>12 years)		Children (1–6 years)		Infants(>6–15 years)	
Pesticide	Maximum level (pg m^{-3})[a]	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}	DIE[d]	HQ_{AOEL}
Abamectin	45,000	1.80E-05	7.19E-03	4.24E-05	1.70E-02	5.00E-05	2.00E-02
Acetamiprid	31	9.19E-09	1.31E-07	2.17E-08	3.10E-07	2.56E-08	3.66E-07
Azoxystrobin	800	2.21E-07	1.10E-06	5.21E-07	2.61E-06	6.14E-07	3.07E-06
Bendiocarb	24	2.06E-06	3.17E-04	4.86E-06	7.48E-04	5.73E-06	8.81E-04
Bifenthrin	80	2.37E-08	3.16E-06	5.60E-08	7.47E-06	6.60E-08	8.80E-06
Bitertanol	800	2.26E-07	1.13E-06	5.34E-07	2.67E-06	6.30E-07	3.15E-06
Buprofezin	3800	2.42E-06	6.05E-05	5.72E-06	1.43E-04	6.74E-06	1.68E-04
Carbendazim	2000	2.35E-04	1.18E-02	5.56E-04	2.78E-02	6.55E-04	3.28E-02
Carbofuran	46	5.21E-08	1.74E-04	1.23E-07	4.10E-04	1.45E-07	4.84E-04
Chlorpyrifos	210	2.21E-06	2.21E-03	5.22E-06	5.22E-03	6.15E-06	6.15E-03
Chlorpyrifos-methyl	10	2.65E-07	2.65E-05	6.25E-07	6.25E-05	7.37E-07	7.37E-05
Chlorothalonil	30	8.70E-07	9.66E-05	2.05E-06	2.28E-04	2.42E-06	2.69E-04
Chlorpropham	10	8.54E-08	1.71E-06	2.02E-07	4.03E-06	2.38E-07	4.75E-06
Diazinon	220	2.95E-06	1.47E-02	6.96E-06	3.48E-02	8.20E-06	4.10E-02
Dichlorvos	910	3.18E-05	6.36E-02	7.52E-05	7.50E-02	8.86E-05	1.77E-01
Diphenylamine	1900	1.02E-04	1.02E-03	2.41E-04	2.41E-03	2.84E-04	2.84E-03
Dimethoate	230	1.34E-07	1.34E-04	3.15E-07	3.15E-04	3.72E-07	3.72E-04
Ethoprophos	1200	3.46E-07	3.46E-04	8.17E-07	8.17E-04	9.62E-07	9.62E-04
Fludioxonil	39	1.12E-08	1.90E-08	2.64E-08	4.48E-08	3.12E-08	5.28E-08
Folpet	160	1.37E-06	1.37E-05	3.24E-06	3.24E-05	3.82E-06	3.82E-05
Hexythiazox	6000	1.61E-04	1.79E-02	3.79E-04	4.22E-02	4.47E-04	4.97E-02
Imazalil	340	2.05E-07	4.10E-06	4.84E-07	9.67E-06	5.70E-07	1.14E-05
Imidacloprid	900	2.54E-07	3.17E-06	5.99E-07	7.48E-06	7.06E-07	8.82E-06
Iprodione	36	1.05E-08	3.49E-08	2.47E-08	8.23E-08	2.91E-08	9.70E-08
Kresoxim-methyl	14	6.00E-09	6.67E-09	1.42E-08	1.57E-08	1.67E-08	1.86E-08
Malathion	90	2.07E-07	6.90E-06	4.89E-07	1.63E-05	5.76E-07	1.92E-05
Metalaxyl	1000	7.31E-05	9.14E-04	1.73E-04	2.16E-03	2.04E-04	2.54E-03
Omethoate	17,000	5.43E-06	1.81E-02	1.28E-05	4.27E-02	1.51E-05	5.03E-02
o-phenylphenol	33	2.49E-06	6.23E-06	5.89E-06	1.47E-05	6.94E-06	1.74E-05
Prohexadione	90	1.47E-03	4.20E-03	3.47E-03	9.92E-03	4.09E-03	1.17E-02
Propanil	900	2.41E-06	1.21E-04	5.70E-06	2.85E-04	6.72E-06	3.36E-04
Pyrimethanil	8	4.93E-08	4.11E-07	1.16E-07	9.69E-07	1.37E-07	1.14E-06
Pyriproxifen	3800	1.28E-06	3.21E-05	3.03E-06	7.58E-05	3.57E-06	8.93E-05
Spinosad	800	2.26E-07	5.66E-06	5.35E-07	1.34E-05	6.30E-07	1.58E-05
Tebuconazole	7000	1.99E-06	1.99E-03	4.69E-06	4.69E-03	5.53E-06	5.53E-03
Terbuthylazine	34,000	4.32E-05	1.35E-02	1.02E-04	3.19E-02	1.20E-04	3.75E-02
Thiabendazole	900	2.58E-07	2.58E-06	6.10E-07	6.10E-06	7.19E-07	7.19E-06

[a] The results are rounded considering the variability of the analytical method (20%).
[b] USEPA (1989), USEPA (2004), USEPA (1991) (DIE: Daily Inhalation Exposure; body weight = 70 kg, inhalation rate = 20 m^3 day).
[c] USEPA (2004), USEPA (1991) (DIE: Daily Inhalation Exposure; body weight = 15 kg, inhalation rate = 10 m^3 day^{-1}, exposure duration.
[d] DIE: Daily Inhalation Exposure; body weight = 10 kg, inhalation rate = 8 m^3 day^{-1}, exposure duration = 24 h.

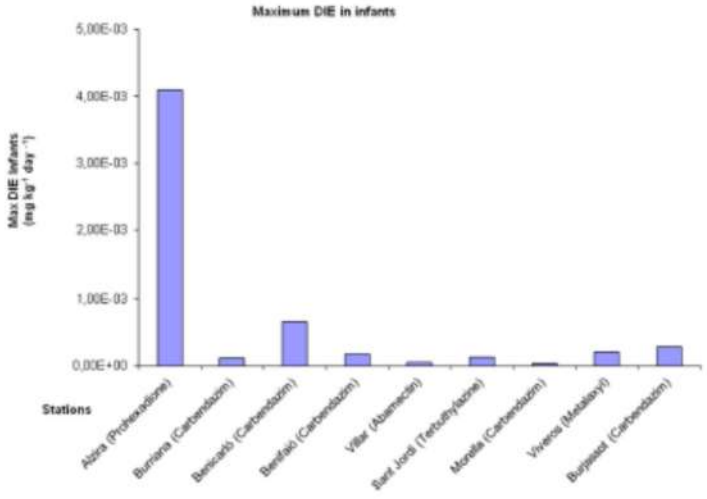


Fig. 1. Maximum daily inhalation exposure levels obtained in infants in Valencia Region (Spain).

presented highest levels of HQs because this is the application period of pesticides in agricultural practices in the Mediterranean region. In addition, winter season normally presented the lowest HQs in all stations, due to this is not a period of application of pesticides in crops. Li et al. (2014) also studied the seasonal variation in risk assessment of pesticides in the air. Evaluation of potential exposure from inhalation of atmospheric CUPs suggested that children, toddlers and infants had the highest exposure, but the risk quotients were low for all age groups when annual average concentrations were used. Exposure risk was higher in summer and fall than the annual average level due to higher atmospheric pesticide concentrations, longer exposure times and more pesticides being in the gaseous form. Yusà et al. (2014) and Lee et al. (2002) did not studied seasonal trends in their risk assessment studies.

The European Food Safety Authority (EFSA) (EFSA, 2013) has defined a Cumulative Assessment Group (CAG) as a group of chemicals that acts by a common mode of action. In this context, pesticides that affect the nervous and thyroid systems which present the same toxicology and have relevant effect on the target organ are CAG, such as organophosphate (OP) and pyrethroid pesticides. The use of the term 'cumulative' is a consequence of the cumulative risk assessment. In our study, the cumulative exposure was estimated using a Hazard Index (HI) approach for pesticides with a common mode of action (Eq. (3)). We obtained a HIs for pesticides that present a similar mode of action (neonicotinoids, benzimidazoles, carbamates, triazoles, organophosphates and micro-organism derived) lower than 1 for all population groups (adults, children and infants) in all stations. Similar results were presented by Yusà et al. (2014).

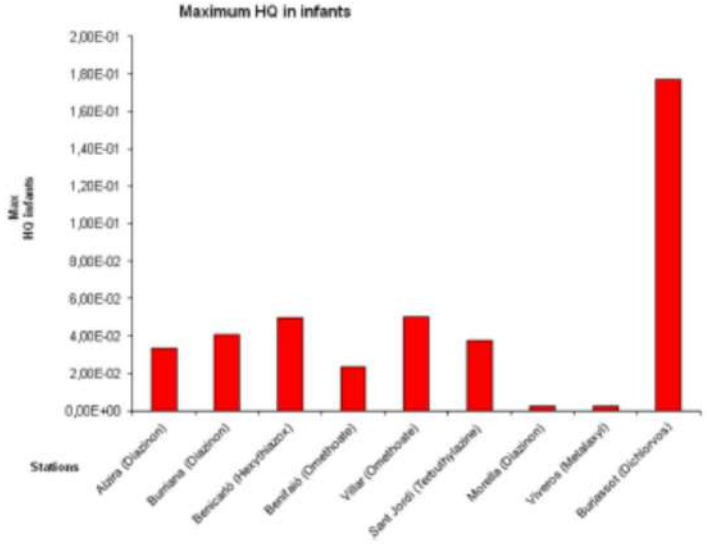


Fig. 2. Maximum Hazard Quotient levels obtained in infants in Valencia Region (Spain).

They calculated that HIs were lower than 2.65E-03, 6.24E-03 and 2.60E-02 for adults, children and infants, respectively, when using the maximum concentration for OPs (chlorpyrifos, diazinon, malathion, omethoate, dimethoate, chlorpyrifos-methyl). Regarding carbamate pesticides (carbofuran and chlorpropham), the HIs were lower than 25.9E-4 for the three groups. HIs for pyrethroids (bifenthrin and permethrin) were also estimated, and values lower than 3.86E-5 were found for infants. When the HI is <1, the cumulative risk from exposure to the compounds is considered to be acceptable. The California Department of Pesticide Regulation from the Environmental Protection Agency also estimated the cumulative exposure for OPs. In this study, the cumulative exposure was estimated using the hazard quotient (HQ) and the HI approach relying on the ratio between detected air concentration and the screening level. The organophosphate cumulative exposures were estimated for each community and for each exposure period. None of the HIs exceeded 1, indicating that the screening levels were not exceeded for all organophosphates combined (Department of Pesticide Regulation California, 2011).

Cancer risk has been calculated for pesticides classified as possible human carcinogenic and the cancer classification of pesticides developed by EPA (USEPA, 2013) was used (Eq. (4)). Table 4 shows the cancer risk of potential carcinogenic compounds. The concern about the cancer risk often occurs when the estimated risk reaches 1E-06 (1/1,000,000) excess lifetime cancer risk. Cancer risk obtained for pesticides classified as possible or potential human carcinogen were lower than 10 E-06 except for the case of carbendazim and hexythiazox in four rural (Alzira, Burriana, Benicarló and Benifaió) and one urban (Burjassot) stations for infants. In contrast, the cancer risk estimated for the detected pesticides classified as Possible or Likely carcinogens by Yusà et al. (2014) were lower than 1.15E-07 in the same region for the most sensitive population (infants).

The present risk assessment only considered inhalation exposures. However, dermal, and especially ingestion are other routes of exposure. Exposure to pesticides residue through the diet is assumed to be between two and five orders to magnitude higher than air exposure (Luo and Zhang, 2009; Juraske et al., 2009). So, in order to deal with safe screening levels of CUPs in the atmosphere (pg m^{-3}) these multiple-exposure pathways need to be considered.

4. Conclusions

A total of 40 pesticides (mainly insecticides and fungicides) were detected in the ambient air of rural, remote and urban stations placed in Valencia Region (Spain). Only two pesticides were detected in all stations (buprofezin and carbendazim). Rural and urban stations presented different profiles depending mainly on the agricultural practices around the sampling site (agricultural practices, seasonal application and climate).

Overall, inhalation exposure to studied pesticides in the different stations and seasons ranged from 3.88 E-09 mg kg^{-1} day^{-1} to 4.09 E-03 mg kg^{-1} day^{-1}. Risk was assessed in different groups of population. Highest HQ obtained from maximum total concentration in adults (6.36 E-02), children (7.50 E-02) and infants (1.77 E-01) do not represent a significant concern for health in the selected stations of Valencia Region.

The HI obtained for pesticides with common mode of action were <1 in all groups studied, so the combined risk resulting from exposure to the compounds was considered to be acceptable. Likewise, for infants the cancer risk estimated for the detected pesticides classified as possible or likely carcinogens were lower than 10 E-06, except for carbendazim and hexythiazox in some sampling sites. However, it will be very important to establish health reference levels for pesticides in air for different populations in the near future to carry out a properly risk studies.

Table 4
Cancer risk of potencial carcinogenic pesticides.

Pesticide	Adults	Children	Infants
Alzira (N = 79)			
Bifenthrine	2.37E-09	5.60E-09	6.60E-09
Carbendazim	4.38E-06	1.04E-05	1.22E-05
Chlorothalonil	8.70E-08	2.05E-07	2.42E-07
Dimethoate	4.64E-09	1.10E-08	1.29E-08
Folpet	7.04E-08	1.66E-07	1.96E-07
Malathion	2.07E-08	4.89E-08	5.76E-08
Tebuconazole	3.88E-10	9.17E-10	1.08E-09
Burriana (N = 48)			
Bifenthrine	5.66E-10	1.34E-09	1.57E-09
Carbendazim	3.80E-06	8.98E-06	1.06E-05
Chlorothalonil	3.38E-08	7.99E-08	9.42E-08
Dimethoate	4.85E-09	1.14E-08	1.35E-08
Ethoprophos	2.25E-07	5.31E-07	6.26E-07
Folpet	3.77E-08	8.90E-08	1.05E-07
Imazalil	1.30E-08	3.06E-08	3.61E-08
Iprodione	1.05E-09	2.47E-09	2.91E-09
Kresoxim-m	3.59E-10	8.47E-10	9.99E-10
Malathion	2.90E-09	6.84E-09	8.06E-09
Pyrimethanil	3.41E-09	8.05E-09	9.49E-09
Tebuconazole	3.15E-10	7.45E-10	8.78E-10
Benicarló (N = 25)			
Carbendazim	2.35E-05	5.56E-05	6.55E-05
Hexythiazox	1.61E-05	3.79E-05	4.47E-05
Imazalil	2.05E-08	4.84E-08	5.70E-08
Tebuconazole	3.76E-09	8.88E-09	1.05E-08
Benifaió (N = 23)			
Carbendazim	6.12E-06	1.44E-05	1.70E-05
Hexythiazox	3.01E-06	7.10E-06	8.37E-06
Imazalil	1.65E-08	3.90E-08	4.59E-08
Tebuconazole	1.84E-08	4.34E-08	5.11E-08
Villar del Arzobispo (N = 9)			
Carbendazim	4.37E-07	1.03E-06	1.22E-06
Hexythiazox	1.08E-06	2.54E-06	3.00E-06
Sant Jordi (N = 43)			
Bifenthrine	6.90E-10	1.63E-09	1.92E-09
Carbendazim	2.39E-06	5.65E-06	6.66E-06
Chlorothalonil	1.88E-08	4.44E-08	5.23E-08
Dimethoate	1.34E-08	3.15E-08	3.72E-08
Ethoprophos	9.60E-08	2.27E-07	2.67E-07
Folpet	1.37E-07	3.24E-07	3.82E-07
Iprodione	8.78E-10	2.07E-09	2.44E-09
Kresoxim-m	6.00E-10	1.42E-09	1.67E-09
Malathion	1.01E-08	2.39E-08	2.82E-08
Tebuconazole	2.71E-08	6.41E-08	7.55E-08
Morella (N = 54)			
Carbendazim	1.13E-06	2.66E-06	3.13E-06
Chlorothalonil	6.16E-08	1.46E-07	1.71E-07
Folpet	4.65E-08	1.10E-07	1.29E-07
Hexythiazox	5.55E-07	1.31E-06	1.54E-06
Kresoxim-m	1.86E-10	4.39E-10	5.17E-10
Tebuconazole	2.80E-10	6.61E-10	7.79E-10
Viveros (Valencia) (N = 48)			
Bifenthrine	8.81E-10	2.08E-09	2.45E-09
Carbendazim	9.31E-07	2.20E-06	2.59E-06
Chlorothalonil	3.27E-08	7.72E-08	9.10E-08
Dimethoate	1.21E-08	2.86E-08	3.38E-08
Ethoprophos	3.46E-08	8.17E-08	9.62E-08
Folpet	5.66E-08	1.34E-07	1.58E-07
Malathion	1.47E-09	3.48E-09	4.10E-09
Pyrimethanil	4.93E-09	1.16E-08	1.37E-08
Tebuconazole	2.37E-10	5.60E-10	6.60E-10
Burjassot (N = 16)			
Carbendazim	1.01E-05	2.39E-05	2.82E-05
Dichlorvos	3.18E-06	7.52E-06	8.86E-06
Hexythiazox	2.63E-06	6.21E-06	7.32E-06
Tebuconazole	1.99E-07	4.69E-07	5.53E-07

Acknowledgements

This study was financed by the Generalitat Valenciana through the DIPA project (GV/2014/005) from the Conselleria d'Educació, the Spanish Ministerio de Economia y Competitividad for IMPLACAVELES project (CGL2013-49093-C2-1-R) and the FISABIO for AIRPEST project (UGP-15-250). The authors wish to acknowledge also Generalitat Valenciana – Spain, for the DESETRES-Prometeo II project. CEAM – is partly supported by Generalitat Valenciana.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.08.149.

References

AEPLA (Asociación Empresarial para la Protección de las Plantas), 2015n. Memoria AEPLA 2014. Available from http://www.aepla.es/tmp/images/publicaciones/P0017_Memoria_AEPLA_2014.pdf accessed November 2015.

Aulagnier, F., Poissant, L., Brunet, D., Beauveais, C., Pilote, M., Deblois, Dassylva, N., 2008. Pesticides measured in air and precipitation in the Yamaska Basin (Québec): occurrence and concentrations in 2004. Sci. Total Environ. 394, 7293–7303.

Avino, P., Cinelli, G., Notardonato, I., Russo, M.V., 2011. Investigation on the behaviour of pesticides in the atmosphere. Aerosol Air Qual. Res. 11, 783–790.

Baraud, L., Tessier, D., Aaron, J.J., Quiseft, J.P., Pinart, J., 2003. A multi-residue method for characterization and determination of atmospheric pesticides measured at two French urban and rural sampling sites. Anal. Bioanal. Chem. 377, 1148–1152.

Borrás, E., Sánchez, P., Muñoz, A., Tortajada-Genaro, L.A., 2011. Development of a gas chromatography–mass spectrometry method for the determination of pesticides in gaseous and particle phases in the atmosphere. Anal. Chim. Acta 699, 57–65.

Cangialosi, F., Intini, G., Liberti, L., Notarnicola, M., Stellacci, P., 2008. Health risk assessment of air emissions from a municipal solid waste incineration plant -case study. Waste Manag. 28, 885–895.

CAPA (Conselleria de Agricultura, Pesca y Alimentación de la Generalitat Valenciana), 2013a. Informe del sector agrario 2012. Available from http://www.agricultura.gva.es/web/c/document_library/get_file?uuid=4f0128d9b-3de1-43d7-a3f2-5bcdac0cf10d&groupId=416 accessed November 2015.

CEPA, California Environmental Protection Agency, 2014. Environmental Monitoring Branco, Air Monitoring Network Results for 2013–2014.

Coscollà, C., Yusà, V., 2016. Chapter 17: pesticides and agricultural air quality. In: De la Guardia, M., Armenta, S. (Eds.), Comprehensive Analytical Chemistry Vol. 73. Elsevier, Amsterdam, pp. 423–490.

Coscollà, C., Hart, E., Pastor, A., Yusà, V., 2013. LC-MS characterization of contemporary pesticides in PM10 of Valencia Region, Spain. Atmos. Environ. 77, 394–403.

Coscollà, C., León, N., Pastor, A., Yusà, V., 2014. Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using Liquid Chromatography – Orbitrap High Resolution Mass Spectrometry. J. Chromatogr. A 1368, 132–142.

Coscollà, C., Yusà, V., Beser, M.J., Pastor, A., 2009. Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM2.5) by microwave-assisted extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1216, 8817–8827.

Department of Pesticide Regulation, California, 2011. Air Monitoring Network Study: Long-term Ambient Air Monitoring for Pesticides in Multiple California Communities.

ECPA (European Crop Protection Association), 2010. Industry statistics 2001–2010. Available from http://www.ecpa.eu/page/industry-statistics.

EFSA (European Food Safety Authority), 2014. Scientific opinion on preparation of a guidance document on pesticide exposure assessment for workers, operators, bystanders and residents. EFSA J. 8 (2), 1501 2010.

EFSA (European Food Safety Autority), 2006. Guidance of the scientific committee on a request from EFSA related to uncertainties in dietary exposure assessment. EFSA J. 438, 1–54.

EFSA (European Food Safety Autority), 2013. International Frameworks Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals.

EPA, IRIS, Environmental Protection Agency, 2012. United States. Integrated Risk Information System, IRIShttp://cfpub.epa.gov/ncea/iris/index.

EU pesticides database, 2016. Available from http://ec.europa.eu/sanco_pesticides/public/?event=homepage accessed March 2016.

Eurostat, 2013. Available from http://ec.europa.eu/eurostat/statistics-explained/index.php/File:Pesticide_sales_by_major_groups,_2013.png#file accessed March 2016.

Eurostat, 2015. Eurostat Statistical Books: Agriculture, Forestry and Fishery Statistics. 2015 edition. ISSN 2363-2488.

Gunier, R., Harnly, M., Reynolds, P., Hertz, A., Von Behren, J., 2001. Agricultural pesticide use in California: pesticide prioritization, use densities, and population distributions for a childhood cancer study. Environ. Health Perspect. 109, 1071–1078.

Hamer, T., Bidleman, T.F., 1998. Octanol-air partition coefficient for describing particle/gas partitioning of aromatic compounds in urban air. Environ. Sci. Technol. 32, 1494–1502.

Hart, E., Coscollà, C., Pastor, A., Yusà, V., 2012. GC-MS characterization of contemporary pesticides in PM10 of Valencia region, Spain. Atmos. Environ. 62, 118–129.

Juraske, R., Mutel, C.L., Stoessel, F., Hellweg, S., 2009. Life cycle human toxicity assessment of pesticides: comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere 77, 939–945.

Lee, S., Mclaughlin, R., Harnly, M., Gunier, R., Kreutzer, R., 2002. Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks. Environ. Health Perspect. 110, 1175–1184.

Li, H., Hongzhu, M., Lidy, M.J., You, J., 2014. Occurrence, seasonal variation and inhalation exposure of atmospheric organophosphate and pyrethroid pesticide in an urban community in South China. Chemosphere 95, 363–369.

London, L., Beseler, C., Bouchard, M.F., Bellinger, D.C., Colosio, C., Grandjean, P., Harari, R., Kootbodien, T., Kromhout, H., Little, F., Meijster, T., Moretto, A., Rohlman, D.S., Stallones, L., 2012. Neurobehavioral and neurodevelopmental effects of pesticide exposures. Neurotoxicology 33 (4), 887–896.

López, A., Yusà, V., Millet, M., Coscollà, C., 2016. Retrospective screening of pesticide metabolites in ambient air using liquid chromatography coupled to high-resolution mass spectrometry. Talanta 150, 27–36.

Luo, Y., Zhang, M., 2009. Multimedia transport and risk assessment of organophosphate pesticides and a case study in the northern San Joaquin Valley of California. Chemosphere 75, 969–978.

Marks, A.R., Harley, K., Bradman, A., Kogut, K., Barr, D.B., Johnson, C., Calderon, N., Eskenazi, B., 2010. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ. Health Perspect. 118 (12), 1768–1774.

Mol, H.G.J., Zomer, P., de Koning, M., 2012. Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry. Anal. Bioanal. Chem. 403, 2891–2908.

Muñoz, A., Le Person, A., Le Calvé, S., Mellouki, A., Borrás, E., Daële, V., Vera, T., 2011a. Studies on atmospheric degradation of diazinon in the EUPHORE simulation chamber. Chemosphere 85, 724–730.

Muñoz, A., Ródenas, M., Borrás, E., Vázquez, M., Vera, T., 2014. The gas-phase degradation of chlorpyrifos and chlorpyrifos-oxon towards OH radical under atmospheric conditions. Chemosphere 111, 522–528.

Muñoz, A., Vera, T., Sidebottom, H., Mellouki, A., Borrás, E., Ródenas, M., Clemente, E., Vázquez, M., 2011b. Studies on the atmospheric degradation of chlorpyrifos-methyl. Environ. Sci. Technol. 45 (5), 1880–1886.

National Center for Biotechnology Information, 2004. PubChem compound database. Available from http://www.ncbi.nlm.nih.gov/pccompound.

Peck, A.M., Hornbuckle, K.C., 2005. Gas-phase concentrations of current-use pesticides in Iowa. Environ. Sci. Technol. 39, 2952–2959.

Raina, R., Sun, L., 2008. Trace level determination of selected organophosphorus pesticides and their degradation products in environmental air samples by liquid chromatography-positive ion electrospray tandem mass spectrometry. J. Environ. Sci. Health B 43, 323–332.

Royal Society of Chemistry, 2011. ChemSpider software. Available from http://www.chemspider.com.

Russo, M.V., Avino, P., Cinelli, G., Notardonato, I., 2012. Sampling of organophosphorus pesticides at trace levels in the atmosphere using XAD-2 adsorbent and analysis by gas chromatography coupled with nitrogen-phosphorus and ion-trap mass spectrometry detectors. Anal. Bioanal. Chem. 404, 1517–1527.

SANCO, 2007. SANCO /2007/3131, 31 /October/ 2007. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed.

SANCO, 2013. SANCO/12571/2013, 19 November 2013. Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed.

Sanusi, A., Mirabel, P., Worthman, H., 2000. Comparison of atmospheric pesticide concentrations measured at three sampling sites: local, regional and long-range transport. Sci. Total Environ. 263, 263–277.

Schummer, C., Mothiron, E., Appenzeller, B.M.R., Rizet, A.L., Wennig, R., Millet, M., 2010. Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France. Environ. Pollut. 158, 576–584.

Scientific, T, 2014b. Mass Frontier 7.0. 2014. Available from http://www.thermoscientific.com/en/product/mass-frontier-7-0-spectral-interpretation-software.html.

Scientific Pesticides, T, 2014a. Database compound library: environmental and food. Available from http://planetorbitrap.com/compoundlibrary#.VBIJ_KMUHAM cited August 2014.

University of Hertfordshire, 2007. PPDB, pesticide property database. Available from http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm cited August 2014.

USEPA, 1989. Risk Assessment Guidance for Superfund (RAGS) (Part A). EPA/540/1-89/002.

USEPA, 1991. RAGS Volume I: Human Health Evaluation Manual. Supplemental Guidance "Standard Default Exposure Factors". OSWER No. 9285.6-03.

USEPA, 1995. Registration Eligibility Decision for Terbuthylazine. U.S. Environmental Protection Agency, Washington DC.

USEPA, 1997a. Tebuconazole - Pesticide Tolerance Petition 3J97. U.S. Environmental Protection Agency, Washington DC.

USEPA, 1997b. Exposure Factors Handbook. U.S. Environmental Protection Agency EPA/600/P-95/002Fa.

USEPA, 2000. EPA's Preliminary Risk Assessment on Diazinon. U.S. Environmental Protection Agency, Washington DC.

USEPA, 2004. Region 9 Preliminary Remediation Goals. Memorandum from Standford Smucker Ph.D., Regional Toxicologist, http://www.epa.gov/region09/waste/sfund/prg/index.html.

10. CAPÍTULO 6. Evaluación del riesgo a la inhalación a plaguicidas en una población rural francesa.

10.1. Introducción.

Una gran variedad de plaguicidas pueden ser aplicados en las actividades agrícolas y su selección depende de un gran número de factores entre los cuales destacan la plaga a combatir y el cultivo al que se aplica el plaguicida. Diferentes estudios en Europa han informado acerca de los niveles de plaguicidas en el aire encontrados en diferentes zonas, oscilando los valores entre los pocos pg m^{-3} hasta los centenares de ng m^{-3} [90, 131, 135, 143, 249]. Los posibles efectos adversos a la exposición y específicamente en los grupos más vulnerables son una preocupación para la salud [10-11]. Además de la ingestión de alimentos y de agua y de la exposición dérmica, la inhalación de plaguicidas en el aire ambiente puede ser una ruta relevante de exposición a plaguicidas.

Durante la aplicación de los plaguicidas en actividades agrícolas, una fracción importante de la dosis aplicada se deposita en zonas adyacentes al área tratada y otra fracción se transfiere a la atmósfera. En los días posteriores a la aplicación, la volatilización desde las plantas y el suelo, contribuye a un aumento de sus niveles en la atmósfera. Además, la presencia de la erosión ocasionada por el viento, transporta partículas del suelo cargadas con plaguicidas (el denominado soil tillage operations) [118]. Como consecuencia de estos procesos, una elevada proporción de los plaguicidas aplicados en una determinada zona son transferidos a la atmósfera. Por lo tanto, una información precisa respecto al uso de sustancias activas y el conocimiento de las emisiones son relevantes para realizar la evaluación de la exposición y del riesgo en la población humana [250].

Como resultado de la actividad agrícola, un gran número de plaguicidas han sido detectados en la atmósfera. Hart et al. (2012) [135] detectaron 24 CUPs (currently used pesticides) en la fase particulada, con concentraciones que oscilan entre 3.2 y 1196 ng m^{-3}, en el aire ambiente de la Comunidad Valenciana. Estos autores también informaron de los niveles obtenidos para los 17 plaguicidas más polares de uso habitual en la misma área de estudio (niveles que oscilaron entre 6.8 y 2898 pg m^{-3}). Además, Moussaoui et al., (2012) [251] evaluaron los niveles de plaguicidas en la atmósfera del norte de Argelia, detectando concentraciones entre los 16 pg m^{-3} y los 11 ng m^{-3}. En un estudio más reciente, Yusà et al. (2014) [91] analizó 56 muestras desde Abril hasta Octubre de 2010 procedentes de una estación rural de la Comunidad Valenciana. Se detectaron un total de 20 plaguicidas, con concentraciones medias que oscilaron entre los 5.75 pg m^{-3} y los 117.01 pg m^{-3}, con concentraciones máximas que oscilan entre los 15.54 y los 758.60 pg m^{-3}. Coscollà et al. (2014) [163] detectaron carbendazim, metalaxil, terbutilazina y miclobutanilo en concentraciones entre 16 pg m^{-3} hasta 174 pg m^{-3} en la atmósfera entre junio y julio del año 2013 en la misma zona rural. Mahmood et al. (2014) [252] evaluaron la contaminación procedente de plaguicidas organoclorados y sus posibles riesgos en la salud humana como cáncer, problemas reproductivos, neurológicos, inmunológicos y otros efectos adversos en muestras recogidas en Pakistán, con niveles que oscilaron entre los 123 y los 635 pg m^{-3}.

Para realizar una evaluación del riesgo debido a la inhalación de plaguicidas, es necesario conocer la concentración global de las sustancias activas (fase particulada + fase gaseosa). En este capítulo (capítulo 6) se presenta la variación temporal (2006-

2013) de los niveles de plaguicidas en la atmósfera en una estación rural de la región Central de Francia. Usando estos valores de concentración, se ha estimado la exposición a la inhalación de plaguicidas y la evaluación del riesgo para la población general que vive próxima a la zona de muestreo.

10.2. Resultados.

10.2.1. Niveles detectados en la zona de estudio.

De las 58 sustancias activas estudiadas en este trabajo, 41 de ellas se han detectado en al menos una de las 134 muestras estudiadas, lo que indica el uso intensivo de plaguicidas en esta zona.

De los 18 herbicidas empleados, 13 de ellos fueron detectados en alguna de las muestras, destacando la presencia de trifluralina, pendimetalina y metolacloro. En el caso de la trifluralina, la frecuencia de detección fue muy alta durante los primeros años (2006-2009) hasta su prohibición en el año 2009. A partir de este instante, sólo en 2010 fue detectado, dejándose de detectar a partir de este instante. Las concentraciones de trifluralina oscilaron entre los 0.16 ng m^{-3} hasta los 25.80 ng m^{-3}. En el caso de la pendimetalina, las concentraciones detectadas oscilaron entre los 0.13 ng m^{-3} y los 117.32 ng m^{-3}, valores superiores a los obtenidos en la región de Alsacia en el año 2010 por Schummer et al. [90] (concentración media de 3.18 ng m^{-3}). El metolacloro, a pesar de estar prohibido su uso desde al año 2003, se detectó en todos los periodos a bajas concentraciones (0.12-1.99 ng m^{-3}), lo que indicaría un uso ilegal de éste.

De los 23 fungicidas estudiados, 16 de ellos fueron detectados en alguna de las muestras, destacando el clorotalonil, con concentraciones que oscilan entre los 0.18 ng m^{-3} y los 1128.38 ng m^{-3}. El clorotalonil es empleado habitualmente en cultivos de maíz, trigo y cebada. Los niveles detectados en la zona de Oysonville son superiores que los detectados en otras zonas como Québec (Canadá) [120], Estrasburgo (Francia) [90], California (USA) [253] o Valencia (España) [135]. Además, el fenpropimorf y el ciprodinil fueron observados en todos los años de estudio.

De los 17 insecticidas estudiados, 12 de ellos fueron detectados en algún momento del estudio. Destaca la presencia del lindano (0.12-1.11 ng m^{-3}) y del α-endosulfan (0.47-9.83 ng m^{-3}), a pesar de estar prohibido su uso (desde el año 1998 el lindano y desde el año 2007 el α-endosulfan). El caso del lindano se debe a que se trata de un plaguicida orgánico persistente, lo que explicaría su presencia en la zona. El α-endosulfan, por su parte, se sigue empleando en el tratamiento del maíz, del trigo y de la cebada.

10.2.2. Exposición y evaluación del riesgo.

Para el estudio de la evaluación del riesgo, se han seleccionado aquellos plaguicidas que se han detectado con mayor frecuencia durante el tiempo del estudio (2006-2013). Los plaguicidas seleccionados han sido los siguientes: cinco herbicidas (acetocloro, metolacloro, pendimetalina, propacloro y trifluralina), cinco fungicidas (clorotalonil, ciprodinil, fenpropidin, fenpropimorf y spiroxamina) y un insecticida (lindano).

Los HQ más elevados obtenidos en bebés (6 meses-1.5 años, grupo más vulnerable) fueron los del acetocloro, clorotalonil, fenpropimorf, trifluralin y spiroxamina. En general, los valores obtenidos son inferiores a 0.0993, valor máximo obtenido de HQ para el clorotalonil en el año 2011. En niños y adultos la tendencia es semejante, obteniéndose valores máximos de HQ de 0.0843 y de 0.0357, respectivamente. Por tanto, como todos los valores obtenidos son inferiores a 1, podemos considerar que para los tres grupos de población no existe riesgo para la población [246]. A pesar de que los valores obtenidos no comportan riesgo en la población, se han obtenido valores de HI mayores que en otros estudios de evaluación del riesgo realizados en China [142] o Valencia [91], pero inferiores a los obtenidos en California [254]. La siguiente Tabla (Tabla 22) muestra los valores medios y máximos de DIE y HQ en niños para los plaguicidas que se han detectado con mayor frecuencia:

Tabla 22. Valores medios y máximos de DIE (mg/kg/dia) y HQ para bebés para aquellos plaguicidas con mayor frecuencia de detección.

Plaguicidas	DIE[1]								HQ AOEL							
	2006	2007	2008	2009	2010	2011	2012	2013	2006	2007	2008	2009	2010	2011	2012	2013
Acetocloro																
Media	6.57E-07	7.44E-07	2.21E-06	5.39E-07	6.65E-07	5.07E-07	3.01E-07	4.59E-07	3.29E-05	3.72E-05	1.10E-04	2.69E-05	3.33E-05	1.78E-05	1.50E-05	2.30E-05
Máximo	2.21E-06	1.62E-06	7.03E-06	9.50E-07	1.11E-06	1.15E-06	5.31E-07	1.24E-06	1.10E-04	8.08E-05	3.51E-04	4.75E-05	5.54E-05	3.52E-05	2.65E-05	6.18E-05
Clorotalonil																
Media	2.36E-05	1.17E-05	2.17E-05	1.24E-05	7.78E-06	1.73E-04	9.42E-07	4.47E-06	2.62E-03	1.30E-03	2.41E-03	1.37E-03	8.64E-04	1.92E-02	1.05E-04	4.96E-04
Máximo	8.55E-05	4.90E-05	5.89E-05	3.51E-05	1.39E-05	8.94E-04	2.46E-06	8.09E-06	9.50E-03	5.44E-03	6.55E-03	3.90E-03	1.55E-03	9.93E-02	2.74E-04	8.98E-04
Ciprodinil																
Media	1.12E-06	2.30E-07	4.20E-07	5.70E-07	1.35E-07	1.82E-07	4.67E-07	2.77E-07	3.72E-05	7.66E-06	1.40E-05	1.90E-05	4.49E-06	6.07E-06	1.56E-05	9.24E-06
Máximo	2.60E-06	3.80E-07	1.30E-06	1.54E-06	1.43E-07	2.69E-07	5.78E-07	2.93E-07	8.68E-05	1.27E-05	4.32E-05	5.12E-05	4.75E-06	8.98E-06	1.93E-05	9.77E-06
Fenpropidin																
Media	9.27E-07	3.01E-07	3.64E-07	4.51E-07	1.66E-07	-	2.22E-07	1.04E-06	4.63E-05	1.50E-05	1.82E-05	2.26E-05	8.32E-06	-	1.11E-05	5.19E-05
Máximo	2.80E-06	6.73E-07	7.76E-07	1.38E-06	1.66E-07	-	2.53E-07	2.14E-06	1.40E-04	3.37E-05	3.88E-05	6.89E-05	8.32E-06	-	1.27E-05	1.07E-04
Fenpropimorf																
Media	2.21E-06	8.08E-07	2.04E-06	6.89E-07	1.16E-06	6.34E-07	1.74E-07	1.98E-07	3.16E-04	1.15E-04	2.91E-04	9.84E-05	1.66E-04	9.05E-05	2.49E-05	2.83E-05
Máximo	1.05E-05	2.27E-06	5.88E-06	1.46E-06	2.87E-06	1.51E-06	2.53E-07	2.53E-07	1.49E-03	3.24E-04	8.41E-04	2.08E-04	4.10E-04	2.16E-04	3.62E-05	3.62E-05
Lindano																
Media	2.46E-07	2.14E-07	3.17E-07	1.50E-07	1.35E-07	1.11E-07	1.43E-07	-	4.91E-05	4.28E-05	6.34E-05	3.01E-05	2.69E-05	2.22E-05	2.85E-05	-
Máximo	8.71E-07	3.64E-07	6.18E-07	3.96E-07	1.66E-07	1.11E-07	1.66E-07	-	1.74E-04	7.29E-05	1.24E-04	7.92E-05	3.33E-05	2.22E-05	3.33E-05	-
Metolaclor																
Media	2.69E-07	3.33E-07	4.04E-07	4.75E-07	2.53E-07	1.58E-07	1.35E-07	2.46E-07	1.80E-06	2.22E-06	2.69E-06	3.17E-06	1.69E-06	1.06E-06	8.98E-07	1.64E-06
Máximo	6.95E-07	5.93E-07	1.58E-06	6.15E-07	6.02E-07	2.77E-07	1.43E-07	3.48E-07	4.64E-06	3.95E-06	1.05E-05	4.10E-06	4.01E-06	1.85E-06	9.50E-07	2.32E-06
Trifluralina																
Media	1.99E-06	8.63E-07	7.05E-07	6.26E-07	2.77E-07	-	-	-	7.65E-05	3.32E-05	2.71E-05	2.41E-05	1.07E-05	-	-	-
Máximo	2.04E-05	3.34E-06	2.38E-06	1.31E-06	3.56E-07	-	-	-	7.86E-04	1.29E-04	9.14E-05	5.06E-05	1.37E-05	-	-	-
Spiroxamina																
Media	1.90E-07	2.15E-06	2.28E-06	1.97E-06	-	3.33E-07	1.19E-07	3.02E-06	3.80E-06	4.29E-05	4.56E-05	3.94E-05	-	6.65E-06	2.38E-06	6.04E-05
Máximo	2.38E-07	7.14E-06	6.44E-06	6.13E-06	-	8.63E-07	1.43E-07	8.51E-06	4.75E-06	1.43E-04	1.29E-04	1.23E-04	-	1.73E-05	2.85E-06	1.70E-04
Pendimetalina																
Media	8.20E-06	4.99E-07	1.12E-06	1.12E-06	6.18E-07	5.07E-07	2.46E-07	1.30E-06	3.50E-05	2.13E-06	4.81E-06	4.81E-06	2.64E-06	2.17E-06	1.05E-06	5.55E-06
Máximo	9.29E-05	1.38E-06	2.88E-06	3.95E-06	1.92E-06	1.15E-06	3.80E-07	3.15E-06	3.97E-04	5.91E-06	1.23E-05	1.69E-05	8.19E-06	4.91E-06	1.62E-06	1.35E-05

[1]USEPA 1996 (DIE: Exposición Inhalatoria Diaria; Peso Corporal=10 kg, Tasa de inhalación =8 m^3/dia, Tiempo de exposición=24 horas)

La exposición acumulada de los plaguicidas organofosforados (máximos de 1.10 E-06, 9.30 E-07 y 3.94 E-07 mg/kg/dia para bebés, niños y adultos, respectivamente) fue inferior al NOAEL del etil clorpririfos (plaguicida organofosforado de referencia), cuyo valor es de 0.1 mg/kg/dia, lo que nos indica que no existe riesgo en la exposición acumulada a organofosforados (ver Tabla SD-5 del Anexo VI).

La exposición acumulada a cloroacetamidas presenta un índice de riesgo máximo en el año 2008 (3.53 E-04, 2.99 E-04 y 1.27 E-04 para bebés, niños y adultos, respectivamente). Como los valores obtenidos son inferiores a 1, valor a partir del cual existe riesgo, podemos considerar que no existe riesgo por la exposición acumulada a las cloroacetamidas.

En el caso del riesgo de cáncer (cancer risk), los valores obtenidos para bebés (empleando el peor escenario posible, un valor de PF de 0.l mg/kg/dia) oscilan entre 1.42 E-08, correspondiente al metolacloro y 8.93 E-05, correspondiente al clorotalonil. Como el valor a partir del cual se considera que existe cierto riesgo es 1 E-06, el valor obtenido para el clorotalonil comporta cierto riesgo. Además, en 2006, valores de trifluralina y pendimetalina superaron el valor umbral, lo que nos indica que existió cierto riesgo por la inhalación de ambos plaguicidas (ver Tabla SD-6 del Anexo VI).

Por último, además, se ha calculado el valor basado en salud para bebés (Health Based Level, HBL en ng m^{-3}), suponiendo que el valor de HQ es 1. Los valores de HBL se calcularon para aquellos plaguicidas que presentaron los HQ más elevados (acetocloro, clorotalonil, fenpropimorf, spiroxamina y trifluralin). El rango de HBL obtenidos osciló entre 8.83 E+03 y 6.30 E+04 ng m^{-3}. Concentraciones de aire por debajo del valor basado en salud no deberían considerarse un problema para la salud y no se sometería a una evaluación posterior, pero tampoco debería considerarse una concentración sin ningún peligro. De igual forma, una concentración superior al HBL no significaría una preocupación significativa, pero si sería necesaria una evaluación más exhaustiva. De igual forma, se ha calculado el 10% del HBL, que corresponde al nivel máximo recomendado (RML, recommended maximum level). Los valores obtenidos oscilan entre 8.83 E+02 y 6.30 E+02, correspondientes al fenpropimorf y la spiroxamina (ver Tabla 23). Sin embargo, actualmente ni los valores de HBL ni los valores de RML están regulados. En un futuro próximo, será importante establecer valores de referencia basados en salud para las concentraciones de plaguicidas en la atmósfera, lo que implicará un esfuerzo complementario para crear una red de control europea y el desarrollo de una nueva legislación para el control de los plaguicidas.

Tabla 23. Valor basado en salud (HBL) (ng m^{-3}) y nivel máximo recomendado (RML) (ng m^{-3}) para varios de los plaguicidas estudiados*

Plaguicidas	**Valor Basado en Salud (HBL) (ng m^{-3})**	**Nivel Máximo Recomendado (RML) (ng m^{-3})**
Acetocloro	2.50E+04	2.50E+03
Clorotalonil	1.10E+04	1.10E+03
Fenpropimorf	8.83E+03	8.83E+02
Spiroxamina	6.30E+04	6.30E+03
Trifluralin	3.30E+04	3.30E+03

*Valores teóricos empleando las ecuaciones (1) y (2) (apartado Metodología) (USEPA, 1997; OMS, 1999; EU pesticide database).

10.3. Conclusiones.

-En este trabajo (capítulo 6), se ha estudiado la exposición a plaguicidas en una zona agrícola de Francia (Región Centro) en tres diferentes grupos de población (adultos, niños y bebés).

-Los plaguicidas que se detectaron con una mayor frecuencia de detección durante el periodo de estudio (2006-2013) fueron los siguientes: los herbicidas trifluralina (0.16-25.8 ng m^{-3}) y pendimetalina (0.13-117.32 ng m^{-3}), el fungicida clorotalonil (0.18-1128.38 ng m^{-3}) y los insecticidas lindano (0.12-1.11 ng m^{-3}) y α-endosulfan (0.47-9.83 ng m^{-3}).

-La exposición inhalatoria diaria en bebés ha oscilado entre 9.41 E-08 mg kg^{-1} dia^{-1} (lindano) y 7.58 E-04 mg kg^{-1} dia^{-1} (clorotalonil), calculados a partir de la concentración máxima detectada. Los valores de HQ obtenidos son inferiores a 3.57 E-02, 8.43 E-02 y 9.93 E-02 para adultos, niños y bebés, respectivamente. Estos valores son inferiores a 1, valor a partir del cual puede existir riesgo para la salud.

-La exposición acumulada para los plaguicidas organofosforados fue menor a 3.94 E-07, 9.30 E-07 y 1.10 E-06 para adultos, niños y bebés, respectivamente. Los valores obtenidos son inferiores a 0.1 mg kg^{-1} dia^{-1} (NOAEL del etil clorpirifos), nivel a partir del cual se considera que existe riesgo para los humanos.

-En el caso de las cloroacetamidas, los índices de peligro (HI) calculados son inferiores a 3.55 E-04 para los tres grupos de población estudiados. Teniendo en cuenta que el valor a partir del cual se considera que hay cierto riesgo es un valor de HI de 1, los niveles obtenidos no implican riesgo en la salud.

10.4. Artículo 6: Human exposure and risk assessment to airborne pesticides in a rural French community.

Science of the Total Environment 584–585 (2017) 856–868

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Human exposure and risk assessment to airborne pesticides in a rural French community

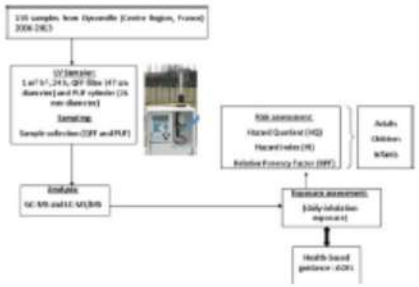
Clara Coscollà [a,d,*], Antonio López [a,c,d], Abderrazak Yahyaoui [b], Patrice Colin [b,**], Corine Robin [b], Quentin Poinsignon [b], Vicent Yusà [a,c,d]

[a] Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020 Valencia, Spain
[b] LIGAIR-Surveillance de la Qualité de l'Air en Région Centre, 3, rue du Carbone, 45100 Orléans, France,
[c] Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain
[d] Atmospheric Pesticide Research Group of Valencia, Spain

HIGHLIGHTS

- Human inhalation risk assessment of outdoor airborne pesticides was performed.
- The levels of 41 detected pesticides ranged from 0.12 ng m^{-3} to 1128 ng m^{-3}.
- All measured reported Hazard Quotients <1
- The cumulative risk for the two pesticide types assessed is acceptable.
- For infants the estimated cancer risk was lower than 8.93×10^{-05}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form 19 January 2017
Accepted 19 January 2017
Available online 1 February 2017

Editor: D. Barcelo

Keywords:
Pesticides
Exposure assessment
Risk assessment
Atmospheric concentrations

ABSTRACT

Outdoor air samples collected during the pesticide agricultural application period (spring and summer) from a rural community in the Centre Region (France) were analyzed to investigate temporal variation of atmospheric pesticide levels (2006–2013) and human inhalation exposure in adults, children and infants.
The most frequently detected pesticides were herbicides (trifluralin, pendimethalin), fungicides (chlorothalonil) and insecticides (lindane and α-endosulfan). The three currently-used pesticides most frequently detected presented concentrations ranging from 0.18 to 1128.38 ng m^{-3}; 0.13 to 117.32 ng m^{-3} and 0.16 to 25.80 ng m^{-3} for chlorothalonil, pendimethalin and trifluralin, respectively.
The estimated chronic inhalation risk, expressed as Hazard Quotient (HQ), for adults, children and infants, was <1 for all measured pesticides. Likewise, the cumulative exposure for detected organophosphorus and chloroacetamide pesticides, was estimated using the Relative Potency Factor (RPF) and Hazard Index (HI) as metrics, which was indicated that no risk was observed. The cancer risk classified as likely or possibly carcinogen was estimated to be < 8.93 E −05 in infants, for the detected pesticides.

* Corresponding author at: Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020, Valencia, Spain.
** Corresponding author.
E-mail addresses: coscolla_cla@gva.es (C. Coscollà), colin@ligair.fr (P. Colin).

http://dx.doi.org/10.1016/j.scitotenv.2017.01.132
0048-9697/

1. Introduction

A wide variety of pesticides can be applied in agriculture and their identity depends on a range of factors including the specific pest and crop of interest. During 2010, about 208.000 t of pesticide active ingredients were used in Europe (EU-15) (ECPA, 2010) and >300 active substances are nowadays authorized by the European Union for their application on various crops according to the Regulation (EC) 1107/2009 (EU, 2014). Several studies in Europe have reported pollution of ambient air by agricultural pesticides in concentrations ranging from few pg m^{-3} to several ng m^{-3} (Schummer et al., 2010; Borràs et al., 2011; Coscollà et al., 2010; Hart et al., 2012; Raeppel et al., 2014). The potentially adverse effects of exposure to pesticides on the general population, and specifically on the more susceptible groups such as infants and children, are a public health concern (Marks et al., 2010; London et al., 2012). Apart from ingestion of foods, drinking water and dermal exposures, inhalation of ambient air could also be a relevant pathway of exposure to pesticides.

A major proportion of the applied pesticides are emitted to the atmosphere. Post-application emissions that involve volatilization from soil and plants, and wind erosion of soil particles containing sorbed pesticides represent further significant pesticide input into the troposphere for several days or weeks after pesticide application (Scheyer et al., 2007). Therefore, accurate information on active substance usage and inventories of emissions are relevant for assessing the exposure and risk to human populations (Sarigiannis et al., 2013).

As a result of agricultural use, a large number of pesticides have been detected in outdoor ambient air. Hart et al. (2012) detected 24 CUPs (currently used pesticides) in the particulate matter, in concentrations between 3.2 and 1196 ng m^{-3} in the atmosphere of the Valencian region (Spain). These authors also reported the levels of 17 more polar CUPs in the PM10 with concentrations ranging from 6.8 to 2898 pg m^{-3} in the same area (Coscollà et al., 2013). Moussaoui et al. (2012) assessed the contamination level of pesticides in ambient air of northern Algeria, detecting concentrations levels from 16 pg m^{-3} to 11 ng m^{-3}. In a more recent study, Yusà et al. (2014) analyzed 56 samples from April to October of 2010 from a rural station in the Valencian region. Twenty out of the 82 pesticides searched were found at this rural site. The average concentrations ranged from 5.75 to 117.01 pg m^{-3}, with maximum concentrations lying between 15.54 and 758.60 pg m^{-3}. Coscollà et al. (2014) mainly found carbendazim, metalaxyl, terbuthylazine and myclobutanil in concentrations from 16 pg m^{-3} to 174 pg m^{-3} in ambient air (June–July 2013) at a rural station located in the same area. Mahmood et al. (2014) assessed the organochlorine pesticide contamination and its probable hazardous effects on human health such as carcinogenic, reproductive, neurological, immunological and other adverse effects. They collected samples from Gujranwala, Punjab Province, Pakistan detecting concentration ranges from 1.23 to 635 pg m^{-3}.

Although a guidance on pesticide exposure and risk assessment for operators, workers, bystanders and residents was recently developed (EFSA, 2014a), the methodologies focused on the exposure and risk assessment of air pesticides on the general population are scarce. Yusà et al. (2014) proposed a screening approach for inhalation chronic risk assessment of CUPs present in ambient air, based on the concentration of these compounds in the inhalable particulate matter (PM10). They applied this approach in a pilot case study for the risk assessment of an agricultural community in Valencia, Spain. In California, the Department of Pesticide Regulation from the Environmental Protection Agency implemented an air monitoring network for measuring pesticides in various agricultural communities to provide data for assessing potential health risks. However they only collect the gas phase fraction (AMN, 2012).

To perform an inhalation risk assessment to airborne pesticides, it is necessary to know the total concentrations of the active substances (gas + particulate phases). In this paper we present the temporal variations (2006–2013) of pesticide levels in the atmosphere of a rural site in the Centre Region (France). Using these concentrations we have estimated the human inhalation exposure and the risk assessment of the general population living near the sampling site.

2. Experimental

2.1. Pesticide selection

In 2011, around 60,000 t of pesticides were sold in France, which is the first consumer country in the European Union (Ministere de l'Écologie, du Developpement durable et de l'énergie, 2014). The farming activities are intensive in the use of pesticides in the Centre Region (France), consuming a total of 4179 tons of active ingredients in 2001 (5.5% of the national consumption) (FREDEC, 2004). Fifty-eight pesticides were studied, of which CUPs and some banned pesticides, constituted the vast majority. A total of 17 insecticides, 23 fungicides and 18 herbicides were investigated. CUPs approved by EU regulations were applied into arable crops such as maize, barley, and wheat, and their selection was based on the amount applied in the Centre Region (France). In addition, some banned persistent pesticides, previously detected in this region, (2,4′ DDD, 4,4′ DDE, α-endosulfan and γ-HCH) were also studied, because some of them could still be present in outdoor ambient air mainly due to their persistence. Table 1 shows the usage and legal status of the measured pesticides.

2.2. Sampling and site characterisation

The present work is part of a broader study on characterisation of contemporary pesticides in the atmosphere of Centre Region (France), which is one of the country's largest regions in France. We previously carried out a monitoring study of five sites (three rurals and two urbans) during three sampling campaigns between 2006 and 2008 (Coscollà et al., 2010). The present work focused on risk assessment and the data from one of the sites (Oysonville) was used with additional data collected at this site between 2009 and 2013. The sampling station was located in a rural area in northern Centre Region, in a town called Oysonville. Oysonville (48°23′35″N, 01°56′57″W) had 501 inhabitants in 2010 and it is situated 55 km from the north of Orléans city (Eure-et-Loir, Région Centre, France). The density population was 52 inhabitants/km^2. The minimum altitude is 143 m and maximum altitude, 154 m, and the total surface is 9.63 km^2. Location map is shown in Fig. 1.

The station is placed in the outskirts of the village, in the countryside, surrounded at many arable crops. About 1 km is the distance between the sampler and the first houses of the town. The most extended crops in this area are wheat, rape and barley. The orchards crop production varies according to the season. There are some spring crops such as wheat, barley, maize and rape; and some summer crops such as beet, peas, potatoes and rape. Samples were collected at the human breathing zone (approximately 1 m from the ground level).

A total of 134 samples were collected (26 in 2006, 13 in 2007, 12 in 2008, 16 in 2009, 13 in 2010, 18 in 2011, 18 in 2012 and 18 in 2013). The sampling weeks coincide with the application period of pesticides for this region (spring and summer seasons) (see Fig. 2).

A low-volume sampler (Partisol 2000) from Thermo Electron Corporation (East Greenbush, NY, USA) sampling both gaseous and particulate (Total Suspended Particles, TSP) phases was employed. Samples were collected using quartz fiber filters (QFFs, 47 mm diameter) (particle phase) and PUF cylinder (polyurethane foam, 26 mm diameter × 76 mm length with 22 mg·cm^{-3}) (gas phase). The samples (QFFs and PUF) ran for 168 h at a flow rate of 1 m^3 h^{-1}. The total volume collected was approximately 168 m^3.

2.3. Sample analysis

QFFs and PUFs samples were extracted together in stainless-steel cells in the same Accelerated Solvent Extraction (ASE) 300 PLE system

Table 1
Agricultural usage, legal situation, limits of quantification for this study, health based reference values and cancer classification of pesticides.

Pesticide	Chemical class	Agricultural usage	legal situation European Union (a)	LOQ ($ng\ m^{-3}$)	AOEL (b)	Cancer classification (c)
Insecticides						
2,4' DDD	Organochlorine	–	Not approved	0.05 (1)		
4,4' DDE	Organochlorine	–	Not approved	0.05 (1)		
α-Endosulfan	Organochlorine	Arable crops, vegetables	Not approved (since 2007)	0.24 (1)		
Chlorpyrifos ethyl	Organophosphate	Vineyard, peach, pear, apple	Approved	0.05 (1)		
Chlorpyrifos methyl	Organophosphate	Vineyards, fruits	Approved	0.05 (1)		
Diazinon	Organophosphate	Vegetables, fruits	Not approved	0.05 (1)		
Ethoprophos	Organophosphate	Potato, vegetables	Approved	0.05 (1)		
Flufenoxuron	Benzoylurea	Vegetables, fruits, vineyard	Not approved	0.05 (2)		
Lambda-cyhalotrin	Pyrethroid	Fruits, vegetables	Approved	0.05 (1)		
Lindane (γ-HCH)	Organochlorine	–	Not approved (since 1998)	0.12 (1)	0.005	4
Malathion	Organophosphate	Vineyard, arable crops, vegetables	Approved	0.05 (1)		
Methidathion	Organophosphate	Fruits	Not approved	0.05 (1)		
Methyl parathion	Organophosphate	–	Not approved (since 2003)	0.24 (1)		
Phosmet	Organophosphate	Pear, apple, potato	Approved	0.05 (2)		
Pirimicarb	Carbamate	Fruits	Approved	0.05 (1)		
Propargite (acaricide)	Sulfite ester	Fruits, flowers	Not approved	0.24 (1)		
Pyriproxifen	Hormone mimic	Apple, vegetables	Approved	0.05 (2)		
Herbicides						
Acetochlor	Chloroacetamide	Arable crops (maize)	Not approved	0.05 (1)	0.02	1
Aclonifen	Diphenyl eter	Arable crops (sunflower, peas), potato	Approved	0.24 (1)		
Alachlor	Chloroacetamide	Arable crops (maize, soybean)	Not approved	0.05 (1)		
Atrazine	Triazine	Arable crops (maize)	Not approved (since 2007)	0.05 (2)		
Dichlobenil	Benzonitrile	Vineyard	Not approved (since 2009)	0.05 (1)		
Diflufenican	Carboxamide	Arable crops (wheat, barley)	Approved	0.05 (1)		
Dimethenamid	Amide	Potato, arable crops (beet), onion	Not approved (since 2008)	0.05 (1)		
Ethofumesate	Alkylsulfonate	Arable crops (beet, beans)	Approved	0.24 (1)		
Mecoprop	Aryloxyalkanoic acid	Arable crops (wheat, barley, rye, oat)	Approved	0.05 (2)		
Metazachlor	Chloroacetamide	Cabbage, arable crops (rape)	Approved	0.05 (1)		
Metolachlor	Organochlorine	–	Not approved (since 2003)	0.05 (1)	0.15	5
Oryzalin	Dinitroaniline	Vineyard, fruit trees, asparagus	Approved	0.05 (1)		
Oxadiazon	Oxadiazole	Vineyard, carrot	Approved	0.05 (1)		
Oxyfluorfen	Diphenyl eter	Vineyard	Approved	0.05 (1)		
Pendimethalin	Dinitroaniline	Arable crops, vegetables, vineyard	Approved	0.05 (1)	0.234	5
Propachlor	Chloroacetamide	Cabbage, onion	Not approved (since 2009)	0.05 (1)		
Prosulfocarb	Thiocarbamate	Arable crops (wheat), potatoes, carrots	Approved	0.05 (1)		
Trifluralin	Dinitroaniline	Arable crops (soybean, sunflower), cabbage	Not approved (since 2009)	0.05 (1)	0.026	5
Fungicides						
Azoxystrobin	Strobilurin	Arable crops, vegetables, vineyard	Approved	0.24 (1)		
Captan	Phtalimide	Vineyard, fruit trees	Approved	0.05 (1)		
Chlorothalonil	Phtalimide	Arable crops, vegetables, fruit trees	Approved	0.27 (1)	0.009	2
Cymoxanil		Potato, lettuce, vineyard	Approved	0.05 (2)		
Cyprodinil	Pyrimidine	Arable crops (wheat, barley), pear, apple	Approved	0.05 (1)	0.03	3
Difenoconazole	Triazole	Arable crops (wheat), apple, vineyard	Approved	0.05 (2)		
Dimethomorph I	Morpholine	Vineyard, flowers	Approved	0.24 (1)		
Dimethomorph II	Morpholine	Vineyard, flowers	Approved	0.24 (1)		
Diphenylamine	Phenylamines	Post-harvesting in fruit	Not approved	0.05 (1)		
Epoxiconazole	Triazole	Arable crops	Approved	0.05 (2)		
Fenpropidin	Piperidine	Arable crops (wheat, barley)	Approved	0.05 (1)	0.02	1
Fenpropimorph	Morpholine	Arable crops (wheat, barley, sunflower)	Approved	0.05 (1)	0.007	3
Fluazinam	Phenylpyridinamine	Vineyard, potato	Approved	0.05 (2)		
Fludioxonil	Phenylpyrrole	Vineyard	Approved	0.05 (1)		
Folpet	Phtalamide	Potato, vineyard, apple, pear, arable crops	Approved	0.24 (1)		
Iprodione	Dicarboximide	Fruits, vineyards	Approved	0.05 (2)		
Kresoxim methyl	Strobilurin	Fruits, vineyards	Approved	0.05 (1)		
Procymidone	Dicarboximide	Cucumbers, plums	Not approved	0.05 (1)		
Pyrimethanil	Anilinopyrimidine	Vegetables, apple trees, vineyard	Approved	0.05 (1)		
Spiroxamine	Morpholine	Arable crops, vineyard	Approved	0.05 (1)	0.05	3
Tolylfluanid	Phenylsulfamide	Almond, hazelnut, apple, pear, vineyard	Not approved	0.05 (1)		
Trifloxystrobin	Strobilurin	Arable crops (wheat), apple, vineyard	Approved	0.05 (1)		
Vinclozolin	Dicarboxamide	Vineyard, vegetables	Not approved (since 2007)	0.05 (1)		

LOQ = Limit of Quantification; 1 = GC-MS and 2 = LC-MS/MS.
AOEL = Acceptable Operator Exposure Level (mg/kg bw/day) from EU Pesticide database (EU, 2014).
a = EU pesticide database (EU, 2014) http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN
b = Only for detected pesticides.
c = Chemicals Evaluated for Carcinogenic Potential Office of Pesticide Programs U.S. Environmental Protection Agency, Annual Cancer Report, USEPA, 2013.
Cancer classification: 1 = suggestive evidence of carcinogenic potential; 2 = likely to be carcinogenic to humans; 3 = not likely to be carcinogenic to humans; 4 = suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential; 5 = group C-possible human carcinogen.

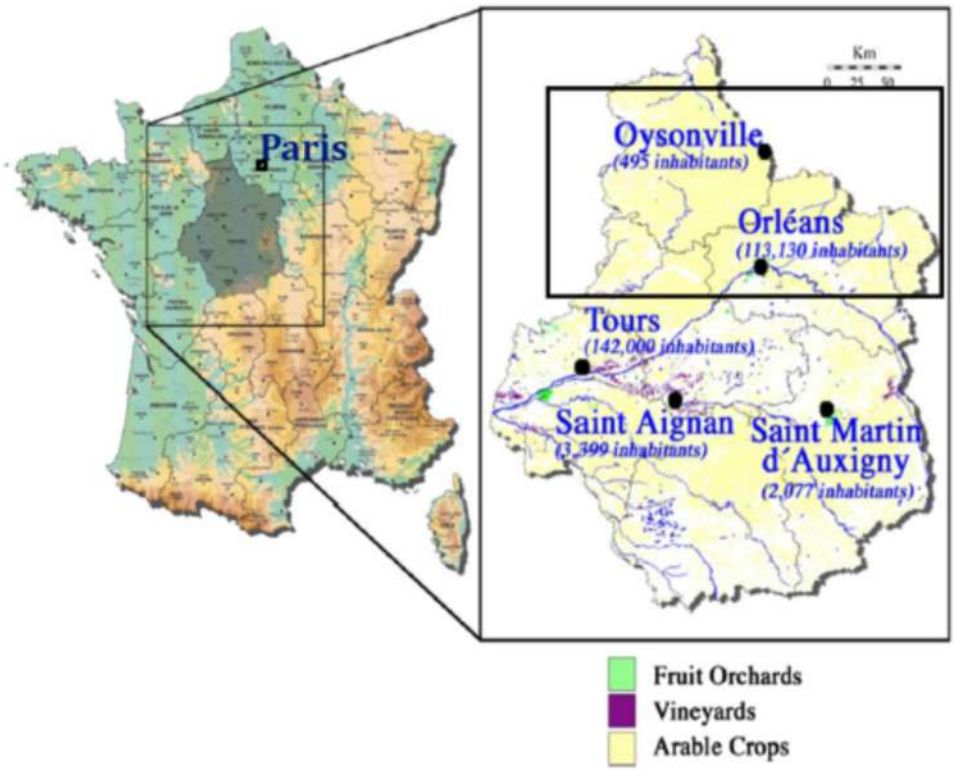


Fig. 1. Location map of the studied area.

(Dionex, Sunnyvale, CA, USA). The extraction conditions were as follows: extraction solvent, dichloromethane; oven temperature, 90 °C; pressure, 100 bars; heat-up time, 5 min; static cycles, 4; static time, 5 min. The flush volume amounted to 90% of the extraction cell volume. The extract was purged from the sample cell using pressurized nitrogen for 150 s. Prior to extraction, each sample was fortified with a mixture containing 8.5 ng each of d^{14} trifluralin and d^{10} chlorpyrifos ethyl, which served as surrogates for assessing method recoveries. Samples were spiked with 170 μL of 50 ng μL^{-1} each of d^{14} trifluralin and d^{10} chlorpyrifos ethyl, with within laboratories recoveries ranging from 75 to 115%.

Afterwards, the extract was divided into two fractions. One fraction was concentrated and redissolved with 1 mL of hexane. This solution was injected by gas chromatography coupled to mass spectrometry (GC–MS) in SIM (Selected Ion Monitoring) mode. Selected ions and limits of detection for GC–MS are shown in Tables 1 and SD-1. The other fraction was solvent exchanged with methanol. This solution was injected by liquid chromatography coupled to mass spectrometry (LC-MS/MS) working in SRM (Selected Reaction Monitoring Mode) mode. Experimental parameters for LC-MS/MS are shown in Table SD-2. All extracts were stored protected from light and below 18 °C.

Separation and identification of 48 pesticides by GC–MS were performed following the XP X 43-059 Normalisation Française (AFNOR, 2007), on an Agilent 6890 coupled to 5973 mass spectrometer. The column used was CPSIL 13CB (25 m × 0.25 mm × 0.4 μm) (Santa Clara, CA, USA). The temperature gradient was 80–140 °C, 20 °C/min; 140–230 °C, 6 °C/min and 230–280 °C, 25 °C/min. The injection type was split/splitless, the injector temperature was 250 °C and the injection volume

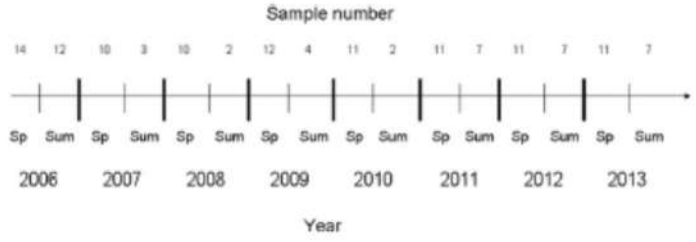


Fig. 2. Sampling timeline including the sampling seasons (Sp = Spring; Su = Summer), year of collection and number of collected samples.

1 μL. The mobile phase was helium with a flow of 1 mL/min. The ionization mode was Electronic Impact with 70 eV.

Analytical determination of 10 pesticides by LC-MS/MS was performed on a Quattro Micro coupled with an Alliance HPLC chain. The column used was Gemini-NX 3u C18 100A, part number 00F-4453-B0, purchased from Phenomenex (Torrance, CA, USA). Eluents were water and methanol with 5 mM of ammonium formate in a gradient mode. The flow rate was 200 μL min^{-1}. Seven pesticides were detected using electrospray ionization in positive mode (ESI+) (atrazine, cymoxanil, difenoconazole, epoxiconazole, flufenoxuron, phosmet and pyriproxyfen) and three of them were detected in negative mode (ESI-) (fluazinam, iprodione and mecoprop) (Coscollà et al., 2010) (see Table SD-2).

2.4. Quality control protocol

Each set of samples was analyzed under quality assurance protocols, including transport blanks and field blanks. In order to determinate pollutant backgrounds, a procedural blank was employed as control and was treated in the same way as the samples. The analysis of samples was performed after sampling or after storage period at −18 °C.

The retention capacity for PUF and the extraction recoveries for PUF and the extraction recoveries for PUF plus filter were tested for all pesticides according to AFNOR (2007), obtaining retention from 60 to 120% and recoveries from 70 to 110%, respectively. The occurrence of breakthrough may affect negatively the sampling efficiency. Breakthrough was evaluated for all pesticides. Breakthrough was not observed.

2.5. Hazard identification and characterisation

For potential non-cancer effects, dose-response information for each compound was reviewed. Health Based Reference Values (HBRV) were chosen for each detected pesticide such as AOEL (Acceptable Operator Exposure Level), that is based on chronic inhalation exposure for operators. At present, there is a lack of reference inhalated exposure levels for the studied target population.

The values for each HBRV were retrieved from data bases of the European Union (EU) (EU, 2014). Table 1 shows the AOELs for each pesticide.

For cancer risk assessment the cancer classification of pesticides developed by EPA (USEPA, 2013) was used. Table 1 shows the cancer classification of the pesticides detected. Cancer Risk has been calculated for those classified as Possible human carcinogen (metolachlor, trifluralin and pendimethalin), likely to be carcinogen to humans (chlorothalonil) or suggestive evidence of carcinogenic potential (acetochlor, fenpropidin and lindane).

2.6. Chronic exposure and risk assessment

Inhalation of atmospheric pesticides is an important route for pesticide exposure. Chronic (>1 year) inhalation exposures were assessed for adults, children and infants. To estimate the inhalation exposure from the atmospheric pesticides, the following equation was used (USEPA, 1997; WHO, 1999):

$$DIE\ (ng/kg/day) = \Sigma\ (C \times IRinh \times ED) \ /\ BW \quad (1)$$

where DIE is the daily inhalation exposure, C is the total (particle + gas phases) concentration of each pesticide in air (ng m^{-3}), IRinh is the inhalation rate per hour ($m^3\ h^{-1}$), ED is the exposure duration (h) to air and BW is the body weight of the subject (kg).

Three groups of population were included in the risk assessment: infants (6 months–1.5 years), children (1–6 years) and adults (>12 years). Two conservative exposure scenarios were considered for the chronic exposure assessment i) using the average total concentration of the detected pesticides during the sampling period, and ii) using the maximum concentration for each pesticide during the sampling period. In both scenarios a conservative ED of 24 h was considered. Likewise, a very conservative assumption was adopted related with the fraction of a year over which the exposure occurs (exposure frequency). We have considered an exposure frequency of 1 which means an exposure over 12 months per year.

IRinh applied was 20 $m^3\ day^{-1}$ for adults, 10 $m^3\ day^{-1}$ for children and 8 $m^3\ day^{-1}$ for infants. BW was 70 kg for adults, 15 kg for children and 10 kg for infants (USEPA, 1989; USEPA, 2004; USEPA, 1991).

The risk assessment was estimated using the Hazard Quotients (HQ) as a risk descriptor, which was calculated as follows:

$$HQ = DIEi/HBRVi \quad (2)$$

where HBRVi stands for Health Based Reference Values.

The HQ level of concern was set to 1.0, thus an HQ > 1 indicated that a potential risk may be present.

The cumulative exposure can be estimated using Hazard Index (HI) or Relative Potency Factors (RPFs) approach. HI could be used for all substances with a same effect on target organs. This approach has been applied to chloroacetamide pesticides, employing the following formula:

$$HI = HQ_1(\text{pesticide 1}) + HQ_2(\text{pesticide 2}) + HQ_3(\text{pesticide 3}) + (\text{and so forth}) \quad (3)$$

The HI level of concern was set to 1.0, thus an HI > 1 indicated that a potential risk may be present.

RPFs have been applied to organophosphate pesticides which have the same mode of action. To estimate cumulative dose from exposure to organophosphate pesticides we have converted each relevant pesticide into its index chemical toxicity equivalent using relative potency factors (RPFs). RPFs are the ratio of the toxic potency of a given chemical to that of an index chemical in the cumulative assessment group. We used NOAEL (No Observed Adverse Effect Values) values for brain cholinesterase inhibition as the measure of potency in our RPF calculations, and we selected chlorpyrifos ethyl as the index compound (Table SD-3). As defined by the U.S. EPA (Castorina et al., 2003),

$$RPFn = \text{Measure of Potency}_{\text{Chlorpyrifos ethyl}} / \text{Measure of Potency}_{\text{Organophosphate}} \quad (4)$$

where chemical n = a member of the cumulative assessment group (organophosphate); index chemical = the chemical selected as the basis for standardization of toxicity of components in a mixture (chlorpyrifos ethyl).

Cumulative exposure for organophosphate pesticides have been calculated using the following formula:

$$\text{Cumulative exposure} = \Sigma\ (DIE \times RPF)\ (mg/kg/day) \quad (5)$$

Cumulative exposure has been compared with the NOAEL of chlorpyrifos ethyl (0.1 mg/kg/day). Levels of cumulative exposure higher than NOAEL, indicated that a potential risk may be present. It was noted that USEPA had used the Benchmark dose (BMD) approach to derive reference values, resulting in slightly lower values (EFSA, 2014b).

For cancer risk the following equation was used:

$$\text{Cancer risk} = DIE\ (mg/kg/day) \times PF\ (mg/kg/day)^{-1} \quad (6)$$

where PF is the Potency Factor. For possible or likely carcinogens the potency factor ranges between >0.01 and 0.1 (Gunier et al., 2001; Lee et al., 2002), so we have used 0.1 for all these pesticides.

Exposure calculation can be affected by numerous sources of uncertainty due to limitations of scientific knowledge including factors related to both the availability of data and the methodology used. Unquantifiable uncertainties associated with exposure assessment

were considered for the interpretation of the results from a qualitative point of view, following the recommendations of the Scientific Committee of EFSA (EFSA, 2006). Table SD-4 shows and briefly explains the factors or sources of uncertainty considered. While it is desirable to provide risk managers of the uncertainties associated with the exposure assessment, it is difficult in some ways to express the impact of uncertainties.

3. Results and discussion

3.1. Measured airborne pesticides at the Oysonville site, 2006–2013

Eight sampling campaigns were conducted on a rural sampling site during 2006–2013. With a few exceptions, the campaigns were conducted from April to mid-July as a consequence of the results of our previous air monitoring studies from 2001 to 2008 (Lig'Air, 2005; Coscollà et al., 2010), which mainly linked the presence of CUPs to the growing season. The overall frequency of detection and the average pesticide concentrations are summarized in Tables 2.1 and 2.2.

Of the 58 active substances monitored during this eight year study, 41 were detected in at least one airborne sample, which highlights the intense use of pesticides in this region. Among herbicides, 13 out of 18 monitored substances were detected, with trifluralin and pendimethalin presenting the highest frequency of detection ranging from 84 to 100% (see Tables 2.1 and 2.2), with overall concentrations ranging from 0.16 to 25.80 ng m^{-3}. Herbicides are generally applied using medium to course droplets. Five herbicides frequently detected could be associated with fine spray droplet sizes derived from inappropriate selection of atomizer or volatilization. These droplets could be easily drifted during application and detected in the air.

During 2010, trifluralin was detected only in 31% of samples, and failed to be detected in the following years. Trifluralin, which was banned in 2009, is a selective soil herbicide commonly used for the control of grass and broad-leaved weeds in arable crops (EFSA, 2009; Coscollà et al., 2010). All sampling sites of Oysonville are surrounded by arable crops, and this setting could be considered an important source for trifluralin in the Centre Region (France). Trifluralin was also detected in other studies in various countries such as France (Bedos et al., 2002; Scheyer et al., 2007), Canada (Waite et al., 2005; Aulagnier et al., 2008), United States (Foreman et al., 2000) and Spain (Hart et al., 2012; Borrás et al., 2011). Scheyer et al. (2007) reported trifluralin in higher concentrations (50 to 90 ng m^{-3}) in Alsace Region (France) during 2007. Trifluralin was also found in 9 out 10 samples in the atmosphere of Strasbourg during 2004, with lower concentrations that ranged from 0.06 to 0.22 ng m^{-3} (Schummer et al., 2010) and in the air of the Centre Region (France) with a frequency of detection of 90% (Coscollà et al., 2010). In Valencia Region (Spain) all samples that tested positive for this pesticide had quantities that were below the limit of quantification (Hart et al., 2012). In another study in Spain developed by Borrás et al. (2011), trifluralin was detected in the particle phase in concentrations ranging from 80 to 300 pg m^{-3}. The Pesticide Air Monitoring Network in California measured pesticides in various agricultural communities and also found trifluralin in a lower average concentration from February to December (2011) of 2 ng m^{-3} (AMN, 2012).

Another frequently detected herbicide is pendimethalin, also used for the treatment of arable crops. Overall concentrations ranged from 0.13 to 117.32 ng m^{-3}. Schummer et al. (2010) studied the presence of pesticides in Alsace (France) and reported the presence of pendimethalin with average concentrations of 3.18 ng m^{-3}. Its relative high volatility and its half life (12 h) permit its medium-range transport (Coscollà et al., 2010).

Apart from these two substances other herbicides such as alachlor, metolachlor, acetochlor, aclonifen and propachlor were also detected. Alachlor is a herbicide applied on maize and soybean crops which was observed during 2006–2008. Metolachlor was detected in all sampling periods, but in low concentrations (0.12–1.99 ng m^{-3}). This indicates that despite its ban since 2003, it is still applied to treat arable crops. Similar concentrations were found in California and Iowa (1.4 ng m^{-3} and 2.3 ng m^{-3}, respectively) (AMN, 2012; Peck and Hornbuckle, 2005). Scheyer et al. (2007) reported metolachlor at a rural site in the Alsace Region (France). In the 2007 study, samples were collected during spring 2003, when this pesticide was still authorized. Acetochlor is another herbicide observed from 2007 to 2013. This pesticide used to be applied to treat maize in the region, but it is nowadays forbidden. In contrast, Schummer et al. (2010) who also searched for this herbicide in 2007 did not find it in the air of the Alsace Region (France).

Aclonifen is authorized for the treatment of arable crops and is used in the area. It was also found by Schummer et al. (2010) with a frequency of detection of 70% in Strasbourg in 2007. Propachlor has been banned since 2009 and used to be applied to cabbage and onion fields in various areas of the Centre Region, so its presence until 2009 is thus explained.

Among fungicides, out of the 23 substances monitored, 16 were detected, with chlorothalonil presenting the highest concentrations ranging from 0.18 to 1128.38 ng m^{-3}. Chlorothalonil is a broad-spectrum fungicide currently applied in maize, wheat and barley. Coscollà et al., 2010 also detected chlorothalonil in air samples collected in other rural and urban sites in the Centre Region (France), with concentrations between 0.11 and 107.93 ng m^{-3} (average concentration of 12.15 ng m^{-3}). It is a relatively persistent substance that can be transported far from its emission source (half life = 4.7 years), which explains its occurrence in urban areas. Apart from its presence at the Oysonville site, its occurrence in the atmosphere was previously reported by Yao et al. (2006) with lower levels ranging from 1.86 to 11.90 ng m^{-3} in the atmosphere of Canada. In another study, Aulagnier et al. (2008) reported levels of this fungicide in an agricultural site of Québec in 2004, in the range of <LQ–0.0015 ng m^{-3} (13% detections) from May to June, and from 0.930 to 1.742 ng m^{-3} (100% detections) from July to September. In a more recent study performed by Schummer et al. (2010), chlorothalonil was found to be present in 7 out 10 samples analyzed in the urban atmosphere of Strasbourg (France), with an average concentration of 0.15 ng m^{-3}, which are lower concentrations than those found in our study. It was also found in the atmosphere of United States (California) with average concentration of 8.8 ng m^{-3} in 2011 (AMN, 2012). Chlorothalonil was also detected in low concentrations (4.38–39.86 pg m^{-3}) at five stations (one remote, one urban and three rural) in the Valencian region (Spain) from January to December 2010. This fungicide was mostly applied on vegetables and fruits in this area (Hart et al., 2012).

Other fungicides such as spiroxamine (0.13–10.75 ng m^{-3}), fenpropimorph (0.14 to 13.19 ng m^{-3}), cyprodinil (0.12–3.29 ng m^{-3}) and fenpropidin (0.12–3.54 ng m^{-3}) were also identified. Spiroxamine and fenpropidin were detected every year except for 2010 and 2011, respectively. Fenpropimorph and cyprodinil were observed throughout the sampling campaign (2006 to 2013). These four substances were applied in agricultural practices for the treatment of wheat, barley and sunflowers.

Among insecticides, out of the 17 substances monitored, 12 were detected, with lindane (0.12–1.11 ng m^{-3}) and α-endosulfan (0.47–9.83 ng m^{-3}) being the most frequently detected. Lindane is forbidden since 1998 and α-endosulfan since 2007. The detection of lindane could be explained by it being a persistent organic pollutant (POP) (Scheyer et al., 2007). α-Endosulfan, in turn, is a pesticide which has been used for the treatment of maize, barley and wheat.

3.2. Exposure and risk assessment

We focused the chronic exposure and risk assessment on the most frequently detected pesticides during 2006–2013. Five herbicides (acetochlor, metolachlor, pendimethalin, propachlor and trifluralin), five fungicides (chlorothalonil, cyprodinil, fenpropidin, fenpropimorph and spiroxamine) and one insecticide (lindane) were selected. Tables 3.1, 3.2, and 3.3 set the average (using the mean concentration of the

Table 2.1
Outdoor concentrations and frequency of detection of detected pesticides during previous studies (2006–2008)[a] and in this study (2009) at Oysonville site.

Pesticide	2006 ($n = 25$)[a]			2007 ($n = 12$)[a]			2008 ($n = 12$)[a]			2009 ($n = 16$)		
	Frequency of detection (%)	Range (ng m^{-3})	Average[b] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[b] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[b] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[b] (ng m^{-3})
4,4′ DDD	–	–	–	–	–	–	25	0.37–0.55	0.44	–	–	–
4,4′ DDE	–	–	–	–	–	–	17	0.27–0.30	0.28	–	–	–
Acetochlor	–	–	–	50	0.45–2.04	0.93	75	0.52–8.87	2.79	56	0.14–1.20	0.68
Aclonifen	24	0.86–4.15	1.78	–	–	–	17	0.67–1.55	1.11	–	–	–
α-Endosulfan	24	0.47–9.83	4.96	25	0.88–1.06	0.96	8	–	0.81	–	–	–
Alachlor	28	0.24–1.06	0.62	83	0.12–1.28	0.46	67	0.15–2.14	0.63	–	–	–
Azoxystrobin	–	–	–	–	–	–	25	0.66–1.79	1.21	–	–	–
Captan	–	–	–	–	–	–	–	–	–	–	–	–
Chlorothalonil	60	0.26–107.93	29.77	67	0.38–61.86	14.78	92	10.67–74.43	27.36	100	0.24–44.36	15.6
Chlorpyriphos ethyl	60	0.18–0.38	0.28	17	0.17–0.26	0.21	–	–	–	–	–	–
Cyprodinil	44	0.18–3.29	1.41	33	0.18–0.48	0.29	58	0.15–1.64	0.53	37	0.16–1.94	0.72
Diazinon	–	–	–	–	–	–	–	–	–	–	–	–
Dichlobenil	12	0.17–0.23	0.19	–	–	–	25	0.21–0.28	0.24	–	–	–
Diflufenican	–	–	–	–	–	–	–	–	–	–	–	–
Dimethenamide	–	–	–	–	–	–	–	–	–	–	–	–
Dimethomorph I	–	–	–	–	–	–	–	–	–	–	–	–
Dimethomorph II	–	–	–	–	–	–	–	–	–	–	–	–
Epoxiconazole	12	0.15–0.35	0.25	67	0.20–3.99	1.02	17	–	0.15	–	–	–
Ethofumesate	8	0.79–1.15	0.97	–	–	–	33	0.54–1.13	0.89	–	–	–
Ethoprophos	–	–	–	–	–	–	17	0.21–0.48	0.34	–	–	–
Fenpropidin	60	0.13–3.54	1.07	58	0.14–0.85	0.38	75	0.19–0.98	0.46	56	0.12–1.74	0.57
Fenpropimorph	48	0.14–13.19	2.79	50	0.16–2.86	1.02	58	0.37–7.43	2.57	37	0.35–1.84	0.87
Fluazinam	–	–	–	45	0.12–0.28	0.19	–	–	–	–	–	–
Fludioxonil	–	–	–	–	–	–	–	–	–	–	–	–
Folpet	12	7.91–10.82	9.02	–	–	–	8	–	1.1	44	1.98–6.81	3.45
γ-HCH (Lindane)	68	0.12–1.11	0.31	83	0.14–0.46	0.27	92	0.12–0.78	0.4	44	0.12–0.50	0.19
Iprodione	–	–	–	–	–	–	–	–	–	–	–	–
Malathion	–	–	–	–	–	–	92	0.12–0.78	0.4	44	0.12–0.50	0.19
Metazachlor	12	0.17–3.13	1.23	–	–	–	1	–	0.41	–	–	–
Methidathion	–	–	–	–	–	–	–	–	–	–	–	–
Metolachlor	32	0.16–0.88	0.34	58	0.15–0.75	0.42	67	0.12–1.99	0.5	37	0.15–0.78	0.6
Oxyfluorfen	–	–	–	8	–	0.76	–	–	–	–	–	–
Parathion methyl	–	–	–	–	–	–	–	–	–	–	–	–
Pendimethalin	84	0.14–117.32	10.35	100	0.18–1.74	0.63	92	0.13–3.64	1.42	94	0.18–4.99	1.41
Phosmet	–	–	–	8	–	0.25	–	–	–	–	–	–
Propachlor	20	0.15–1.66	1.29	25	0.20–0.36	0.29	58	0.14–3.48	1.22	19	0.22–0.72	0.5
Propargite	–	–	–	–	–	–	–	–	–	–	–	–
Spiroxamine	16	0.20–0.31	0.24	67	0.16–9.02	2.71	92	0.36–8.13	2.88	56	0.20–7.74	2.49
Tolylfluanid	4	–	0.1	17	0.65–0.68	0.67	–	–	–	–	–	–
Trifluralin	88	0.16–25.80	2.51	92	0.16–4.22	1.09	92	0.30–3.0	0.89	100	0.23–1.66	0.79
Vinchlozolin	16	0.14–0.19	0.16	–	–	–	–	–	–	–	–	–

(–) means not detected.

[a] Clara Coscollà, Patrice Colin, Abderrazak Yahyaoui, Olivier Petrique, Vicent Yusà, Abdelwahid Mellouki and Agustin Pastor. Occurrence of currently used pesticides in ambient air of centre region (France). Atmospheric Environment (2010) 44; 3915-3925.

[b] The average was calculated from the arithmetic mean of samples with concentration above LOD.

quantified samples) and maximum DIE for each pesticide, derived from the application of Eq. (1) (Section 2.6), for adults, children and infants. The risk assessment, calculated as HQ for the AOEL health-based reference values (HQ_a) (Eq. (2), Section 2.6) is also provided in Tables 3.1, 3.2, and 3.3 for the three groups of populations.

Five pesticides (acetochlor, trifluralin, spiroxamine, chlorothalonil and fenpropimorph) obtained the highest HQs. Fig. 3 shows the temporal evolution of HQs for these pesticides in infants (6 months–1.5 years). Chlorothalonil presented the highest levels of HQs throughout the 2006–2013 period with a maximum in 2011. HQs were similar for fenpropimorph, ranging from 2.08 E−04 to 1.49 E−03 in 2006–2011, with lower values in 2012–2013. Acetochlor HQs were constant throughout the sampling (2.65 E−05 to 3.51 E−04). Lower HQs were found for spiroxamine in 2006 and 2012. Trifluralin was detected with HQs from 1.37 E−05 to 7.86 E−04 in the 2006–2010 period.

A similar pattern was observed for children (1–6 years). Fig. SD-1 shows the temporal evolution of HQs for these pesticides for this population group. Chlorothalonil also presented the highest levels of HQs throughout the period with a maximum in 2011. HQs were also similar for fenpropimorph, ranging from 1.77 E−04 to1.27 E−03 in 2006–2011, with a lower value in the period 2012–2013. Acetochlor HQs were constant for all periods (2.00 E−04), but was not detected in year 2006. Lower HQs were also found for spiroxamine ranging from 2.42 E−06 to 5.46 E−06 in the years 2006, 2008 and 2012. Trifluralin was detected with HQs from 1.16 E−05 to 6.67 E−04 only in the period 2006–2010.

These five pesticides showed the same profile in the adult population (>12 years). Among these substances, chlorothalonil presented the highest HQ. HQs were regular for fenpropimorph in 2006–2011, with a lower value in the period 2012–2013. HQs for acetochlor were similar throughout time. Spiroxamine presented two lower peaks in 2006 and 2012 (see Fig. SD-1).

In general, HQ_a were always lower than 3.57 E−02, 8.43 E−02 and 9.93 E−02 in adults, children and infants, respectively. Chlorothalonil presented the highest levels of HQs during the 2006–2013 sampling period with a maximum in the year 2011 in the three population groups.

Table 2.2
Outdoor concentrations and frequency of detection of detected pesticides (2010 – 2013) at Oysonville site.

Pesticide	2010 (n = 13)			2011 (n = 18)			2012 (n = 18)			2013 (n = 18)		
	Frequency of detection (%)	Range (ng m^{-3})	Average[a] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[a] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[a] (ng m^{-3})	Frequency of detection (%)	Range (ng m^{-3})	Average[a] (ng m^{-3})
4,4′ DDD	–	–	–	–	–	–	–	–	–	–	–	–
4,4′ DDE	–	–	–	–	–	–	–	–	–	–	–	–
Acetochlor	23	0.32–1.40	0.85	22	0.20–0.89	0.44	33	0.12–0.67	0.38	33	0.17–1.56	0.58
Aclonifen	–	–	–	–	–	–	–	–	–	–	–	–
α-Endosulfan	–	–	–	–	–	–	–	–	–	–	–	–
Alachlor	–	–	–	–	–	–	–	–	–	–	–	–
Azoxystrobin	–	–	–	–	–	–	–	–	–	–	–	–
Captan	–	–	–	–	–	–	–	–	–	–	–	–
Chlorothalonil	92	0.38–17.59	9.8	61	5.85–1128.38	218.25	50	0.18–3.11	1.18	28	1.6–10.21	5.64
Chlorpyriphos ethyl	–	–	–	11	0.14–0.16	0.15	–	–	–	5	–	0.12
Cyprodinil	15	0.15–0.18	0.16	11	0.12–0.34	0.23	11	0.45–0.73	0.59	11	0.32–0.37	0.34
Diazinon	–	–	–	–	–	–	–	–	–	–	–	–
Dichlobenil	–	–	–	–	–	–	–	–	–	–	–	–
Diflufenican	–	–	–	–	–	–	–	–	–	–	–	–
Dimethenamide	–	–	–	–	–	–	–	–	–	–	–	–
Dimethomorph I	–	–	–	–	–	–	–	–	–	–	–	–
Dimethomorph II	–	–	–	–	–	–	–	–	–	–	–	–
Epoxiconazole	–	–	–	–	–	–	–	–	–	–	–	–
Ethofumesate	–	–	–	–	–	–	–	–	–	–	–	–
Ethoprophos	–	–	–	–	–	–	11	0.18–0.25	0.21	–	–	–
Fenpropidin	8	–	0.21	–	–	–	11	0.23–0.32	0.27	28	0.17–2.7	1.31
Fenpropimorph	61	0.22–3.62	1.47	44	0.31–1.91	0.8	17	0.15–0.32	0.22	33	0.17–0.33	0.24
Fluazinam	–	–	–	–	–	–	–	–	–	–	–	–
Fludioxonil	–	–	–	–	–	–	–	–	–	–	–	–
Folpet	–	–	–	5	–	2.17	–	–	–	–	–	–
γ-HCH (Lindane)	38	0.12–0.21	0.16	5	–	0.14	22	0.13–0.21	0.18	–	–	–
Iprodione	–	–	–	–	–	–	–	–	–	–	–	–
Malathion	–	–	–	–	–	–	–	–	–	–	–	–
Metazachlor	–	–	–	–	–	–	–	–	–	5	–	0.24
Methidathion	–	–	–	–	–	–	–	–	–	–	–	–
Metolachlor	61	0.13–0.76	0.32	28	0.14–0.35	0.2	11	0.16–0.18	0.17	33	0.19–0.44	0.31
Oxyfluorfen	–	–	–	–	–	–	–	–	–	–	–	–
Parathion methyl	–	–	–	–	–	–	–	–	–	5	–	1.39
Pendimethalin	85	0.13–2.42	0.78	33	0.16–1.45	0.64	44	0.13–0.48	0.31	39	0.46–3.98	1.64
Phosmet	–	–	–	–	–	–	–	–	–	–	–	–
Propachlor	–	–	–	–	–	–	–	–	–	–	–	–
Propargite	–	–	–	–	–	–	–	–	–	–	–	–
Spiroxamine	–	–	–	28	0.17–1.09	0.42	17	0.14–0.18	0.16	22	0.13–10.75	3.81
Tolylfluanid	–	–	–	–	–	–	–	–	–	–	–	–
Trifluralin	31	0.25–0.45	0.35	–	–	–	–	–	–	–	–	–
Vinchlozolin	–	–	–	–	–	–	–	–	–	–	–	–

(–) means not detected.
[a] The average was calculated from the arithmetic mean of samples with concentration above LOD.

According to Yusà et al. (2014) the risks for infants are greater than those for adults and children because they have a greater inhalation-to-body weight ratio. However, HQs were <1 for all pesticides for the three groups of populations at every stage of the 2006–2013 period studied, which is generally considered to be health protective (Cangialosi et al., 2008).

To date, there are few studies published on the inhalation risk assessment of CUPs in outdoor ambient air. In a study carried out in an urban community in South China, Li et al. (2014) studied the inhalation exposure of atmospheric organophosphate and pyrethroid pesticides. Similarly, they found HQs from 7.93 E – 04 to 15.4 E – 04 for these pesticides. Similar results were obtained on a risk assessment study in the population of Valencia region (Spain), in which the HQs were always lower than 1.90 E – 03, 1.55 E – 03 and 1.40 E – 02 in adults, children and infants, respectively (Yusà et al., 2014). Following the present study, all HQs were significantly smaller than 1.0.

However, in another study carried out by Luo and Zhang (2009) on risk assessment of organophosphate pesticides in the northern San Joaquin Valley in California, higher HQs were found. As an example, HQ for chlorpyrifos ethyl was 4.0 for the median of children's acute exposures. In contrast, in our study HQ for chlorpyrifos ethyl was calculated to range from 8.06 E – 06 to 2.55 E – 05 for the maximum exposure of children during 2006–2013. Chlorpyrifos ethyl is probably extensively used in California for the treatment of citrus and fruit crops but its use in arable crops of the Centre Region (France) is not as intensive. It is important to highlight, that the HQ calculated in California was using the acute exposure to children, but in the case of the Centre Region it was calculated using the maximum chronic exposure during a long period 2006–2013.

The cumulative exposure was estimated using the RPF approach for organophosphate pesticides and the hazard index for chloroacetamides. Organophosphates are a group of pesticides with a common mechanism based on the ability of inhibiting acetylcholinesterase by phosphorylation. And chloroacetamide pesticides are another group of pesticides which produce tumors on rat nasal olfactory epithelium via their cytotoxic action (EFSA, 2013).

Organophosphates presented cumulative risk with ranges from 3.28 E – 10 to 1.10 E – 06 mg/kg/day in infants, from 2.78 E – 10 to 9.30 E – 07 mg/kg/day in children and from 1.18 E – 10 to 3.94 E – 07 mg/kg/day in adults. Comparing these expositions with chlorpyrifos ethyl

Table 3.1
Daily inhalation exposure (mg/kg/day) for average and maximum concentrations and Hazard Quotient (HQ) for the highest detected pesticides in adults.

Pesticides	DIE[1]								HQ_{AOEL}							
	2006	2007	2008	2009	2010	2011	2012	2013	2006	2007	2008	2009	2010	2011	2012	2013
Acetochlor																
Average	2.36E−07	2.67E−07	7.94E−07	1.94E−07	2.39E−07	1.28E−07	1.08E−07	1.65E−07	1.18E−05	1.34E−05	3.97E−05	9.68E−06	1.20E−05	6.40E−06	5.41E−06	8.25E−06
Maximum	7.94E−07	5.81E−07	2.52E−06	3.41E−07	3.98E−07	2.53E−07	1.91E−07	4.44E−07	3.97E−05	2.90E−05	1.26E−04	1.71E−05	1.99E−05	1.27E−05	9.53E−06	2.22E−05
Chlorothalonil																
Average	8.47E−06	4.21E−06	7.79E−06	4.44E−06	2.79E−06	6.21E−05	3.39E−07	1.60E−06	9.42E−04	4.68E−04	8.65E−04	4.93E−04	3.10E−04	6.90E−03	3.76E−05	1.78E−04
Maximum	3.07E−05	1.76E−05	2.12E−05	1.26E−05	5.01E−06	3.21E−04	8.85E−07	2.91E−06	3.41E−03	1.96E−03	2.35E−03	1.40E−03	5.56E−04	3.57E−02	9.83E−05	3.23E−04
Cyprodinil																
Average	4.01E−07	8.25E−08	1.51E−07	2.05E−07	4.84E−08	2.18E−06	1.68E−07	9.96E−08	1.34E−05	2.75E−06	5.03E−06	6.83E−06	1.61E−06	2.18E−06	5.60E−06	3.32E−06
Maximum	9.36E−07	1.37E−07	4.66E−07	5.52E−07	5.12E−08	3.23E−06	2.08E−07	1.05E−07	3.12E−05	4.55E−06	1.55E−05	1.84E−05	1.71E−06	3.23E−06	6.92E−06	3.51E−06
Fenpropidin																
Average	3.33E−07	1.08E−07	1.31E−07	1.62E−07	5.99E−08	–	7.97E−08	3.73E−07	1.66E−05	5.41E−06	6.53E−06	8.09E−06	3.00E−06	–	3.98E−06	1.86E−05
Maximum	1.01E−06	2.42E−07	2.79E−07	4.95E−07	5.98E−08	–	9.11E−08	7.68E−07	5.04E−05	1.21E−05	1.39E−05	2.48E−05	2.99E−06	–	4.55E−06	3.84E−05
Fenpropimorph																
Average	7.94E−07	2.90E−07	7.31E−07	2.48E−07	4.18E−07	2.28E−07	6.26E−08	7.11E−08	1.13E−04	4.15E−05	1.04E−04	3.54E−05	5.98E−05	3.25E−05	8.94E−06	1.02E−05
Maximum	3.76E−06	8.14E−07	2.11E−06	5.24E−07	1.03E−06	5.44E−07	9.11E−08	9.39E−08	5.36E−04	1.16E−04	3.02E−04	7.48E−05	1.47E−04	7.76E−05	1.30E−05	1.34E−05
Lindane																
Average	8.82E−08	7.68E−08	1.14E−07	5.41E−08	4.84E−08	3.98E−08	1.02E−05	–	1.76E−05	1.54E−05	2.28E−05	1.08E−05	9.68E−06	7.97E−06	1.02E−05	–
Maximum	3.13E−07	1.31E−07	2.22E−07	1.42E−07	5.98E−08	3.98E−08	1.20E−05	–	6.26E−05	2.62E−05	4.44E−05	2.85E−05	1.20E−05	7.97E−06	1.20E−05	–
Metolachlor																
Average	9.68E−08	1.20E−07	1.45E−07	1.71E−07	9.11E−08	5.69E−08	4.84E−08	8.82E−08	6.45E−07	7.97E−07	9.68E−07	1.14E−06	6.07E−07	3.79E−07	3.23E−07	5.88E−07
Maximum	2.50E−07	2.13E−07	5.66E−07	2.21E−07	2.16E−07	9.96E−08	5.12E−08	1.25E−07	1.67E−06	1.42E−06	3.78E−06	1.47E−06	1.44E−06	6.64E−07	3.41E−07	8.35E−07
Trifluralin																
Average	7.14E−07	3.10E−07	2.53E−07	2.25E−07	9.96E−08	–	–	–	2.75E−05	1.19E−05	9.74E−06	8.65E−06	3.83E−06	–	–	–
Maximum	7.34E−06	1.20E−06	8.54E−07	4.72E−07	1.28E−07	–	–	–	2.82E−04	4.62E−05	3.28E−05	1.82E−05	4.93E−06	–	–	–
Spiroxamine																
Average	6.83E−08	7.71E−07	8.20E−07	7.09E−07	–	1.20E−07	4.27E−08	1.08E−06	1.37E−06	1.54E−05	1.64E−05	1.42E−05	–	2.39E−06	8.54E−07	2.17E−05
Maximum	8.54E−08	2.57E−06	2.31E−06	2.20E−06	–	3.10E−07	5.12E−08	3.06E−06	1.71E−06	5.13E−05	4.63E−05	4.41E−05	–	6.20E−06	1.02E−06	6.12E−05
Pendimethalin																
Average	2.95E−06	1.79E−07	4.04E−07	4.04E−07	2.22E−07	1.82E−07	8.82E−08	4.67E−07	1.26E−05	7.66E−07	1.73E−06	1.73E−06	9.49E−07	7.78E−07	3.77E−07	1.99E−06
Maximum	3.34E−05	4.97E−07	1.04E−06	1.42E−06	6.89E−07	4.13E−07	1.37E−07	1.13E−06	1.43E−04	2.12E−06	4.43E−06	6.07E−06	2.94E−06	1.76E−06	5.84E−07	4.84E−06

[1] USEPA, 1989, USEPA, 2004, USEPA, 1991 (DIE: Daily Inhalation Exposure; body weight = 70 kg, inhalation rate = 20 m 3/day, exposure duration = 24 h).

Table 3.2
Daily inhalation exposure (mg/kg/day) for average and maximum concentrations and Hazard Quotient (HQ) for the highest detected pesticides in children.

Pesticides	DIE[a]								HQ_{AOEL}							
	2006	2007	2008	2009	2010	2011	2012	2013	2006	2007	2008	2009	2010	2011	2012	2013
Acetochlor																
Average	–	6.32E−07	1.87E−06	4.57E−07	5.64E−07	3.56E−07	2.55E−07	3.90E−07	–	3.16E−05	9.37E−05	2.28E−05	2.82E−05	1.51E−05	1.28E−05	1.95E−05
Maximum	–	1.37E−06	5.96E−06	8.06E−07	9.41E−07	7.05E−07	4.50E−07	1.05E−06	–	2.00E−04	2.00E−04	2.00E−04	2.00E−04	2.00E−04	2.00E−04	2.00E−04
Chlorothalonil																
Average	2.00E−05	9.94E−06	1.84E−05	1.05E−05	6.60E−06	1.47E−04	8.00E−07	3.79E−06	2.22E−03	1.10E−03	2.04E−03	1.16E−03	7.33E−04	1.63E−02	8.89E−05	4.21E−04
Maximum	7.25E−05	4.16E−05	5.00E−05	2.98E−05	1.18E−05	7.58E−04	2.09E−06	6.86E−06	8.06E−03	4.62E−03	5.56E−03	3.31E−03	1.31E−03	8.43E−02	2.32E−04	7.62E−04
Cyprodinil																
Average	9.48E−07	1.95E−07	3.56E−07	4.84E−07	1.14E−07	1.55E−07	3.96E−07	2.35E−07	3.16E−05	6.50E−06	1.19E−05	1.61E−05	3.81E−06	5.15E−06	1.32E−05	7.84E−06
Maximum	2.21E−06	3.23E−07	1.10E−06	1.30E−06	1.21E−07	2.28E−07	4.91E−07	2.49E−07	7.36E−05	1.08E−05	3.67E−05	4.35E−05	4.03E−06	7.62E−06	1.64E−05	8.29E−06
Fenpropidin																
Average	7.86E−07	2.55E−07	3.09E−07	3.83E−07	1.41E−07	–	1.88E−07	8.80E−07	3.93E−05	1.28E−05	1.55E−05	1.92E−05	7.06E−06	–	9.41E−06	4.40E−05
Maximum	2.38E−06	5.71E−07	6.59E−07	1.17E−06	1.41E−07	–	2.15E−07	1.81E−06	1.19E−04	2.86E−05	3.29E−05	5.85E−05	7.06E−06	–	1.08E−05	9.07E−05
Fenpropimorph																
Average	1.87E−06	6.85E−07	1.73E−06	5.85E−07	9.88E−07	5.38E−07	1.48E−07	1.68E−07	2.68E−04	9.79E−05	2.47E−04	8.35E−05	1.41E−04	7.68E−05	2.11E−05	2.40E−05
Maximum	8.87E−06	1.92E−06	4.99E−06	1.24E−06	2.43E−06	1.28E−06	2.15E−07	2.15E−07	1.27E−03	2.75E−04	7.13E−04	1.77E−04	3.48E−04	1.83E−04	3.07E−05	3.07E−05
Lindane																
Average	2.08E−07	1.81E−07	2.69E−07	1.28E−07	1.14E−07	9.41E−08	1.21E−07	–	4.17E−05	3.63E−05	5.38E−05	2.55E−05	2.28E−05	1.88E−05	2.42E−05	–
Maximum	7.39E−07	3.09E−07	5.24E−07	3.36E−07	1.41E−07	9.41E−08	1.41E−07	–	1.48E−04	6.18E−05	1.05E−04	6.72E−05	2.82E−05	1.88E−05	2.82E−05	–
Metolachlor																
Average	2.28E−07	2.82E−07	3.43E−07	4.03E−07	2.15E−07	1.34E−07	1.14E−07	2.08E−07	1.52E−06	1.88E−06	2.28E−06	2.69E−06	1.43E−06	8.96E−07	7.62E−07	1.39E−06
Maximum	5.90E−07	5.03E−07	1.34E−06	5.22E−07	5.11E−07	2.35E−07	1.21E−07	2.96E−07	3.93E−06	3.35E−06	8.92E−06	3.48E−06	3.40E−06	1.57E−06	8.06E−07	1.97E−06
Trifluralin																
Average	1.69E−06	7.32E−07	5.98E−07	5.31E−07	2.35E−07	–	–	–	6.49E−05	2.82E−05	2.30E−05	2.04E−05	9.05E−06	–	–	–
Maximum	1.73E−05	2.84E−06	2.02E−06	1.12E−06	3.02E−07	–	–	–	6.67E−04	1.09E−04	7.75E−05	4.29E−05	1.16E−05	–	–	–
Spiroxamine																
Average	1.61E−07	1.82E−06	1.94E−06	1.67E−06	–	2.82E−07	1.01E−07	2.56E−06	3.23E−06	3.64E−05	1.94E−06	3.35E−05	–	5.64E−06	2.02E−06	5.12E−05
Maximum	2.02E−07	6.06E−06	5.46E−06	5.20E−06	–	7.32E−07	1.21E−07	7.22E−06	4.03E−06	1.21E−04	5.46E−06	1.04E−04	–	1.46E−05	2.42E−06	1.44E−04
Pendimethalin																
Average	6.96E−06	4.23E−07	9.54E−07	9.54E−07	5.24E−07	1.84E−06	2.08E−07	1.10E−06	2.97E−05	1.81E−06	4.08E−06	4.08E−06	2.24E−06	1.84E−06	8.90E−07	4.71E−06
Maximum	7.88E−05	1.17E−06	2.45E−06	3.35E−06	1.63E−06	4.16E−06	3.23E−07	2.67E−06	3.37E−04	5.02E−06	1.05E−05	1.43E−05	6.95E−06	4.16E−06	1.38E−06	1.14E−05

[a] USEPA, 2004, USEPA, 1991 (DIE: Daily Inhalation Exposure; body weight = 15 kg, inhalation rate = 10 m3/day, Exposure duration = 24 h).

C. Coscollà et al. / Science of the Total Environment 584–585 (2017) 856–868

Table 3.3
Daily inhalation exposure (mg/kg/day) for average and maximum concentrations and Hazard Quotient (HQ) for the highest detected pesticides in infants.

Pesticides	DIE[a]								HQ_{AOEL}							
	2006	2007	2008	2009	2010	2011	2012	2013	2006	2007	2008	2009	2010	2011	2012	2013
Acetochlor																
Average	6.57E−07	7.44E−07	2.21E−06	5.39E−07	6.65E−07	5.07E−07	3.01E−07	4.59E−07	3.29E−05	3.72E−05	1.10E−04	2.69E−05	3.33E−05	1.78E−05	1.50E−05	2.30E−05
Maximum	2.21E−06	1.62E−06	7.03E−06	9.50E−07	1.11E−06	1.15E−06	5.31E−07	1.24E−06	1.10E−04	8.08E−05	3.51E−04	4.75E−05	5.54E−05	3.52E−05	2.65E−05	6.18E−05
Chlorothalonil																
Average	2.36E−05	1.17E−05	2.17E−05	1.24E−05	7.78E−06	1.73E−04	9.42E−07	4.47E−06	2.62E−03	1.30E−03	2.41E−03	1.37E−03	8.64E−04	1.92E−02	1.05E−04	4.96E−04
Maximum	8.55E−05	4.90E−05	5.89E−05	3.51E−05	1.39E−05	8.94E−04	2.46E−06	8.09E−06	9.50E−03	5.44E−03	6.55E−03	3.90E−03	1.55E−03	9.93E−02	2.74E−04	8.98E−04
Cyprodinil																
Average	1.12E−06	2.30E−07	4.20E−07	5.70E−07	1.35E−07	1.82E−07	4.67E−07	2.77E−07	3.72E−05	7.66E−06	1.40E−05	1.90E−05	4.49E−06	6.07E−06	1.56E−05	9.24E−06
Maximum	2.60E−06	3.80E−07	1.30E−06	1.54E−06	1.43E−07	2.69E−07	5.78E−07	2.93E−07	8.68E−05	1.27E−05	4.32E−05	5.12E−05	4.75E−06	8.98E−06	1.93E−05	9.77E−06
Fenpropidin																
Average	9.27E−07	3.01E−07	3.64E−07	4.51E−07	1.66E−07	–	2.22E−07	1.04E−06	4.63E−05	1.50E−05	1.82E−05	2.26E−05	8.32E−06	–	1.11E−05	5.19E−05
Maximum	2.80E−06	6.73E−07	7.76E−07	1.38E−06	1.66E−07	–	2.53E−07	2.14E−06	1.40E−04	3.37E−05	3.88E−05	6.89E−05	8.32E−06	–	1.27E−05	1.07E−04
Fenpropimorph																
Average	2.21E−06	8.08E−07	2.04E−06	6.89E−07	1.16E−06	6.34E−07	1.74E−07	1.98E−07	3.16E−04	1.15E−04	2.91E−04	9.84E−05	1.66E−04	9.05E−05	2.49E−05	2.83E−05
Maximum	1.05E−05	2.27E−06	5.88E−06	1.46E−06	2.87E−06	1.51E−06	2.53E−07	2.53E−07	1.49E−03	3.24E−04	8.41E−04	2.08E−04	4.10E−04	2.16E−04	3.62E−05	3.62E−05
Lindane																
Average	2.46E−07	2.14E−07	3.17E−07	1.50E−07	1.35E−07	1.11E−07	1.43E−07	–	4.91E−05	4.28E−05	6.34E−05	3.01E−05	2.69E−05	2.22E−05	2.85E−05	–
Maximum	8.71E−07	3.64E−07	6.18E−07	3.96E−07	1.66E−07	1.11E−07	1.66E−07	–	1.74E−04	7.29E−05	1.24E−04	7.92E−05	3.33E−05	2.22E−05	3.33E−05	–
Metolachlor																
Average	2.69E−07	3.33E−07	4.04E−07	4.75E−07	2.53E−07	1.58E−07	1.35E−07	2.46E−07	1.80E−06	2.22E−06	2.69E−06	3.17E−06	1.69E−06	1.06E−06	8.98E−07	1.64E−06
Maximum	6.95E−07	5.93E−07	1.58E−06	6.15E−07	6.02E−07	2.77E−07	1.43E−07	3.48E−07	4.64E−06	3.95E−06	1.05E−05	4.10E−06	4.01E−06	1.85E−06	9.50E−07	2.32E−06
Trifluralin																
Average	1.99E−06	8.63E−07	7.05E−07	6.26E−07	2.77E−07	–	–	–	7.65E−05	3.32E−05	2.71E−05	2.41E−05	1.07E−05	–	–	–
Maximum	2.04E−05	3.34E−06	2.38E−06	1.31E−06	3.56E−07	–	–	–	7.86E−04	1.29E−04	9.14E−05	5.06E−05	1.37E−05	–	–	–
Spiroxamine																
Average	1.90E−07	2.15E−06	2.28E−06	1.97E−06	–	3.33E−07	1.19E−07	3.02E−06	3.80E−06	4.29E−05	4.56E−05	3.94E−05	–	6.65E−06	2.38E−06	6.04E−05
Maximum	2.38E−07	7.14E−06	6.44E−06	6.13E−06	–	8.63E−07	1.43E−07	8.51E−06	4.75E−06	1.43E−04	1.29E−04	1.23E−04	–	1.73E−05	2.85E−06	1.70E−04
Pendimethalin																
Average	8.20E−06	4.99E−07	1.12E−06	1.12E−06	6.18E−07	5.07E−07	2.46E−07	1.30E−06	3.50E−05	2.13E−06	4.81E−06	4.81E−06	2.64E−06	2.17E−06	1.05E−06	5.55E−06
Maximum	9.29E−05	1.38E−06	2.88E−06	3.95E−06	1.92E−06	1.15E−06	3.80E−07	3.15E−06	3.97E−04	5.91E−06	1.23E−05	1.69E−05	8.19E−06	4.91E−06	1.62E−06	1.35E−05

[1] USEPA 1996 (DIE: Daily Inhalation Exposure; body weight = 10 kg, inhalation rate = 8 m3/day, Exposure duration = 24 h).

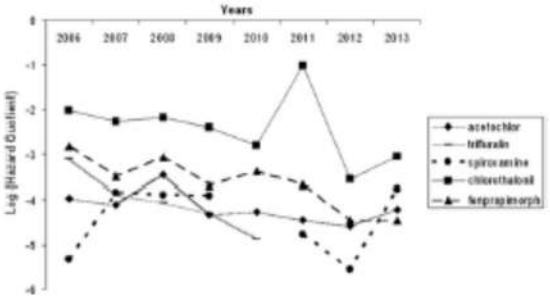


Fig. 3. Temporal evolution of Hazard Quotient (HQ) for infant population.

NOAEL (0.1 mg/kg/day), the cumulative risk from inhalation exposure of organophosphates is considered to be acceptable (see Table SD-5).

Chloroacetamide pesticides presented the highest HI for the three groups of population in 2008. During this year the HI obtained was 3.53 E−04 for infants, 2.99 E−04 for children and 1.27 E−04 for adults (see Fig. SD-2). Regarding chloroacetamide pesticides (alachlor, metazachlor, propachlor and acetochlor), the HIs were lower than 3.53 E−04 for the three groups during all the sampling period. When the HI is <1, the cumulative risk from exposure to the compounds is considered to be acceptable.

Lifetime cancer risk for infants (using a worst-case scenario PF of 10^{-1} (mg/kg day^{-1}) ranged from 1.42 E−08 for metolachlor to 8.93 E−05 for chlorothalonil. The concern about cancer risk often occurs when the estimated risk reaches 1.0 E−06 (1/1,000,000) excess lifetime cancer risk. Lifetime cancer risks that reached or exceeded 1.0 E−06 of the exposed population include chlorothalonil, and trifluralin and pendimethalin, in 2006. Chlorothalonil is listed by the U.S. EPA as likely to be carcinogen to humans, while trifluralin and pendimethalin are possible human carcinogens (Tables 1 and SD-6).

The Department of Pesticide Regulation in California has developed health screening levels for the monitored pesticides to place the results in a health-based context (AMN, 2012). Health screening levels were calculated in air concentrations based on a chemical's toxicity that was used to evaluate the possible health effects of exposure to the chemical.

The present risk assessment only considered human inhalation exposures in outdoor ambient air. However, dermal (Xue et al., 2007), and especially ingestion are other routes of exposure. Exposure to pesticides residue through the diet is assumed to be between two and five orders to magnitude higher than air exposure (Luo and Zhang, 2009; Juraske et al., 2009). So, in order to deal with safe screening levels of CUPs in the atmosphere (pg m^{-3}) these multiple-exposure pathways need to be considered.

Table 4 shows the Health Based Levels (HBLs) for the five pesticides with the highest HQs (acetochlor, trifluralin, spiroxamine, chlorothalonil and fenpropimorph) in infants. HBLs was calculated as air concentrations assuming an HQ = 1. A measured air concentration below the HBL would not be considered a significant health concern and would generally not undergo further evaluation, but should also not automatically be considered "safe" and could undergo further evaluation. A measured concentration that is above the HBL would not necessarily indicate a significant health concern, but would indicate the need for a further, more refined evaluation. Recommended Maximum Levels (RMLs) were calculated as 10% of the HBL and could be considered as a safer value. In the infant population, HBLs ranged from 8.83 E+03 to 6.30 E+04 ng m^{-3}, and RMLs ranged from 8.83 E+02 to 6.30 E+03 ng m^{-3} for fenpropimorph and spiroxamine, respectively.

However, HBLs and RMLs are not regulatory standards. In the near future it will be very important to establish health reference levels for pesticides in air. These reference values should be based on previous risk assessment studies. These findings suggest that more efforts

Table 4
Health Based Levels (HBL) (ng m^{-3}) and Recommended Maximum Levels (RML) (ng m^{-3}) for some detected pesticides in infants[a].

Pesticides	Health Based Level (HBL) (ng m^{-3})	Recommended Maximum level (RML) (ng m^{-3})
Acetochlor	2.50E+04	2.50E+03
Trifluralin	3.30E+04	3.30E+03
Spiroxamine	6.30E+04	6.30E+03
Chlorothalonil	1.10E+04	1.10E+03
Fenpropimorph	8.83E+03	8.83E+02

[a] Theoretical values using Eqs. (1) and (2) (USEPA, 1997;WHO, 1999; EU pesticide database).

are required to implement an extensive air monitoring network in Europe for pesticide control and to develop regulations or recommendations regarding pesticide levels in outdoor ambient air.

4. Conclusions

Pesticide exposure in adults, children and infants was studied following pesticide application in an agricultural community in the Centre Region (France).

The pesticides most frequently detected during the application period, 2006–2013, were herbicides such as trifluralin (0.16–25.8 ng m^{-3}) and pendimethalin (0.13–117.32 ng m^{-3}); fungicides like chlorothalonil (0.18–1128.38 ng m^{-3}); insecticides such as lindane (0.12–1.11 ng m^{-3}) and α-endosulfan (0.47–9.83 ng m^{-3}).

Estimated DIE in children ranged from 9.41 E−08 mg kg^{-1}/day^{-1} for lindane to 7.58 E−04 mg/kg/day^{-1} for chlorothalonil, when maximum concentrations were applied. HQ_{AOEL} were always lower than 3.57 E−02, 8.43 E−02 and 9.93 E−02 in adults, children and infants, respectively, assuming that this work was an outdoor sampling project.

Using the maximum concentration for the detected organophosphate pesticides, the cumulative exposure were lower than 3.94 E−07, 9.30 E−07 and 1.10 E−06 for adults, children and infants, respectively. Regarding chloroacetamide pesticides, the HIs were lower than 3.53 E−04 for the three groups during all the sampling period.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.01.132.

References

AFNOR (Association Française de Normalisation), 2007. Dosage des substances phytosanitaires (pesticides) dans l'air ambient. Prélèvement actif. XP X 43-058 (La Plaine Saint-Denis, France, 17 pp. www.afnor.org).
AMN, 2012. In: Vidrio, E., Wofford, P., Segawa, R., Schreider (Eds.), Air Monitoring Network Results for 2011, California.
Aulagnier, F., Poissant, L., Brunet, D., Beauvais, C., Pilote, M., Deblois, C., Dassylva, N., 2008. Pesticides measured in air and precipitation in the Yamaska Basin (Québec): occurrence and concentrations in 2004. Sci. Total Environ. 394, 338–348.
Bedos, C., Cellier, P., Calvet, R., Barriuso, E., Gabrielle, B., 2002. Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: over-view. Agronomie 22, 21-33.
Bordas, E., Sanchez, P., Muñoz, A., Tortajada-Genaro, L.A., 2011. Development of a gas chromatography-mass spectrometry method for the determination of pesticides in gaseous and particulate phases in the atmosphere. Anal. Chim. Acta 699, 57–65.
Cangialosi, F., Intini, G., Liberti, L., Notarnicola, M., Stellacci, P., 2008. Health risk assessment of air emissions from a municipal solid waste incineration plant e a case study. Waste Manag. 28, 885–895.
Castorina, R., Bradman, A., McKone, T.E., Barr, D.B., Harnly, M.E., Eskenazi, B., 2003. Assessing cumulative organophosphate pesticide exposure and risk among pregnant women living in an agricultural community. Environ. Health Perspect. 111 (13), 1640–1648.
Coscollà, C., Colin, P., Yahyaoui, A., Petrique, O., Yusà, V., Mellouki, A., Pastor, A., 2010. Occurrence of currently used pesticides in ambient air of Centre Region (France). Atmos. Environ. 44, 3915–3925.
Coscollà, C., Hart, E., Pastor, A., Yusà, V., 2013. LC-MS characterization of contemporary pesticides in PM10 of Valencia Region, Spain. Atmos. Environ. 77, 394–403.

Cascollà, C., León, N., Pastor, A., Yusà, V., 2014. Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using liquid chromatography-Orbitrap high resolution mass spectrometry. J. Chromatogr. A 1368, 132–142.
ECPA (European Crop Protection Association), 2010. Industry Statistics 2001–2010. http://www.ecpa.eu/page/industry-statistics.
EFSA (European Food Safety Authority), 2006. Guidance of the scientific committee on a request from EFSA related to uncertainties in dietary exposure assessment. EFSA J. 438, 1–54.
EFSA (European Food Safety Authority), 2009. Conclusion on the peer review of trifluralin. Sci. Rep. 327, 1–111.
EFSA (European Food Safety Authority), 2013. Scientific opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA J. 11 (7), 3293.
EFSA (European Food Safety Authority), 2014a. Scientific opinion on preparation of a guidance document on pesticide exposure assessment for workers, operators, bystanders and residents. EFSA J. 8 (2), 1501 (2010).
EFSA (European Food Safety Authority), 2014b. Conclusion on the peer review of chlorpyrifos. EFSA J. 12 (4), 3640 (2014).
EU, 2014. EU Pesticides Database. (Retrieved Jun 2, 2014, from: http://ec.europa.eu/sanco_pesticides/public/?event=homepage).
Foreman, W.T., Majewski, M.S., Goolsby, D.A., Wiebe, F.W., Coupe, R.H., 2000. Pesticides in the atmosphere of the Mississippi River Valley, part II e air. Sci. Total Environ. 248, 213–216.
FRDEC, 2004. Fédération Regionale de Défense contre les ennemies des cultures.
Gunier, R., Harnly, M., Reynolds, P., Hertz, A., Von Behren, J., 2001. Agricultural pesticide use in California: pesticide prioritization, use densities, and population distributions for a Childhood Cancer Study. Environ. Health Perspect. 109, 1071–1078.
Hart, E., Coscollà, C., Pastor, A., Yusà, V., 2012. GC–MS characterization of contemporary pesticides in PM10 of Valencia Region, Spain. Atmos. Environ. 62, 118–129.
Juraske, R., Mutel, C.L., Stoessel, F., Hellweg, S., 2009. Life cycle human toxicity assessment of pesticides: comparing fruit and vegetable diets in Switzerland and the United States. Chemosphere 77, 939–945.
Lee, S., McLaughlin, R., Harnly, M., Gunier, R., Kreutzer, R., 2002. Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks. Environ. Health Perspect. 110, 1175–1184.
Li, H., Ma, H., Lydy, M.J., You, J., 2014. Occurrence, seasonal variation and inhalation exposure of atmospheric organophosphate and pyrethroid pesticides in an urban community in South China. Chemosphere 95, 363–369.
Lig'Air, 2005. Contamination de l'air par les produits phytosanitaires en région Centre. Rapport Final. Lig'Air-Réseau de surveillance de la qualité de l'air en région Centre, Orléans. http://www.ligair.fr.
London, L., Beseler, C., Bouchard, M.F., Bellinger, D.C., Colosio, C., Grandjean, P., Harari, R., Kootbodien, T., Kromhout, H., Little, F., Meijster, T., Moretto, A., Rohlman, D.S., Stallones, L., 2012. Neurobehavioral and neurodevelopmental effects of pesticide exposures. Neurotoxicology 33 (4), 887–896.
Luo, Y., Zhang, M., 2009. Multimedia transport and risk assessment of organophosphate pesticides and a case study in the northern San Joaquin Valley of California. Chemosphere 75, 969–978.
Mahmood, A., Malik, R.N., Li, J., Zhang, G., 2014. Human health risk assessment and dietary intake of organochlorine pesticides through air, soil and food crops (wheat and rice) along two tributaries of river Chenab, Pakistan. Food Chem. Toxicol. 71, 17–25.
Marks, A.R., Harley, K., Bradman, A., Kogut, K., Barr, D.B., Johnson, C., Calderon, N., Eskenazi, B., 2010. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ. Health Perspect. 118 (12), 1768–1774.
Ministere de l'Écologie, du Developpement durable et de l'énergie, 2014é. L'environment en France. 2014 ed. (www.statistiques.developpement-durable.gouv.fr, visited November 2016).
Moussaoui, Y., Tuduri, L., Kerchich, Y., Meklati, B.Y., Eppe, G., 2012. Atmospheric concentrations of PCDD/Fs, dl-PCBs and some pesticides in northern Algeria using passive air sampling. Chemosphere 88, 270–277.
Peck, A.M., Hornbuckle, K.C., 2005. Gas-phase concentrations of current-use pesticides in Iowa. Environ. Sci. Technol. 39, 2952–2959.
Raeppel, C., Fabritius, M., Nief, M., Appenzeller, B.M., Millet, M., 2014. Coupling ASE, silylation and SPME-GC/MS for the analysis of current-used pesticides in atmosphere. Talanta 121, 24–29.
Sarigiannis, D.A., Kontoroupis, P., Solomou, E.S., Nikolaki, S., Karabelas, A.J., 2013. Inventory of pesticide emissions into the air in Europe. Atmos. Environ. 75, 6–14.
Scheyer, A., Morville, S., Mirabel, P., Millet, M., 2007. Variability of atmospheric pesticide concentrations between urban and rural areas during intensive pesticide application. Atmos. Environ. 41 (17), 3604–3618.
Schummer, C., Mothiron, E., Appenzeller, B.M.R., Rinet, A., Wennig, R., Millet, M., 2010. Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France. Environ. Pollut. 158, 576–584.
USEPA, 1989. Risk Assessment Guidance for Superfund (RAGS) (Part A) (EPA/540/1-89/002).
USEPA, 1991. RAGS Volume I: Human Health Evaluation Manual. Supplemental Guidance "Standard Default Exposure Factors" (OSWER No. 9285.6-03).
USEPA, 1997. Exposure Factors Handbook. U.S. Environmental Protection Agency (EPA/600/P-95/002/Fa).
USEPA, 2004. Region 9 Preliminary Remediation Goals. Memorandum From Standford Smucker, PhD., Regional Toxicologist. (http://www.epa.gov/region09/waste/sfund/prg/index.html).
USEPA, 2013. Chemicals Evaluated for Carcinogenic Potential (Office of Pesticide Programs).
Waite, D.T., Bailey, P., Sproull, J.F., Quiring, D.V., Chau, D.F., Bailey, J., Cessna, A.J., 2005. Atmospheric concentrations and dry and wet deposits of some herbicides currently used on the Canadian Prairies. Chemosphere 58, 693–703.
WHO (World Health Organization), 1999. Principle for the assessment of risks to human health from exposure to chemicals. Environ. Health Criteria 210.
Xue, J., Zartaria, V., Moya, J., Freeman, N., Beamer, P., Black, K., Tulve, N., Shalat, S., 2007. A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. Risk Anal. 27 (2), 411–420.
Yao, Y., Tuduri, L., Harner, T., Blanchard, P., Waite, D., Poissant, L., Murphy, C., Belzer, W., Aulagnier, F., Li, Y.F., Sverko, E., 2006. Spatial and temporal distribution of pesticide air concentrations in Canadian agricultural regions. Atmos. Environ. 40, 4339–4351.
Yusà, V., Coscollà, C., Millet, M., 2014. New screening approach for risk assessment of pesticides in ambient air. Atmos. Environ. 96, 322–330.

V. CONCLUSIONES

11. CONCLUSIONES FINALES

Las principales conclusiones a las cuáles se han llegado después de la realización de los seis estudios descritos en esta Tesis Doctoral son las siguientes:

- El enorme potencial que presenta el uso de la alta resolución (HRMS), gracias a su alta sensibilidad y su elevado poder de resolución (50000 FWHM), permite el desarrollo de nuevas metodologías analíticas adecuadas para el análisis de plaguicidas y sus metabolitos en la atmósfera, tanto en la fase particulada (filtro PM10) como en la fase gaseosa (adsorbente PUF-XAD2-PUF). Además, el empleo de la HRMS permite desarrollar métodos target, post-target y non-target para los plaguicidas y metabolitos estudiados, dependiendo del conocimiento previo de estos compuestos (compuestos prioritarios previamente definidos, compuestos conocidos no prioritarios o compuestos totalmente desconocidos).

- Los estudios de evaluación del riesgo debido a la inhalación de plaguicidas realizados en diferentes grupos de población nos han indicado que, en general, los niveles inhalados por la población estudiada en la Comunidad Valenciana y en Francia no son peligrosos para la salud de las personas. Estos estudios de evaluación realizados son un primer paso para el establecimiento a nivel europeo de un umbral límite de concentración de plaguicidas detectados en la atmósfera y un valor de referencia basado en salud.

Por otra parte, las conclusiones de cada capítulo ya han sido descritas previamente y se encuentran detalladas seguidamente:

Capítulo 1:

-La aplicación del análisis retrospectivo para la determinación de metabolitos de plaguicidas en la atmósfera empleando la espectrometría de masas de alta resolución ha resultado ser muy adecuada, puesto que tras la creación de una base de datos de 250 metabolitos de plaguicidas, 34 de ellos fueron detectados en alguna de las 31 muestras analizadas. Además, once de ellos fueron confirmados mediante el uso de patrones.

- Por otro lado, el empleo de la estrategia de fragmentación-degradación para el análisis de compuestos desconocidos permitió la detección de dos productos de transformación procedentes del malaoxon y de la fenhexamida.

Capítulo 2:

- Se ha desarrollado un método de determinación de la fase particulada 35 de los plaguicidas más empleados habitualmente mediante una etapa de extracción mediante una cafetera, y una posterior etapa de preconcentración mediante salting out, optimizándose para obtener unas condiciones de extracción y preconcentración adecuadas. Este método desarrollado se trata de un método rápido, de bajo coste y sostenible con el medio ambiente debido al poco volumen de disolvente empleado.

-Los límites de cuantificación obtenidos son de 6.5 pg m^{-3} para la mayoría de los plaguicidas estudiados, obteniéndose recuperaciones del método entre 70 % y 129 % en los niveles de fortificación estudiados (entre 5 y 100 ng).

-Además, se ha empleado la metodología de análisis retrospectivo descrita en el capítulo 1 para la determinación de metabolitos de plaguicidas.

Capítulo 3:

- Para la determinación de la fase gaseosa de los plaguicidas se han evaluado tanto el muestreo, la etapa de extracción y la etapa de análisis para los adsorbentes empleados de forma habitual en la captación de los plaguicidas. El sándwich PUF-XAD2-PUF fue seleccionado como el adsorbente más adecuado para el muestreo de la fase gaseosa de 34 plaguicidas (incluyendo persistentes y de uso habitual en la agricultura).

-Los límites de cuantificación obtenidos oscilan entre 16.1 y 322.6 pg m^{-3}, obteniéndose recuperaciones del método entre 75 % y 110 % en los niveles de fortificación estudiados (entre 2.5 y 50 ng).

- La metodología desarrollada puede ser empleada tanto para ambientes exteriores como para ambientes interiores. Así, se analizaron 10 muestras procedentes de viviendas de la Comunidad Valenciana donde seis plaguicidas fueron detectados en al menos una muestra, con concentraciones entre 1.46 y 22.02 ng m^{-3}. En un futuro próximo, esta metodología podrá ser aplicada para el control de los niveles de plaguicidas tanto en ambientes interiores como en la atmósfera.

Capítulo 4:

- El sándwich PUF-XAD2-PUF fue seleccionado como el adsorbente más adecuado para el muestreo de la fase gaseosa de 28 plaguicidas polares. La estrategia analítica ha incluido una extracción mediante MAE y una posterior determinación empleando UHPLC-HRMS, con LOQ que variaban desde 32.2 hasta 129.0 pg m^{-3}. Estos valores son adecuados para su aplicación en programas de vigilancia y control.

- Empleando la metodología desarrollada, se analizaron 15 muestras recogidas en una zona rural de la Comunidad Valenciana. Se detectaron un total de 10 plaguicidas en al menos una de las muestras, con concentraciones que oscilaron entre 411.16 pg m^{-3} hasta 11011.45 pg m^{-3}.

-El empleo de la espectrometría de masas de alta resolución puede permitirnos realizar un análisis retrospectivo de las muestras.

Capítulo 5:

-Se han estudiado nueve localizaciones diferentes (rurales, remotas y urbanas) dentro de la Comunidad Valenciana, detectándose un total de 40 plaguicidas (insecticidas y fungicidas, mayoritariamente). Sólo dos plaguicidas fueron detectados en todas las estaciones (buprofezin y carbendazim). Las zonas rurales y urbanas presentan diferentes perfiles característicos según su localización (prácticas agrícolas, tiempo de aplicación y clima).

- La exposición debido a la inhalación (DIE) de plaguicidas oscila entre 3.88 E-09 mg kg^{-1} dia $^{-1}$ a 4.09 E-09 mg kg^{-1} dia^{-1}. Además, el índice de peligro (HQ) fue evaluado para tres grupos de población principales (bebés, niños y adultos). Los HQ máximos obtenidos en adultos (6.36 E-02), niños (7.50 E-02) y bebés (1.77 E-01), no representan un riesgo significativo para la salud en los grupos estudiados.

-Los índices de peligro acumulados (HI) obtenidos fueron inferiores 1 en todos los grupos de población, no representando un riesgo significativo los valores obtenidos.

-Los valores de riesgo de cáncer para bebés calculados fueron menor que 10^{-6} (valor indicativo a partir del cual existe cierto riesgo) excepto para la carbendazima y el hexitiazox en varias de las estaciones empleadas.

Capítulo 6:

-En la Región Centro francesa, en el período descrito entre 2006 y 2013, los principales plaguicidas detectado fueron los herbicidas trifluralina (0.16-25.8 ng m^{-3}) y pendimetalina (0.13-117.32 ng m^{-3}), el fungicida clorotalonil (0.18-1128.38 ng m^{-3}) y los insecticidas lindano (0.12-1.11 ng m^{-3}) y α-endosulfan (0.47-9.83 ng m^{-3}).

-La exposición inhalatoria diaria en bebés osciló entre 9.41 E-08 mg kg^{-1} dia^{-1} (lindano) y 7.58 E-04 mg kg^{-1} dia^{-1} (clorotalonil), calculados a partir de la concentración máxima detectada. Por otra parte, los valores de HQ obtenidos son inferiores a 3.57 E-02, 8.43 E-02 y 9.93 E-02 para adultos, niños y bebés, respectivamente. Estos valores son inferiores a 1, valor a partir del cual puede existir riesgo para la salud.

-La exposición acumulada para los plaguicidas organofosforados fue menor a 3.94 E-07, 9.30 E-07 y 1.10 E-06 para adultos, niños y bebés, respectivamente. En el caso de las cloroacetamidas, los índices de peligro (HI) calculados son inferiores a 3.55 E-04 para los tres grupos de población estudiados. Teniendo en cuenta los valores a partir del cual se considera que existe cierto riesgo en la salud (0.1 mg kg^{-1} dia^{-1} y 1, respectivamente para organofosforados y cloroacetamidas), los niveles obtenidos no implican riesgo en la salud de las personas de la zona estudiadas.

VI. BIBLIOGRAFÍA

12. REFERENCIAS

[1] F.P. Garcia, S.Y. Cortés-Ascensio, J.C. Gaytan-Oyarzun, A. Ceruelo-Hernández, P. Vázquez-Alavarado, Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks, *Int. J. Environ. Sci. Technol.* 1(11) (2012) 279-293.
[2] Eurostat, 2016. Eurostat Statistical Books. Agriculture, Forestry and Fishery Statistics. 2016 edition. ISSN 2363-2488.
[3] R. Coscollà, Introducción a la Protección Integrada, Valencia, 2004. ISBN: 8493205656
[4] D. Pimentel, Pesticides and Pest Control: Integrated Pest Management: Innovation-Development Process, volume 1 (2009) pp 83-89.
[5] EU Pesticide Database, 2017. Última visita: Mayo 2017. Disponible en: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN
[6] ECPA (European Crop Protection Association), 2010. Industry statistics 2001-2010. Available from hhtp://www.ecpa.eu/page/industry-statistics.
[7] AEPLA (Asociación Empresarial para la Protección de las Plantas), 2015. Memoria AEPLA 2015. Disponible en: http://www.aepla.es/tmp/images/publicaciones/P0017_Memoria_AEPLA_2014.pdf
[8] Portal Estadístico de la Generalitat Valenciana, datos 2015. Última visita: Mayo 2017. Disponible en: http://www.pegv.gva.es/60101cultivo
[9] 2009/128/EC, Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides, *Offic. J. Eur. Commun*. L309 (2009) 71.
[10] A.R. Marks, K. Harley, A. Bradman, K. Kogut, D.B. Barr, C. Johnson, N. Calderón, B. Eskenazi, Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study, *Environ. Health Perspect.* 118 (2010) 1768-1774.
[11] L. London, C. Beseler, M.F. Bouchard, D.C. Bellinger, C. Colosio, P. Grandjean, R. Harari, T. Kootbodien, H. Kromhout, F. Little, T. Meijster, A. Moretto, D.S. Rohlman, L. Stallones, Neurobehavioral and neurodevelopmental effects of pesticide exposures, *Neurotoxicol.* 33 (2012) 887-896.
[12] S.E. Anderson, B.J. Meade, Potential health effects associated with dermal exposure to occupational chemicals, *Environ. Health Insights.* 8 (2014) 51–62.
[13] C.A. Damalas, G.E. Eleftherohorinos, Pesticide exposure, safety issues, and risk assessment indicators, *Int. J. Environ. Res. Public Health* 8 (5) (2011) 1402–1419.
[14] Meenakshi, P. Sharon, M. Bhawana, S. Anita, G. V.K., A short review on how pesticides affect human health, *Int. J. Ayurvedic Herbal Medic.* 5 (2012) 935–946.
[15] A.L. Salvatore, A. Bradman, R. Castorina, J. Camacho, J. López, D.B. Barr, J. Snyder, N.P. Jewell, B. Eskenazi, Occupational behaviors and farmworkers' pesticide exposure: findings from a study in Monterey County, California, *Am. J. Ind. Med.* 51 (2008) 782–794.
[16] A.F.S. Amaral, Pesticides and Asthma: Challenges for Epidemiology, *Front Public Health* 2 (2014) 6-8.
[17] K. Jaga, C. Dharmani, Ocular toxicity from pesticide exposure: a recent review. *Environ. Health Prev. Med.* 11 (3) (2006)102–107.
[18] G.Van Maele-Fabry, A.C. Lantin, P. Hoet, D. Lison, Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis, *Cancer Causes Control* 21 (6) (2010) 787–809.

[19] EFSA, Literature review on epidemiological studies linking to exposure to pesticides and health effects, in EFSA supporting publication 2013: EN-497. 2013.
[20] I. Baldi, A. Gruber, V. Rondeau, P. Lebailly, P. Brochard, C. Fabrigoule, Neurobehavioral effects of long-term exposure to pesticides: results from the 4-year followup of the PHYTONER study, *Occup. Environ. Med.* 68 (2) (2010) 108–115.
[21] E.L. Wickerham, B. Lozoff, J. Shao, N. Kaciroti, Y. Xia, J.D. Meeker, Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants, *Environ. Int.* 47 (2012) 80–85.
[22] R. Raanan, K.G. Harley, J.R. Balmes, A. Bradman, M. Lipsett, B. Eskenazi, Early-life exposure to organophosphate pesticides and pediatric respiratory symptoms in the CHAMACOS cohort, *Environ. Health Perspect*. 119 (8) (2015)1196-1201.
[23] P.K. Henneberger, X. Liang, S.J. London, D.M. Umbrach, D.P. Sandler, J.A. Hoppin, Exacerbation of symptoms in agricultural pesticide applicators with asthma, *Int. Arch. Occup. Environ. Health* 87 (4) (2014) 423-432.
[24] V. Ndlovu, M.A. Dalvie, M.F. Jeebhay, Asthma associated with pesticide exposure among women in rural Western Cape of South Africa, *Am. J. Ind. Med.* 57 (12) (2014) 1331-1343.
[25] A.F. Hernández, O. López, G. Pena, J.L. Serrano, T. Parrón, L. Rodrigo, F. Gil, A. Pla, Implications of Paraoxonase-1 (PON1) Activity and Polymorphisms on Biochemical and Clinical Outcomes in Workers Exposed to Pesticides. In: B. Mackness, M. Mackness, M. Aviram, G. Paragh (eds) The Paraoxonases: Their Role in Disease Development and Xenobiotic Metabolism. Proteins And Cell Regulation, vol 6. Springer, Dordrecht.
[26] J.A. Hoppin, D.M. Umbach, S.J. London, P.K. Henneberger, G.J. Kullman, M.C. Alavanja, D.P. Sandler Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study, *Am. J. Respir. Crit. Care Med.* 177 (1) (2008) 11-18.
[27] P.D. Stivaktakis, M.P. Kavvalakis, M.N. Tzatzarakis, A.K. Alegakis, M.N. Panagiotakis, P. Fragkiadaki, E. Vakonaki, E. Ozcagli, W.A. Hayes, V.N. Rakitskii, A.M. Tsatsakis, Long-term exposure of rabbits to imidaclorpid as quantified in blood induces genotoxic effect, *Chemosphere* 149 (2016) 108-113.
[28] S. Amr, R. Dawson, D.A. Saleh, L.S. Magder, D.M. St George, M. El-Daly, K. Squibb, N.N. Mikhail, M. Abdel-Hamid, H. Khaled, C.A. Loffredo, Pesticides, gene polymorphisms, and bladder cancer among Egyptian agricultural workers, Arch. *Environ. Occup. Health* 70 (1) (2015) 19-26.
[29] J.P. Arrebola, H. Belhassen, F. Artacho-Cordón, R. Ghali, H. Gorbel, H. Boussen, F.M. Pérez-Carrascosa, J. Expósito, A. Hedhili, N. Olea, Risk of female breast cancer and serum concentrations of organochlorine pesticides and polychlorinated biphenyls: a case-control study in Tunisia, *Sci. Total. Environ.* 520 (2015) 106-113.
[30] S. Koutros, D.T. Silverman, M.C. Alavanja, G. Andreotti, C.C. Lerro, S. Heltshe, C.F. Lynch, D.P. Sandler, A. Blair, L.E. Beane Freeman, Occupational exposure to pesticides and bladder cancer risk, *Int. J. Epidemiol.*45 (3) (2015) 792-805.
[31] C.C. Lerro, S. Koutros, G. Andreotti, M.C. Friesen, M.C. Alavanja, A. Blair, J.A. Hoppin, D.P. Sandler, J.H. Luppin, X. Ma, Y. Zhang, L.E. Beane Freeman, Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study, *Occup. Environ. Med.* 72 (10) (2015a) 736-744.
[32] C.C. Lerro, S. Koutros, G. Andreotti, C.J. Hines, A. Blair, J. Lubin, X. Ma, Y. Zhang, L.E. Freeman, Use of acetochlor and cancer incidence in the Agricultural Health Study, *Int. J. Cancer* 137 (5) (2015b) 1167-1175.

[33] J. Rivero, O.P. Luzardo, L.A. Henríquez-Hernández, R.P. Machín, J. Pestano, M. Zumbado, L.D. Boada, M. Camacho, P.F. Valerón, In vitro evaluation of oestrogenic/androgenic activity of the serum organochlorine pesticide mixtures previously described in a breast cancer case-control study, *Sci. Total Environ.* 537 (2015) 197-202.
[34] M. Luqman, M.M. Javed, S. Daud, N. Raheem, J. Ahmad, A.U. Khan, Risk factors for lung cancer in the Pakistani population, *Asian Pac. J. Cancer Prev.* 15 (7) (2014) 3035-3039.
[35] S. El-Zaemey, J. Heyworth, L.Fritschi, Noticing pesticide spray drift from agricultural pesticide application areas and breast cancer: a case-control study, *Aust. N. Z.J. Public Health* 37 (6) (2013) 547–555.
[36] G.P. Ortega-Jacome, R.J. Koifman, G.T. Rego-Monteiro, S. Koifman, Environmental exposure and breast cancer among young women in Rio de Janeiro, Brazil, *J. Toxicol. Environ Health A* 73 (13-14) 858-865.
[37] S. Koutros, C.F. Lynch, X. Ma, W.J. Lee, J.A. Hoppin, Aromatic amine pesticide use and human cancer risk: results from the U.S. Agricultural Health Study, *Int. J. Cancer* 124 (5) 1206-1212.
[38] C.M. Samanic, A.J. De Roos, P.A. Stewart, P. Rajaraman, M.A. Waters, P.D. Inskip, Occupational exposure to pesticides and risk of adult brain tumors, *Am. J. Epidemiol.* 167 (8) 976-985.
[39] D. Provost, A. Cantagrel, P. Lebailly, A. Jaffré, V. Loyant, H. Loiseau, A. Vital, P. Brouchard, I. Baldi, Brain tumors and exposure to pesticides: a case-control study in southwestern France, *Occup. Environ. Med* 64 (8) (2007) 509-514.
[40] F. Giordano, V. Dell'Orco, F. Giannandrea, L. Lauria, P. Valente, I. Figà-Talamanca, Mortality in a cohort of pesticide applicators in an urban setting: sixty years of follow-up, *Int.J. Immunopathol. Pharmacol.* 19 (2006) 61-65.
[41] L.M. Jaacks, L.R. Staimez, Association of persistent organic pollutants and non-persistent pesticides with diabetes and diabetes-related health outcomes in Asia: a systematic review, *Environ. Int.* 76 (2015) 57-70.
[42] M.R. Hansen, E. Jørs, F. Lander, G. Condarco, V. Schlünssen, Is cumulated pyrethroid exposure associated with prediabetes? A cross-sectional study, *J. Agromedicine* 19 (4) (2014) 417-426.
[43] A.P. Starling, D.M. Umbrach, F. Kamel, S. Long, D.P. Sandler, J.A. Hoppin, Pesticide use and incident diabetes among wives of farmers in the Agricultural Health Study, *Occup. Environ. Med.* 71 (9) (2014) 629-635.
[44] C.S. Azandjeme, M. Bouchard, B. Fayomi, F. Dyrolo, D. Houinato, H. Delisle, Growing burden of diabetes in sub-saharian Africa: contribution of pesticides? *Curr. Diabetes Rev.* 9 (6) (2013) 437-449.
[45] M. Turyk, H. Anderson, L. Knobeloch, P. Imm, V. Persky, Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers, *Environ. Health Perspect.* 117 (2009) 1076-1082.
[46] C.J. Everett, I.L. Frithsen, V.A. Diaz, R.J. Koopman, W.M. Simpson, Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and DDT with diabetes in the 1999-2002 National Health and Nutrition Examination Survey, *Environ. Res.* 103 (2007) 413-418.
[47] T.M. Saldana, O. Basso, J.A. Hopin, D.D. Baird, C. Knott, A. Blair, M.C. Alavanja, D.P. Sandler, Pesticide exposure and self-reported gestational diabetes mellitus in the Agricultural Health Study, *Diabetes Care* 30 (3) (2007) 529-534.
[48] D.H. Lee, P. Lind, D.R. Jacobs, S. Salihovic, B. van Babel, L. Lind, Association between background exposure to organochlorine pesticides and the risk of cognitive

impairment: a prospective study that accounts for weight change, *Environ. Int.* 89-90 (2016) 179-184.
[49] S.-A. Kim, Y.M. Lee, H.-W. Lee, D.R. Jacobs, D.H. Lee, Greater cognitive decline with aging among elders with high serum concentrations of organochlorine pesticides, *PLoS One* 10 (6) (2015) e0130623.
[50] J.-F. Viel, C. Warembourg, G. LeManner-Idrissi, A. Lacroix, G. Limon, F. Rouget, C. Monfort, G. Durand, S. Cordier, Pyrethroid insecticide exposure and cognitive developmental dis-abilities in children : the PELAGIE mother-child cohort, *Environ. Int.* 82 (0) (2015) 69-75.
[51] M.F. Bouchard, J. Chevrier, K.G. Harley, K. Kogut, M. Vedar, N. Calderon, C. Trujillo, C. Johnson, A. Bradman, D.B. Barr, B. Eskenazi, Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children, *Environ. Health. Perspect*. 119 (2011) 1189-1195.
[52] S.M. Engel, J. Wetmur, J. Chen, C. Zhu, D.B. Barr, R.L. Canfield, M.S. Woldd, Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood, *Environ. Health. Perspect* 119 (8) (2011) 1182-1188.
[53] V. Rauh, S. Arunajadai, M. Horton, F. Perera, L. Hoepner, D.B. Barr, R. Whyatt, Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide, *Environ. Health Perspect.* 119 (8) (2011) 1196-2001.
[54] I. Baldi, A. Gruber, V. Rondeau, P. Lebailly, P. Brochard, C. Fabrigoule, Neurobehavioral effects of long-term exposure to pesticides: results from the 4-year follow-up of the PHYTONER study, *Occup. Environ. Med.* 68 (2) (2010) 108-115.
[55] H.D. Bailey, C. Infante-Rivard, C. Metayer, J. Clavel, T. Lightfoot, P. Kaatsch, E. Roman, C. Magnani, L.G. Spectror, E. Th. Peridou, E. Milne, J.D. Dockerty, L. Miligi, B.K. Armstrong, J. Rudant, L. Fritschi, J. Simpson, L. Zhang, R. Rondelli, M. Baka, L. Orsi, M. Moschovi, A.Y. Kang, J. Schüz, Home pesticide exposures and risk of childhood leukemia: findings from the childhood leukemia international consortium, *Int.J. Cancer* 137 (11) (2015) 2644-2663.
[56] Z. Maryam, A. Sajad, N. Maral, L. Zahra, P. Sima, A. Zeinab, M. Zahra, E. Fariba, H. Sezaneh, M. Davood, Relationship between exposure to pesticide and occurrence of acute leukemia in Iran, *Asian Pac.J. Cancer Prev.* 16 (1) (2015) 239-244.
[57] M. Brouwer, T. Koeman, P.A. van den Bradt, H. Krombout, L.J. Schouten, S. Peters, A. Huss, R. Vermeulen, Occupational exposures and Parkinson's disease mortality in a prospective Dutch cohort, *Occup. Environ. Med* 72 (6) (2015) 448-455.
[58] K.A. James, D.A. Hall, Groundwater pesticide levels and the association with Parkison disease, *Int. J. Toxicol.* 34 (3) (2015) 266-273.
[59] F. Moisan, J. Spinsosi, L. Delabre, V. Gourlet, J.L. Mazurie, I. Bénatru, M. Goldberg, M.G. Weisskopf, E. Imbernon, C. Tzourio, A. Eibaz, Association of Parkisnon's disease and its subtypes with agricultural pesticide exposures in men: a case-control study in France, *Environ. Health Perspect*. 123 (11) (2015) 1123-1129.
[60] M.H. Ratner, D.H. Farb, J. Ozer, R.G. Feldman, R. Durso, Younger age at onset of sporadic Parkisnon's disease among subjects occupationally exposed to metals and pesticides, *Interdiscip. Toxicol.* 7 (3) (2014) 123-133.
[61] S. Narayan, Z. Liew, K. Paul, P.C. Lee, J.S. Sinsheimer, J.M. Bronstein, B. Ritz, Household organophosphorus pesticide use and Parkinson's disease, Int. J. Epidemiol.42 (5) (2013) 1476-1485.
[62] F.R. Van der Berg, W.G. Kubiak, M.S. Benley, S.R. Majewski, S.R. Yates, G.L. Reeves, Emission of Pesticides into the Air, *Water, Air, Soil Pollut.* 115 (1999) 195-218.

[63] European Commission Health & Consumer Protection Directorate-General. FOCUS Working Group On Pesticides in Air, Pesticides in air: Considerations for exposure assessment 2008, 327 pp.
[64] Y. Gil, C. Sinfort, Emission of pesticides to the air during sprayer application: A bibliographic review, *Atmos. Environ.* 39 (2005) 5183-5193.
[65] C. Bedos, M.F. Rousseau-Djabri, D. Flura, S. Masson, E. Barriuso, P. Cellier, Rate of pesticide volatilization from soil: An experimental approach with a wind tunnel system applied to trifluralin, *Atmos. Environ.* 36 (2002) 5917-5925.
[66] W.A.H. Asman, A. Jorgensen, R. Bossi, K.V. Vejrup, B. Búgel-Mogensen, M. Glasius, Wet deposition of pesticides and nitrophenols at two sites in Denmark: measurements and contributions from regional sources, *Chemosphere* 59(7) (2005) 1023-1031.
[67] T.F. Bidleman, Atmospheric processes, *Environ. Sci. Technol.* 22 (1998) 361-367.
[68] A. Sanusi, P. Mirabel, H. Wortham, Gas–particle partitioning of pesticides in atmospheric samples, *Atmos. Environ.* 33 (1999) 4941-4951.
[69] H.F.G. Van Dijk, P. De Voogt, Fate of pesticides in the atmosphere; implications for environmental risk assessment, *Water, Air, Soil Pollut.* 115 (1999) 3-4.
[70] E. Atkinson, R.A. Hites, W. Palm, J.N. Seiber, P. De Voogt, Transformations of pesticides in the atmosphere: A state of the art, *Water, Air, Soil Pollut.* 115 (1999) 219-243.
[71] M. Al Rashidi, A. Chakir, E. Roth, Heterogeneous oxidation of folpet and dimethomorph by OH radicals: A kinetic and mechanism study, *Atmos. Environ.* 82 (2014) 164-171.
[72] M. Bavcon-Kralj, M. Franko, P.Trebse, Photodegradation of organophosphorus insecticides investigations of products and their toxicity using GC-MS and AChE-termal lens spectrometry bioassay, *Chemosphere* 67 (1) (2007) 99-107.
[73] A. Muñoz, T. Vera, M. Ródenas, E. Borrás, W. Mellouki, J. Treacy, H. Sidebottom, Gas-phase degradation of the herbicide ethafluralin under atmospheric conditions, *Chemosphere* 95 (2014) 395-401.
[74] A. Muñoz, T. Vera, H. Sidebottom, M. Ródenas, E. Borrás, M. Vázquez, M. Raro, W. Mellouki, Studies on the atmospheric fate of propachlor in the gase phase, *Atmos. Environ.* 49 (2012) 33-40.
[75] Q. Zhou, X. Shi, F. Xu, Q. Zhang, Mechanism of OH-initiated atmospheric photooxidation of the organophosphorus insecticide $(C_2H_5O)_3PS$, *Atmos. Environ.* 43 (27) (2009) 4163-4170.
[76] T. Vera, A. Muñoz, M. Ródenas, M. Vázquez, E. Borrás, M. Marqués, W. Mellouki, J. Treacy, H. Sidebottom, Atmospheric fate of hymexazol (5-methylisoxazol-3-ol): Simulation chamber studies, *Atmos. Environ.* 45 (2011) 3704-3710.
[77] A. Muñoz, M. Ródenas, E. Borràs, M. Vázquez, T. Vera, The gas-phase degradation of chlorpyrifos and chlorpyrifos-oxon towards OH radical under atmospheric conditions, *Chemosphere* 111 (2014b) 522-528.
[78] J.L. Armstrong, R.A. Fenske, M.G. Yost, K. Galvin, M. Tchong-French, J. Yu, Presence of organophosphorus pesticide oxygen analogs in air sample, *Atmos. Environ.* 66 (2013) 145-150.
[79] V. Yusà, C. Coscollà, W. Mellouki, A. Pastor, M. de la Guardia, Sampling and analysis of pesticides in ambient air, *J. Chromatogr. A* 1216 (15) (2009) 2972-2983.
[80] N. Sauret, H. Wortham, J.P. Putaud, P. Mirabel, Study of the effects of environmental parameters on the gas/particle partitioning of current-use pesticides in urban air, *Atmos. Environ.* 42(3) (2008) 544-553.

[81] C. Bedos, Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: overview, *Agronomie* 22 (2002) 21-23.
[82] E. Voutsas, C. Vavva, K. Magoulas, D. Tassios, Estimation of the volatilization of organic compounds from soil surfaces, *Chemosphere* 58 (2005) 751-758.
[83] C. Coscollà, A. Yahyaoui, P. Colin, C. Robin, L. Martinon, S. Val, A. Baeza-Squiban, A. Mellouki, V. Yusà, Particle size distributions of currently used pesticides in a rural atmosphere of France, *Atmos. Environ.* 81 (2013) 32-38.
[84] C. Coscollà, A. Muñoz, E. Borràs, T. Vera, M. Ródenas, V. Yusà, Particle size distributions of currently used pesticides in ambient air of an agricultural Mediterranean sea, *Atmos. Environ.* 95 (2014) 29-35.
[85] J.F. Pankow, Review and comparative analysis of the theories on partitioning between the gas and aerosol particulates phase in the atmosphere, *Atmos. Environ.* 22 (1987) 2775-2783.
[86] A. Finizio, T. Bidleman, T. Harner, Octanol-air partition coefficient as a predictor of partitioning of semi-volatile organic chemicals to aerosols, *Atmos. Environ.* 31 (15) (1997) 2289-2296.
[87] T. Harner, T. Bidleman, Octanol-air partition coefficient for describing particle/gas partitioning of aromatic compounds in ambient air, *Environ. Sci. Technol.* 32 (1998) 1494-1502.
[88] A. Sufouglu, E. Cetin, S.S. Bozacioglu, G.D. Sener, M. Odabasi, Short-term variation in ambient concentrations and gas/particle partitioning of organochlorine pesticides in Izmir, Turkey, *Atmos. Environ.* 38 (2004) 4483-4493.
[89] A. Scheyer, S. Morville, P. Mirabel, M. Millet, Gas/particle partitioning of lindane and current-used pesticides and their relationship with temperature in urban and rural air in Alsace region (east of France), *Atmos. Environ.* 42 (2008) 7695-7705.
[90] C. Schummer, E. Monthiron, B.M.R. Appenzeller, A.L. Rizet, R. Wennig, M. Millet, Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France, *Environ. Pollut.* 158 (2010) 576-584.
[91] V. Yusà, C. Coscollà, M. Millet, New screening approach for risk assessment of pesticides in ambient air, *Atmos. Environ.* 96 (2014) 322-330.
[92] J.L. Tadeo, Analysis of pesticides in food and environmental samples, CRC Press, 2008.
[93] F.A. Esteve-Turrillas, A. Pastor, V. Yusà, M. de la Guardia, Using semi-permeables membrane devices as passive samplers, *TrAC* 26 (2007) 703-712.
[94] V. Yusà, C. Coscollà, W. Mellouki, A. Pastor, M. de la Guardia, Sampling and analysis of pesticides in ambient air, *J. Chromatogr. A* 1216 (2009) 2972-2983.
[95] H.A. Alegria, T.F. Bidleman, T.J. Shaw, Organochlorine Pesticides in Ambient Air of Belize, Central America, *Environ. Sci. Technol.* 34 (2000) 1953-1958.
[96] A.J. Cessna, D.T. Waite, L.A. Kerr, R. Grover, Duplicate Sampling Reproducibility of Atmospheric Residues of Herbicides for Paired Pan and High-Volume Air Samplers, *Chemosphere* 40 (2000) 795-802.
[97] A. Sanusi, P. Mirabel, H. Wortham, Comparison of atmospheric pesticide concentrations measured at three sampling sites: local, regional and long-range transport, *Sci. Total Environ.* 263 (2000) 263-277.
[98] S. Buehler, I. Basu, R.A. Hites, A comparison of PAH, PCB, and pesticide concentrations in air at two rural sites on Lake Superior, *Environ. Sci. Technol.* 35 (2001) 2417-2422.
[99] A. Sofuoglu, M. Odabasi, Y. Tasdemir, R.N. Khalili, M.T. Holsen, Temperature dependence of gas-phase polycyclic aromatic hydrocarbon and organochlorine pesticide concentrations in Chicago air, *Atmos. Environ.* 35 (2001) 6503-6510.

[100] D.T. Waite, N.P. Gurprasad, J. F. Sproull, D.V. Quiring, M. W. Kotylak, Atmospheric Movements of Lindane (γ-Hexachlorocyclohexane) from Canola Fields Planted with Treated Seed, *J. Environ. Qual.* 30 (2001) 768–775.
[101] L. Baraud, D. Tessier, J.J. Aaron, J.P. Quisefit, J. Pinart, A multi-residue method for characterization and determination of atmospheric pesticides measured at two French urban and rural sampling sites, *Anal. Bioanal. Chem.* 377 (2003) 1148-1152.
[102] S. Buehler, I. Basu, R.A. Hites, Causes of Variability in Pesticide and PCB Concentrations in Air near the Great Lakes, *Environ. Sci. Technol.* 38 (2004) 414-422.
[103] F. Ferrari, A. Sanusi, M. Millet, F. Bertrand, M. Clement, M. Monutry, Multiresidue method using SPME for the determination of various pesticides with different volatility in confined atmospheres, *Anal. Bioanal. Chem.* 379 (3) (2004) 476-483.
[104] S. Harrad, H. Mao, Atmospheric PCBs and organochlorine pesticides in Birmingham, UK: concentrations, sources, temporal and seasonal trends, *Atmos. Environ.* 38 (2004) 1437-1445.
[105] D.T. Waite, R. Grover, L.A. Kerr, A.D. Snihura, Environmental Concentrations of Agricultural Herbicides: 2,4-D and Triallate, *J. Environ. Qual. 33 (2004) 1616-1628.*
[106] R. Gioia, J.H. Offenberg, C.L. Gigliotti, L.A. Totten, S. Du, S.J. Eisenreich, Atmospheric concentrations and deposition of organochlorine pesticides in the US Mid-Atlantic region, *Atmos. Environ.* 39 (2005) 2309-2322.
[107] A.M. Peck, K.C. Hornbuckle, Gas-Phase Concentrations of Current-Use Pesticides in Iowa, *Environ. Sci. Technol.* 39 (2005) 2952-2959.
[108] A. Scheyer, C. Graeff, S. Morville, P. Mirabel, M. Millet, Analysis of Some Organochlorine Pesticides in an Urban Atmosphere (Strasbourg, East of France), *Chemosphere* 58 (2005) 1517-1524.
[109] D.T. Waite, P. Bailey, J.F. Sproull, D.V. Quiring, D.F. Chau, J. Bailey, A.J. Cessna, Environmental Concentrations of Agricultural Herbicides: 2,4-D and Triallate, *Chemosphere* 58 (2005) 693-703.
[110] D. Xu, M. Dan, Y. Song, Z. Chai, G. Zhuang, Concentration characteristics of extractable organohalogens in PM2.5 and PM10 in Beijing, China, *Atmos. Environ.* 39 (2005) 4119-4128.
[111] H. Alegria, T.F. Bidleman, M.S. Figueroa, Organochlorine pesticides in the ambient air of Chiapas, Mexico, *Environ. Pollut.* 140 (2006) 483-491.
[112] Lig´Air, Qualité de l´air. Contamination de l´air par les produits phytosanitaires en Région Center, 2006.
[113] L.M. White, W.R. Ernst, G. Julien, C. Garron, M. Leger, Ambient air concentrations of pesticides used in potato cultivation in Prince Edward Island, Canada, *Pest. Manag. Sci.* 62 (2006) 126-136.
[114] Y. Yao, L. Tuduri, T. Harner, P. Blanchard, D. Waite, L. Poissant, C. Murphy, W. Belzer, F. Aulagnier, Y.F. Li, E. Sverko, Spatial and temporal distribution of pesticide air concentrations in Canadian agricultural regions, *Atmos. Environ.* 40 (2006) 4339-4351.
[115] R. Bailey, W. Belzer, Large Volume Cold On-Column Injection for Gas Chromatography−Negative Chemical Ionization−Mass Spectrometry Analysis of Selected Pesticides in Air Samples, *J. Agric. Food Chem.* 55 (2007) 1150-1155.
[116] G. Lammel, Y.S. Ghim, A. Grados, H. Gao, H. Hühnerfuss, R. Lohmann, Levels of persistent organic pollutants in air in China and over the Yellow Sea, *Atmos. Environ.* 41 (2007) 452-464.

[117] J. Li, G. Zhang, L. Guo, W. Xu, X. Li, C.S.L. Lee, A. Ding, T. Wang, Organochlorine pesticides in the atmosphere of Guangzhou and Hong Kong: Regional sources and long-range atmospheric transport, *Atmos. Environ.* 41 (2007) 3889-3903.
[118] A. Scheyer, S. Morville, P. Mirabel, M. Millet, Variability of atmospheric pesticide concentrations between urban and rural areas during intensive pesticide application, Atmos. Environ. 41 (2007) 3604-3618.
[119] H. Alegria, F. Wong, L.M. Jantunen, T.F. Bidleman, M.S. Figueroa, G.G. Bouchot, V.C. Moreno, S.M. Waliszewski, R. Infanzon, Organochlorine pesticides and PCBs in air of southern Mexico (2002–2004), *Atmos. Environ.* 42 (2008) 8810-8818.
[120] F. Aulagnier, L. Poissant, D. Brunet, C. Beauvais, M. Pilote, C. Deblois, N. Dassylva, Pesticides Measured in Air and Precipitation in the Yamaska Basin (Québec): Occurrence and Concentrations in 2004, *Sci. Total Environ.* 394 (2008) 338-348.
[121] R. Bossi, H. Skov, K. Vorkamp, J. Christensen, S.C. Rastogi, A. Egeløv, D. Petersen, Atmospheric concentrations of organochlorine pesticides, polybrominated diphenyl ethers and polychloronaphthalenes in Nuuk, South-West Greenland, *Atmos. Environ.* 42 (2008) 7293-7303.
[122] A. Dvorska, G. Lammel, J. Klánova, I. Holoubek, Kosetice, Czech Republic--Ten Years of Air Pollution Monitoring and Four Years of Evaluating the Origin of Persistent Organic Pollutants, *Environ. Pollut.* 156 (2008) 403-408.
[123] R. Raina, L. Sun, Trace level determination of selected organophosphorus pesticides and their degradation products in environmental air samples by liquid chromatography-positive ion electrospray tandem mass spectrometry, *J. Environ. Sci. Health B* 43 (2008) 323-332.
[124] Y. Yang, D. Li, D. Mu, Levels, seasonal variations and sources of organochlorine pesticides in ambient air of Guangzhou, China, *Atmos. Environ.* 42 (2008) 677-687.
[125] M.P. Choi, Y.H. Kang, X.L. Peng, K.W. Ng, M.H. Wong, Stockholm Convention Organochlorine Pesticides and Polycyclic Aromatic Hydrocarbons in Hong Kong Air, *Chemosphere* 77 (2009) 714-719.
[126] S. Fu, H.X. Cheng, Y.H. Liu, X.B. Xu, Levels and Distribution of Organochlorine Pesticides in Various Media in a Mega-City, China, *Chemosphere* 75 (2009) 588-594.
[127] S. Ozcan, M.E. Aydin, Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides in urban air of Konya, Turkey, *Atmos. Research* 93 (2009) 715-722.
[128] C. Coscollà, P. Colin, A. Yahyaoui, O. Petrique, V. Yusà, W. Mellouki, A. Pastor, Determination of 40 Currently Used Pesticides in Airborne Particulate Matter (PM 10) by Microwave-Assisted Extraction and Gas Chromatography Coupled to Triple Quadrupole Mass Spectrometry, *Atmos. Environ.* 44 (2010) 3915-3925.
[129] J. Hayward, T. Gouin, F. Wania, Levels and Seasonal Variability of Pesticides in the Rural Atmosphere of Southern Ontario, *J. Agric. Food Chem*. 58 (2010) 1077-1084.
[130] M. Morshed, D. Omar, R. Mohamad, S. Wahed, M.A. Rahman, Airborne Paraquat measurement and its exposure to spray operators in treated field environment, *Int. J. Agri. Biol.* 12 (2010) 679-684.
[131] E. Borràs, P. Sánchez, A. Muñoz, L.A. Tortajada-Genaro, Development of gas chromatography – mass spectrometry method for the determination of pesticides in gaseous and particulate phases in the atmosphere, *Anal. Chim. Acta* 699 (2011) 57-65.
[132] Department of Pesticide Regulation, Environmental Monitoring Branch, California. Air monitoring network study: Long-term ambient air monitoring for pesticides in multiple California communities, 2011.
[133] S.S. Cindoruk, Atmospheric organochlorine pesticide (OCP) levels in a metropolitan city in Turkey, *Chemosphere* 82 (2011) 78-87.

[134] J.S. Park, S.K. Shin, W.I. Kim, B.H. Kim, Residual levels and identify possible sources of organochlorine pesticides, *Atmos. Environ.* 45 (2011) 7496-7502.
[135] E. Hart, C. Coscollà, A. Pastor, V. Yusà, GC-MS characterization of contemporary pesticides in PM10 of Valencia Region, Spain, *Atmos. Environ.* 62 (2012) 118-129.
[136] M.V. Russo, P. Avino, G. Cinelli, I. Notardonato, Sampling of organophosphorus pesticides at trace levels in the atmosphere using XAD-2 adsorbent and analysis by Gas Chromatography coupled with Nitrogen-Phosphorus and Ion-Trap Mass Spectrometry Detectors, *Anal. Bioanal. Chem.* 404 (2012) 1517-1527.
[137] California Environmental Protection Agency, Environmental Monitoring Branch, Air Monitoring Network results for 2013-2014.
[138] C. Coscollà, E. Hart, A. Pastor, V. Yusà, LC-M S characterization of contemporary pesticides in PM10 of Valencia Region, Spain, *Atmos. Environ.* 77 (2013) 394-403.
[139] G.Z. Jin, S.M. Kim, S.Y. Lee, J.S. Park, D.H. Kim, M.J. Lee, K.T. Sim, H.G. Kang, I.G. Kim, S.K. Shin, K.S. Seok, S.R. Hwang, Levels and potential sources of atmospheric organochlorine pesticides at Korea background sites, *Atmos. Environ.* 68 (2013) 333-342.
[140] J.L. Armstrong, M.G. Yost, R.A. Fenske, Development of a passive air sampler to measure airborne organophosphorus pesticides and oxygen analogs in an agricultural community, *Chemosphere* 111 (2014) 135-143.
[141] M. Hengel, P. Lee, Community air monitoring for pesticides—part 2: multiresidue determination of pesticides in air by gas chromatography, gas chromatography–mass spectrometry, and liquid chromatography–mass spectrometry, *Environ. Monit. Assess* 186 (2014) 1343-1353.
[142] J. Li, H. Lydy, M.J. You, Occurrence, Seasonal Variation and Inhalation Exposure of Atmospheric Organophosphate and Pyrethroid Pesticides in an Urban Community in South China, *Chemosphere* 95 (2014) 363-369.
[143] C. Raeppel, M. Fabritius, M. Nief, B.M. Appenzeller, M. Millet, Coupling ASE, sylilation and SPME–GC/MS for the analysis of current-used pesticides in atmosphere, *Talanta* 121 (2014) 24-29.
[144] T. Ji, T. Lin, F. Wang, Y. Li, Z. Gao, Seasonal variation of organochlorine pesticides in the gaseous phase and aerosols over the East China Sea, *Atmos. Environ.* 109 (2015) 31-41.
[145] C. Degrendele, K. Okonski, L. Melymuk, L. Landlová, P. Kukučka, O. Audy, J. Kohoutek, P. Čupr, J. Klánova, Pesticides in the atmosphere: a comparison of gas-particle partitioning and particle size distribution of legacy and current-use pesticides, *Atmos. Chem. Phys.* 16 (2016) 1531-1544.
[146] L. Melymuk, P. Bohlin-Nizzeto, R. Prokes, P. Kukučka, J. Klánova, Sampling artifacts in active air sampling of semivolatile organic contaminants: Comparing theoretical and measured artifacts and evaluating implications for monitoring networks, *Environ. Pollut* 217 (2016) 97-106.
[147] A. Carratalá, R. Moreno-González, V.M. León, Occurrence and seasonal distribution of polyciclic aromatic hydrocarbons and legacy and current-use pesticide in air from a Mediterranean coastal lagoon (Mar Menor, SE Spain), *Chemosphere* 167 (2017) 382-395.
[148] M. Púcko, G.A. Stern, A.E. Burt, L.M. Jantunen, T.F. Bidleman, R.W. Macdonald, D.G. Barber, N.X. Geilfus, Søren Rysgaard, Current use pesticide and legacy organochlorine pesticide dynamics at the ocean-sea ice-atmosphere interface in

resolute passage, Canadian Arctic, during winter-summer transition, *Sci. Total Environ.* 580 (2017) 1460-1469.
[149] J. Namiesnik, B. Zabielagala, A. Kot-Wasik, M. Partyka, A. Wasik, Passive sampling and/or extraction techniques in environmental analysis: a review, *Anal. Bioanal. Chem.* 381 (2005) 279-301.
[150] S. Bayen, H.K. Lee, J.P. Obbard, Determination of polybrominated diphenyl ethers in marine biological tissues using microwave-assisted extraction*, J. Chromatogr. A* 1035 (2004) 291-294.
[151] O. Wurl, J.P. Obbard, Organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in Singapore's coastal marine sediments, *Chemosphere* 58 (2005) 925-933.
[152] V. Yusà, G. Quintás, O. Pardo, A. Pastor, M. de la Guardia, Determination of PAHs in airborne particles by accelerated solvent extraction and large-volume injection–gas chromatography–mass spectrometry, *Talanta* 69 (2006) 807-815.
[153] J. Patsias, E.N. Papadakis, E. Papadopoulou-Mourkidou, Analysis of phenoxyalkanoic acid herbicides and their phenolic conversion products in soil by microwave assisted solvent extraction and subsequent analysis of extracts by on-line solid-phase extraction–liquid chromatography, *J. Chromatogr. A* 959 (2002) 153-161.
[154] E. Fuentes, M.E. Báez, R. Labra, Parameters affecting microwave-assisted extraction of organophosphorus pesticides from agricultural soil, *J. Chromatogr. A* 1169 (2007) 40-46.
[155] P.N. Carvalho, P.N.R. Rodrigues, F. Alves, R. Evangelista, M.C. Basto, M.T. Vasconcelos, An expeditious method for the determination of organochlorine pesticides residues in estuarine sediments using microwave assisted pre-extraction and automated headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry, *Talanta* 76 (2008) 1124-1129.
[156] A. Sanusi, V. Guillet, M. Montury, Advanced method using microwaves and solid-phase microextraction coupled with gas chromatography–mass spectrometry for the determination of pyrethroid residues in strawberries, *J. Chromatogr. A* 1046 (2004) 35-40.
[157] S.B. Singh, G.D. Foster, S.U. Khan, Microwave-assisted extraction for the simultaneous determination of thiamethoxam, imidaclorpid, and carbendazim residues in fresh and cooked vegetable samples, *J. Agric. Food Chem.* 52 (2004) 105-109.
[158] F.M. Jaward, N.J. Farrar, T. Harner, A.J. Sweetman, K.C. Jones, Passive Air Sampling of PCBs, PBDEs, and Organochlorine Pesticides Across Europe, *Environ. Sci. Technol.* 38 (2004) 34-41.
[159] K. Pozo, M. Shoeib, R. Urrutia, R. Barra, O. Parra, S. Focardi, Passive-Sampler Derived Air Concentrations of Persistent Organic Pollutants on a North−South Transect in Chile, *Environ. Sci. Technol.* 38 (2004) 6529-6537.
[160] N. J. Farrar, T. Harner, M. Shoeib, A. Sweetman, K.C. Jones, Field Deployment of Thin Film Passive Air Samplers for Persistent Organic Pollutants: A Study in the Urban Atmospheric Boundary Layer, *Environ. Sci. Technol.* 39 (2005) 42-48.
[161] H.T. Gouin, P. Blanchard, D. Mackay, Passive and Active Air Samplers as Complementary Methods for Investigating Persistent Organic Pollutants in the Great Lakes Basin, *Environ. Sci. Technol.* 39 (2005) 9115-9122.
[162] T. Gouin, M. Shoeib, T. Harner, Atmospheric concentrations of current-use pesticides across south-central Ontario using monthly-resolved passive air samplers, *Atmos. Environ.* 34 (2008) 8096-8104.
[163] C. Coscollà, N. León, A. Pastor, V. Yusà, Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using Liquid

Chromatography-Orbitrap High Resolution Mass Spectrometry, *J Chromatogr A* 1368 (2014) 132-142.
[164] V.H. Estellano, K. Pozo, C. Efstathiou, K. Pozo, S. Corsolini, S. Focardi, Assessing levels and seasonal variations of current-use pesticides (CUPs) in the Tuscan atmosphere, Italy, using polyurethane foam disks (PUF) passive air samplers, *Environ. Pollut.* 205 (2015) 52-59.
[165] Q. Li, Y. Lu, J. Jin, G. Li, P. Li, C. He, Y. Wang, Comparison of using polyurethane foam passive samplers and tree bark samples from Western China to determine atmospheric organochlorine pesticide, *J. Environ. Sci.* 41 (2016) 90-98.
[166] K. Pozo, M. Palmeri, V. Palmeri, V.H. Estellano, M.D. Mulder, C.I. Efstathiou, G.L. Sará, T. Romeo, G. Lammel, S. Focardi, Assessing persistent organic pollutants (POPs) in the Sicily Island atmosphere, Mediterranean, using PUF disk passive air samplers, *Environ. Sci. Pollut. Res* 23 (2016) 20796-20804.
[167] K. Pozo, S. Kumar Sarkar, V.H. Estellano, S. Mitra, O. Audi, P. Kukucka, P. Přiyova, J. Klánová, S. Corsolini, Passive air sampling of persistent organic pollutants (POPs) and emerging compounds in Kolkata megacity and rural mangrove wetland Sundarban in India: An approach to regional monitoring, *Chemosphere* 168 (2017) 1430-1438.
[168] C. Coscollà, V. Yusà, P. Martí, A. Pastor, Analysis of currently used pesticides in fine airborne particulate matter (PM 2.5) by pressurized liquid extraction and liquid chromatography–tandem mass spectrometry, *J. Chromatogr. A* 1200 (2008) 100-107.
[169] Principios de análisis instrumental, D. A. Skoog, F.J. Haller, T.A. Nieman, 5º Edición, Ed: McGrawHill, Madrid (2001) 558-559.
[170] G. Bouvier, O. Blanchard, I. Momas, N. Seta, Pesticide exposure of non-occupationally exposed subjects compared to some occupational exposure: A French pilot study, *Sci. Total Environ.* 366 (2006) 74-91.
[171] F. Hernández, J.V. Sancho, M. Ibáñez, E. Abad, T. Portolés, L. Mattioli, Current use of high-resolution in the environment sciences, *Anal. Bioanal. Chem* 403 (5) (2012) 1251-1264.
[172] T. Scientific. Mass Frontier 7.0, 2014. Available from: http:://www.thermoscientific.com/en/product/mass-frontier-7-0-spectral-interpretation-software.html
[173] S. Wolf, S. Schmidt, M. Müller-Hannemann, S. Neumann, In silico fragmentation for computer assisted identification of metabolite mass spectra, *BMC Bioinformatics* 11 (2010) 148-160.
[174] J.F. Garcia Reyes, M.D. Hernando, A. Molina–Diaz, A. Fernandez-Alba, Comprehensive screening of target, non-target and unknown pesticides in food by LC-TOF-MS, *TrAC* 26 (8) (2007) 828-841.
[175] C. Hug, N. Ulrich, T. Schulze, W. Brack, M. Krauss, Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening, *Environ. Pollut.* 184 (2014) 25-32.
[176] T. Pluskal, S. Castillo, A. Villar-Briones, M. Oresic, MzMine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecule profile data, *BMC Bioinformatics* 11 (2010) 395-406.
[177] ChemSpider software http://www.chemspider.com/
[178] PubChem compound database http://www.ncbi.nlm.nih.gov/pccompound
[179] L.C. Menikarachi, S. Cawley, D.W. Hill, L.M. Hall, L. Hall, S. Lai, J. Wilder, D.F. Grant, MolFind: a software package enabling HPLC-MS based identification of unknown chemical structures, *Anal. Chem.* 84(21) (2012) 388-394.

[180] N. Cech, C. Enke, Practical implications of some recent studies in electrospray ionization fundamentals, *Mass Spectrom. Rev.* 20 (6) (2001) 362-387.
[181] R.H. Perry, R.G. Cooks, R.J. Noll. Orbitrap mass spectrometry: Instrumentation, ion motion and applications, *Mass Spectrom. Rev.* 27 (6) (2008) 661-699.
[182] A. Makarov, M. Scigelova, Coupling liquid chromatography to orbitrap mass spectrometry, *J. Chromatogr. A* 1217 (25) (2010) 3938-3945.
[183] K.K. Murray, R.K. Boyd, M.N. Eberlin, G.J. Langley, L. Li, Y. Naito, Definitions of terms relating to mass spectrometry (IUPAC Recommendations 2013), *Pure Appl. Chem.* 85 (2013) 1515-1609.
[184] M.P. Jedrychowski, E.L. Huttlin, W. Haas, M.E. Sowa, R. Rad, S.P. Gygi, Evaluation of HCD- and CID-type fragmentation within their respective detection platforms for murine phosphoproteomics, *Mol. Cell Proteomics* 10 (12) (2011) 1-9.
[185] N. Gai, J. Pan, H. Tang, K.Y. Tan, D.Z. Chen, X.H. Zhu, G.H. Lu, S. Chen, Y. Huang, Y.L. Yang, Selected organochlorine pesticides and polychlorinated biphenyls in atmosphere at Ruoergai high altitude prairie in eastern edge of Qinghai-Tibet Plateau and their source identifications, *Atmos. Environ.* 95 (2014) 89-95.
[186] S. Newton, T. Bidleman, M. Bergknut, J. Racine, H. Laudon, R. Giesler, K. Wiberg, Atmospheric deposition of persistent organic pollutants and chemicals of emerging concern at two sites in northern Sweden, *Environ. Sci. Processes Impacts* 16 (2014) 298-306.
[187] S. Grimalt, J.V. Sancho, O.J. Pozoa, F.E. Hernandez, Quantification, confirmation and screening capability of UHPLC coupled to triple quadrupole and hybrid quadrupole time-of-flight mass spectrometry in pesticide residue analysis, *J. Mass Spectrom.* 45 (2010) 421-436.
[188] C. Ferrer, A. Lozano, A. Aguera, A.J. Giron, A.R. Fernandez-Alba, Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables, *J. Chromatogr. A* 1218 (2011) 7634-7639.
[189] O. Malato, A. Lozano, M. Mezcua, A. Aguera, A.R. Fernandez-Alba, Benefits and pitfalls of the application of screening methods for the analysis of pesticide residues in fruits and vegetables, *J. Chromatogr. A* 1218 (2011) 7615-7626.
[190] T. Schwarz, T.A. Snow, C.J. Santee, C.C. Mulligan, T. Class, M.P. Wadsley, S.C. Nanita, QuEChERS Multiresidue Method Validation and Mass Spectrometric Assessment for the Novel Anthranilic Diamide Insecticides Chlorantraniliprole and Cyantraniliprole, *J. Agr. Food Chem.* 59 (2011) 814.
[191] E. De Dominicis, I. Commissati, M. Suman, Targeted screening of pesticides, veterinary drugs and mycotoxins in bakery ingredients and food commodities by liquid chromatography-high-resolution single-stage Orbitrap mass spectrometry, *J. Mass. Spectrom.* 47 (2012) 1232-1241.
[192] A. Kaufmann, The current role of high-resolution mass spectrometry in food analysis, *Anal. Bioanal. Chem*. 403 (2012) 1233-1249.
[193] H.G.J. Mol, P. Zomer, M. De Koning, Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry, *Anal. Bioanal. Chem.* 403 (2012) 2891-2908.
[194] L. Polgár, J.F. García Reyes, P. Fodor, A. Gyepes, M. Dernovics, L. Abrankó, B. Gilbert-López, A. Molina-Diaz, Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions, *J. Chromatogr. A* 1249 (2012) 83-91.

[195] F. Wode, C. Reilich, P. van Baar, U. Dünnbier, M. Jekel, T. Reemtsma, Multiresidue analytical method for the simultaneous determination of 72 micropollutants in aqueous samples with ultra high performance liquid chromatography–high resolution mass spectrometry, *J. Chromatogr. A* 1270 (2012) 118-126.
[196] M. Roca, N. León, A. Pastor, V. Yusà, Comprehensive analytical strategy for biomonitoring of pesticide in urine by liquid chromatography-Orbitrap High Resolution Mass Spectrometry, *J. Chromatogr. A* 1374 (2014) 66-76.
[197] M.L. Gómez-Pérez, R. Romero-González, J.L. Martínez-Vidal, A. Garrido-Frenich, Identification of transformation products of pesticides and veterinary drugs in food and related matrices: Use of retrospective analysis, *J. Chromatogr. A* 1389 (2015) 133-138.
[198] O.K. Ulutaş, I. Çok, F. Darendelider, B. Aydin, A. Çoman, B. Henkelmann, K.W. Schramm, Blood concentrations and risk assessment of persistent organochlorine compounds in newborn boys in Turkey. A pilot study, *Environ Sci Pollut Res* 22 (2015) 19896–19904.
[199] Q. Zhang, Z. Chen, Y. Li, P. Wang, C. Zhu, G. Gao, K. Xiao, S. Zheng, Y. Liang, G. Jiang, Occurrence of organochlorine pesticides in the environmental matrices from King George Island, west Antarctica, *Environ. Pollut* 206 (2015) 142-149.
[200] J. Cotton, F. Leroux, S. Broudin, M. Poirel, B. Corman, C. Junot, C. Decruix, Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry, *Water Res*. 104 (2016) 20-27.
[201] N. León, A. Pastor, V. Yusà, Target analysis and retrospective screening of veterinary drugs, ergot alkaloids, plant toxins and other undesirable substances in feed using liquid chromatography–high resolution mass spectrometry, Talanta 149 (2016) 43-52.
[202] G. Martínez-Domínguez, R. Romero-González, A. Garrido-Frenich, Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry, *Food Chem.* 197 (2016) 907-915.
[203] H.G.J. Mol, M. Tienstra, P. Zomer, Evaluation of gas chromatography-electron ionization-full scan high resolution Orbitrap mass spectrometry for pesticide residue analysis, *Anal. Chim. Acta* 935 (2016) 161-172.
[204] P. Pérez-Ortega, FJ. Lara-Ortega, J.F. García-Reyes, B. Gilbert-López, M. Trojanowicz, A. Molina-Diaz, A feasibility study of UHPLC-HRMS accurate-mass screening methods for multiclass testing of organic contaminants in food, *Talanta* 160 (2016) 704-712.
[205] I. Reinholds, I. Pugajeva, V. Bartkevics, A reliable screening of mycotoxins and pesticide residue in paprika using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry, *Food Control* 60 (2016) 683-689.
[206] A.A. Deeb, S. Stephan, O.J. Schmitz, T.C. Schmidt, Suspect screening of micropollutants and their transformation products in advanced wastewater treatment, *Sci. Total. Environ.* 601-602 (2017) 1247-1253.
[207] F.J. Lara, D. Chan, M. Dickinson, A.S. Lloyd, S.J. Adams, Evaluation of direct analysis in real time for the determination of highly polar pesticides in lettuce and celery using modified QuickPolar Pesticides Extraction method, *J. Chromatogr. A* 1496 (2017) 37-44.

[208] N.I. Rousis, R. Bade, L. Bijlsma, E. Zuccato, J.V. Sancho, F. Hernández, S. Castiglioni, Monitoring a large number of pesticides and transformation products in water samples from Spain and Italy, Environ. Res. 156 (2017) 31-38.
[209] SANTE/11945/2015.Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed.
[210] R. Castorina, A. Bradman, T.E. McKone, D.B. Barr, M.E. Harnly, B. Eskenazi, Assessing Cumulative Organophosphate Pesticide Exposure and Risk among Pregnant Women Living in an Agricultural Community, *Environ. Health Perspect.* 111 (13) (2013) 1640-1648.
[211] EFSA (European Food Safety Authority), Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment, *EFSA Journal* 438 (2006) 1-54.
[212] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, Liquid chromatography-high resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: screening and quantitative studies, *J. Chromatogr. A* 1287 (2013) 24-37.
[213] A. Muñoz, T. Vera, H. Sidebottom, A. Mellouki, E. Borrás, M. Ródenas, E. Clemente, M. Vázquez, Studies on the atmospheric degradation of chlorpyrifos-methyl, *Environ. Sci. Technol.* 45(5) (2011) 1880-1886.
[214] Y. Yang, D. Li, D. Mu, Levels, seasonal variations and sources of organochlorine pesticides in ambient air of Guangzhou, China, *Atmos. Environ.* 42 (2008) 677-687.
[215] C. Coscollà, V. Yusà, M.I. Beser, A. Pastor, Multi-residue analysis of 30 currently used pesticides in fine airborne particulate matter (PM 2.5) by microwave-assisted extraction and liquid chromatography–tandem mass spectrometry, *J. Chromatogr. A* 1216 (2009) 8817-8827.
[216] J. He, R. Balasubramanian, S. Karthikeyan, U.M. Joshi, Determination of semi-volatile organochlorine compounds in the atmosphere of Singapore using accelerated solvent extraction, *Chemosphere* 75 (2009) 640-648.
[217] J. Cheng, M. Liu, X. Zhang, L. Ding, Y. Yu, X. Wang, H. Jin, H. Zhang, Determination of triazine herbicides in sheep liver by microwave-assisted extraction and high performance liquid chromatography, *Anal. Chim. Acta* 590 (2007) 34-39.
[218] K.L. Smalling, K.M. Kuivila, Multi-residue method for the analysis of 85 current-use and legacy pesticides in bed and suspended sediments, *J. Chromatogr. A* 1210 (2008) 8-18.
[219] D. Gallart-Mateu, A. Pastor, M. de la Guardia, S. Armenta, F.A. Esteve-Turrillas, Hard cap espresso extraction-stir bar preconcentration of polychlorinated biphenyls in soil and sediments, *Anal. Chim. Acta* 952 (2017) 41-49.
[220] M.T. Martinez-Sena, M. de la Guardia, F.A. Esteve-Turrillas, S. Armenta, Hard cap espresso extraction and liquid chromatography determination of bioactive compounds in vegetables and spices, *Food Chem* 237 (2017) 75-82.
[221] S. Armenta, M. de la Guardia, F.A. Esteve-Turrillas, Hard Cap Espresso Machines in Analytical Chemistry: What Else?, *Anal. Chem.* 88 (2016) 6570−6576.
[222] S.J. Lehotay, Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study, *J. AOAC Int.* 90 (2007) 485-520.
[223] A. Lozano, L. Rajski, N. Belmonte-Valles, A. Uclés, S. Uclés, M. Mezcua, A.R. Fernández-Alba, Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: validation and pilot survey in real samples, *J. Chromatogr. A* 1268 (2012) 109–122.

[224] J. F. Pankow, An absorption model of gas/particle partitioning in the atmosphere, *Atmos. Environ.* 28 (1994) 185-188.
[225] W. Hadnagy, G. Leng, D. Sugiri, U. Ranft, H. Idel, Pyrethroids used indoors–immune status of humans exposed to pyrethroids following a pest control operation–a one year follow-up study, *Int. J. Hyg. Environ. Health* 206 (2003) 93–102.
[226] Y. Zhang, J. Mo, C.J. Weschler, Reducing health risks from indoor exposures in rapidly developing urban China, *Environ. Health Perspect.*121 (2013) 751–755.
[227] D. Zhang, X. Zhan, X. Wu, X. Yang, G. Liang, Z. Zheng, Z. Li, Y. Wu, X. Zheng, A field survey for Wolbchia and phage WO infections of Aedes albopictus in Guangzhou City, China, *Parasitol.Res.*113 (2014) 399–404.
[228] L. G. Dos Santos, L. Lourencetti, A. A. Pinto, W. A. Pignati, E. F. G. C Dores, Validation and application of an analytical method for determining pesticides in the gas phase of ambient air, *J. Environ. Sci. Heal. B* 46 (2011) 150-162.
[229] R. Dobson, A. Scheyer, A. L. Rizet, P. Mirabel, M. Millet, Comparison of the efficiencies of different types of adsorbents at trapping currently used pesticides in the gaseous phase using the technique of high-volume sampling, *Anal. Bioanal. Chem.* 386 (2006) 1781-1789.
[230] P. Avino, G. Cinelli, I. Notardonato, M. V. Russo, Investigation on the behaviour of pesticides in the atmosphere, *Aerosol Air Qual.* Res 11 (2011) 783-790.
[231] J. L. Freixo, E. F. G. C. Dores, R. D. Villa, Sampling and analysis of pesticides in the gas phase of air: method validation, *Anal. Methods 7* (2015) 8255-8261.
[232] S. Y. Baek, J. Jung, Y. S. Chang, Spatial distribution of polychlorinated biphenyls, organochlorine pesticides, and dechlorane plus in Northeast Asia, *Atmos. Environ.* 64 (2013) 40-46.
[233] Q. Li, X. Wang, J. Song, H. Sui, L. Huang, L. Li, Seasonal and diurnal variation in concentrations of gaseous and particles phase endosulfan, *Atmos. Environ.* 61 (2012) 620-626.
[234] N. G. Tsirapoulos, E. B. Bakeas, V. Raptis, S. S. Batistatou, Evaluation of solid sorbents for the determination of fenhexamid, metalaxyl-M, pyrimethanil, malathion and myclobutanil residues in air samples: Application to monitoring malathion and fenhexamid dissipation in greenhouse air using C-18 or Supelpak-2 for sampling, *Anal Chim. Acta.* 573-574 (2006) 209-215.
[235] C. Coscollà, V. Yusà, Chapter 17: Pesticides and agricultural air quality in: M.de la Guardia, S. Armenta (Eds.), The Quality of Air, *Elsevier*, Amsterdam, 2016, pp. 423–490.
[236] P. Pentamwa, N. Kanaratanadilok, N.T.K. Oanh, Indoor pesticide application practices and levels in homes of Bangkok Metropolitan Region, *Environ. Monit. Assess.* 181 (2011) 363-372.
[237] S. Laborie, E. Moreau-Guignon, F. Alliot, A. Desportes, L. Oziol, M. Chevrauil, A new analytical protocol for the determination of 62 endocrine-disrupting compounds in indoor air, *Talanta* 147 (2016) 132-141.
[238] H. Li, M.J. Lidy, J. You, Pyrethroids in indoor air during application of various mosquito repellents: Occurrence, dissipation and potential exposure risk, *Chemosphere* 144 (2016) 2427-2435.
[239] C. Lu, G. Adamkiewicz, K.R. Attfield, M. Kapp, J.D. Spengler, L. Tao, S.H. Xie, Household Pesticide Contamination from Indoor Pest Control Applications in Urban Low-Income Public Housing Dwellings: A Community-Based Participatory Research, *Environ. Sci. Technol.* 47 (2013) 2018-2025.
[240] A. Bradman, D. Whitaker, L. Quirós, R. Castorina, B. C. Henn, M. Nishioka, J. Morgan, D. B. Barr, M. Harnly, J. A. Brisbin, L. S. Sheldon, T. E. Mckone, B.

Eskenazi, Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. J. Expo. Sci. Environ. Epidemiol. 17 (2007) 331-349.
[241] P. Pentamwa, Nguyen Thi Kim Oanh, Levels of pesticides and polychlorinated biphenyls in selected homes in the Bangkok Metropolitan Region, Thailand, *Ann N Y Acad Sci.* 1140 (2008) 91-112.
[242] C. Mai, N. Theobald, G. Lammel, H. Hühnerfuss, Spatial, seasonal and vertical distributions of currently-used pesticides in the marine boundary layer of the North Sea, Atmos. Environ. 75 (2013) 92-102.
[243] H. P. Li, J. H. Li, G. C. Li, J. F. Jen, Simultaneous determination of airborne carbamates in workplace by high performance liquid chromatography with fluorescence detection, Talanta 63 (2004) 547-553.
[244] EFSA (European Food Safety Authority), Scientific opinion on preparation of a guidance document on pesticide exposure assessment for workers, operators, bystanders and residents, *EFSA* J. 8 (2) (2014) 1501.
[245] S. Lee, R. McLaughlin, M. Harnly, R. Gunier, R. Kreutzer, Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks, *Environ. Health Perspect.* 110 (2002) 1175-1184.
[246] F. Cangialosi, G. Intini, L. Liberti, M. Notarnicola, P. Stellacci, Health risk assessment of air emissions from a municipal solid waste incineration plant -case study, *Waste Manag.* 28 (2008) 885-895.
[247] Department of Pesticide Regulation, California 2011. Air Monitoring Network Study: Long-term Ambient Air Monitoring for Pesticides in Multiple California Communities.
[248] USEPA, 2013. Chemicals Evaluated for Carcinogenic Potential (Office of pesticide programs).
[249] C. Coscollà, P. Colin, A. Yahyaoui, O. Petrique, V. Yusà, W. Mellouki, A. Pastor, Occurrence of currently used pesticides in ambient air of Centre Region (France), *Atmos. Environ.* 44 (2010) 3915-3925.
[250] D.A. Sarigiannis, P. Kontoroupis, E.S. Solomou, S. Nikolaki, A.J. Karabelas, Inventory of pesticide emissions into the air in Europe, *Atmos. Environ.* 75 (2013) 6-14.
[251] Y. Moussaoui, L. Tuduri, Y. Kerchich, B.Y. Meklati, G. Eppe, Atmospheric concentrations of PCDD/Fs, dl-PCBs and some pesticides in northern Algeria using passive air sampling, *Chemosphere* 88 (2012) 270-277.
[252] A. Mahmood, R.N. Malik, J. Li, G. Zhang, Human health risk assessment and dietary intake of organochlorine pesticides through air, soil and food crops (wheat and rice) along two tributaries of river Chenab, Pakistan, *Food and Chemical Toxicol.* 71 (2014) 17-25.
[253] AMN, E. Vidrio, P. Wofford, R. Segawa, R. Schreider. (Eds), Air Monitoring Network Results for 2011, California (2012).
[254] Y. Luo, M. Zhang, Multimedia transport and risk assessment of organophosphate pesticides and a case study in the northern San Joaquin Valley of California, *Chemosphere* 75 (2009) 969-978.
[255] T. Reemstma, L. Alder, U. Banasiak, Determination of 150 pesticide metabolites in surface water and groundwater by a multimethod using direct injection liquid chromatography-mass spectrometry, *J. Chromatogr. A* 1271 (2013) 95-104.
[256] PPDB, pesticide property database. University of Hertfordshire. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm.
[257] T. Scientific. Pesticides Database Compound Library: Environmental and Food http://planetorbitrap.com/compoundlibrary#.VBlJ_KMUfAM

[258] A. Muñoz, A. Le Person, S. LeCalvé, A. Mellouki, E. Borràs, V. Deale, T. Vera, Studies on atmospheric degradation of diazinon in EUPHORE simulation chamber, *Chemosphere* 85 (2011) 724-730.
[259] USEPA, Risk Assessment Guidance for Superfund (RAGS) (Part A) (1989) EPA/540/1-89/002.
[260] USEPA, Region 9 Preliminary Remediation Goals (2004) Memorandum from Standford Smucker, Ph.D., Regional Toxicologist. http://www.epa.gov/region09/ waste/sfund/prg/index.html.
[261] USEPA, RAGS Volume I: Human Health Evaluation Manual. Supplemental Guidance "Standard Default Exposure Factors". (1991) OSWER No. 9285.6-03.
[262] EFSA (European Food Safety Authority), Conclusion on the peer review of chlorpyrifos, EFSA J. 12 (4) (2014) 3640, 2014.

VII. ANEXOS

13. ANEXOS: INFORMACIÓN SUPLEMENTARIA

13.1. Anexo I. Información suplementaria del artículo científico 1.

Supplementary Data for

Retrospective screening analysis of pesticide metabolites in ambient air using liquid chromatography coupled to high resolution mass spectrometry.

Antonio López, Vicent Yusà, Maurice Millet, Clara Coscollà.

Table of Contents :

Table SI-1. Database of pesticide metabolites used for post-run target screening (suspect screening).

Nº	Compound	CAS number	Elemental composition	Monitored ion	Monitored Mass	Frag. 1	Structure	Frag. 2	Structure	Frag.3	Structure	Matrix(*)	Ref
1	Abamectin metabolite (5-O-demethyl-Avermectin A1a)	113665-89-7	$C_{48}H_{72}O_{14}$	M+H	873.49948	831.452533	$C_{45}H_{67}O_{14}$	641.36841	$C_{37}H_{53}O_9$	295.17513	$C_{13}H_{27}O_7$	W	[172], [255], [256]
2	Abamectin metabolite (5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-Avermectin A1a)	65195-56-4	$C_{47}H_{70}O_{14}$	M+H	859.48383	583.326544	$C_{34}H_{47}O_8$	333.19078	$C_{16}H_{29}O_7$	175.096485	$C_8H_{15}O_4$	W	[172], [255], [256]
3	Acetamiprid metabolite[N-methyl-(6-chloro-3-pyridyl)methylamine]	120739-62-0	$C_7H_9ClN_2$	M+H	157.0527	126.010503	C_6H_5ClN	121.076025	$C_7H_9N_2$	111.994853	C_5H_3NCl	S	[172],[256]
4	Acetochlor metabolite [N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide]	34256-82-1	$C_{14}H_{21}NO_2$	M+H	236.16450	177.114815	$C_{11}H_{15}NO$	136.112076	$C_9H_{14}N$	121.101177	C_9H_{13}	W	[172], [255], [256]
5	Acetochlor metabolite [Acetic acid, [(ethoxymethyl)(2-ethyl-6-methylphenyl)-amino]oxo]		$C_{14}H_{19}NO_4$	M+H	266.13868	236.091734	$C_{12}H_{14}NO_4$	119.085527	C_9H_{11}	192.138291	$C_{12}H_{18}NO$	W	[172], [255], [256]
6	Acetochlor metabolite [Acetic acid, {2-[(ethoxymethyl)(2-ethyl-6-methylphenyl)-amino]-2-oxoethyl}sulfinyl]		$C_{16}H_{23}NO_5S$	M+H	342.13697	313.097844	$C_{14}H_{19}NO_5S$	296.095105	$C_{14}H_{18}NO_4S$	190.122641	$C_{12}H_{16}NO$	W	[172], [255], [256]
7	Acetochlor metabolite [2-[(2-ethyl-6-methylphenyl)amino]-2-oxoethanesulfonic acid; [(2-Ethyl-6-methylphenyl)-carbamoyl]-methanesulfonic acid]		$C_{11}H_{15}NO_4S$	M+H	258.07945	148.112076	$C_{10}H_{14}N$	140.001205	$C_2H_6NO_4S$	226.053241	$C_{10}H_{12}NO_3S$	W	[172], [255], [256]
8	Acetochlor metabolite [Ethanesulfonic acid, 2-[(2-ethyl-6-methylphenyl)(ethoxymethyl)amino]-2-oxo]		$C_{14}H_{21}NO_5S$	M+H	316.12132	270.079455	$C_{12}H_{16}NO_4S$	240.068891	$C_{11}H_{14}NO_3S$	190.122641	$C_{12}H_{16}NO$	W	[172], [255], [256]
9	Ametryn metabolite[Deethyl ametrin]	4147-57-3	$C_9H_{17}N_5S$	M+H	228.12774	212.096442	$C_8H_{14}N_5S$	170.049493	$C_5H_8N_5S$	136.061772	$C_5H_6N_5$	S	[172],[256]
10	Amitraz metabolite(N-2 4-Dimethylphenyl-N'methylformamidine)	33089-74-6	$C_{10}H_{14}N_2$	M+H	163.12297	132.080775	$C_9H_{10}N$	134.096426	$C_9H_{12}N$	147.091675	$C_9H_{11}N_2$	W	[193]
11	Atrazine metabolite (6-deisopropyl atrazine)	1007-28-9	$C_5H_8ClN_5$	M+H	174.0541	157.027550	$C_5H_6ClN_4$	146.022799	$C_3H_5ClN_5$	111.053947	$C_3H_5N_5$	S	[172],[256]
12	Atrazine metabolite (Deethyl atrazine)	6190-65-4	$C_6H_{10}ClN_5$	M+H	188.06975	146.0228	$C_3H_5N_5Cl$	136.061772	$C_5H_6N_5$	128.99625	$C_3H_2ClN_4$	S	[257]
13	Azinphos-methyl metabolite (phosphorodithioic O,O,S-trimethyl ester)	2953-29-9	$C_3H_9O_2PS_2$	M+H	172.98543	140.959219	$C_2H_6OPS_2$	124.982063	$C_2H_6O_2PS$	93.0099925	$C_2H_6O_2P$	W	[72]
14	Azinphos-methyl metabolite (phosphorothioic O,O,S-trimethyl ester)	152-20-5	$C_3H_9O_3PS$	M+H	157.00827	124.982063	$C_2H_6O_2PS$	109.004907	$C_2H_6O_3P$			W	[72]
15	Azinphos-methyl metabolite (Benzamide)	55-21-0	C_7H_7NO	M+H	122.06004	105.033491	C_7H_5O	77.0385768	C_6H_5			W	[72]
16	Azinphos-methyl metabolite (Pyrido[3,4-d] pyrimidin-4-ol)	19178-25-7	$C_7H_5N_3O$	M+H	148.05053	130.039973	$C_7H_4N_3$					W	[72]
17	Azinphos-methyl metabolite (3-methyl-benzotriazin-4-one)	22305-44-8	$C_8H_7N_3O$	M+H	162.06618	134.071273	$C_7H_8N_3$	146.071274	$C_8H_8N_3$			W	[72]
18	Azoxystrobin metabolite [4-(2-cyanophenoxy)-6-hydroxypyrimidine]		$C_{11}H_7N_3O_2$	M+H	214.0611	196.050538	$C_{11}H_6N_3O$	187.050204	$C_{10}H_7N_2O_2$	118.02874	C_7H_4NO	S,W	[172],[256]
19	Azoxystrobin metabolite [2-[6-(2-cyanophenoxy)pyrimidin-4yloxy]benzoic acid]		$C_{18}H_{11}N_3O_4$	M+H	334.08223	318.087317	$C_{18}H_{12}N_3O_3$	307.071333	$C_{17}H_{11}N_2O_4$	171.055289	$C_{10}H_7N_2O$	S,W	[172],[256]
20	Bensulfuron methyl metabolite[methyl 2-({[(4-hydroxy-6-methoxypyrimidin-2-yl)carbamoyl]sulfamoyl}methyl)benzoate]		$C_{15}H_{16}N_4O_7S$	M+H	397.08124	367.070681	$C_{14}H_{15}N_4O_6S$	339.075767	$C_{13}H_{15}N_4O_5S$	185.066917	$C_6H_9N_4O_3$	W	[172], [255], [256]
21	Bensulfuron methyl metabolite (4,6-	36315-01-2	$C_6H_9N_3O_2$	M+H	156.07675	124.050538	$C_5H_6N_3O$	139.050204	$C_6H_7N_2O_2$			W	[172], [255],

	Dimethoxy-2-pyrimidinamine)												[256]
22	Bensulfuron methyl metabolite [methyl 2-(sulfamoylmethyl)benzoate]	112941-26-1	$C_9H_{11}NO_4S$	M+H	230.04815	199.005955	$C_8H_7O_4S$	170.027026	$C_7H_8NO_2S$	135.044056	$C_8H_7O_2$	W	[172], [255], [256]
23	Bensulfuron methyl metabolite [(4,6-dimethoxypyrimidin-2-yl)urea]		$C_7H_{10}N_4O_3$	M+H	199.08256	156.076753	$C_6H_{10}N_3O_2$	124.050538	$C_5H_6N_3O$	139.050204	$C_6H_7N_2O_2$	W	[172], [255], [256]
24	Bensulfuron methyl metabolite [{[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]sulfamoyl}acetic acid]		$C_9H_{12}N_4O_7S$	M+H	321.04995	289.023731	$C_8H_9N_4O_6S$	182.056018	$C_7H_8N_3O_3$	156.076753	$C_6H_{10}N_3O_2$	W	[172], [255], [256]
25	Bensulfuron methyl metabolite [2-(sulfamoylmethyl)benzoic acid]		$C_8H_9NO_4S$	M+H	216.03250	180.995391	$C_8H_5O_3S$	170.027026	$C_7H_8NO_2S$	121.028406	$C_7H_5O_2$	W	[172], [255], [256]
26	Bifenox metabolite (Bifenox acid)	59024-05-4	$C_{13}H_7Cl_2NO_5$	M+H	327.97740	292.000726	$C_{13}H_7ClNO_5$	236.986847	$C_{12}H_7Cl_2O$	166.013484	$C_7H_4NO_4$	W	[172], [255], [256]
27	Bifenox metabolite[2-Amino-5-(2,4-dichlorophenoxy)-benzoic acid]	59216-76-1	$C_{13}H_9Cl_2NO_3$	M+H	298.00322	280.976676	$C_{13}H_7Cl_2O_3$	251.997746	$C_{12}H_8Cl_2NO$	236.986847	$C_{12}H_7Cl_2O$	W	[172], [255], [256]
28	Bitertanol metabolite (1,2,4-triazole)	288-88-0	$C_2H_3N_3$	M+H	70.039970							S, GW, Pl, An	[172],[256]
29	Bitertanol metabolite (1H-1,2,4-triazol-1-ylacetic acid)	28711-29-7	$C_4H_5N_3O_2$	M+H	128.04545	98.0348883	$C_3H_4N_3O$	82.0399737	$C_3H_4N_3$	112.050538	$C_4H_6N_3O$	Soil, Pl, An	[172],[256]
30	Bromopropylate metabolite(4,4-dibromobenzilic acid)	30738-49-9	$C_{14}H_{10}Br_2O_3$	M+H	384.90695	338.901466	$C_{13}H_9Br_2O$	156.964739	C_6H_6Br	184.959654	C_7H_6BrO	S	[172],[256]
31	Cadusafos metabolite (Methyl-2-butyl sulfone)		$C_8H_{18}O_2S$	M+H	179.11003	109.031776	$C_3H_9O_2S$	91.0212121	C_3H_7OS			S,W	[172],[256]
32	Captan metabolite (THPAM)	4795-29-3	$C_5H_{11}NO$	M+H	102.09134	85.0647915	C_5H_9O	71.0491414	C_4H_7O			W	[172], [255], [256]
33	Captan metabolite (THPI)	85-40-5	$C_8H_9NO_2$	M+H	152.07060	131.000179	$C_7H_{10}NO$	109.064792	C_7H_9O			W	[172], [255], [256]
34	Carbaryl metabolite (1-naphtol)	90-15-3	$C_{10}H_8O$	M+H	145.06479	127.0752	$C_{10}H7$					S,Hu	[172],[256]
35	Carbaryl metabolite (Methylamine)	200-820-0	CH_5N	M+H	32.04948							S	[172],[256]
36	Carbendazim metabolite(2-aminobenzimidazole)	934-32-7	$C_7H_7N_3$	M+H	134.07127	133.0634	$C_7H_7N_3$	105.0578	C_7H_7N	117.044724	$C_7H_5N_2$	S	[172],[256]
37	Carbofuran metabolite (3-Keto-Carbofuran)	16709-30-1	$C_{12}H_{13}NO_4$	M+H	236.09173	208.060434	$C_{10}H_{10}NO_4$	186.990009	$C_9H_{10}NO_3$	151.07536	$C_9H_{11}O_2$	W	[193]
38	Carbofuran metabolite (Carbofuran-7-phenol)	1563-38-8	$C_{10}H_{12}O_2$	M+H	165.09100	135.044056	$C_8H_7O_2$	107.049141	C_7H_7O			W	[172], [255], [256]
39	Carboxin metabolite (Carboxin sulfoxide)	17757-70-9	$C_{12}H_{13}NO_3S$	M+H	252.06889	220.079061	$C_{12}H_{14}NOS$	196.042676	$C_9H_{10}NO_2S$	120.04439	C_7H_6NO	W	[172], [255], [256]
40	Chloridazon metabolite (Desphenyl Chloridazon)	6339-19-1	$C_4H_4ClN_3O$	M+H	146.01156	128.985017	$C_4H_2ClN_2O$	110.034888	$C_4H_4N_3O$	130.016651	$C_4H_5ClN_3$	W	[172], [255], [256]
41	Chloridazon metabolite (Methyl-desphenyl-chloridazon)	17254-80-7	$C_5H_6ClN_3O$	M+H	160.02721	143.000667	$C_5H_4ClN_2O$	124.050538	$C_5H_6N_3O$			W	[172], [255], [256]
42	Chlorpropham metabolite (HSA-4)		$C_{10}H_{12}ClNO_6S$	M+H	310.01466	292.004097	$C_{10}H_{11}ClNO_5S$	274.037984	$C_{10}H12NO_6S$	251.972797	$C_7H_7ClNO_5S$	W	[193]
43	Chlorthalonil metabolite [2-amido-3,5,6-trichlo-4-cyanobenzenesulphonic acid]		$C_8H_3O_4Cl_3N_2S$	M+H	328.895186	311.868637	$C_8HCl_3NO_4S$	301.884288	$C_7H_3Cl_3NO_4S$	292.918509	$C_8H_3Cl_2N_2O_4S$	W	[172], [255], [256]
44	Chlorthalonil metabolite [2,4-bis-amido-3,5,6-trichloro benzenesulfonic acid]		$C_8H_5Cl_3N_2O_5S$	M+H	346.90575							W	[172], [255], [256]
45	Chlorthalonil metabolite [4-amido-2,5-dichloro-6-cyano benzene-1,3-disulfonic acid]		$C_8H_4Cl_2N_2O_7S_2$	M+H	374.89097							W	[172], [255], [256]
46	Chlorthalonil metabolite [2,5-dichloro-4,6-dicyano-benzene-1,3-disulfonic acid]		$C_8H_2Cl_2N_2O_6S_2$	M+H	356.88040							W	[172], [255], [256]
47	Chlorthalonil (3-carbamyl-2,4,5-	142733-37-7	$C_8H_4Cl_3NO_3$	M+H	267.93295							W	[172], [255],

	trichlorobenzoic acid)												[256]
48	Chlorsulfuron metabolite (2-chlorobenzenesulfonamide)	6961-82-6	$C_6H_6ClNO_2S$	M+H	191.98805	156.011375	$C_6H_6NO_2S$	142.971675	C_6H_4ClS	110.999604	C_6H_4Cl	W	[172], [255], [256]
49	Chlorsulfuron metabolite [2-amino-4-methoxy-6-methyl-1,3,5-triazine]	1668-54-8	$C_5H_8N_4O$	M+H	141.07708	124.050538	$C_5H_6N_3O$	109.050873	$C_4H_5N_4$			W	[172], [255], [256]
50	Chlorsulfuron metabolite [N-[(N-carbamoylcarbamimidoyl)carbamoyl]-2-chlorobenzenesulfonamide]		$C_5H_8N_4O$	M+H	141.07708	124.050538	$C_5H_6N_3O$	109.050873	$C_4H_5N_4$			W	[172], [255], [256]
51	Chlorotoluron metabolite [3-(3-chloro-p-tolyl)-1-methylurea]	22175-22-0	$C_{10}H_{13}ClN_2O$	M+H	213.07892	168.021068	C_8H_7ClNO	163.08659	$C_9H_{11}N_2O$	161.07094	$C_9H_9N_2O$	S,W	[172],[256]
52	Chlorotoluron metabolite [2-Chloro-4-(3,3-dimethyl-ureido)-benzoic acid; 1-(3-Chlor-4-carboxyphenyl)-3,3-dimethylharnstoff]	59587-01-8	$C_{10}H_{11}ClN_2O_3$	M+H	243.05309	225.042531	$C_{10}H_{10}ClN_2O_2$	207.076419	$C_{10}H_{11}N_2O_3$	193.060769	$C_9H_9N_2O_3$	W	[172], [255], [256]
53	Chlorotoluron metabolite [Desmethylchlortoluron]	22175-22-0	$C_9H_{11}ClN_2O$	M+H	199.06326	168.021068	C_8H_7ClNO	163.08659	$C_9H_{11}N_2O$	142.041804	C_7H_9ClN	W	[172], [255], [256]
54	Chlorpyrifos metabolite (3,5,6-trichloro-2-pyridinol)	6515-38-4	$C_5H_2Cl_3NO$	M+H	197.92747	179.916908	C_5HCl_3N	161.950796	$C_5H_2Cl_2NO$	145.955881	$C_5H_2Cl_2N$	S	[172],[256]
55	Chlorpyrifos metabolite (dimethyl 3,5,6-trichloro-2-pyridylphosphate)	5598-52-7	$C_7H_7NO_4Cl_3P$	M+H	305.92510	273.89889	$C_6H_4O_3NCl_3P$	269.94843	$C_7H_7O_4NPCl_2$	179.91691	C_5HNCl_3	A	[172],[213]
56	Chlorpyrifos metabolite (Chlorpyrifos oxon)	5598-15-2	$C_9H_{11}Cl_3NO_4P$	M+H	333.95640	179.916908	C_5HCl_3N	297.979726	$C_9H_{11}Cl_2NO_4P$	241.917126	$C_5H_3Cl_2NO_4P$	W	[78], [172]
57	Chlorpyrifos-methyl metabolite(Dimethyl 3,5,6-trichloro-2-pyridinyl phosphate)		$C_7H_7Cl_3NO_4P$	M+H	305.92104	273.898889	$C_6H_4Cl_3NO_3P$	269.948426	$C_7H_7Cl_2NO_4P$	179.916909	C_5HCl_3N	A	[172],[213]
58	Chlorpyrifos-methyl metabolite(3,5,6-Trichloro-2(1H)-pyridinone)	6515-38-4	$C_5H_2NOCl_3$	M+H	197.92744	179.916908	C_5HCl_3N	161.950796	$C_5H_2Cl_2NO$	145.955881	$C_5H_2Cl_2N$	A	[172],[213]
59	Chlorpyrifos-methyl metabolite(Desmethyl chlorpyrifos)		$C_6H_5NO_4Cl_3P$	M+H	291.90945	259.883239	$C_5H_2Cl_3NO_3P$	255.932776	$C_6H_5Cl_2NO_4P$	179.916909	C_5HCl_3N	A	[172],[213]
60	Cymoxanil metabolite [3-ethyl-4-(methoxyamino)-2,5-dioxoimidazolidine-4-carbonitrile]	644972-55-4	$C_7H_{10}N_4O_3$	M+H	199.08257	172.071667	$C_6H_{10}N_3O_3$	153.040702	$C_5H_5N_4O_2$	152.045453	$C_6H_6N_3O_2$	S,W	[172],[256]
61	Cymoxanil metabolite[3-ethyl-4-(methoxyamino)-2,5-dioxoimidazolidine-4-carboxamide]	644972-61-2	$C_7H_{12}N_4O_4$	M+H	217.09313	200.066582	$C_7H_{10}N_3O_4$	170.056018	$C_6H_8N_3O_3$	127.050204	$C_5H_7N_2O_2$	S,W ,Sed	[172],[256]
62	Cyromazine metabolite (Ammelide)	645-93-2	$C_3H_4N_4O_2$	M+H	129.04070	112.014152	$C_3H_2N_3O_2$	111.030137	$C_3H_3N_4O$	112.014152	$C_3H_2N_3O_2$	W	[172], [255], [256]
63	Cyromazine metabolite (Ammeline)	645-92-1	$C_3H_5N_5O$	M+H	128.05668	111.030137	$C_3H_3N_4O$	110.046122	$C_3H_4N_5$	111.030137	$C_3H_3N_4O$	W	[172], [255], [256]
64	Cyromazine metabolite (Melamine)	108-78-1	$C_3H_6N_6$	M+H	127.07267	110.046121	$C_3H_4N_5$	68.0243237	$C_2H_2N_3$	110.046121	$C_3H_4N_5$	W	[172], [255], [256]
65	Cyproconazole metabolite (1,2,4-triazole)	288-88-0	$C_2H_3N_3$	M+H	70.039970							S, GW, Pl, An	[172],[256]
66	Cyproconazole metabolite(1H-1,2,4-triazol-1-ylacetic acid)	28711-29-7	$C_4H_5N_3O_2$	M+H	128.0455	110.034888	$C_4H_4N_3O$	98.0348883	$C_3H_4N_3O$	82.0399737	$C_3H_4N_3$	S, Pl, An	[172],[256]
67	Deltamethrin metabolite (Decamethrinic acid)	53179-78-5	$C_8H_{10}Br_2O_2$	M+H	296.91203	250.906552	$C_7H_9Br_2$	216.985869	$C_8H_{10}BrO_2$	113.059706	$C_6H_9O_2$	S	[172],[256]
68	Deltamethrin metabolite (3-phenoxybenzoic acid)	3739-38-6	$C_{13}H_{10}O_3$	M+H	215.07027	105.033491	C_7H_5O	169.064792	$C_{12}H_9O$	121.028406	$C_7H_5O_2$	S,W	[172],[256]
69	Desmedipham metabolite (EHPC)	6641-13-0	$C_9H_{11}NO_3$	M+H	182.081169	170.995094	$C_9H_{10}NO_2$	136.039305	$C_7H_6NO_2$	93.0334914	C_6H_5O	W	[172], [255], [256]
70	Diazinon metabolite (Pyrimidol)	557-01-7	$C_4H_4N_2O$	M+H	97.03964	79.0290747	$C_4H_3N_2$					W	[172],[256]

71	Diazinon metabolite (4-hydroxy-2-isopropyl-6-methylpyrimidine)	2814-20-2	$C_8H_{12}N_2O$	M+H	153.10224	137.070939	$C_7H_9N_2O$	135.091675	$C_8H_{11}N_2$	119.060375	$C_7H_7N_2$	An	[172],[256]
72	Diazinon metabolite(Diethylphosphate)	598-02-7	$C_4H_{11}O_4P$	M+H	155.04677	143.960696	$C_4H_{10}O_3P$	110.984172	CH_4O_4P	98.9841716	H_4O_4P	A	[172],[258]
73	Diazinon metabolite (2-Isopropyl-6-methyl-pyrimidinol)	2814-20-2	$C_8H_{12}N_2O$	M+H	153.10224	137.070939	$C_7H_9N_2O$	135.091675	$C_8H_{11}N_2$	121.039639	$C_6H_5N_2O$	W	[172], [255], [256]
74	Diazinon metabolite [2-(1-hydroxy-1-methyl)-ethyl-4-methyl-6-hydroxpyrimidine]	28175-97-5	$C_8H_{12}N_2O_2$	M+H	169.09715	151.086589	$C_8H_{11}N_2O$	109.039639	$C_5H_5N_2O$			W	[172], [255], [256]
75	Dicamba metabolite (3,6-dichloro-2-hydroxy benzoic acid)	3401-80-7	$C_7H_4Cl_2O_3$	M+H	206.96102	188.950461	$C_7H_3Cl_2O_2$	170.984348	$C_7H_4ClO_3$	160.955547	$C_6H_3Cl_2O$	W	[172], [255], [256]
76	Dichlobenil metabolite (2,6-Dichlorobenzamide)	2008-58-4	$C_7H_5Cl_2NO$	M+H	189.98209	154.005418	C_7H_5ClNO	144.960632	$C_6H_3Cl_2$	110.999604	C_6H_4Cl	W	[172], [255], [256]
77	Dichlofluanid metabolite [N,N-Dimethylaminosulfanilide]	4710-17-2	$C_8H_{12}N_2O_2S$	M+H	201.06922	156.011375	$C_6H_6NO_2S$	169.04301	$C_7H_9N_2OS$			W	[172], [255], [256]
78	Dichlorvos metabolite (2,2-Dichloroacetic acid)	79-43-6	$C_2H_2Cl_2O_2$	M+H	128.95046	110.939896	C_2HCl_2O	90.981943	C_2H_2ClNO			W	[172], [255], [256]
79	Dichlorvos metabolite (2,2 dichloro-acetaldehyde)	79-02-7	$C_2H_2Cl_2O$	M+H	112.95554	110.939896	C_2HCl_2O					W	[172], [255], [256]
80	Diclofop-methyl metabolite (diclofop-phenol)	40843-73-0	$C_{12}H_8Cl_2O_2$	M+H	254.99741	236.986846	$C_{12}H_7Cl_2O$	219.020734	$C_{12}H_8ClO_2$	160.955547	$C_6H_3Cl_2O$	W	[172], [255], [256]
81	Dimethachlor metabolite[N-(2,6-dimethylphenyl)-N-(2-methoxyethyl)oxalamic acid]	1086384-49-7	$C_{13}H_{17}NO_4$	M+H	252.12303	234.112469	$C_{13}H_{16}NO_3$	204.101905	$C_{12}H_{14}NO_2$	105.069877	C_8H_9	W	[172], [255], [256]
82	Dimethachlor metabolite [(2,6-Dimethylphenyl)-(2-methoxyethyl)-carbamoyl]-methanesulfonic acid]		$C_{13}H_{19}NO_5S$	M+H	302.10567	240.068890	$C_{11}H_{14}NO_3S$	220.133205	$C_{13}H_{18}NO_2$	192.138291	$C_{12}H_{18}NO$	W	[172], [255], [256]
83	Dimethachlor metabolite [(2,6-Dimethylphenylcarbamoyl)-methanesulfonic acid]		$C_{10}H_{13}NO_4S$	M+H	244.06380	162.091340	$C_{10}H_{12}NO$	134.096426	$C_9H_{12}N$	105.069877	C_8H_9	W	[172], [255], [256]
84	Dimethachlor metabolite [(2,6-Dimethyl-phenyl)-(2-sulfo-acetyl)-amino]-acetic acid]		$C_{12}H_{15}NO_6S$	M+H	302.06928	284.058719	$C_{12}H_{14}NO_5S$	240.068891	$C_{11}H_14NO_3S$	220.09682	$C_{12}H_{14}NO_3$	W	[172], [255], [256]
85	Dimethachlor metabolite [2-[(2-hydroxy-acetyl)-(2-methoxy-ethyl)-amino]3-methyl-benzoic acid]		$C_{13}H_{17}NO_5$	M+H	268.11794	250.107384	$C_{13}H_{16}NO_4$	192.06552	$C_{10}H_{10}NO_3$	135.044056	$C_8H_7O_2$	W	[172], [255], [256]
86	Dimethachlor metabolite [3-{2-[(2,6-dimethyl-phenyl)-(2-hydroxyacetyl)-amino]-ethylsulfanyl}-2-hydroxy-propionic acid]		$C_{15}H_{21}NO_5S$	M+H	328.12132	306.079455	$C_{15}H_{16}NO_4S$	134.096426	$C_9H_{12}N$	280.100191	$C_{14}H_{18}NO_3S$	W	[172], [255], [256]
87	Dimethachlor metabolite [(2,6-dimethylphenyl)-2-methoxyethyl)carbamoyl]methanesulfonic acid sodium salt]		$C_{13}H_{18}O_5NaNS$	M+H	324.08761	284.095105	$C_{13}H_{18}NO_4S$	268.100191	$C_{13}H_{18}NO_3S$	192.138291	$C_{12}H_{18}NO$	W	[172], [255], [256]
88	Dimethachlor metabolite [(2,6-dimethylphenylcarbamoyl)-methanesulfonic acid sodium salt]		$C_{10}H_{12}NNaO_4S$	M+H	266.04574	226.053240	$C_{10}H_{12}NO_3S$	210.058326	$C_{10}H_{12}NO_2S$	134.096426	$C_9H_{12}N$	W	[172], [255], [256]
89	Dimethenamid metabolite [sodium[(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]methanesulfonate]		$C_{12}H_{19}NO_5S_2$	M+H	322.07774							W	[172], [255], [256]

90	Dimethenamid metabolite [N-(2,4-dimethyl-thiophen-3-yl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid]	380412-59-9	$C_{12}H_{17}NO_4S$	M+H	272.09510							W	[172], [255], [256]
91	Dimethoate Metabolite(Omethoate)	1113-02-6	$C_5H_{12}NO_4PS$	M+H	214.02974	124.9821	$C_2H_6O_2PS$	154.9926	$C_3H_8O_3PS$	182.987542	$C_4H_8O_4PS$	S	[193]
92	Dimethomorph metabolite (4-chlorophenyl)(3,4-dimethoxyphenyl)methanone)	116412-84-1	$C_{15}H_{13}O_3Cl$	M+H	277.06259	245.036383	$C_{14}H_{10}ClO_2$	227.070271	$C_{14}H_{11}O_3$	215.025819	$C_{13}H_8ClO$	W	[172], [255], [256]
93	Dimethomorph metabolite (4-formylmorpholine)	113009-82-8	$C_5H_9O_2N$	M+H	116.07060	98.0600404	C_5H_8NO	71.0491414	C_4H_7O			W	[172], [255], [256]
94	Dimoxystrobin metabolite [(E)-0-[(2-hydroxycarbonyl-5-methyl)phenoxymethyl]-2-methoxyimino-Nmethylphenylacetamide]		$C_{19}H_{20}N_2O_5$	M+H	357.14449							W	[172], [255], [256]
95	Diuron metabolite [1-(3,4-dichlorophenyl)-3-methylurea]	3567-62-2	$C_8H_8Cl_2N_2O$	M+H	219.0086	204.992994	$C_7H_7Cl_2N_2O$	187.966446	$C_7H_4Cl_2NO$	159.97272		S	[172], [256]
96	Diuron metabolite (3,4-dichlorophenyl urea)	2327-02-8	$C_7H_6Cl_2N_2O$	M+H	204.99299	169.016317	$C_7H_6ClN_2O$	144.960632	$C_6H_3Cl_2$	161.987181	$C_6H_6Cl_2N$	S	[172], [256]
97	Diuron metabolite(3,4-dichloroaniline)	95-76-1	$C_6H_5Cl_2N$	M+H	161.98718	144.960632	$C_6H_3Cl_2$	126.010504	C_6H_5ClN	91.04165	C_6H_5N	S,An	[172], [256]
98	Ethiofencarb metabolite (Ethiofencarb-sulfoxide)	53380-22-6	$C_{11}H_{15}NO_3S$	M+H	242.08454	107.0491	C_7H_7O	185.0631	$C_9H_{13}O_2S$			S	[193]
99	Fenamiphos metabolite (fenamiphos-sulfone-phenol)		$C_8H_{10}O_3S$	M+H	187.04234	169.031776	$C_8H_9O_2S$	155.016127	$C_7H_7O_2S$	107.049141	C_7H_7O	S	[172], [255], [256]
100	Fenhexamid metabolite(Deschlorofenhexamid)	1335041-78-5	$C_{14}H_{19}NO_2$	M+H	234.14886	216.138290	$C_{14}H_{18}NO$	142.122641	$C_8H_{16}NO$	125.096092	$C_8H_{13}O$	S,W	[172], [256]
101	Fenhexamid metabolite(Biphenyl-fenhexamid)		$C_{28}H_{32}Cl_4N_2O_4$	M+H	601.11889	565.142217	$C_{28}H_{32}Cl_3N_2O_4$	317.904151	$C_{12}H_4Cl_4NO$	175.966446	$C_6H_4Cl_2NO$	S	[172], [256]
102	Fenitrothion metabolite(3-methyl-4-nitrophenol)	2581-34-2	$C_7H_7NO_3$	M+H	154.04987	136.039305	$C_7H_6NO_2$	124.051881	$C_7H_8O_2$	107.049141	C_7H_7O	S	[172], [256]
103	Fenitrothion metabolite(Dimethyl-3-methyl-4-nitrophenyl phosphate)	2255-17-6	$C_8H_{10}NO_6P$	M+H	248.03185	215.046771	$C_9H_{12}O_4P$	201.031122	$C_8H_{10}O_4P$	138.01857	$C_6H_4NO_3$	A	[73], [172]
104	Fenitrothion metabolite(Methyl 3-methyl-4-nitrophenyl hydrogen phosphate)	15930-84-4	$C_9H_{12}NO_6P$	M+H	262.04750	152.034219	$C_7H_6NO_3$	230.021285	$C_8H_9NO_5P$	201.031122	$C_8H_{10}O_4P$	A	[73], [172]
105	Fipronil metabolite(Fipronil amide)		$C_{12}H_6Cl_2F_6N_4O_2S$	M+H	454.95655	418.979869	$C_{12}H_6ClF_6N_4O_2S$	368.983063	$C_{11}H_6ClF_4N_4O_2S$	336.986527	$C_{11}H_6Cl_2F_3N_4O$	S	[172], [256]
106	Fipronil metabolite(Fipronil sulphide)	120067-83-6	$C_{12}H_4Cl_2F_6N_4S$	M+H	420.95107	403.924519	$C_{12}H_2Cl_2F_6N_3S$	393.940169	$C_{11}H_4Cl_2F_6N_3S$	318.975963	$C_{11}H_4Cl_2F_3N_4$	S	[172], [256]
107	Fipronil metabolite (Fipronil sulfone)	120068-36-2	$C_{12}H_4Cl_2F_6N_4O_2S$	M+H	452.94090	435.914348	$C_{12}H_2Cl_2F_6N_3O_2S$	425.929998	$C_{11}H_4Cl_2F_6N_3O_2S$	212.948017	$C_7H_2Cl_2F_3$	S	[193]
108	Fludioxonil meyabolite [3-Carbamoyl-2-cyano-3-(2,2-difluorobenzo[1,3]-dioxo-4-yl)-oxirane-2-carboxylic acid]		$C_{12}H_6F_2N_2O_6$	M+H	313.02666	296.000119	$C_{12}H_4F_2NO_6$	286.01577	$C_{11}H_6F_2NO_6$	268.005205	$C_{11}H_4F_2NO_5$	W	[172], [255], [256]
109	Fludioxonil meyabolite [2,2-difluorobenzo[1,3]dioxole-4-carboxylic acid]	126120-85-2	$C_8H_4F_2O4$	M+H	203.01504	185.004476	$C_8H_3F_2O_3$	157.009562	$C_7H_3F_2O_2$	137.023321	$C_7H_5O_3$	W	[172], [255], [256]
110	Fludioxonil meyabolite [4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile]		$C_{12}H_6F_2N_2O_4$	M+H	281.03686	254.025940	$C_{11}H_6F_2NO_4$	238.031026	$C_{11}H_6F_2NO_3$	215.045119	C11H7N2O3	W	[172], [255], [256]
111	Flufenacet metabolite [[(4-Fluorophenyl)isopropylcarbamoyl]-methanesulfonic acid]		$C_{11}H_{14}FNO_4S$	M+H	276.07003	242.064554	$C_{11}H_{14}NO_4S$	124.055704	C_7H_7FN			W	[172], [255], [256]
112	Flufenacet metabolite [1,3,4,-Thiadiazol-2(3h)-one, 5-(trifluoromethyl)-]	84352-75-0	$C_3HF_3N_2OS$	M+H	170.98344	142.98853	$C_2H_2F_3N_2S$	112.966732	C_2F_3S			W	[172], [255], [256]
113	Fluopicolide metabolite (3-Sulfo-5-trifluoromethylpyridine-2-carboxylic acid)		$C_7H_4F_3NO_5S$	M+H	271.98350	201.980469	$C_7H_3F_3NO_4S$	190.01104	$C_6H_4NO_5S$			W	[172], [255], [256]

114	Fluopicolide metabolite (dichlorbenzamide)	4659-54-4	$C_7H_5Cl_2NO$	M+H	189.98209	162.971197	$C_7H_3Cl_2O$	154.005418	$C_6H_5Cl_2O$			W	[172], [255], [256]
115	Fluopicolide metabolite (3-Chloro-5-trifluoromethylpyridine-2-carboxylic acid)	80194-18-9	$C_7H_5ClF_3NO_2$	M+H	228.00337	209.992802	$C_7H_4ClF_3NO$	192.02669	$C_7H_5F_3NO_2$	155.984683	$C_6H_3ClNO_2$	W	[172], [255], [256]
116	Flupyrsulfuron metabolite (Flupyrsulfuron-methyl)	144740-53-4	$C_{15}H_{14}F_3N_5O_7S$	M+H	466.06387	434.037664	$C_{14}H_{11}F_3N_5O_6S$	406.04275	$C_{13}H_{11}F_3N_5O_5S$	267.98859	$C_8H_5F_3N0_4S$	W	[172], [255], [256]
117	Fluquinconazole metabolite [3-(2,4-dichlorophenyl)-6-fluoro-quinazolin-2,4(3H)-dione]	168900-02-5	$C_{14}H_7Cl_2FN_2O_2$	M+H	324.99414	289.017459	$C_{14}H_7ClFN_2O_2$	281.988324	$C_{13}H_7Cl_2FNO$	144.960632	$C_6H_3Cl_2$	S	[172], [256]
118	Fluquinconazole metabolite (1,2,4-triazole)	288-88-0	$C_2H_3N_3$	M+H	70.03997							S,GW,Pl, An	[172], [256]
119	Flurtamone metabolite (2,2,2-Trifluoroacetic acid)	76-05-1	$C_2HF_3O_2$	M+H	115.00014	96.9895758	C_2F_3O	94.9939122	$C_2HF_2O_2$			W	[172], [255], [256]
120	Flusilazole metabolite [(bis(4-fluorophenyl)methyl silanol]	156162-13-9	$C_{13}H_{12}SiOF_2$	M+H	251.06982	233.059259	$C_{13}H_{11}F_2Si$	231.063596	$C_{13}H_{12}OFSi$	113.03972	C_6H_6FO	S,An	[172], [256]
121	Glufosinate metabolite (3-methylphosphinico-propionic acid)	15090-23-0	$C_4H_9O_4P$	M+H	153.03112	135.020557	$C_4H_8O_3P$	107.025643	$C_3H_8O_2P$	93.0099925	$C_2H_6O_2P$	W	[172], [255], [256]
122	Glyphosphate metabolite (Aminomethylphosphonic acid)	1066-51-9	CH_6NO_3P	M+H	112.01580	94.989257	CH_4O_3P	94.0052415	CH_5NO_2P			W	[172], [255], [256]
123	Haloxyfop metabolite (DE-535 pyridinol)	76041-71-9	$C_6H_3ClNOF_3$	M+H	197.99280	179.982238	$C_6H_2ClF_3N$	162.016125	$C_6H_3F_3NO$			W	[172], [255], [256]
124	Imazalil metabolite[1-(2,4-dichlorophenyl)2-imidazol-1-ylethanol]	24155-42-8	$C_{11}H_{10}Cl_2N_2O$	M+H	257.02429	239.013730	$C_{11}H_9Cl_2N_2$	227.972107	$C_{11}H_{10}ClN_2O$	188.986847	$C_8H_7Cl_2O$	S,W	[172], [256]
125	Imazalil metabolite (Methyl isothiocyanate)	556-61-6	C_2H_3NS	M+H	74.0059							S	[172], [256]
126	Imazosulfuron (2-Chloroimidazo[1,2-a]pyridin-3-sulfonamid)		$C_7H_6ClN_3O_2S$	M+H	231.99420	214.967652	$C_7H_4ClN_2O_2S$	196.017524	$C_7H_6N_3O_2S$	151.005752	$C_7H_4ClN_2$	W	[172], [255], [256]
127	Imidaclorprid metabolite [1-[(6-chloro-3-pyridinyl)methyl]N-nitro-1H-imidazol-2-amine]	115086-54-9	$C_9H_8ClN_5O_2$	M+H	254.04393	218.067251	$C_9H_8N_5O_2$	192.032302	$C_9H_7ClN_3$	126.010504	C_6H_5ClN	S,An , Pl	[172], [256]
128	Imidaclorprid metabolite (6-chloronicotinic acid)	5326-23-8	$C_6H_4ClNO_2$	M+H	158.00033	139.989768	C_6H_3ClNO	122.023655	$C_6H_4NO_2$	111.994853	C_5H_3ClN	W	[172], [256]
129	Iprodione metabolite [N-(3,5-dichlorophenyl)3-isopropyl-2,4-dioxoimidazoline-1-carboxamide]	63637-89-8	$C_{13}H_{13}Cl_2N_3O_3$	M+H	330.04067	294.063995	$C_{13}H_{13}ClN_3O_3$	283.998808	$C_{11}H_8Cl_2N_3O_2$	270.967174	$C_{10}H_5Cl_2N_2O_3$	W	[172], [255], [256]
130	Iprovalicarb metabolite(p-methyl-phenethylamine)	3261-62-9	$C_9H_{13}N$	M+H	136.11208	91.05393	C_7H_7					S	[172], [256]
131	Isoproturon metabolite (p-methyl-phenethylamine)	3261-62-9	$C_9H_{13}N$	M+H	136.11208	119.085526	C_9H_{11}	118.065126	C_8H_8N	105.069877	C_8H_9	S	[172], [256]
132	Isoproturon metabolite (Desmethylisoproturon)	34123-57-4	$C_{11}H_{16}N_2O$	M+H	193.13354	119.085527	C_9H_{11}	179.11789	$C_{10}H_{15}N_2O$	151.08650	$C_8H_{11}N_2O$	W	[256]
133	Kresoxim methyl metabolite [(E)-methoxyamino(alpha-(o-tolyloxy)-o-tolyl)acetic acid]		$C_{17}H_{17}NO_4$	M+H	300.12303							S,W	[172], [256]
134	Linuron metabolite (3,4-dichloroaniline)	95-76-1	$C_6H_5Cl_2N$	M+H	161.9872	144.960632	$C_6H_3Cl_2$	126.010504	C_6H_5ClN	91.0416508	C_6H_5N	S,An	[172], [256]
135	Malathion metabolite(ethyl ester-2-hydroxyl-3-thionyl 2-butenoic acid)		$C_8O_5SH_{12}$	M+H	221.04782	187.060099	$C_8H_{11}O_5$	161.026691	$C_6H_9O_3S$	151.005956	$C_4H_7O_4S$	W	[72], [172]
136	Malathion metabolite(Ethyl malate)	7554-12-3	$C_8H_{14}O_5$	M+H	221.04782	161.044449	$C_6H_9O_5$	157.085921	$C_8H_{13}O_3$	145.049535	$C_6H_9O_4$	W	[72], [172]
137	Malathion metabolite[(7S,8R,9S)-7,8,9-Trihydroxy-6-oxaspiro[4.5]dec-7-yl]methyl dihydrogen phosphate)		$C_{10}H_{19}O_8P$	M+H	299.08903	267.062815	$C_9H_{16}O_7P$	253.047166	$C_8H_{14}O_7P$	209.020951	$C_6H_{10}O_6P$	W	[72], [172]

138	Malathion metabolite(Diethylsuccinate)	123-25-1	$C_8H_{14}O_4$	M+H	175.09664	145.049535	$C_6H_9O_4$	121.085921	$C_5H_{13}O_3$			W	[72], [172]
139	Malathion metabolite(Dimethyl 2,3-dihydroxy-2,3-dimethylsuccinate)	15309-47-4	$C_8H_{14}O_6$	M+H	207.08631	177.039364	$C_6H_9O_6$	157.085921	$C_8H_{13}O_3$	145.049535	$C_6H_9O_4$	W	[72], [172]
140	Malathion metabolite (diethylmaleate)	141-05-9	$C_8H_{12}O_4$	M+H	173.08085	143.033885	$C_6H_7O_4$	129.054621	$C_6H_9O_3$	127.038971	$C_6H_7O_3$	W	[72], [172]
141	Malathion metabolite (Malathion dicarboxylic acid)	1190-28-9	$C_6H_{11}O_6PS_2$	M+H	274.98074	256.970177	$C_6H_{10}O_5PS_2$	226.959613	$C_5H_8O_4PS_2$	200.980349	$C_4H_{10}O_3PS_2$	S	[172], [256]
142	Malathion metabolite (Malathion monocarboxylic acid)	35884-76-5	$C_8H_{15}O_6PS_2$	M+H	303.01204	285.001477	$C_8H_{14}O_5PS_2$	270.985828	$C_7H_{12}O_5PS_2$	257.006563	$C_7H_{14}O_4PS_2$	S	[172], [256]
143	Malathion metabolite (Isomalathion)	3344-12-5	$C_{10}H_{19}O_6PS_2$	M+H	331.04334	124.982063	$C_2H_6O_2PS$	173.080835	$C_8H_{13}O_4$	236.998107	$C_7H_{10}O_5SP$	PChl	[172], [256]
144	Malathion metabolite [diethyl (dimethoxy-phosphoryl) succinate]		$C_{10}H_{19}O_8P$	M+H	299.08903	267.062815	$C_9H_{16}O_7P$	253.047166	$C_8H_{14}O_7P$	209.020951	$C_6H_{10}O_6P$	W	[72], [172]
145	Malathion metabolite [diethyl (methyl sulphonyl) succinate]		$C_9O_6H_{16}S$	M+H	253.07404	223.027085	$C_7H_{11}O_6S$	219.032171	$C_8H_{11}O_5S$	203.037256	$C_8H_{11}O_4S$	W	[72], [172]
146	Malathion metabolite [2-hydroxyl-3-thionyl-2-butene-diethylester]		$C_8H_{12}O_5S$	M+H	221.04782	203.037256	$C_8H_{11}O_4S$	187.0601	$C_8H_{11}O_5$	179.037256	$C_6H_{11}O_4S$	W	[72], [172]
147	Malathion metabolite (malaoxon)	1634-78-2	$C_{10}H_{19}O_7PS$	M+H	315.06618	129.054621	$C_6H_9O_3$	142.992628	$C_2H_8O_3PS$	127.01542	$C_2H_8O_4P$	A	[72], [172]
148	Mancozeb metabolite (Ethylenethiourea)	13966-32-0	$C_3H_6N_2S$	M+H	103.03244	77.0167955	CH_5N_2S					W	[172], [255], [256]
149	Mancozeb metabolite (Ethyleneurea)	120-93-4	$C_3H_6N_2O$	M+H	87.055289	61.0396394	CH_5N_2O					W	[172], [255], [256]
150	Metalaxyl metabolite[N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine]	467430-42-8	$C_{14}H_{19}NO_4$	M+H	266.13868	180.1015	$C_{10}H_{12}O_3$	95.08516	C_7H_{11}	213.15	$C_{14}H_{19}NO_4$	S	[172], [256]
151	Metalaxyl metabolite [(R)-2-[(2,6-dimethyl-phenyl)-methoxyacetyl-amino]-propionic acid]	75596-99-5	$C_{14}H_{19}NO_4$	M+H	266.13868	248.128119	$C_{14}H_{18}NO_3$	206.117555	$C_{12}H_{16}NO_2$	164.106991	$C_{10}H_{14}NO$	W	[172], [255], [256]
152	Metalaxyl metabolite [2-[(1-carboxyethyl)-methoxyacetyl-amino]-3-methyl-benzoic acid]	104390-56-9	$C_{14}H_{17}NO_6$	M+H	296.11286	278.102299	$C_{14}H_{16}NO_5$	252.123035	$C_{13}H_{18}NO_4$			W	[172], [255], [256]
153	Metamitron metabolite [4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one]	36993-94-9	$C_{10}H_9N_3O$	M+H	188.08183	162.066188	$C_8H_8N_3O$	145.076025	$C_9H_9N_2$			W	[172], [255], [256]
154	Metazachlor metabolite [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)aminocarbonylmethylsulfonic acid]	172960-62-2	$C_{14}H_{17}N_3O_4S$	M+H	324.10125	290.095774	$C_{14}H_{16}N_3O_2S$	256.063805	$C_{11}H_{14}NO_4S$			W	[172], [255], [256]
155	Metazachlor metabolite [methyl N-(2,6-dimethylphenyl)-N-(1Hpyrazol-1-ylmethyl)aminocarbonylmethylsulfoxide]		$C_{15}H_{19}N_3O_2S$	M+H	306.12707	242.128788	$C_{14}H_{16}N_3O$	238.089626	$C_{12}H_{16}NO_2S$			W	[172], [255], [256]
156	Metazachlor metabolite [N-[(2-hydroxycarbonyl-6-methyl)phenyl]-N-(1-H-pyrazol-1-ylmethyl)oxalamide]		$C_{14}H_{13}N_3O_5$	M+H	304.09279	286.082232	$C_{14}H_{12}N_3O_4$	244.071668	$C_{12}H_{10}N_3O_3$			W	[172], [255], [256]
157	Metazachlor metabolite [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)aminocarbonylmethylsulfinyl acid]		$C_{16}H_{19}N_3O_4S$	M+H	350.11690	322.121988	$C_{15}H_{20}N_3O_3S$	304.111424	$C_{15}H_{18}N_3O_2S$			W	[172], [255], [256]
158	Metazachlor metabolite [N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)oxalamide]		$C_{14}H_{15}N_3O_3$	M+H	274.11861	256.108053	$C_{14}H_{14}N_3O_2$	230.128789	$C_{13}H_{16}N_3O$			W	[172], [255], [256]
159	Methiocarb Metabolite(Methiocarb-sulfone)	2179-25-1	$C_{11}H_{15}NO_4S$	M+H	258.07945	201.058	$C_9H_{13}O_3S$	122.0726	$C_8H_{10}O$			S	[193]
160	Methiocarb Metabolite(Methiocarb-	2635-10-1	$C_{11}H_{15}NO_3S$	M+H	242.08454	185.0631	$C_9H_{13}O_2S$	170.0396	$C_8H_{10}O_2S$			S	[193]

	sulfoxide)												
161	Methyl-Thiophanate metabolite (Carbendazim)	10605-21-7	$C_9H_9N_3O_2$	M+H	192.07675	160.05035	$C_8H_6N_3O$					S	[193], [256]
162	Metolachlor metabolite(Metolachlor ethane sulfonic acid)	171118-09-5	$C_{15}H_{23}NO_5S$	M+H	330.13697	316.12132	$C_{14}H_{22}NO_5S$	310.110755	$C_{15}H_{20}NO_4S$	268.100191	$C_{13}H_{18}NO_3S$	S	[172], [256]
163	Metolachlor metabolite(Metolachlor oxanilic acid)	152019-73-3	$C_{15}H_{21}NO_4$	M+H	280.15433	262.14377	$C_{15}H_{20}NO_3$	246.11247	$C_{14}H_{16}NO_3$	194.08117	$C_{10}H_{12}NO_3$	S,W	[172], [256]
164	Metolachlor metabolite [(S)-2-[(Oxalyl)(2-ethyl-6-methylphenyl)amino]propionic acid]		$C_{14}H_{17}NO_5$	M+H	280.11794	266.102299	$C_{13}H_{16}NO_5$	244.09682	$C_{14}H_{14}NO_3$	218.117555	$C_{13}H_{16}NO_2$	W	[172], [255], [256]
165	Metolachlor metabolite [(2-Ethyl-6-methylphenyl)-carbamoyl]-methanesulfonic acid]		$C_{11}H_{15}NO_4S$	M+H	258.07945	244.063805	$C_{10}H_{14}NO4S$	224.073976	$C_{11}H_{14}NO_2S$	176.106991	$C_{11}H_{14}NO$	W	[172], [255], [256]
166	Metolachlor metabolite [N-(2-Ethyl-6-methylphenyl)-2-hydroxyacetamide]	97055-05-5	$C_{11}H_{15}NO_2$	M+H	194.11755	176.106990	$C_{11}H_{14}NO$	148.112076	$C_{10}H_{14}N$	136.112076	$C_9H_{14}N$	W	[172], [255], [256]
167	Metolachlor metabolite [2-[((S)-1-Carboxyethyl)(2-ethyl-6-methylphenyl)amino]2-oxo-ethanesulfonic acid]		$C_{14}H_{19}NO_6S$	M+H	330.10058	316.084934	$C_{13}H_{18}NO_6S$	312.09002	$C_{14}H_{18}NO_5S$	232.133205	$C_{14}H_{18}NO_2$	W	[172], [255], [256]
168	Metolachlor metabolite [N-(2-Ethyl-6-methylphenyl)-L-alanine]	82508-03-0	$C_{12}H_{17}NO_2$	M+H	208.13320	194.117555	$C_{11}H_{16}NO_2$	148.112076	$C_{10}H_{14}N$	135.10420	$C_9H_{13}N$	W	[172], [255], [256]
169	Metolachlor metabolite [N-(2-Ethyl-6-methylphenyl)-oxalamic acid]	152019-74-4	$C_{11}H_{13}NO_3$	M+H	208.09682	190.086255	$C_{11}H_{12}NO_2$	164.106991	$C_{10}H_{14}NO$	136.112076	$C_9H_{14}N$	W	[172], [255], [256]
170	Metolachlor metabolite [2-[((S)-1-Carboxyethyl)(2-ethyl-6-methylphenyl)amino]-2-oxo-ethanesulfonic acid disodium salt]	1418095-19-8	$C_{14}H_{17}NNa_2O_6S$	M+H	374.06447	356.053909	$C_{14}H_{16}NNa_2O_5S$	346.033174	$C_{12}H_{14}NNa_2O_6S$	280.0614	$C_{11}H_{15}NNaO_4S$	W	[172], [255], [256]
171	Myclobutanil metabolite(1,2,4-triazole)	288-88-0	$C_2H_3N_3$	M+H	70.03997							S, GW, Pl, An	[256]
172	Myclobutanil metabolite (1H-1,2,4-triazol-1-ylacetic acid)	28711-29-7	$C_4H_5N_3O_2$	M+H	128.04545	98.0348883	$C_3H_4N_3O$	82.0399737	C3H4N3	112.050538	C4H6N3O	S,An ,Pl	[172], [256]
173	Molinate metabolite (Molinate sulfoxide)	52236-29-0	$C_9H_{17}NO_2S$	M+H	204.10528	174.058325	$C_7H_{12}NO_2S$	147.034851	$C_5H_9NO_2S$	126.091341	$C_7H_{12}NO$	S	[172], [256]
174	Napropamide metabolite [α-naphthol-2-methyl-naphthol(1,2-b)-2Hfuran-3-one]		$C_{13}H_{10}O_2$	M+H	199.07535	161.059706	$C_{10}H_9O_2$	143.049141	$C_{10}H_7O$	127.054227	$C_{10}H_7$	W	[172], [255], [256]
175	Napropamide metabolite [(N,N,N',N',Tetraethyl -4.4'-dihydroxyalpha,alpha'-2'-dimethyl[1,1-binaphthalene]-3,3'-diacetamide]		$C_{34}H_{40}N_2O_4$	M+H	541.30608	495.264219	$C_{32}H_{35}N_2O_3$	468.216935	$C_{30}H_{30}NO_4$	414.20637	$C_{27}H_{28}NO_3$	W	[172], [255], [256]
176	Napropamide metabolite [N,N-diethyl-4-hydroxy-α-methyl-2-naphthaleneacetamide]		$C_{17}H_{21}NO_2$	M+H	272.16450	256.133205	$C_{16}H_{18}NO_2$	254.153941	$C_{17}H_{20}NO$	199.075356	$C_{13}H_{11}O_2$	W	[172], [255], [256]
177	Napropamide metabolite [N,N-diethyl-4-hydroxy-α-methyl-1-naphthaleneacetamide]		$C_{17}H_{21}NO_2$	M+H	272.16450	254.15394	$C_{17}H_{20}NO$	216.10191	$C_{13}H_{14}NO_2$	199.07536	$C_{13}H_{11}O_2$	W	[172], [255], [256]
178	Paclobutrazol metabolite [4H-1,2,4-triazol-3-ol]	122442-66-4	$C_2H_3N_3O$	M+H	86.034888	68.0243237	$C_2H_2N_3$					W	[172], [255], [256]
179	Paclobutrazol metabolite [(2RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 triazol-1-yl) pentan-3-one]	63190-87-4	$C_{15}H_{18}ClN_3O$	M+H	292.12111	262.074166	$C_{13}H_{13}ClN_3O$	256.144439	$C_{15}H_{18}N_3O$	223.088419	$C_{13}H_{16}ClO$	W	[172], [255], [256]
180	Pethoxamid metabolite [N-(2-Ethoxyethyl)-N-(2-methyl-1-		$C_{16}H_{23}NO_5S$	M+H	342.13697	308.131490	$C_{16}H_{22}NO_3S$	300.09002	$C_{13}H_{18}NO_5S$	270.079455	$C_{12}H_{16}NO_4S$	W	[172], [255], [256]

	phenylpropenyl)-2-sulfoacetamide]												
181	Phenmedipham metavbolite [Methyl-N-(3-hydropxyphenyl)-carbamate]	13683-89-1	$C_8H_9NO_3$	M+H	168.06551	150.054955	$C_8H_8NO_2$	110.06004	C_6H_8NO	135.03148	$C_7H_5NO_2$	W	[172], [255], [256]
182	Picolinafen metabolite [6-[(3-trifluoromethylphenoxy)picolinic acid]	137640-84-7	$C_{13}H_8F_3NO_3$	M+H	284.05290	237.0396	$C_{12}H_6F_3NO$	214.04987	$C_{12}H_8NO_3$	198.054955	$C_{12}H_8NO_2$	W	[172], [255], [256]
183	Pinoxaden metabolite [8-(2,6-diethyl-4-methyl-phenyl)-tetrahydropyrazolo[1,2-d][1,4,5]oxadiazepine-7,9-dione]	314020-44-5	$C_{18}H_{24}N_2O_4$	M+H	333.180883	315.170319	$C_{18}H_{23}N_2O_3$	275.139019	$C_{15}H_{19}N_2O_3$	177.127392	$C_{12}H_{17}O$	W	[172], [255], [256]
184	Pirmicarb metabolite [2-amino-5,6-dimethylpyrimidin-4-yldimethylcarbamate]		$C_9H_{14}N_4O_2$	M+H	211.11895	195.087652	$C_8H_{11}N_4O_2$	182.092403	$C_8H_{12}N_3O_2$	124.050538	$C_5H_6N_3O$	W	[172], [255], [256]
185	Pirmicarb metabolite [5,6-dimethyl-2-(methylamino)pyrimidin-4-ol]	78195-30-9	$C_7H_{11}N_3O$	M+H	154.09748	152.081838	$C_7H_{10}N_3O$	123.055289	$C_6H_7N_2O$	135.079099	$C_7H_9N_3$	W	[172], [255], [256]
186	Pirmicarb metabolite [2-dimethylamino-5,6-dimethylpyrimidin-4-ol]	40778-16-3	$C_8H_{13}N_3O$	M+H	168.11313	150.102574	$C_8H_{12}N_3$	136.050538	$C_6H_6N_3O$	124.063114	$C_6H_8N_2O$	W	[172], [255], [256]
187	Propachlor metabolite (propachlor sulphonic acid)		$C_{11}H_{15}NO_4S$	M+H	258.07945	224.073975	$C_{11}H_{14}NO_2S$	176.106991	$C_{11}H_{14}NO$	160.112076	$C_{11}H_{14}N$	W	[172], [255], [256]
188	Propachlor metabolite (propachlor sulphinylacetic acid)		$C_{13}H_{17}NO_4S$	M+H	284.09510							W	[172], [255], [256]
189	Propachlor metabolite (propachlor oxanilic acid)	70628-36-3	$C_{11}H_{13}NO_3$	M+H	208.09682	190.086255	$C_{11}H_{12}NO_2$	150.054955	$C_8H_8NO_2$	132.06552	$C_5H_{10}NO_3$	W	[172], [255], [256]
190	Propachlor metabolite (propachlor alcohol)	42404-06-8	$C_{11}H_{15}NO_2$	M+H	194.11756	152.070605	$C_8H_{10}NO_2$	163.06278	$C_9H_9NO_2$	132.080776	$C_9H_{10}N$	W	[172], [255], [256]
191	Propaquizafop metabolite (Quizalofop)	76578-12-6	$C_{17}H_{13}ClN_2O_4$	M+H	345.06366	327.053096	$C_{17}H_{12}ClN_2O_3$	309.086983	$C_{17}H_{13}N_2O_4$	165.054621	$C_9H_9O_3$	W	[193]
192	Propazine Metabolite(hydroxypropazine)	7374-53-0	$C_9H_{17}N_5O$	M+H	212.15059	196.00		112.00				S	[172], [256]
193	Propineb metabolite (4-Methyl-imidazolidin-2-thione)	2122-19-2	$C_4H_8N_2S$	M+H	117.04809	101.016795	$C_3H_5N_2S$	82.0525498	C_4H_6N2			W	[172], [255], [256]
194	Propyzamide metabolite (2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methylene-2-oxazoline)		$C_{12}H_{11}Cl_2NO$	M+H	256.02904	220.052368	$C_{12}H_{11}ClNO$	201.982096	$C_8H_6Cl_2NO$	162.971197	$C_6H_5Cl_2O$	W	[172], [255], [256]
195	Propyzamide metabolite [3,5-dichloro-N-(1,1-dimethyl)-2-oxo-npropyl)benzamide]	29939-97-7	$C_{12}H_{13}Cl_2NO_2$	M+H	274.03961	252.066006	$C_{12}H_{13}ClN_2O_2$	230.013396	$C_{10}H10Cl_2NO$	203.997746	$C_8H_8Cl_2NO$	W	[172], [255], [256]
196	Prothioconazole metabolite (1H-1,2,4-Triazole-1-ethanol, alpha-(1-chlorocyclopropyl)-alpha-(2-chlorophenyl)methyl)		$C_{14}H_{15}Cl_2N_3O$	M+H	312.06649	294.055929	$C_{14}H_{14}Cl_2N_3$	284.035194	$C_{12}H_{12}Cl_2N_3O$	211.007582	$C_{11}H_9Cl_2$	W	[172], [255], [256]
197	Pyraflufen-ethyl metabolite [2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetate]	129630-17-7	$C_{13}H_9Cl_2F_3N_2O_4$	M+H	384.99642	366.985858	$C_{13}H_8Cl_2F_3N_2O_3$	349.019746	$C_{13}H_9ClF_3N_2O_4$	316.989052	$C_{12}H_8Cl_2FN_2O_3$	W	[172], [255], [256]
198	Pyraflufen-ethyl metabolite [2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazole-3yl)-4-fluorophenol]		$C_{11}H_7Cl_2F_3N_2O_2$	M+H	326.99094							W	[172], [255], [256]
199	Pyridaben metabolite [2-tert-butyl-4-(4-tertbutylbenzoyl)pyridazin-3(2H)-one-5-sulfonic acid]		$C_{19}H_{24}N_2O_5S$	M+H	393.14786							W	[172], [255], [256]
200	Pyridaben metabolite [2-tert-butyl-5-(4-tert-butylbenzylsulfinyl)-4-chloropyridazin-3(2H)-one]		$C_{19}H_{25}ClN_2O_2S$	M+H	381.13980	307.066638	$C_{15}H_{16}ClN_2OS$	247.030253	$C_9H_{12}ClN_2O_2S$	233.014603	$C_8H_{10}ClN_2O_2S$	W	[172], [255], [256]
201	Pyridaben metabolite [2-tert-butyl-5-[4-(1-carboxy-1-methylethyl)benzylthio]-4-		$C_{19}H_{23}ClN_2O_3S$	M+H	395.11906							W	[172], [255], [256]

	chloropyridazin-3(2H)-one]												
202	Pyridate metabolite (6-chloro-3-phenylpyridazin-4-ol)	40020-01-7	$C_{10}H_7ClN_2O$	M+H	207.03197	104.0495	C_7H_6N	189.021403	$C_{10}H_6ClN_2$	171.055289	$C_{10}H_7N_2O$	W	[73], [172]
203	Pyrimiphos methyl metabolite [Phosphoric acid, 2-(diethylamino)-5-methyl-4-pyrimidinyl dimethyl ester]		$C_{11}H_{20}N_3O_4P$	M+H	290.12641	274.095119	$C_{10}H_{17}N_3O_4P$	258.100204	$C_{10}H_{17}N_3O_3P$	219.05292	$C_7H_{12}N_2O_4P$	A	[172],[213]
204	Pyrimiphos methyl metabolite[2-diethylamino-6-methyl-4-pyrimidinol]	42487-72-9	$C_9H_{15}N_3O$	M+H	182.12879	166.097488	$C_8H_{12}N_3O$	164.118224	$C_9H_{14}N_3$	150.102574	$C_8H_{12}N_3$	A	[172],[213]
205	Pyrimiphos methyl metabolite (4-[4-(Diethoxyphosphino)-1H-1,2,3-triazol-1-yl]butanoic acid)		$C_{10}H_{18}N_3O_4P$	M+H	276.11077	244.084554	$C_9H_{15}N_3O_3P$	228.053254	$C_8H_{11}N_3O_3P$	215.045429	$C_7H_{10}N_3O_3P$	A	[172],[213]
206	Pyroxsulam metabolite (Aminotriazole)	61-82-5	$C_9H_9F_3N_6O_3S$	M+H	339.04817	319.041941	$C_9H_9F_2N_6O_3S$	292.047442	$C_9H_9F_3N_5OS$	172.017524	$C_5H_6N_3O_2S$	W	[172], [255], [256]
207	Pyroxsulam metabolite (5,7-Dihydroxy-Pyroxsulam)		$C_{12}H_9F_3N_6O_5S$	M+H	407.03799	375.011784	$C_{11}H_6F_3N_6O_4S$	337.034965	$C_{11}H_9N_6O_5S$	225.978025	$C_6H_3F_3NO_3S$	W	[172], [255], [256]
208	Pyroxsulam metabolite (7-Hydroxy-Pyroxsulam)		$C_{13}H_{11}F_3N_6O_5S$	M+H	421.05363	389.027434	$C_{12}H_8F_3N_6O_4S$	351.050615	$C_{12}H_{11}N_6O_5S$	208.975285	$C_6H_2F_3NO_2S$	W	[172], [255], [256]
209	Quinmerac metabolite (7-chloro-2-hydroxy-3-methylquinoline-8-carboxylic acid)		$C_{11}H_8ClNO_3$	M+H	238.02654	220.015982	$C_{11}H_7ClNO_2$	202.04987	$C_{11}H_8NO_3$	192.021068	$C_{10}H_7ClNO$	W	[172], [255], [256]
210	Quinmerac metabolite (7-chloro-3,8-quinoline-dicarboxylic acid)	90717-07-0	$C_{11}H_6ClNO_4$	M+H	252.00581	233.995247	$C_{11}H_5ClNO_3$	216.029134	$C_{11}H_6NO_4$	154.989434	$C_7H_4ClO_2$	W	[172], [255], [256]
211	Simazine metabolite (6-deisopropyl atrazine)	1007-28-9	$C_5H_8ClN_5$	M+H	174.05410	157.027550	$C_5H_6ClN_4$	146.022799	$C_3H_5ClN_5$	111.053947	$C_3H_5N_5$	S	[172], [256]
212	Simazine metabolite (2-hydroxy-4,6-bis(ethlyamino)-triazine)	2599-11-3	$C_7H_{13}N_5O$	M+H	184.11929	166.108722	$C_7H_{12}N_5$	139.061437	$C_5H_7N_4O$	111.030137	$C_3H_3N_4O$	S	[172], [256]
213	Spirodiclofen metabolite (3-(2,4-dichlorophenyl)-4-hydroxy-1-oxaspiro[4,5]dec-3-en-2-one)	148476-22-6	$C_{15}H_{14}Cl_2O_3$	M+H	313.03927	277.062598	$C_{15}H_{14}ClO_3$	242.961026	$C_{10}H_5Cl_2O_3$	158.976282	$C_7H_5Cl_2$	W	[172], [255], [256]
214	Sulcotrione metabolite (2-chloro-4-(methylsulfonyl)-benzoic acid)	53250-83-2	$C_8H_7ClO_4S$	M+H	234.98263	199.005955	$C_8H_7O_4S$	188.977154	$C_7H_6ClO_2S$	171.011041	$C_7H_7O_3S$	W	[172], [255], [256]
215	Tebuconazole metabolite (1,2,4-triazole)	288-88-0	$C_2H_3N_3$	M+H	70.03997							S, GW, Pl, An	[172], [256]
216	Tebufenozide metabolite[4-(N'-(3,5-dimethylbenzoyl-N-(1,1-dimethylethyl)hydrazinocarbonyl)phenyl acetic acid]		$C_{22}H_{26}N_2O_4$	M+H	383.19653	323.175404	$C_{20}H_{23}N_2O_2$	281.128454	$C_{17}H_{17}N_2O_2$	165.054621	$C_9H_9O_3$	S,W, Pl	[172], [256]
217	Tebufenozide metabolite [N-(1,1-dimethyethyl)-N-(4-acetylebenzoyl)-3,5-dimethylbenzohydrazine]		$C_{22}H_{26}N_2O_3$	M+H	367.20162	323.175404	$C_{20}H_{23}N_2O_2$	309.123369	$C_{18}H_{17}N_2O_3$	149.059706	$C_9H_9O_2$	S, GW, Pl, An	[172], [256]
218	Tepraloxydim metabolite [(RS)-2-ethyl-6,7-dihydro-6-perhydropyran-4-ylbenzoxazol-4-(5H)-one]		$C_{14}H_{21}NO_3$	M+H	252.15492	209.153606	$C_{13}H_{21}O_2$	197.117221	$C_{11}H_{17}O_3$	179.106656	$C_{11}H_{15}O_2$	W	[172], [255], [256]
219	Tepraloxydim metabolite [3-hydroxy-2-(1-iminopropyl)-5-perhydropyran-4-ylcyclohex-2-en-1-one]		$C_{14}H_{21}NO_3$	M+H	252.15942	235.132871	$C_{14}H_{19}O_3$	224.12812	$C_{12}H_{18}NO_3$	179.106656	$C_{11}H_{15}O_2$	W	[172], [255], [256]
220	Terbuthylazine Metabolite(Terbuthylazine-2-hydroxy)	66753-07-9	$C_9H_{17}N_5O$	M+H	212.15059	156.08802	$C_5H_{10}N_5O$	114.06604	$C_4H_8N_3O$	86.03456	$C_2H_4ON_3$	S,Sed,W	[192], [256]
221	Terbuthylazine Metabolite(Desethyl-terbuthylazine)	30125-63-4	$C_7H_{12}ClN_5$	M+H	202.0854	185.058850	$C_7H_{10}ClN_4$	166.108722	$C_7H_{12}N_5$	128.99625	$C_3H_2ClN_4$	S,Sed,W	[172], [256]
222	Tetraconazole metabolite [2-(2,4-		$C_{13}H_{12}Cl_2N_6O$	M+H	339.05224	254.024629	$C_{11}H_{10}Cl_2N_3$	236.058516	$C_{11}H_{11}ClN_3O$	172.991932	$C_8H_7Cl_2$	S,An	[172], [256]

	dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-1-ol]												
223	Tetraconazole metabolite [2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propanoic acid]		$C_{11}H_9Cl_2N_3O_2$	M+H	286.01446	268.003893	$C_{11}H_8Cl_2N_3O$	250.037781	$C_{11}H_9ClN_3O_2$	216.981761	$C_9H_7Cl_2O_2$	S,An	[172], [256]
224	Tetraconazole metabolite [1H-1,2,4-triazol-1-ylacetic acid]	28711-29-7	$C_4H_5N_3O_2$	M+H	128.04545	98.0348883	$C_3H_4N_3O$	82.0399737	$C_3H_4N_3$	112.050538	$C_4H_6N_3O$	S,An, Pl	[172], [256]
225	Thiacloprid metabolite (Thiacloprid sulfonic acid)		$C_{10}H_{13}ClN_4SO_5$	M+H	440.97241	303.031315	$C_{10}H_{12}ClN_4O_3S$	301.060117	$C_{10}H_{13}N_4SO_5$	229.04868	$C_8H_{10}ClN_4O_2$	S	[172], [256]
226	Thiacloprid metabolite (Thiacloprid-amide)	676228-91-4	$C_{10}H_{11}ClN_4OS$	M+H	271.04149	235.064808	$C_{10}H_{11}N_4OS$	167.037053	$C_8H_8ClN_2$	158.038259	$C_5H_8N_3OS$	S	[172], [256]
227	Thiamethoxam metabolite (Clothianidim)	210880-92-5	$C_6H_8ClN_5O_2S$	M+H	250.01600	131.96660	C_4H_3NClS	217.9897847	$C_5H_5ClN_5OS$	214.039322	$C_6H_8N_5O_2S$	S	[193]
228	Tolclofos-methyl metabolite (2,6-Dichloro-4-methylphenyl dimethyl phosphate)		$C_9H_{11}Cl_2O_4P$	M+H	284.98447	252.958262	$C_8H_8Cl_2O_3P$	249.0078	$C_9H_{11}ClO_4P$	160.955547	$C_6H_3Cl_2O$	A	[74], [172]
229	Tolclofos-methyl metabolite (1,4-dichloro-3-methylphenol)	17788-00-0	$C_7H_6Cl_2O$	M+H	176.98685	158.976282	$C_7H_5Cl_2$	126.994519	C_6H_4ClO	141.010169	C_7H_6ClO	A	[74], [172]
230	Tolclofos-methyl metabolite (Desmethyl tolclofos)		$C_8H_9Cl_2O_4P$	M+H	270.98685	234.992149	$C_8H_9ClO_4P$	202.965935	$C_7H_5ClO_3P$	158.976282	$C_7H_5Cl_2$	A	[74], [172]
231	Tolyfluanid metabolite(DMST)	66840-71-9	$C_9H_{14}N_2O_2S$	M+H	215.08487	170.027025	$C_7H_8NO_2S$	183.05866	$C_8H_{11}N_2O_S$	91.0542268	C_7H_7	W	[193]
232	Tolyfluanid metabolite (N,N-Dimethylsulfamide)	4315-09-7	$C_2H_8N_2O_2S$	M+H	125.03792	108.011375	$C_2H_6NO_2S$	93.9957256	CH_4NO_2S			W	[172], [255], [256]
233	Triclorfom Metabolite(Dichlorvos)	62-73-7	$C_4H_7Cl_2O_4P$	M+H	220.95318	127.0155	$C_2H_8O_4P$	109.0049	$C_2H_6O_3P$	188.926962	$C_3H_4Cl_2O_3P$	S	[193]
234	Triclorfom Metabolite(Desmethyl dichlorvos)	17650-82-7	$C_2H_3Cl_2O_4P$	M+H	192.92188	174.911312	$C_2H_2Cl_2O_3P$	138.934635	C_2HClO_3P	80.973607	H_2O_3P	S,W	[172], [256]
235	Triclorfom metabolite (Dichlorivinylphosphate)		$C_2HCl_2O_4P$	M+H	190.90623							S	[172], [256]
236	Triclorfom metabolite (Dichloroethanol)	598-39-8	$C_2H_4Cl_2O$	M+H	114.97120	61.9917794	C_2H_3Cl	78.994519	C_2H_4ClO			S	[172], [256]
237	Trifluralin metabolite [3-nitro-N2,N2-dipropyl-5-(trifluoromethyl)benzene-1,2-diamine]	2078-04-8	$C_{13}H_{18}F_3N_3O_2$	M+H	306.14239	289.115838	$C_{13}H_{16}F_3N_2O_2$	259.14166	$C_{13}H_{18}F_3N_2$	205.021939	$C_7H_4F_3N_2O_2$	S,W, Sed	[172], [256]
238	Tritosulfuron metabolite (2-trifluoromethyl-benzenesulfonamide)	1869-24-5	$C_7H_6F_3NO_2S$	M+H	226.01441	208.987861	$C_7H_4F_3O_2S$	156.011376	$C_6H_6NO_2S$	145.025961	$C_7H_4F_3$	W	[172], [255], [256]
239	Vinclozolin metabolite (3,5-dichlorophenylcarbamic acid-(1-carboxyl-1-methyl)-2-propenyl-ester)		$C_{12}H_{11}Cl_2NO_4$	M+H	304.01378	268.037112	$C_{12}H_{11}ClNO_4$	254.021462	$C_{11}H_9ClNO_4$	205.97701	$C_7H_6Cl_2NO_2$	W	[172], [255], [256]
240	Vinclozolin metabolite (N-(3,5-dichlorophenyl)-2-hydroxy-2-methyl-3-butenic acid-amide)		$C_{11}H_{11}Cl_2NO_2$	M+H	260.02396	230.013395	$C_{10}H_{10}Cl_2NO$	224.047283	$C_{11}H_{11}ClNO_2$	154.005418	C_7H_5ClNO	W	[172], [255], [256]

(*) Matrix where the metabolite was found in the literature
A= Air
W=Water
S=Soil
Pl=Plants
An=Animals
Hu=Humans
GW=Groundwater
PhChI= Photochemical Indicator

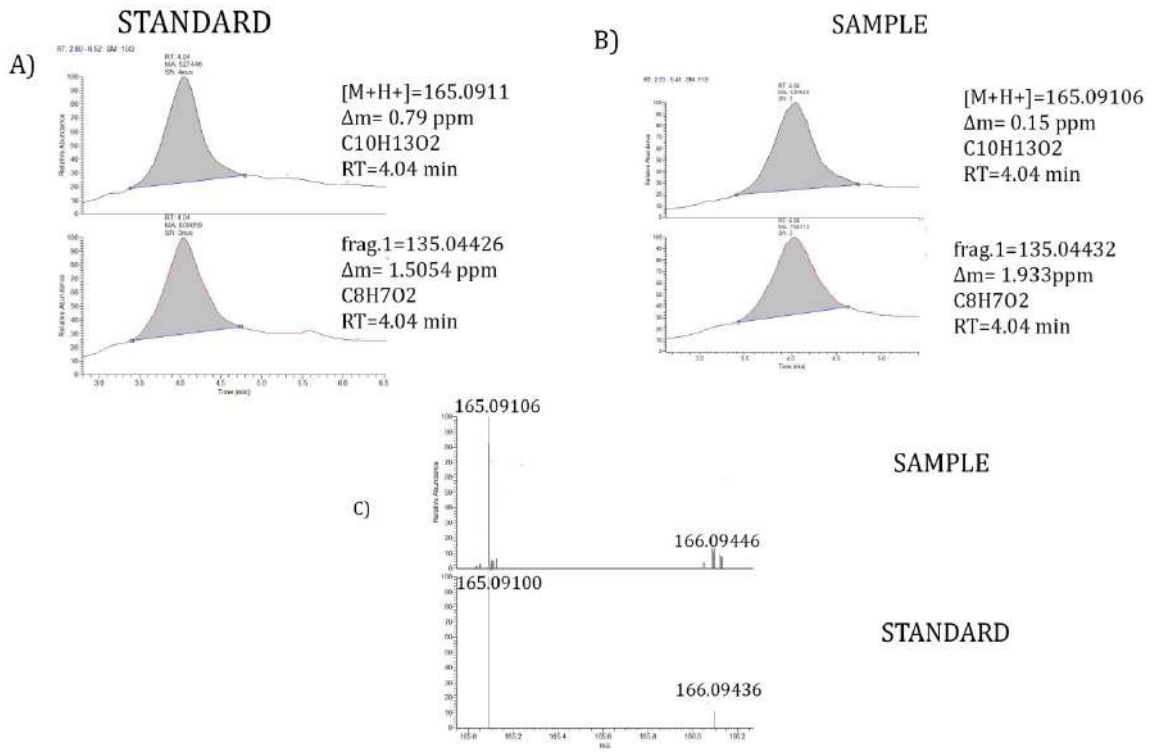


Figure SI-1. a) Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of carbofuranphenol a) standard; b) in air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

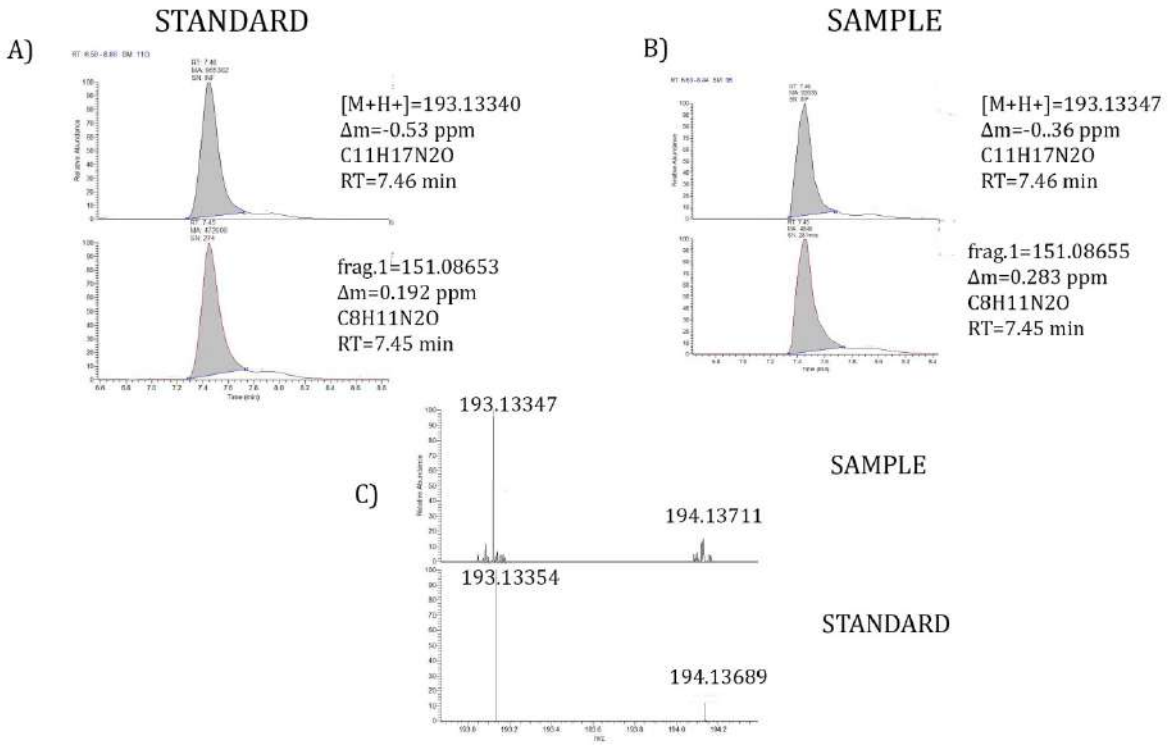


Figure SI-2. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of desmethylisoproturon a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

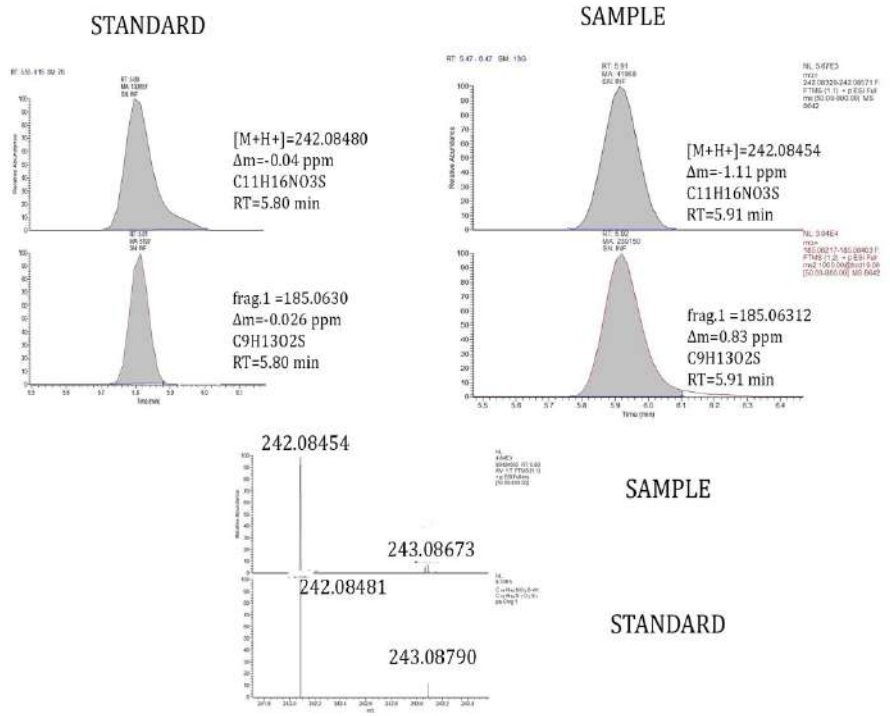


Figure SI-3. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of ethiofencarb-sulfoxide a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

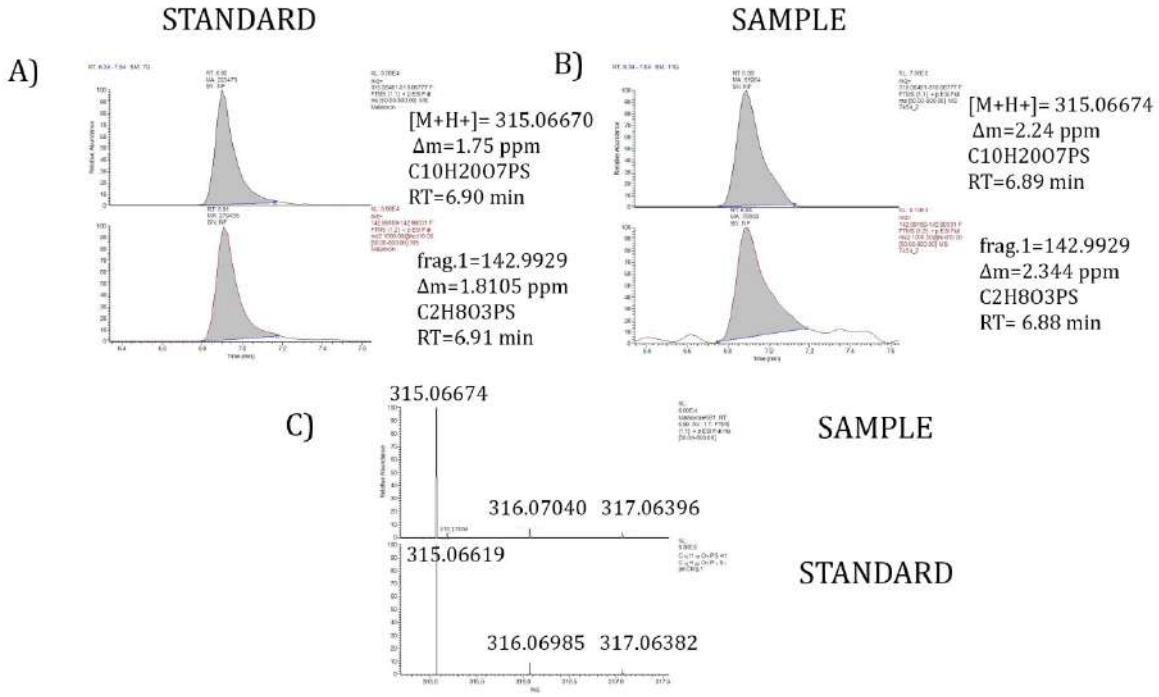


Figure SI-4. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of malaoxon a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

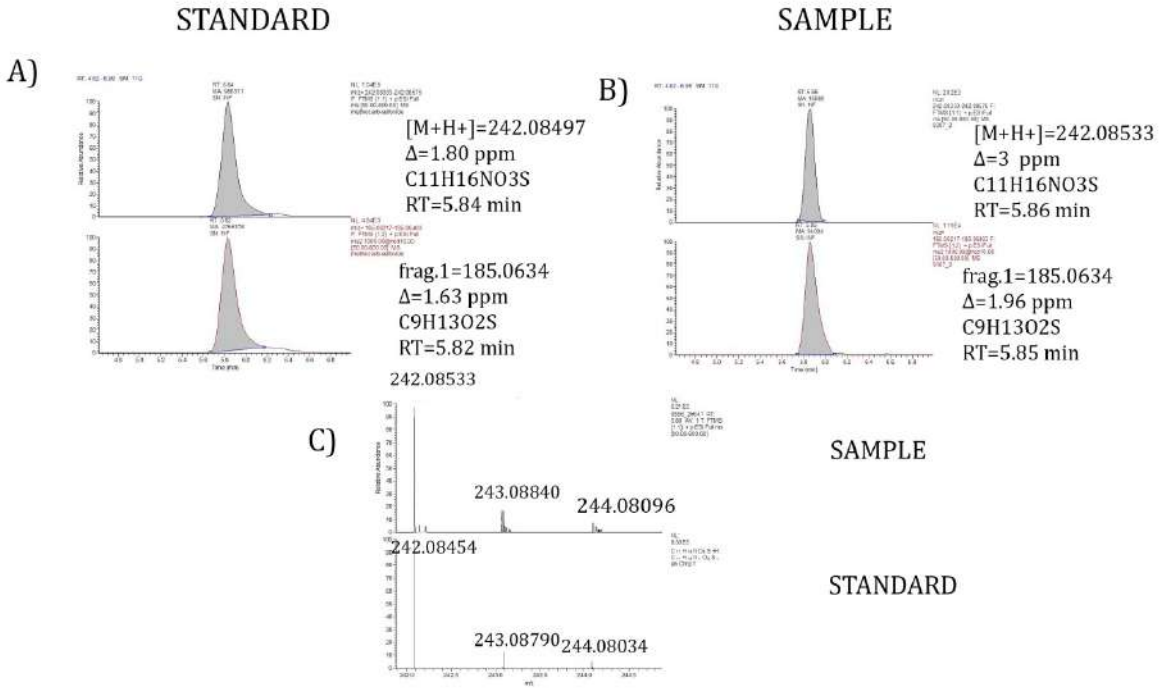


Figure SI-5. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of methiocarb-sulfoxide a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

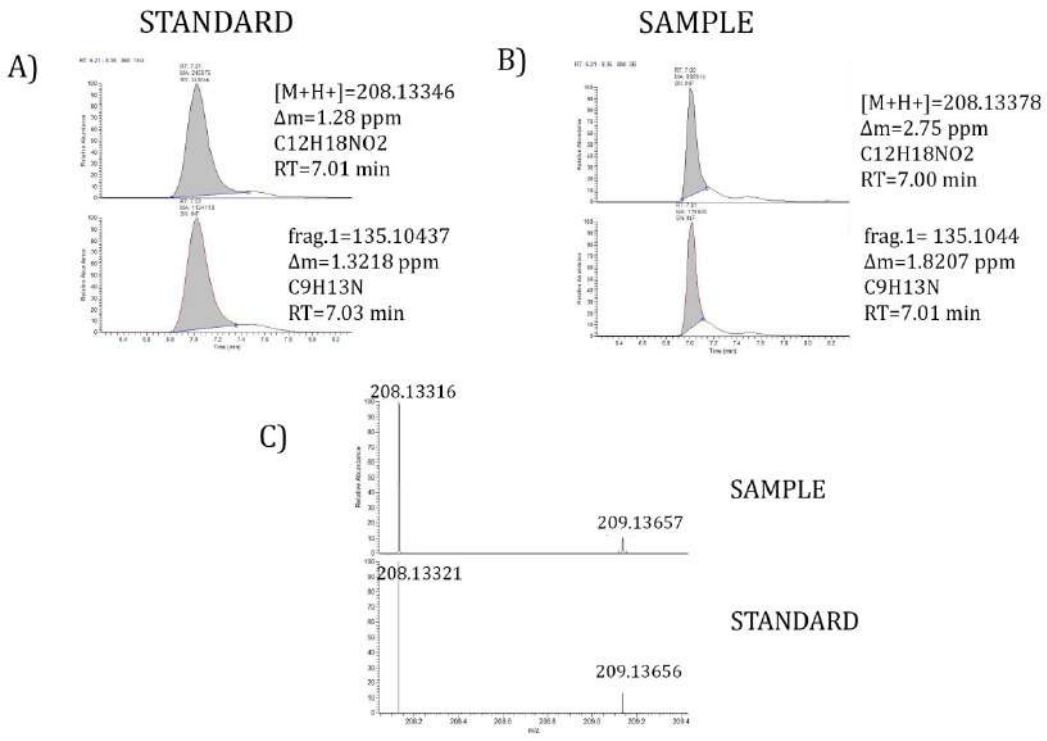


Figure SI-6. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of N-(2-ethyl-6-methylphenyl)-L-alanine a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

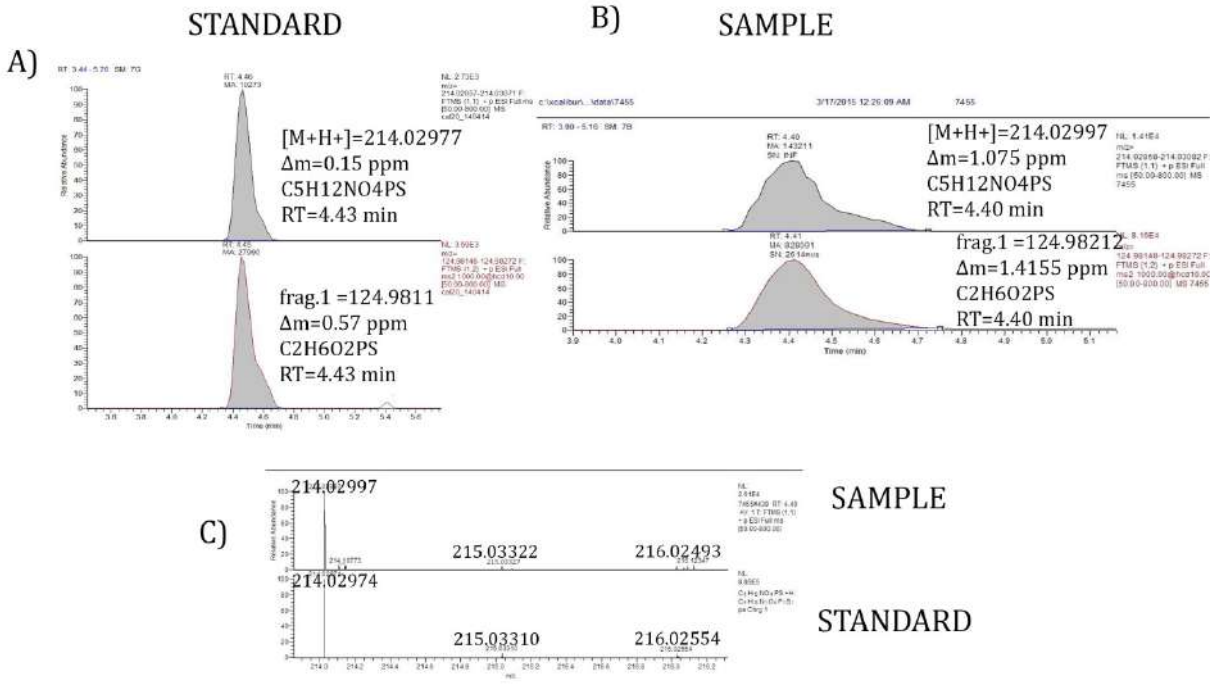


Figure SI-7. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of Omethoate a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

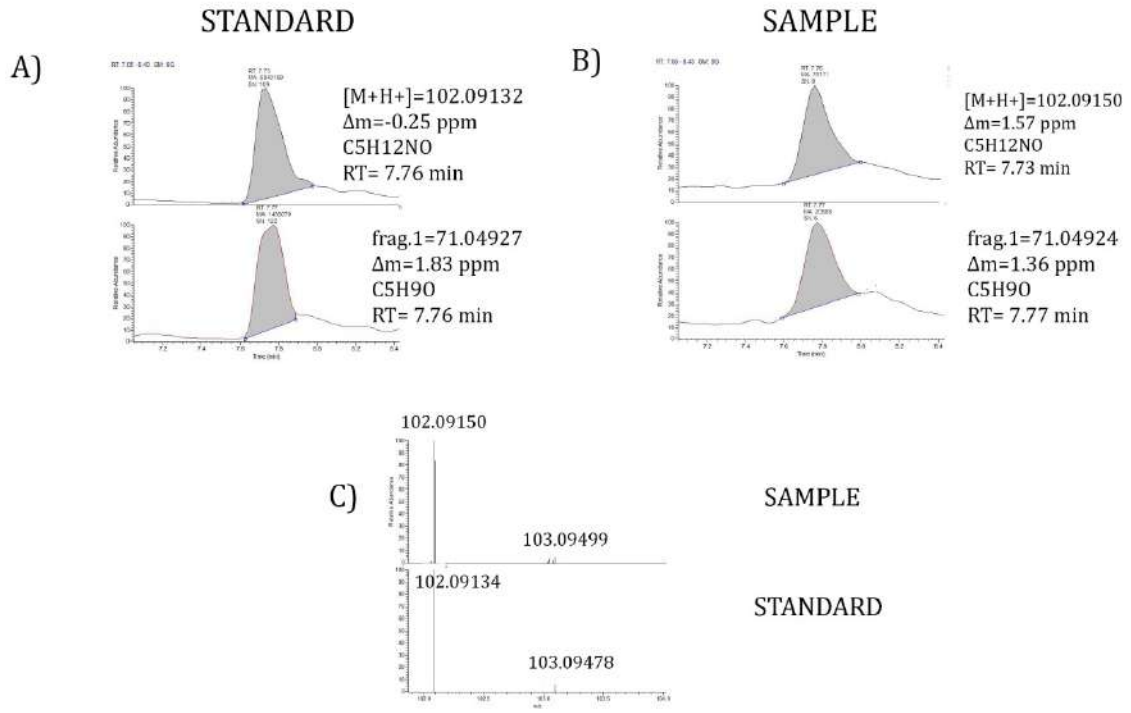


Figure SI-8. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of THPAM a) standard; b) air sample; c) Isotopic patterns of the molecular ion of the sample and standard.

13.2. Anexo II. Información suplementaria del artículo científico 2.

Supplementary Data for

Comprehensive analysis of airborne pesticides using hard cap espresso extraction-liquid chromatography-high-resolution mass spectrometry.

Antonio López, Clara Coscollà, Vicent Yusà, Sergio Armenta, Miguel de la Guardia, Francesc A. Esteve-Turrillas.

Table of Contents :

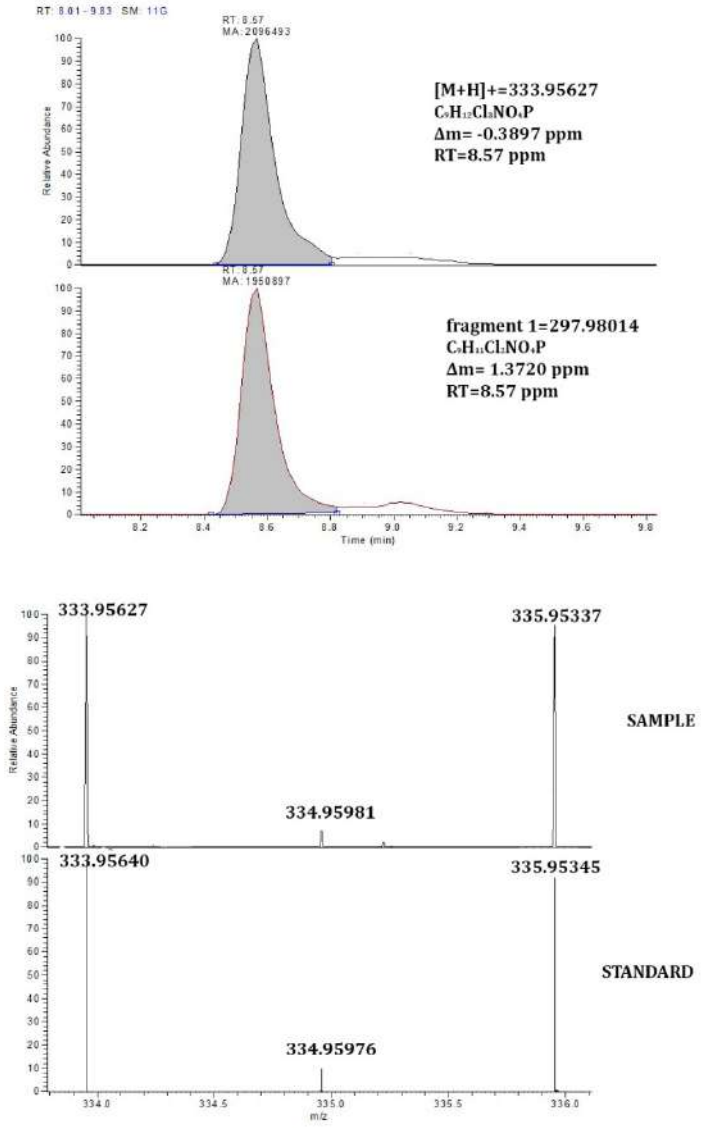


Figure SI-1. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of chlorpyrifos-oxon a) air sample; b) Isotopic patterns of the molecular ion of the sample and standard.

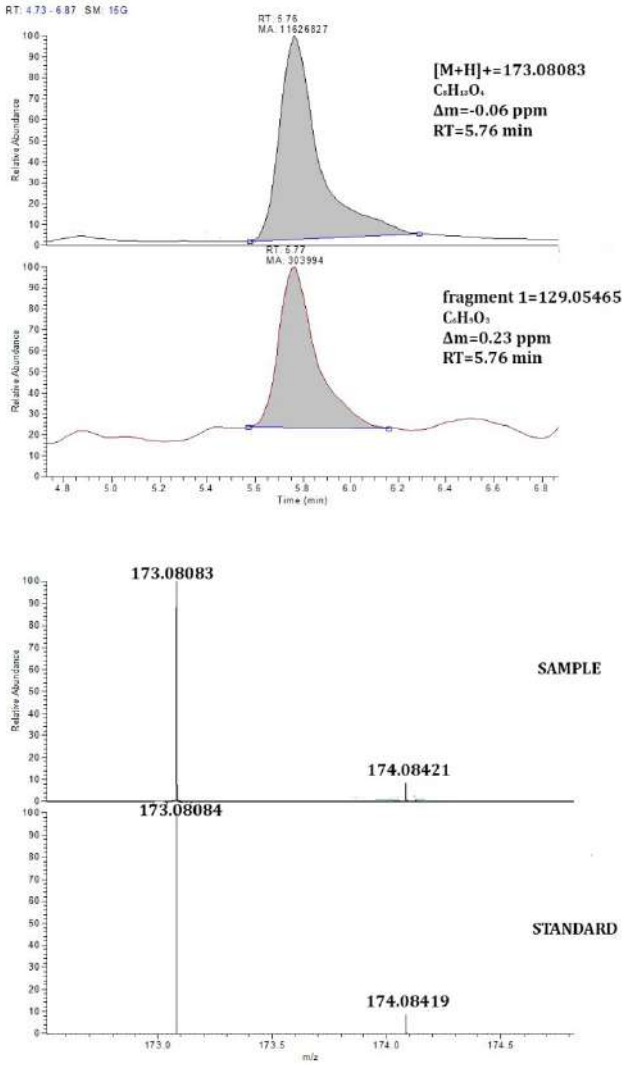


Figure SI-2. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of diethylmaleate a) air sample; b) Isotopic patterns of the molecular ion of the sample and standard.

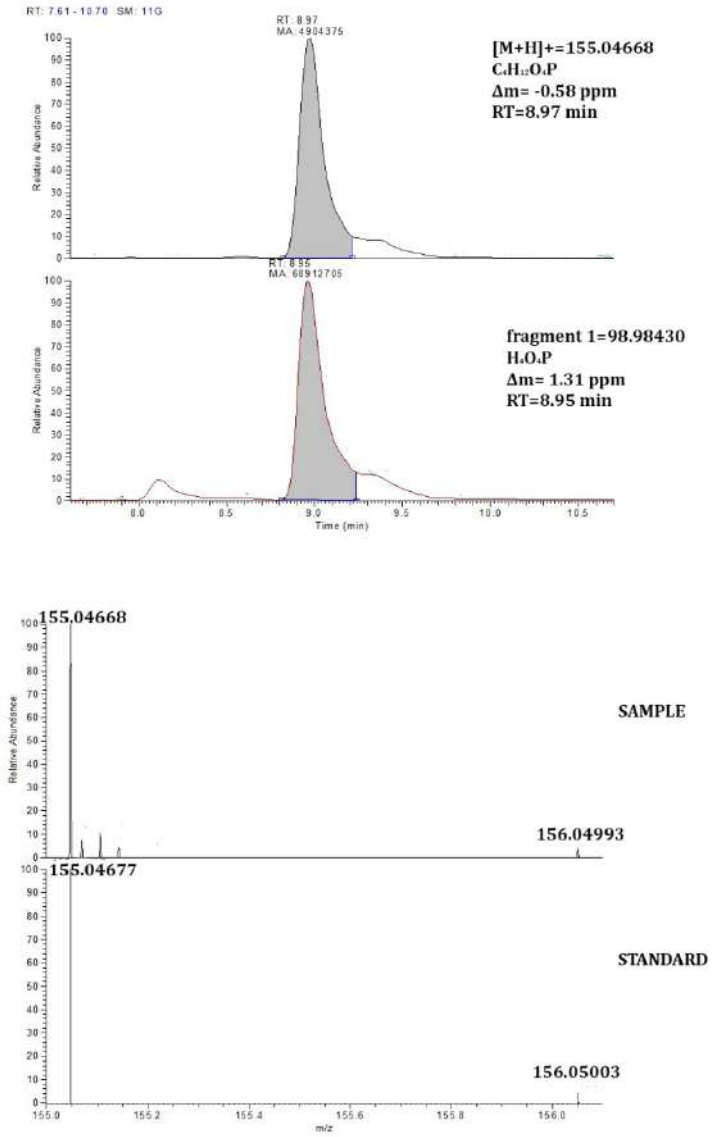


Figure SI-3. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of diethylphosphate a) air sample; b) Isotopic patterns of the molecular ion of the sample and standard.

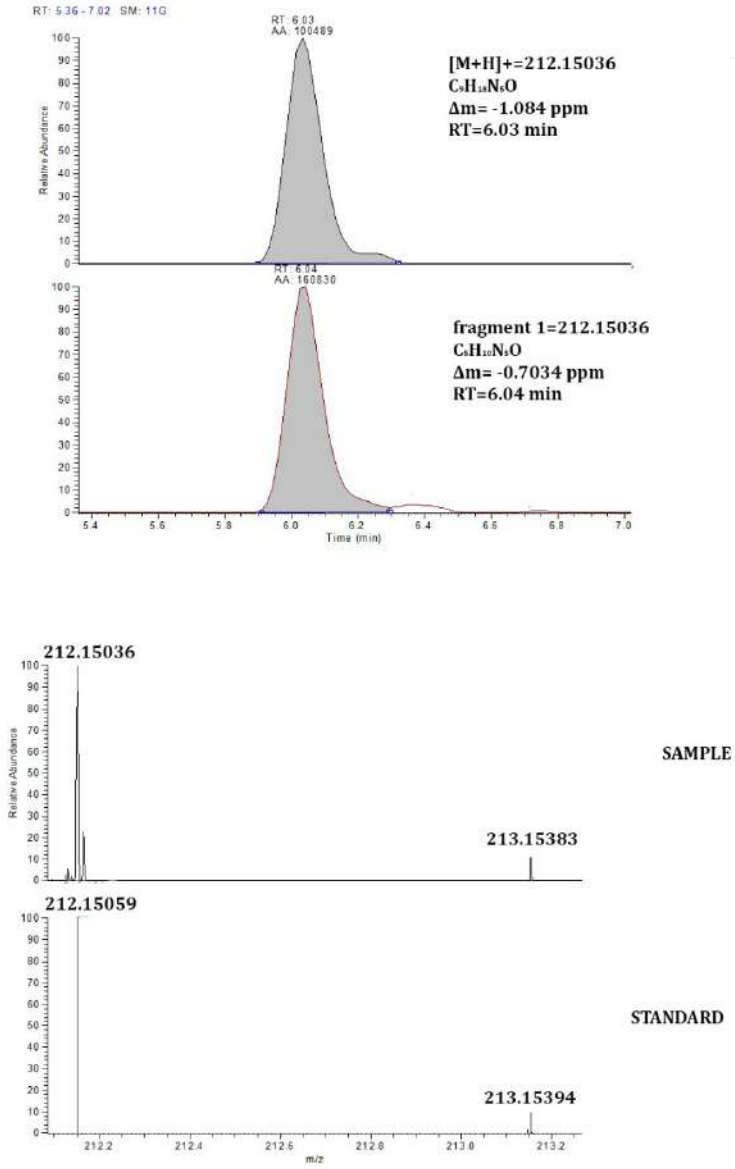


Figure SI-4. Accurate mass extracted ion chromatograms of the molecular ion and a characteristic fragment of terbuthylazine-2-OH a) air sample; b) Isotopic patterns of the molecular ion of the sample and standard.

13.3. Anexo III. Información suplementaria del artículo científico 3.

Supplementary Data for

Selection of sampling adsorbents and optimisation and validation of a GC-MS/MS method for airborne pesticides.

Antonio López, Clara Coscollà, Vicent Yusà.

Type	Captions
Table SI-1	Experimental conditions for the Plackett-Burman design for studying the influence of the different parameters in the GC-IT-MS/MS
Table SI-2	Experimental conditions of the Central Composite Design (CCD) used for the GC-IT-MS/MS optimization
Table SI-3	Estimated effects and p-values (α=0.05) of the four main factors obtained from Plackett-Burman design used in the optimization of GC-IT-MS/MS
Table SI-4	Optimized composition of the CCD (Central Composite Design)
Table SI-5	Matrix effect observed for each pesticide
Table SI-6	Recoveries of the pesticides using XAD-2 as solid sorbent (n=3)
Table SI-7	Recoveries of the pesticides using XAD-4 as solid sorbent (n=3)

Table SI-1.Experimental conditions for the Plackett-Burman design for studying the influence of the different parameters in the GC-IT-MS/MS

Run Order	Ressonance excitation voltage (EV)	Excitation time (ET)	Ion Source Temperature (IST)	Isolation time (IT)
1	0.1	5	250	34
2	2	50	250	4
3	0.1	5	150	4
4	2	5	250	34
5	2	5	250	4
6	2	50	150	34
7	0.1	50	250	34
8	1.05	27.5	200	19
9	2	50	150	34
10	1.05	27.5	200	19
11	0.1	50	250	4
12	2	5	150	4
13	1.05	27.5	200	19
14	0.1	50	150	4
15	0.1	5	150	34

The minimum, central and maximum values used in the P-B were: EV (0.1-1.05-2 V), ET (5-27.5-50 ms), IST (150-200-250 ºC) and IT (4-19-34 ms)

Table SI-2. Experimental conditions of the Central Composite Design (CCD) used for the GC-IT-MS/MS optimization

Run Order	EV	ET	IT
1	1.05	27.5	19
2	0.48513	14.1214	27.9191
3	2	27.5	19
4	1.61487	14.1214	27.9191
5	1.61487	40.8786	10.0809
6	1.61487	40.8786	27.9191
7	1.05	5	19
8	1.05	27.5	19
9	1.05	27.5	19
10	0.48513	14.1214	10.0809
11	1.05	27.5	19
12	0.48513	40.8786	27.9191
13	0.48513	40.8786	10.0809
14	1.61487	14.1214	10.0809
15	1.05	50	19
16	0.1	27.5	19
17	1.05	27.5	4
18	1.05	27.5	19
19	1.05	27.5	19
20	1.05	27.5	34

EV(Excitation Voltage), ET (Excitation Time) and IT (Isolation Time)

The minimum and maximum values used in the CCD were: EV (0.1-2 V), ET (5-50 ms), and IT (4-34 ms)

Table SI-3. Estimated effects and p-values (α=0.05) of the four main factors obtained from Plackett-Burman design used in the optimization of GC-IT-MS/MS

Compounds	Factors							
	EV		ET		IST		IT	
	EV Effect	EV p-value	ET Effect	ET p-value	IST effect	IST p-value	IT Effect	IT p-value
Ethoprophos	65.62	0.171	5.87	0.897	24.29	0.595	-33.44	0.467
Diphenylamine	118.74	0.108	-145.60	0.056	18.65	0.786	-140.85	0.063
Trifluralin	812.5	0.002	-586.9	0.014	177.1	0.382	-245.6	0.235
Chlorpropham	154197	0.001	47507	0.199	-16470	0.642	-13507	0.702
Diazinon	209.67	0.003	-27.00	0.609	34.00	0.521	-37.67	0.478
Pyrimethanil	-42.21	0.596	11.05	0.889	63.84	0.427	-176.49	0.047
Chlorothalonil	4763	0.010	0.637	0.671	2160	0.171	-4363	0.015
Chlorpyrifos-methyl	235.98	0.000	15.65	0.730	11.65	0.797	-25.02	0.583
Vinclozolin	221.21	0.001	-50.58	0.279	-7.22	0.873	12.10	0.789
Tolclofos-methyl	1406.37	0.000	-76.18	0.750	95.97	0.688	-169.70	0.482
Fenitrothion	156.79	0.000	-25.07	0.290	-5.79	0.801	-17.13	0.462
Malathion	291.29	0.000	-23.57	0.539	-42.92	0.275	-41.63	0.289
Chlorpyrifos-ethyl	427.17	0.000	-21.83	0.526	48.17	0.179	-65.50	0.079
Triadimefon	45.37	0.001	-22.36	0.045	21.26	0.054	-8.62	0.393
Fipronil	36.2267	0.001	11.9367	0.170	5.2133	0.531	-1.8133	0.826
Penconazole	191345	0.010	64692	0.296	31645	0.601	-0.398	0.995
Folpet	39262	0.000	-5102	0.266	11895	0.022	-7285	0.124
Fludioxonil	139.96	0.000	0.79	0.964	32.58	0.089	-30.25	0.111
Kresoxim-methyl	115.43	0.000	24.69	0.237	18.09	0.378	-22.98	0.269
Quinoxyfen	505.9	0.104	204.8	0.483	38.1	0.895	-310.0	0.297
Propargite	170.51	0.010	-67.19	0.232	97.49	0.096	-47.86	0.386
Bifenthrin	784.1	0.000	-58.6	0.545	206.0	0.054	-210.1	0.051
Iprodione	49.05	0.042	-36.00	0.117	20.59	0.347	-31.52	0.163
Dicofol	86.04	0.086	25.57	0.580	66.33	0.171	-59.47	0.215
Permethrin	126.73	0.006	-46.53	0.224	40.08	0.289	-54.18	0.162

The minimum, central and maximum values used in the P-B were: EV (0.1-1.05-2 V), ET (5-27.5-50 ms), IST (150-200-250 ºC) and IT (4-19-34 ms)

EV (Excitation Voltage), ET (Excitation Time), IST (Ion Source Temperature) and IT (Isolation Time)

Table SI-4. Optimized composition of the CCD (Central Composite Design)

Compound	EV	ET	IT	Desirability
Ethoprophos	0.89	5	24	0.943
Diphenylamine	1.56	5	18	0.639
Trifluralin	1.02	33.11	20	0.915
Chlorpropham	1.07	50	14	1
Diazinon	1.21	32.83	20	0.913
Pyrimethanil	2.0	5	30	0.708
Chlorpyrifos-methyl	1.22	32.83	20	0.910
Vinclozolin	1.26	31.42	20	0.865
Tolclofos-m	1.26	31.42	20	0.947
Fenitrothion	1.0	5	14	0.788
Malathion	1.43	32.26	18	0.846
Chlorpyrifos-ethyl	2.0	30.29	18	0.963
Triadimefon	1.03	31.98	16	0.837
Fipronil	1.18	30.29	18	0.830
Penconazole	1.26	30.29	20	0.766
Folpet	1.43	50	34	1
Fludioxonil	1.26	31.70	20	0.727
Kresoxim-methyl	0.92	5	4	0.846
Quinoxyfen	1.28	35.08	20	0.813
Propargite	1.0	34.24	18	0.756
Bifenthrin	1.77	50	28	0.918
Iprodione	1.06	32.26	20	0.582
Dicofol	1.11	37.90	22	0.592
Permethrin	1.21	35.08	20	0.628

EV (Excitation voltage), ET (Excitation time) and IT (Isolation time)

The minimum and maximum values used in the CCD were: EV (0.1-2 V), ET (5-50 ms), and IT (4-34 ms)

Table SI-5. Matrix effect observed for each pesticide

Analyte	*Matrix effect (%)*
Ethoprophos	25
Trifluralin	20
Diphenylamine	20
Chlorpropham	25
Diazinon	25
Lindane	35
Pyrimethanil	25
Chlorpyrifos-methyl	35
Vinclozolin	30
Tolclofos-methyl	30
Fenitrothion	40
Malathion	60
Chlorpyrifos-ethyl	40
Aldrin	35
Triadimefon	55
Fipronil	30
Penconazole	40
Folpet	40
Alfa-endosulfan	55
Dieldrin	45
Kresoxim-methyl	35
Fludioxonil	25
Beta-endosulfan	55
Quinoxyfen	30
Endosulfan-sulfate	65
Propargite	25
Bifenthrin	60
Iprodione	65
Dicofol	35
Lambda-cyhalothrin	75
Permethrin	70
Cyfluthrin	100
Cypermethrin	80
Deltamethrin	80

Table SI-6. Recoveries of the pesticides using XAD-2 as solid sorbent (n=3)

Pesticide	Recovery (%) ± SD		
	2.5 ng (equivalent to 16.1 pg m^{-3})	10 ng (equivalent to 64.5 pg m^{-3})	50 ng (equivalent to 322.6 pg m^{-3})
Diphenylamine	55±5	59±5	110±10
Chlorpropham	48±4	64±4	110±10
Diazinon	34±4	50±2	78±1
Pyrimethanil	104±8	94±2	85±5
Chlorpyrifos-methyl	38±7	61±3	83±3
Tolclofos-methyl	46±9	57±8	78±9
Malathion	110±5	110±5	107±4
Chlorpyrifos-ethyl	100±10	110±10	110±10
Triadimefon	97±7	95±5	90±10
Penconazole	104±5	90±7	108±6
Folpet	107±8	105±10	109±6
Alfa-endosulfan	109±5	110±10	110±4
Dieldrin	96±4	90±10	90±10
Kresoxim-methyl	50±10	70±10	100±10
Endosulfan-sulfate	53±8	67±7	83±8
Propargite	110±5	105±3	108±9
Bifenthrin	48±8	61±6	82±4
Iprodione	39±6	59±3	109±1
Lambda-cyhalothrin	62±8	71±3	100±10
Permethrin	28±7	35±5	43±4
Cyfluthrin	80±5	85±3	85±1
Cypermethrin	31±7	58±4	110±5
Deltamethrin	29±6	51±4	110±8

Table SI-7. Recoveries of the pesticides using XAD-4 as solid sorbent (n=3)

Pesticide	Recovery (%) ± SD		
	2.5 ng (equivalent to 16.1 pg m^{-3})	10 ng (equivalent to 64.5 pg m^{-3})	50 ng (equivalent to 322.6 pg m^{-3})
Ethoprophos	106±4	95±4	91±2
Trifluralin	51±7	67±4	111±2
Chlorpropham	84±5	80±3	89±2
Diazinon	106±4	90±3	103±2
Lindane	80±3	83±3	82±3
Pyrimethanil	56±7	105±10	108±4
Chlorpyrifos-methyl	108±4	96±4	101±5
Tolclofos-methyl	109±2	89±3	96±1
Fenitrothion	62±6	90±6	98±3
Chlorpyrifos-ethyl	34±7	109±3	101±6
Aldrin	43±6	68±4	97±2
Triadimefon	57±4	89±5	98±3
Fipronil	48±5	76±3	80±2
Alfa-endosulfan	41±3	63±4	89±3
Kresoxim-methyl	36±5	54±6	94±4
Beta-endosulfan	52±6	81±4	95±3
Bifenthrin	36±4	51±3	100±3
Iprodione	61±3	102±4	96±1
Lambda-cyhalothrin	57±5	89±3	91±3
Cypermethrin	54±7	98±5	101±3

13.4. Anexo IV. Información suplementaria del artículo científico 4.

Supplementary Data for

Evaluation of sampling adsorbents and validation of a LC-HRMS method for determination of 28 airborne pesticides

Antonio López, Clara Coscollà, Vicent Yusà.

Type	Captions
Table SI-1	Matrix effect observed for each pesticide
Table SI-2	Recoveries of the pesticides using XAD-2 as solid sorbent (n=3)
Table SI-3	Recoveries of the pesticides using XAD-4 as solid sorbent (n=3)
Table SI-4	Quantification limits of the different studied gaseous phase pesticides

Table SI-1. Matrix effect observed for each pesticide

Analyte	Matrix effect (%)
Acetamiprid	64
Azoxystrobin	1
Benalaxyl	1
Carbendazim	83
Carbofuran	34
Cyproconazole	13
Cyprodinil	1
Difenoconazole	1
Dimethoate	63
Diuron	23
Fenbuconazole	4
Fenhexamid	3
Fluazifop	8
Flusilazole	2
Imazalil	11
Imidacloprid	67
Iprovalicarb	18
Metalaxyl	61
Myclobutanil	11
Omethoate	60
Pirimicarb	72
Pirimicarb-desmethyl	94
Pyrimethanil	23
Tebuconazole	5
Tebufenpyrad	7
Terbuthylazine	11
Thiabendazole	79
Thiamethoxam	41

Table SI-2. Recoveries of the pesticides using XAD-2 as solid sorbent (R%±SD) (n=3)

Pesticide	Recovery (%) ± SD		
	5 ng (equivalent to 32.2 pg m^{-3})	**20 ng (equivalent to 129.0 pg m^{-3})**	**100 ng (equivalent to 645.2 pg m^{-3})**
Acetamipird	98±4	103±3	110±3
Azoxystrobin	93±4	97±2	103±8
Benalaxyl	95±5	96±4	98±5
Carbendazim	80±3	95±2	104.4±0.8
Carbofuran	96±5	94±7	103±1
Cyproconazole	59±5	67±3	96±2
Cyprodinil	90±3	92±4	95±1
Difenoconazole	50±7	67±3	91±1
Dimethoate	68±3	90±2	97±1
Diuron	98±5	95±3	94±1
Fenhexamid	57±1	62±4	88±1
Flusilazole	71±3	90±2	95±3
Imazalil	92±3	95±3	97±2
Imidacloprid	54±5	89±4	97±1
Iprovalicarb	90±2	93±3	96±2
Metalaxyl	94±4	96±4	99±3
Myclobutanil	62±5	90±3	94±2
Omethoate	66±3	91±4	97±3
Pirimicarb	58±4	90±5	96±2
Pirimicarb-desmethyl	92±3	94±3	98±2
Pyrimethanil	88±4	91±4	93±2
Tebuconazole	62±3	88±4	94±2
Terbuthylazine	89±3	92±4	94±2
Thiabendazole	93±3	95±5	104±3
Thiamethoxam	88±2	92±3	94±2

Table SI-3. Recoveries of the pesticides using XAD-4 as solid sorbent (R%±SD) (n=3)

	Recovery (%) ± SD		
Pesticide	**5 ng (equivalent to 32.2 pg m^{-3})**	**20 ng (equivalent to 129.0 pg m^{-3})**	**100 ng (equivalent to 645.2 pg m^{-3})**
Acetamipird	93±3	95±5	96±3
Azoxystrobin	52±6	94±2	98±3
Bitertanol	51±4	60±3	96±5
Buprofezin	87±3	90±2	95±4
Carbendazim	84±2	90±3	95±4
Carbofuran	93±2	95±2	95±2
Cyproconazole	79±4	88±2	92±3
Difenoconazole	58±5	64±3	90±2
Dimethoate	96±3	95±3	96±4
Fenbuconazole	58±2	65±4	92±3
Fluquinconazole	49±3	68±2	90±2
Flusilazole	55±4	92±2	94±3
Imazalil	90±3	92±2	95±3
Imidacloprid	82±4	90±2	91±3
Iprovalicarb	84±3	92±3	97±3
Metalaxyl	92±2	94±4	97±2
Methidathion	43±4	62±4	90±3
Omethoate	53±6	80±3	90±2
Pyriproxifen	47±3	55±8	90±4
Tebuconazole	83±4	88±3	91±3
Terbuthylazine	84±3	89±4	92±3
Thiabendazole	90±1	92±3	94±4

Table SI-4. Quantification limits of the different studied gaseous phase pesticides

		LOD/LOQ (pg m^{-3})						
			Reference					
Pesticide	**Status ***	**Present study**	**[101]**	**[123]**	**[130]**	**[140]**	**[141]**	**[145]**
2,4-D	Approved	-	2500	-	-	-	-	-
Acetamipird	Approved	32.2	-	-	-	-	-	-
Acetochlor	Not Approved	-	-	-	-	-	-	14
Alachlor	Not Approved	-	-	-	-	-	-	56
Atrazine	Not Approved	-	700	-	-	-	-	5.6
Azinphos-ethyl	Not Approved	-	-	0.88	-	-	-	-
Azinphos-methyl	Not Approved	-	-	0.60	-	700	7600	14
Azinphos-methyl-oxon	Metabolite	-	-	-	-	700	-	-
Azoxystrobin	Approved	129.0	-	-	-	-	-	-
Benalaxyl	Approved	32.2	-	-	-	-	-	-
Carbendazim	Not Approved	32.2	-	-	-	-	-	2.8
Carbofuran	Not Approved	32.2	-	-	-	-	-	-
Chlorpyrifos-ethyl	Approved	-	-	0.35	-	700	-	28
Chlorpyrifos-methyl	Approved	-	-	0.50	-	-	-	-
Chlorpyrifos-oxon	Metabolite	-	-	-	-	700	2916	-
Chlorotoluron	Approved	-	-	-	-	-	-	28
Coumaphos	Not Approved	-	-	0.30	-	-	-	-
Cyproconazole	Approved	129.0	-	-	-	-	-	-
Cyprodinil	Approved	32.2	-	-	-	-	-	-
DEF	Not Approved	-	-	-	-	-	1760	-
Deltamethrin	Approved	-	13800	-	-	-	-	-
Diazinon	Not Approved	-	-	0.15	-	-	1160	14
Diazinon oxon	Metabolite	-	-	-	-	-	2080	-

Pesticide	Status *	Present study	[101]	[123]	[130]	[140]	[141]	[145]
Diclofop methyl	Approved	-	3900	-	-	-	-	-
Difenoconazole	Approved	129.0	-	-	-	-	-	-
Dimethachlor	Approved	-	-	-	-	-	-	28
Dimethoate	Approved	32.2	-	0.18	-	-	2320	1.67
Dimethoate oxon	Metabolite	-	-	-	-	-	1945	-
Diquat	Approved	-	2200	-	-	-	-	-
Disulfoton	Not Approved	-	-	-	-	-	-	1.12
Diuron	Approved	32.2	-	-	-	-	5140	14
EPTC	Not Approved	-	-	-	-	-	1670	-
Fenbuconazole	Approved	32.2	-	-	-	-	-	-
Fenclorphos	Not Approved	-	-	28.80	-	-	-	-
Fenhexamid	Approved	129.0	-	-	-	-	-	-
Fenitrothion	Not Approved	-	-	-	-	-	-	1110
Fenpropimorph	Approved	-	-	-	-	-	-	2.8
Flamprop isopropyl	Not Approved	-	5100	-	-	-	-	-
Fluazifop	Not Approved	32.2	-	-	-	-	-	-
Flusilazole	Not Approved	129.0	-	-	-	-	-	-
Fonofos	Not Approved	-	-	-	-	-	-	14
Imazalil	Approved	32.2	-	-	-	-	-	-
Imidacloprid	Approved	32.2	-	-	-	-	-	-
Iprovalicarb	Approved	32.2	-	-	-	-	-	-
Isoproturon	Not Approved	-	1200	-	-	-	-	7
Linuron	Not Approved	-	4500	-	-	-	-	-
Malathion	Approved	-	13000	0.40	-	-	2180	28
Malathion oxon	Metabolite	-	-	-	-	-	1300	-
MCPA	Approved	-	2300	-	-	-	-	-

Pesticide	Status *	Present study	[101]	[123]	[130]	[140]	[141]	[145]
MCPB	Approved	-	5000	-	-	-	-	-
MCPP	Approved	-	3900	-	-	-	-	-
Mefenpyr methyl	NPPP	-	9200	-	-	-	-	-
Metalaxyl	Approved	32.2	-	-	-	-	-	-
Metamitron	Approved	-	-	-	-	-	-	28
Metazachlor	Approved	-	-	-	-	-	-	5.6
Metolachlor	Not Approved	-	-	-	-	-	2730	-
Metribuzin	Approved	-	-	-	-	-	-	14
Metsulfuron methyl	Approved	-	1700	-	-	-	-	-
Myclobutanil	Approved	32.2	-	-	-	-	-	-
Molinate	Not Approved	-	-	-	-	-	1800	-
Norflurazon	Not Approved	-	-	-	-	-	3750	-
Omethoate	Not Approved	32.2	-	-	-	-	-	-
Oryzalin	Approved	-	-	-	-	-	1390	-
Paraquat	Not Approved	-	1700	-	33300	-	-	-
Phorate	Not Approved	-	-	0.68	-	-	-	-
Phosmet	Approved	-	-	-	-	-	-	-
Pirimicarb	Approved	32.2	-	-	-	-	-	-
Pirimicarb-desmethyl	Approved	32.2	-	-	-	-	-	-
Prochloraz	Approved	-	-	-	-	-	-	1.67
Propanil	Pending	-	-	-	-	-	2315	-
Pyrazon	Approved	-	-	-	-	-	-	7
Pyrimethanil	Approved	32.2	-	-	-	-	-	-
Simazine	Not Approved	-	-	-	-	-	1200	5.6
S-metolachlor	Approved	-	-	-	-	-	-	14
Tebuconazole	Approved	32.2	-	-	-	-	-	-
Tebufenpyrad	Approved	129.0	-	-	-	-	-	-

Pesticide	Status *	Present study	[101]	[123]	[130]	[140]	[141]	[145]
Temephos	Not Approved	-	-	-	-	-	-	2.8
Terbufos	Not Approved	-	-	-	-	-	-	5.6
Terbuthylazine	Approved	32.2	-	-	-	-	-	5.6
Thiabendazole	Approved	32.2	-	-	-	-	-	-
Thiamethoxam	Approved	32.2	-	-	-	-	-	-
Thiobencarb	Not Approved	-	-	-	-	-	5600	-

NPPP=Not Plant Protection Product

*Under Regulation 1107/2009 (EC) http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN

13.5. Anexo V. Información suplementaria del artículo científico 5.

Supplementary Data for

Risk assessment of airborne pesticides in a Mediterranean region of Spain.

Antonio López, Vicent Yusà, Amalia Muñoz, Teresa Vera, Esther Borràs, Milagros Ródenas, Clara Coscollà.

Table of Contents:

Table SI-1 Sources of uncertainty associated with the estimation of exposure to pesticides in the ambient air

Sources of uncertainty	Brief description	Uncertainty impact
A. Uncertainties of pesticide concentration in PM10	Losses of volatile pesticides during sampling	(-)
	Analytical methods: RSD(%) ≤ 20% for all instrumental techniques	(+/-)
B. Uncertainties related to the theoretical G-P partitioning model	The validation of the method used was performed previously (Yusà et al, 2014) [89]. However, it is difficult to quantify the uncertainties linked to this model for each pesticide. Yusà et al, (2014) demonstrated that most of the experimental data on the pesticide G/P distribution agree with the absorption model. However there were some pesticides that presented a higher or lower percentage in the particulate phase than predicted by the model.	(+/-)
C. Exposure assessment	In the equation for DIE calculation we have used the tabulated average of IR_{inh} and body weight. However, these values present a variability inside the population.	(+/-)

RSD: Relative Standard Deviation
(+): could increase the final exposure and risk
(-): could decrease the final exposure and risk

Table SI-2. Spatial distribution of detected pesticides in rural stations

Station	Alzira (N=79)			Burriana (N=48)			Benicarló (N=25)		
Pesticide	Frequency of detection (%) > LD	Range [a] (pg m^{-3})	Average [a] (pg m^{-3})*	Frequency of detection (%) > LD	Range [a] (pg m^{-3})	Average [a] (pg m^{-3})*	Frequency of detection (%) > LD	Range [a] (pg m^{-3})	Average [a] (pg m^{-3})*
Abamectin	-	-	-	-	-	-	16	<LQ-36000	36000
Acetamiprid	-	-	-	21	<LQ-31	16	-	-	-
Azoxystrobin	-	-	-	-	-	-	-	-	-
Bendiocarb	-	-	-	10	16-24	19	-	-	-
Bifenthrin	35	<LQ-80	29	42	<LQ-20	16	-	-	-
Bitertanol	-	-	-	-	-	-	24	<LQ-120	70
Buprofezin	16	<LQ-42	20	21	<LQ-130	60	28	120-900	410
Carbendazim	53	<LQ-370	100	58	<LQ-320	60	68	21-2000	300
Carbofuran	14	<LQ-16	8	4	<LQ-29	19	-	-	-
Chlorpyrifos	46	<LQ-210	90	58	<LQ-44	44	-	-	-
Chlorpyrifos-methyl	6	<LQ-10	10	31	<LQ	-	-	-	-
Chlorothalonil	15	<LQ-28	13	31	<LQ-11	11	-	-	-
Chlorpropham	4	<LQ-9	9	31	<LQ-9	9	-	-	-
Diazinon	33	<LQ-170	36	58	<LQ-220	29	-	-	-
Dichlorvos	-	-	-	-	-	-	-	-	-
Diphenylamine	6	<LQ-7	7	35	<LQ	-	-	-	-
Dimethoate	16	<LQ-80	19	10	<LQ-80	32	-	-	-
Dioxacarb	-	-	-	6	13-41	26	-	-	-
Endothal	-	-	-	8	26	26	-	-	-
Ethoprophos	-	-	-	31	<LQ-150	150	-	-	-
Fludioxonil	13	<LQ-39	18	31	<LQ-19	19	-	-	-
Folpet	13	<LQ-80	30	33	<LQ-45	35	-	-	-
Hexythiazox	-	-	-	-	-	-	64	20-6000	800
Imazalil	-	-	-	6	<LQ-220	110	24	39-340	180
Imidacloprid	1	<LQ-15	15	4	<LQ-33	24	32	29-900	140
Iprodione	-	-	-	31	<LQ-36	36	-	-	-
Kresoxim methyl	-	-	-	31	<LQ-8	8	-	-	-
Malathion	27	<LQ-90	12	38	<LQ-13	13	-	-	-
Metalaxyl	78	<LQ-130	43	38	<LQ-180	45	16	<LQ-600	600
Omethoate	65	<LQ-500	45	79	<LQ-1600	190	16	4000-9000	7000
o-phenylphenol	3	11-33	22	-	-	-	-	-	-
Prohexadione	13	13-90	56	-	-	-	-	-	-
Propanil	-	-	-	-	-	-	16	30-370	160
Pyrimethanil	-	-	-	31	<LQ	-	-	-	-
Pyriproxifen	-	-	-	-	-	-	20	15-360	130
Spinosad	-	-	-	-	-	-	-	-	-
Tebuconazole	8	<LQ-14	12	6	<LQ-11	11	4	130	130

Terbuthylazine	44	<LQ-800	100	94	<LQ-500	90	-	-	-
Thiabendazole	-	-	-	6	<LQ-80	50	20	140-900	500
Tricyclazole	1	20.00-29.00	24.50	2	15-24.5	18	-	-	-

N= Total samples * The average was calculated from the arithmetic mean of samples with concentration above LOQ.
[a] The results are rounded considering the variability of the analytical method (20%).

Table SI-2. Spatial distribution of detected pesticides in rural stations

Station	Benifaió (N=23)			Villar del Arzobispo (N=9)			Sant Jordi (N=43)		
Pesticide	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*
Abamectin	13	<LQ-30000	30000	33	<LQ-45000	35000	-	-	-
Acetamiprid	-	-	-	-	-	-	-	-	-
Azoxystrobin	35	<LQ-400	46	11	<LQ	-	-	-	-
Bendiocarb	-	-	-	-	-	-	-	-	-
Bifenthrin	-	-	-	-	-	-	21	<LQ-24	15
Bitertanol	17	<LQ-220	120	11	<LQ	-	-	-	-
Buprofezin	22	33-3800	800	22	100-1800	900	5	<LQ	-
Carbendazim	52	27-500	140	11	37	37	40	<LQ-200	50
Carbofuran	-	-	-	-	-	-	7	<LQ-46	27
Chlorpyrifos	-	-	-	-	-	-	23	<LQ	-
Chlorpyrifos-methyl	-	-	-	-	-	-	-	-	-
Chlorothalonil	-	-	-	-	-	-	2	<LQ	-
Chlorpropham	-	-	-	-	-	-	-	-	-
Diazinon	-	-	-	-	-	-	40	<LQ-18	11
Dichlorvos	-	-	-	-	-	-	-	-	-
Diphenylamine	-	-	-	-	-	-	-	-	-
Dimethoate	-	-	-	-	-	-	44	<LQ-230	43
Dioxacarb	-	-	-	-	-	-	-	-	-
Endothal	-	-	-	-	-	-	-	-	-
Ethoprophos	-	-	-	-	-	-	2	<LQ-60	60
Fludioxonil	-	-	-	-	-	-	-	-	-
Folpet	-	-	-	-	-	-	26	<LQ-160	60
Hexythiazox	74	8-1100	220	33	20-390	150	-	-	-
Imazalil	22	37-270	140	-	-	-	-	-	-
Imidacloprid	26	<LQ-230	110	11	90	90	-	-	-
Iprodione	-	-	-	-	-	-	2	<LQ-30	30
Kresoxim methyl	-	-	-	-	-	-	7	<LQ-14	11
Malathion	-	-	-	-	-	-	16	<LQ-46	19
Metalaxyl	22	<LQ-800	600	-	-	-	19	<LQ-19	11
Omethoate	9	8000-8000	8000	22	16000-17000	17000	93	<LQ-2700	330
o-phenylphenol	-	-	-	-	-	-	-	-	-
Prohexadione	-	-	-	-	-	-	-	-	-
Propanil	22	24-370	240	11	50	50	-	-	-
Pyrimethanil	-	-	-	-	-	-	-	-	-
Pyriproxifen	44	<LQ-3800	500	-	-	-	-	-	-
Spinosad	-	-	-	-	-	-	-	-	-
Tebuconazole	13	120-640	310	-	-	-	23	<LQ-900	15
Terbuthylazine	-	-	-	-	-	-	98	<LQ-34000	270
Thiabendazole	17	60-500	300	22	160-380	270	-	-	-

Tricyclazole	-	-	-	-	-	-	-	-	-

Table SI-3. Spatial distribution of detected pesticides in urban and remote sites

Station	Morella (N=54)			Viveros (Valencia) (N=48)			Burjassot (N=16)		
Pesticide	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*	Frequency of detection (%)>LD	Range (pg m^{-3})	Average (pg m^{-3})*
Abamectin	2	<LQ	-	-	-	-	-	-	-
Acetamiprid	-	-	-	-	-	-	-	-	-
Azoxystrobin	6	<LQ-60	36	4	7-8	8	19	180-800	500
Bendiocarb	-	-	-	-	-	-	-	-	-
Bifenthrin	-	-	-	48	<LQ-31	14	-	-	-
Bitertanol	4	<LQ-80	80	-	-	-	25	170-800	500
Buprofezin	6	<LQ-70	50	17	<LQ-18	16	63	210-600	340
Carbendazim	20	<LQ-90	50	48	<LQ-80	33	63	110-800	500
Carbofuran	-	-	-	-	-	-	-	-	-
Chlorpyrifos	13	<LQ	-	40	<LQ	-	-	-	-
Chlorpyrifos-methyl	-	-	-	-	-	-	-	-	-
Chlorothalonil	7	<LQ-20	19	6	<LQ-11	9	-	-	-
Chlorpropham	-	-	-	-	-	-	-	-	-
Diazinon	11	<LQ-12	11	67	<LQ-40	15	-	-	-
Dichlorvos	-	-	-	-	-	-	100	120-900	400
Diphenylamine	2	<LQ	-	6	<LQ-10	8	38	900-1900	1200
Dimethoate	-	-	-	4	<LQ-210	100	-	-	-
Dioxacarb	-	-	-	-		-	-	-	-
Endothal	-	-	-	-		-	-	-	-
Ethoprophos	-	-	-	46	<LQ-1200	100	-	-	-
Fludioxonil	-	-	-	-	-	-	-	-	-
Folpet	20	<LQ-60	39	17	<LQ-70	29	-	-	-
Hexythiazox	2	200	200	-	-	-	75	140-1000	410
Imazalil	-	-	-	-	-	-	-	-	-
Imidacloprid	-	-	-	2	<LQ-12	12	63	150-500	370
Iprodione	-	-	-	-	-	-	-	-	-
Kresoxim methyl	4	<LQ	-	-	-	-	-	-	-
Malathion	-	-	-	4	<LQ-7	7	-	-	-
Metalaxyl	4	<LQ-13	13	88	<LQ-80	22	100	<LQ-1000	700
Omethoate	44	<LQ-46	14	73	<LQ-150	31	-	-	-
o-phenylphenol	-	-	-	-	-	-	-	-	-
Prohexadione	-	-	-	-	-	-	-	-	-
Propanil	-	-	-	-	-	-	6	900	900
Pyrimethanil	-	-	-	4	<LQ-8	8.	-	-	-
Pyriproxifen	2	110	110	-	-	-	69	140-1000	500
Spinosad	-	-	-	-	-	-	44	190-800	500
Tebuconazole	17	<LQ-10	9	4	<LQ-8	8	6	7000	7000
Terbuthylazine	48	<LQ-50	19	81	<LQ-90	25	-	-	-
Thiabendazole	2	600	600	-	-	-	19	600-600	600
Tricyclazole	-	-	-	-	-	-	-	-	-

N= Total samples * The average was calculated from the arithmetic mean of samples with concentration above LOQ.
[a] The results are rounded considering the variability of the analytical method (20%).

Table SI-4. Maximum total (particulate and gaseous) concentrations (pg m^{-3}), Daily inhalation exposure (mg kg^{-1} day^{-1}) and Hazard Quotient (HQ AOEL) for the detected pesticides

Alzira (N=79)							
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Bifenthrin	80	2.37E-08	3.16E-06	5.60E-08	7.47E-06	6.60E-08	8.80E-06
Buprofezin	40	2.66E-08	6.65E-07	6.29E-08	1.57E-06	7.41E-08	1.85E-06
Carbendazim	370	4.38E-05	2.19E-03	1.04E-04	5.18E-03	1.22E-04	6.10E-03
Carbofuran	16	1.76E-08	5.87E-05	4.16E-08	1.39E-04	4.90E-08	1.63E-04
Chlorpyrifos	210	2.21E-06	2.21E-03	5.22E-06	5.22E-03	6.15E-06	6.15E-03
Chlorpyrifos-methyl	10	2.65E-07	2.65E-05	6.25E-07	6.25E-05	7.37E-07	7.37E-05
Chlorothalonil	28	8.70E-07	9.66E-05	2.05E-06	2.28E-04	2.42E-06	2.69E-04
Chlorpropham	9	8.54E-08	1.71E-06	2.02E-07	4.03E-06	2.38E-07	4.75E-06
Diazinon	180	2.39E-06	1.20E-02	5.66E-06	2.83E-02	6.67E-06	3.33E-02
Diphenylamine	7	3.75E-07	3.75E-06	8.86E-07	8.86E-06	1.04E-06	1.04E-05
Dimethoate	80	4.64E-08	4.64E-05	1.10E-07	1.10E-04	1.29E-07	1.29E-04
Fludioxonil	39	1.12E-08	1.90E-08	2.64E-08	4.48E-08	3.12E-08	5.28E-08
Folpet	80	7.04E-07	7.04E-06	1.66E-06	1.66E-05	1.96E-06	1.96E-05
Imidacloprid	15	4.23E-09	5.29E-08	9.99E-09	1.25E-07	1.18E-08	1.47E-07
Malathion	100	2.07E-07	6.90E-06	4.89E-07	1.63E-05	5.76E-07	1.92E-05
Metalaxyl	130	9.53E-06	1.19E-04	2.25E-05	2.81E-04	2.65E-05	3.32E-04
Omethoate	500	1.46E-07	4.87E-04	3.45E-07	1.15E-03	4.07E-07	1.36E-03
o-phenylphenol	33	2.49E-06	6.23E-06	5.89E-06	1.47E-05	6.94E-06	1.74E-05
Prohexadione	90	1.47E-03	4.20E-03	3.47E-03	9.92E-03	4.09E-03	1.17E-02
Tebuconazole	14	3.88E-09	3.88E-06	9.17E-09	9.17E-06	1.08E-08	1.08E-05
Terbuthylazine	800	9.60E-07	3.00E-04	2.27E-06	7.08E-04	2.67E-06	8.35E-04
Burriana (N=48)							
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Acetamiprid	31	9.19E-09	1.31E-07	2.17E-08	3.10E-07	2.56E-08	3.66E-07
Bendiocarb	24	2.06E-06	3.17E-04	4.86E-06	7.48E-04	5.73E-06	8.81E-04
Bifenthrin	20	5.66E-09	7.54E-07	1.34E-08	1.78E-06	1.57E-08	2.10E-06
Buprofezin	130	8.59E-08	2.15E-06	2.03E-07	5.07E-06	2.39E-07	5.98E-06
Carbendazim	320	3.80E-05	1.90E-03	8.98E-05	4.49E-03	1.06E-04	5.29E-03
Carbofuran	29	3.24E-08	1.08E-04	7.65E-08	2.55E-04	9.02E-08	3.01E-04
Chlorpyrifos	44	4.59E-07	4.59E-04	1.08E-06	1.08E-03	1.28E-06	1.28E-03

Chlorothalonil	11	3.38E-07	3.76E-05	7.99E-07	8.88E-05	9.42E-07	1.05E-04
Chlorpropham	9	7.96E-08	1.59E-06	1.88E-07	3.76E-06	2.21E-07	4.43E-06
Diazinon	220	2.95E-06	1.47E-02	6.96E-06	3.48E-02	8.20E-06	4.10E-02
Dimethoate	80	4.85E-08	4.85E-05	1.14E-07	1.14E-04	1.35E-07	1.35E-04
Ethoprophos	150	2.25E-06	2.25E-03	5.31E-06	5.31E-03	6.26E-06	6.26E-03
Fludioxonil	19	5.57E-09	9.45E-09	1.32E-08	2.23E-08	1.55E-08	2.63E-08
Folpet	45	3.77E-07	3.77E-06	8.90E-07	8.90E-06	1.05E-06	1.05E-05
Imazalil	220	1.30E-07	2.59E-06	3.06E-07	6.13E-06	3.61E-07	7.22E-06
Imidacloprid	33	9.25E-09	1.16E-07	2.18E-08	2.73E-07	2.57E-08	3.22E-07
Iprodione	36	1.05E-08	3.49E-08	2.47E-08	8.23E-08	2.91E-08	9.70E-08
Kresoxim methyl	8	3.59E-09	3.99E-09	8.47E-09	9.41E-09	9.99E-09	1.11E-08
Malathion	13	2.90E-08	9.65E-07	6.84E-08	2.28E-06	8.06E-08	2.69E-06
Metalaxyl	180	1.28E-05	1.61E-04	3.03E-05	3.79E-04	3.58E-05	4.47E-04
Omethoate	1600	4.93E-07	1.64E-03	1.17E-06	3.88E-03	1.37E-06	4.58E-03
Tebuconazole	11	3.15E-09	3.15E-06	7.45E-09	7.45E-06	8.78E-09	8.78E-06
Terbuthylazine	500	6.22E-07	1.95E-04	1.47E-06	4.59E-04	1.73E-06	5.41E-04
Thiabendazole	80	2.40E-08	2.40E-07	5.68E-08	5.68E-07	6.69E-08	6.69E-07

Benicarló (N=25)

Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Abamectin	36000	1.42E-05	5.69E-03	3.36E-05	1.34E-02	3.96E-05	1.58E-02
Bitertanol	120	3.53E-08	1.76E-07	8.33E-08	4.17E-07	9.82E-08	4.91E-07
Buprofezin	900	5.76E-07	1.44E-05	1.36E-06	3.40E-05	1.60E-06	4.01E-05
Carbendazim	2000	2.35E-04	1.18E-02	5.56E-04	2.78E-02	6.55E-04	3.28E-02
Hexythiazox	6000	1.61E-04	1.79E-02	3.79E-04	4.22E-02	4.47E-04	4.97E-02
Imazalil	340	2.05E-07	4.10E-06	4.84E-07	9.67E-06	5.70E-07	1.14E-05
Imidacloprid	900	2.54E-07	3.17E-06	5.99E-07	7.48E-06	7.06E-07	8.82E-06
Metalaxyl	600	4.17E-05	5.21E-04	9.85E-05	1.23E-03	1.16E-04	1.45E-03
Omethoate	9000	2.93E-06	9.78E-03	6.93E-06	2.31E-02	8.17E-06	2.72E-02
Propanil	380	9.91E-07	4.96E-05	2.34E-06	1.17E-04	2.76E-06	1.38E-04
Pyriproxifen	360	1.23E-07	3.07E-06	2.90E-07	7.24E-06	3.41E-07	8.54E-06
Tebuconazole	130	3.76E-08	3.76E-05	8.88E-08	8.88E-05	1.05E-07	1.05E-04
Thiabendazole	900	2.58E-07	2.58E-06	6.10E-07	6.10E-06	7.19E-07	7.19E-06

Benifaió (N=23)							
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Abamectin	28000	1.09E-05	4.36E-03	3.36E-05	1.34E-02	3.96E-05	1.58E-02
Azoxystrobin	400	1.16E-07	5.80E-07	2.74E-07	1.37E-06	3.23E-07	1.61E-06
Bitertanol	370	1.05E-07	5.27E-07	2.49E-07	1.25E-06	2.94E-07	1.47E-06
Buprofezin	3800	2.42E-06	6.05E-05	5.72E-06	1.43E-04	6.74E-06	1.68E-04
Carbendazim	500	6.12E-05	3.06E-03	1.44E-04	7.22E-03	1.70E-04	8.51E-03
Hexythiazox	1100	3.01E-05	3.34E-03	7.10E-05	7.89E-03	8.37E-05	9.30E-03
Imazalil	270	1.65E-07	3.30E-06	3.90E-07	7.79E-06	4.59E-07	9.18E-06
Imidacloprid	230	6.63E-08	8.29E-07	1.57E-07	1.96E-06	1.85E-07	2.31E-06
Metalaxyl	800	5.59E-05	6.99E-04	1.32E-04	1.65E-03	1.56E-04	1.95E-03
Omethoate	8000	2.51E-06	8.37E-03	5.93E-06	1.98E-02	6.99E-06	2.33E-02
Propanil	370	9.91E-07	4.96E-05	2.34E-06	1.17E-04	2.76E-06	1.38E-04
Pyriproxifen	3800	1.28E-06	3.21E-05	3.03E-06	7.58E-05	3.57E-06	8.93E-05
Tebuconazole	600	1.84E-07	1.84E-04	4.34E-07	4.34E-04	5.11E-07	5.11E-04
Thiabendazole	500	1.63E-07	1.63E-06	3.85E-07	3.85E-06	4.54E-07	4.54E-06
Villar del Arzobispo (N=9)							
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Abamectin	45000	1.80E-05	7.19E-03	4.24E-05	1.70E-02	5.00E-05	2.00E-02
Buprofezin	1800	1.12E-06	2.80E-05	2.64E-06	6.60E-05	3.11E-06	7.78E-05
Carbendazim	37	4.37E-06	2.19E-04	1.03E-05	5.16E-04	1.22E-05	6.08E-04
Hexythiazox	400	1.08E-05	1.20E-03	2.54E-05	2.82E-03	3.00E-05	3.33E-03
Imidacloprid	90	2.47E-08	3.09E-07	5.84E-08	7.29E-07	6.88E-08	8.60E-07
Omethoate	17000	5.43E-06	1.81E-02	1.28E-05	4.27E-02	1.51E-05	5.03E-02
Propanil	50	1.37E-07	6.88E-06	3.25E-07	1.62E-05	3.83E-07	1.92E-05
Thiabendazole	380	1.15E-07	1.15E-06	2.72E-07	2.72E-06	3.20E-07	3.20E-06
Sant Jordi (N=43)							
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Bifenthrine	24	6.90E-09	9.20E-07	1.63E-08	2.17E-06	1.92E-08	2.56E-06
Carbendazim	200	2.39E-05	1.20E-03	5.65E-05	2.82E-03	6.66E-05	3.33E-03
Carbofuran	46	5.21E-08	1.74E-04	1.23E-07	4.10E-04	1.45E-07	4.84E-04
Diazinon	18	2.43E-07	1.21E-03	5.74E-07	2.87E-03	6.76E-07	3.38E-03

Dimethoate	230	1.34E-07	1.34E-04	3.15E-07	3.15E-04	3.72E-07	3.72E-04
Ethoprophos	60	9.60E-07	9.60E-04	2.27E-06	2.27E-03	2.67E-06	2.67E-03
Folpet	160	1.37E-06	1.37E-05	3.24E-06	3.24E-05	3.82E-06	3.82E-05
Iprodione	30	8.78E-09	2.93E-08	2.07E-08	6.91E-08	2.44E-08	8.14E-08
Kresoxim methyl	14	6.00E-09	6.67E-09	1.42E-08	1.57E-08	1.67E-08	1.86E-08
Malathion	45	1.01E-07	3.37E-06	2.39E-07	7.97E-06	2.82E-07	9.39E-06
Metalaxyl	19	1.36E-06	1.70E-05	3.22E-06	4.02E-05	3.79E-06	4.74E-05
Omethoate	2700	8.50E-07	2.83E-03	2.01E-06	6.69E-03	2.37E-06	7.88E-03
Tebuconazole	1000	2.71E-07	2.71E-04	6.41E-07	6.41E-04	7.55E-07	7.55E-04
Terbuthylazine	34000	4.32E-05	1.35E-02	1.02E-04	3.19E-02	1.20E-04	3.75E-02

Morella (N=54)

Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Azoxystrobin	60	1.83E-08	9.15E-08	4.32E-08	2.16E-07	5.09E-08	2.55E-07
Bitertanol	80	2.25E-08	1.13E-07	5.31E-08	2.66E-07	6.26E-08	3.13E-07
Buprofezin	70	4.61E-08	1.15E-06	1.09E-07	2.72E-06	1.28E-07	3.20E-06
Carbendazim	90	1.13E-05	5.63E-04	2.66E-05	1.33E-03	3.13E-05	1.57E-03
Chlorothalonil	20	6.16E-07	6.85E-05	1.46E-06	1.62E-04	1.71E-06	1.91E-04
Diazinon	12	1.61E-07	8.07E-04	3.81E-07	1.90E-03	4.49E-07	2.24E-03
Folpet	50	4.65E-07	4.65E-06	1.10E-06	1.10E-05	1.29E-06	1.29E-05
Hexythiazox	200	5.55E-06	6.17E-04	1.31E-05	1.46E-03	1.54E-05	1.72E-03
Metalaxyl	13	9.37E-07	1.17E-05	2.21E-06	2.77E-05	2.61E-06	3.26E-05
Omethoate	50	1.44E-08	4.79E-05	3.39E-08	1.13E-04	4.00E-08	1.33E-04
Pyriproxifen	110	3.82E-08	9.56E-07	9.03E-08	2.26E-06	1.06E-07	2.66E-06
Tebuconazole	10	2.80E-09	2.80E-06	6.61E-09	6.61E-06	7.79E-09	7.79E-06
Terbuthylazine	50	6.60E-08	2.06E-05	1.56E-07	4.87E-05	1.84E-07	5.74E-05
Thiabendazole	600	1.76E-07	1.76E-06	4.15E-07	4.15E-06	4.89E-07	4.89E-06

Viveros (Valencia) (N=48)

Pesticide	Maximum Level (pg m^{-3}) [d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Azoxystrobin	8	2.36E-09	1.18E-08	5.56E-09	2.78E-08	6.56E-09	3.28E-08
Bifenthrine	31	8.81E-09	1.17E-06	2.08E-08	2.77E-06	2.45E-08	3.27E-06
Buprofezin	18	1.13E-08	2.82E-07	2.66E-08	6.65E-07	3.13E-08	7.83E-07
Carbendazim	80	9.31E-06	4.65E-04	2.20E-05	1.10E-03	2.59E-05	1.30E-03
Chlorothalonil	11	3.27E-07	3.63E-05	7.72E-07	8.57E-05	9.10E-07	1.01E-04
Diazinon	40	5.40E-07	2.70E-03	1.28E-06	6.38E-03	1.50E-06	7.52E-03

Diphenylamine	10	5.17E-07	5.17E-06	1.22E-06	1.22E-05	1.44E-06	1.44E-05
Dimethoate	200	1.21E-07	1.21E-04	2.86E-07	2.86E-04	3.38E-07	3.38E-04
Ethoprophos	1200	3.46E-07	3.46E-04	8.17E-07	8.17E-04	9.62E-07	9.62E-04
Folpet	70	5.66E-07	5.66E-06	1.34E-06	1.34E-05	1.58E-06	1.58E-05
Imidacloprid	12	3.52E-09	4.40E-08	8.31E-09	1.04E-07	9.80E-09	1.22E-07
Malathion	7	1.47E-08	4.91E-07	3.48E-08	1.16E-06	4.10E-08	1.37E-06
Metalaxyl	80	5.96E-06	7.45E-05	1.72E-04	2.15E-03	2.03E-04	2.53E-03
Pyrimethanil	8	4.93E-08	4.11E-07	1.16E-07	9.69E-07	1.37E-07	1.14E-06
Tebuconazole	8	2.37E-09	2.37E-06	5.60E-09	5.60E-06	6.60E-09	6.60E-06
Terbuthylazine	90	1.08E-07	3.37E-05	2.55E-07	7.96E-05	3.00E-07	9.38E-05

Burjassot (N=16)

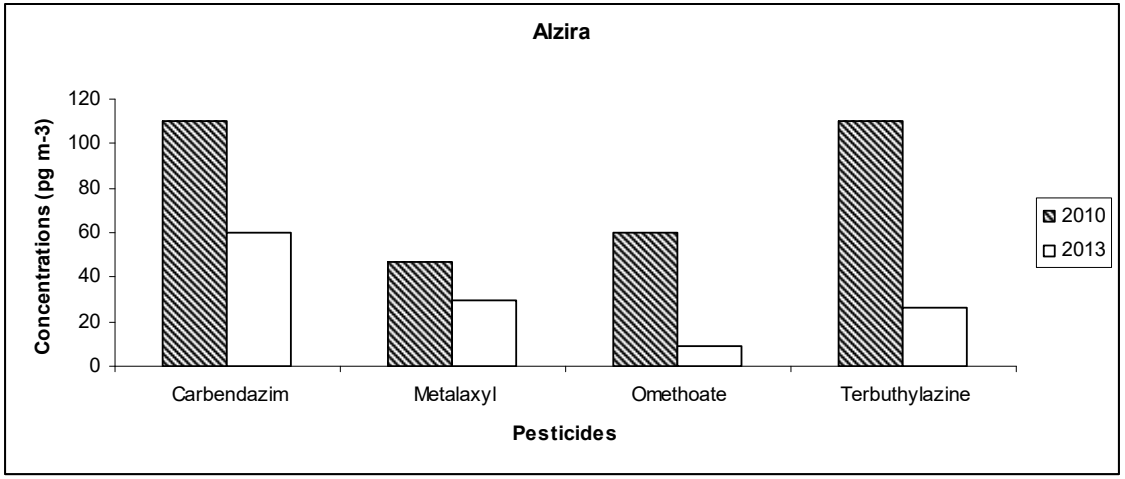
Pesticide	Maximum Level (pg m^{-3})[d]	Adults (>12 years)		Children (1-6 years)		Infants(>6-1.5 years)	
		DIE[a]	HQ_{AOEL}	DIE[b]	HQ_{AOEL}	DIE[c]	HQ_{AOEL}
Azoxystrobin	800	2.21E-07	1.10E-06	5.21E-07	2.61E-06	6.14E-07	3.07E-06
Bitertanol	800	2.26E-07	1.13E-06	5.34E-07	2.67E-06	6.30E-07	3.15E-06
Buprofezin	600	3.78E-07	9.44E-06	8.92E-07	2.23E-05	1.05E-06	2.63E-05
Carbendazim	800	1.01E-04	5.07E-03	2.39E-04	1.20E-02	2.82E-04	1.41E-02
Dichlorvos	900	3.18E-05	6.36E-02	7.52E-05	7.50E-02	8.86E-05	1.77E-01
Diphenylamine	1900	1.02E-04	1.02E-03	2.41E-04	2.41E-03	2.84E-04	2.84E-03
Hexythiazox	1000	2.63E-05	2.92E-03	6.21E-05	6.90E-03	7.32E-05	8.13E-03
Imidacloprid	500	2.72E-07	3.40E-06	6.42E-07	8.03E-06	7.57E-07	9.46E-06
Metalaxyl	1000	7.31E-05	9.14E-04	1.73E-04	2.16E-03	2.04E-04	2.54E-03
Propanil	900	2.41E-06	1.21E-04	5.70E-06	2.85E-04	6.72E-06	3.36E-04
Pyriproxifen	1000	3.23E-07	8.09E-06	7.64E-07	1.91E-05	9.00E-07	2.25E-05
Spinosad	800	2.26E-07	5.66E-06	5.35E-07	1.34E-05	6.30E-07	1.58E-05
Tebuconazole	7000	1.99E-06	1.99E-03	4.69E-06	4.69E-03	5.53E-06	5.53E-03
Thiabendazole	600	1.78E-07	1.78E-06	4.20E-07	4.20E-06	4.96E-07	4.95E-06

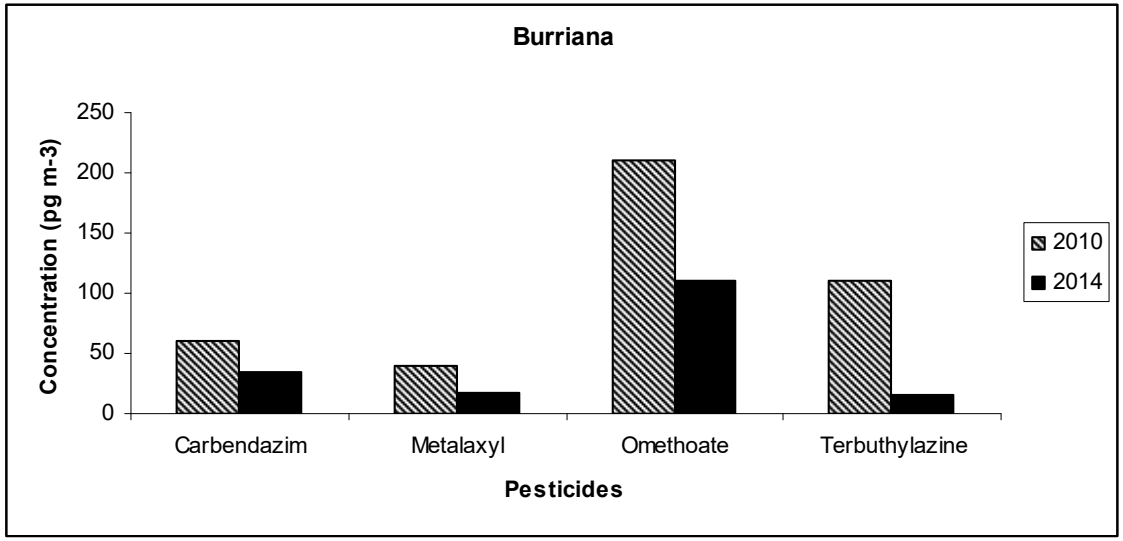
[a] [259], [260], [261] (DIE: Daily Inhalation Exposure; body weight=70 kg, inhalation rate=20 m^3 day).

[b] [260], [261] (DIE: Daily Inhalation Exposure; body weight=15 kg, inhalation rate=10 m^3 day^{-1}, exposure duration.

[c] DIE: Daily Inhalation Exposure; body weight=10 kg, inhalation rate=8 m^3 day^{-1}, exposure duration= 24 h.

[d] The results are rounded considering the variability of the analytical method (20%).


Alzira
Concentrations (pg m-3)
120
100
80
60
40
20
0
Carbendazim
Metalaxyl
Omethoate
Terbuthylazine
Pesticides
2010
2013


Burriana
Concentration (pg m-3)
250
200
150
100
50
0
Carbendazim
Metalaxyl
Omethoate
Terbuthylazine
Pesticides
2010
2014

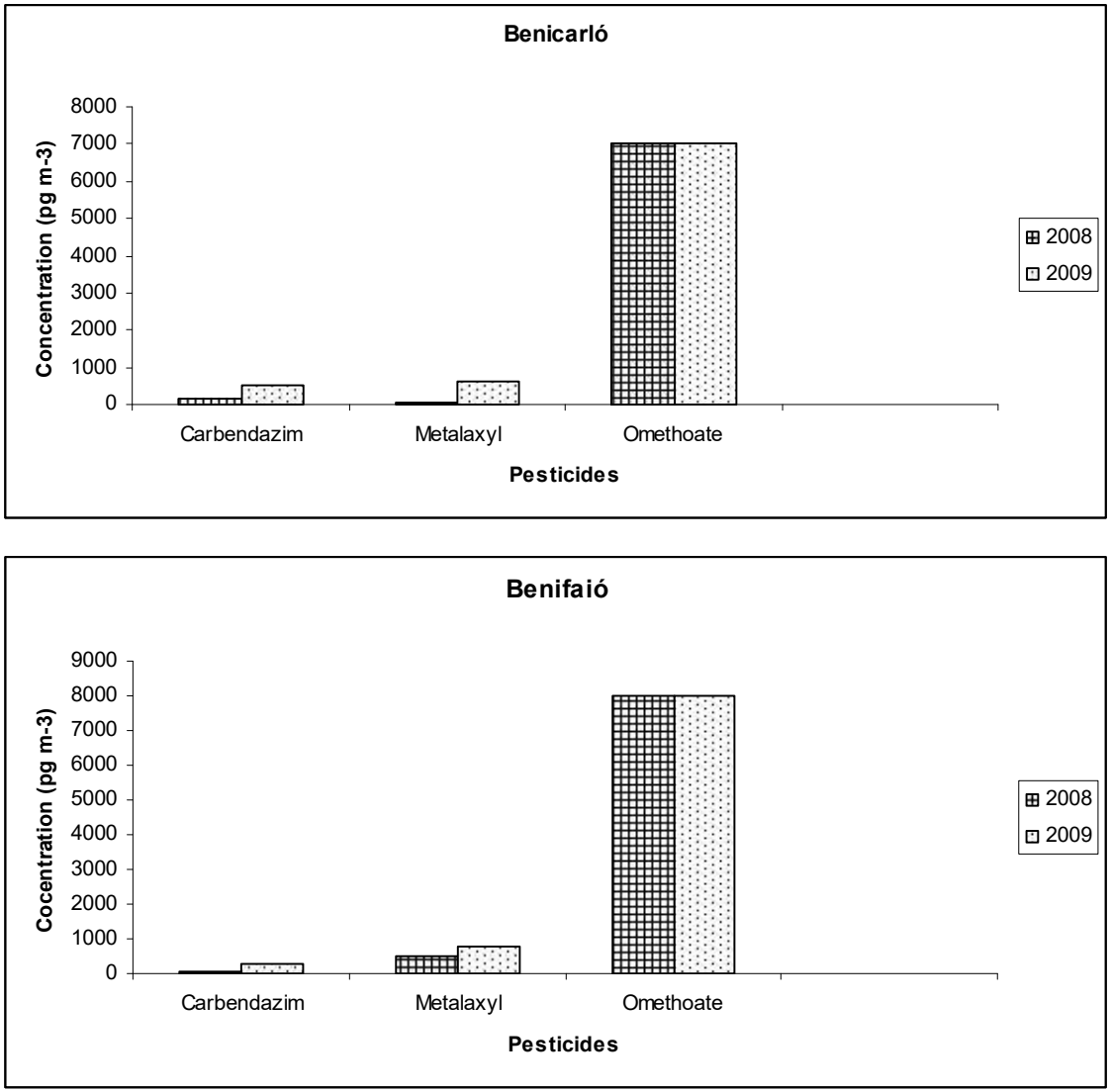


Figure SI-1. Temporal trends for some pesticides during sampling period in rural stations.

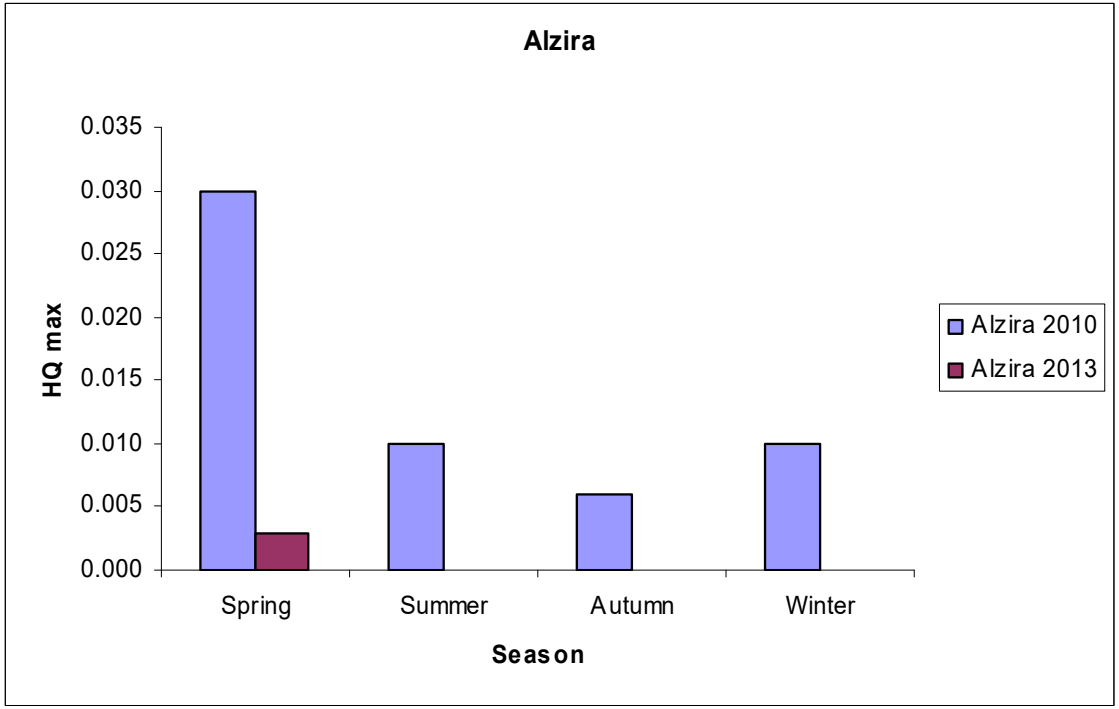


Figure SI-2. Seasonal distribution of HQ in Alzira.

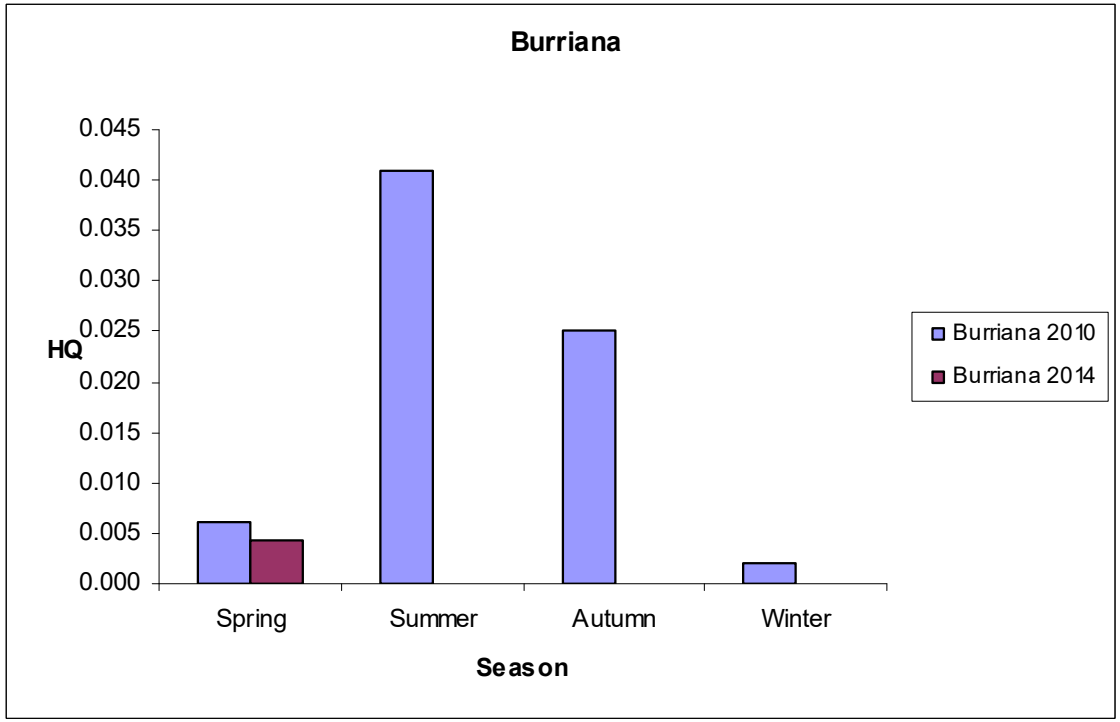


Figure SI-3. Seasonal distribution of HQ in Burriana.

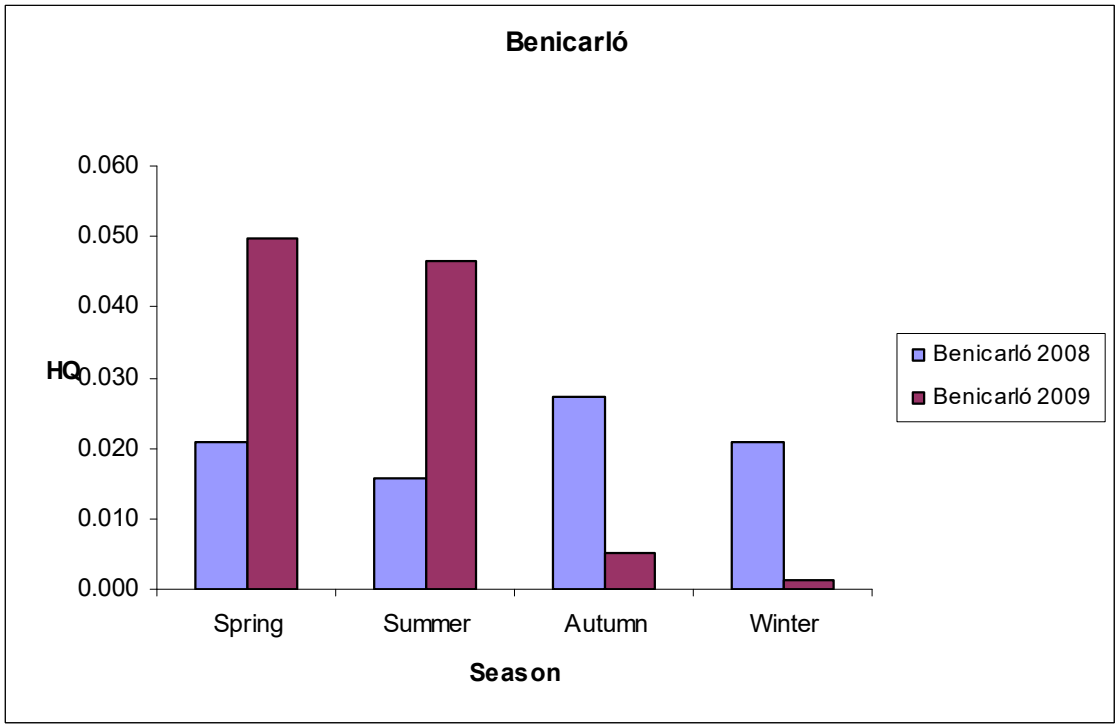


Figure SI-4. Seasonal distribution of HQ in Benicarló.

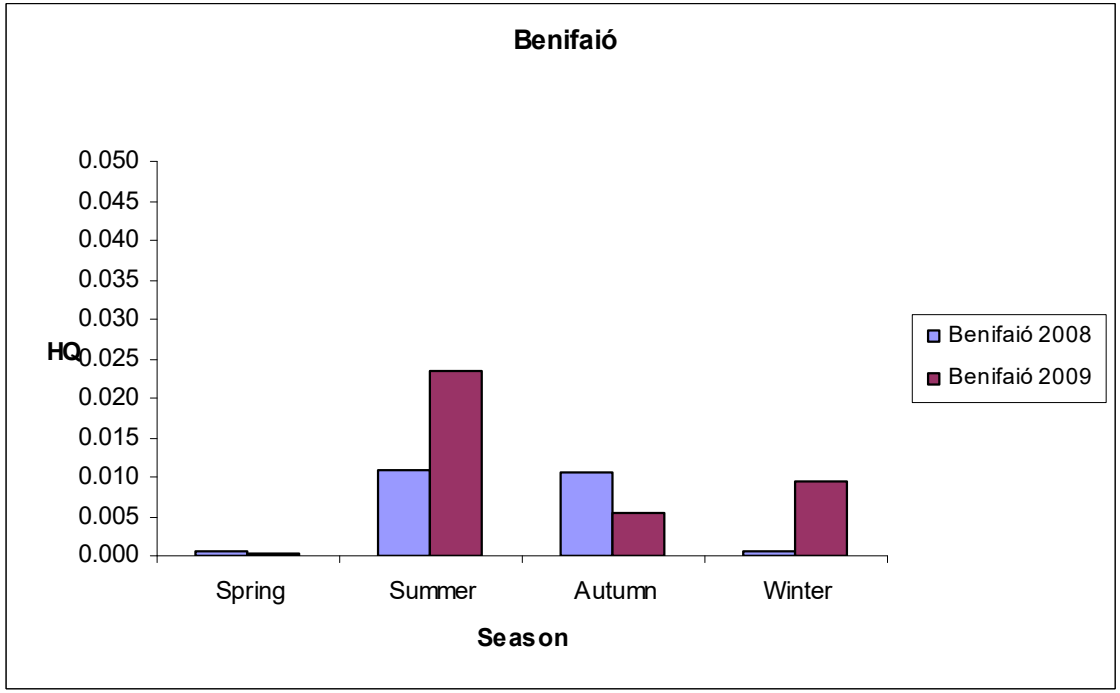


Figure SI-5. Seasonal distribution of HQ in Benifaió.

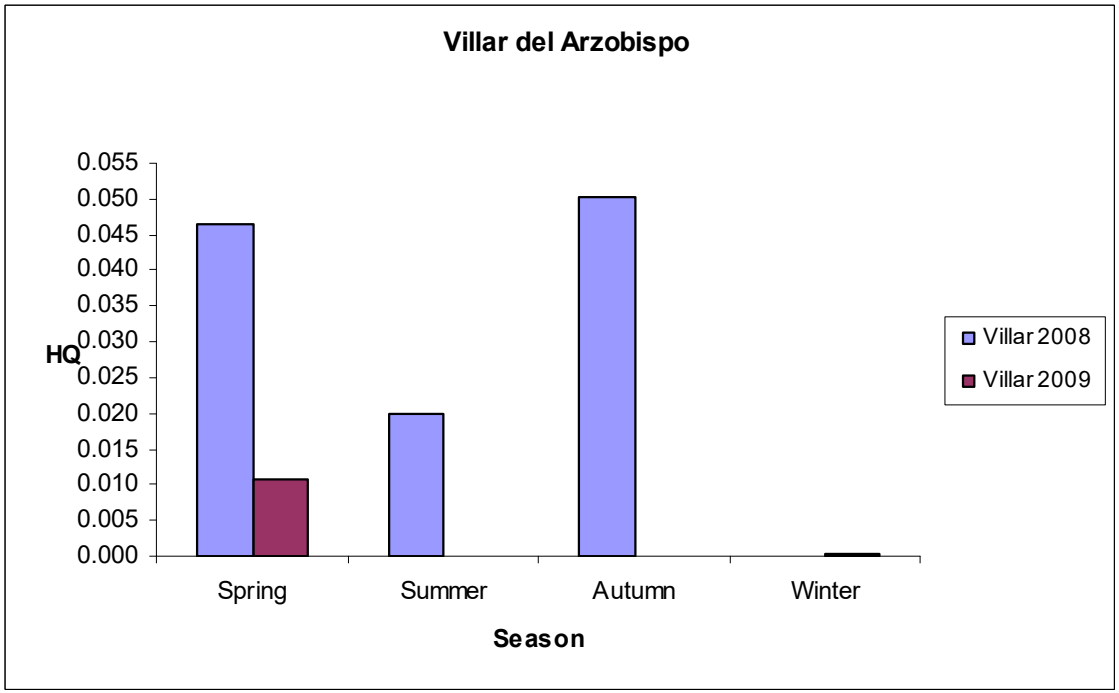


Figure SI-6. Seasonal distribution of HQ in Villar del Arzobispo.

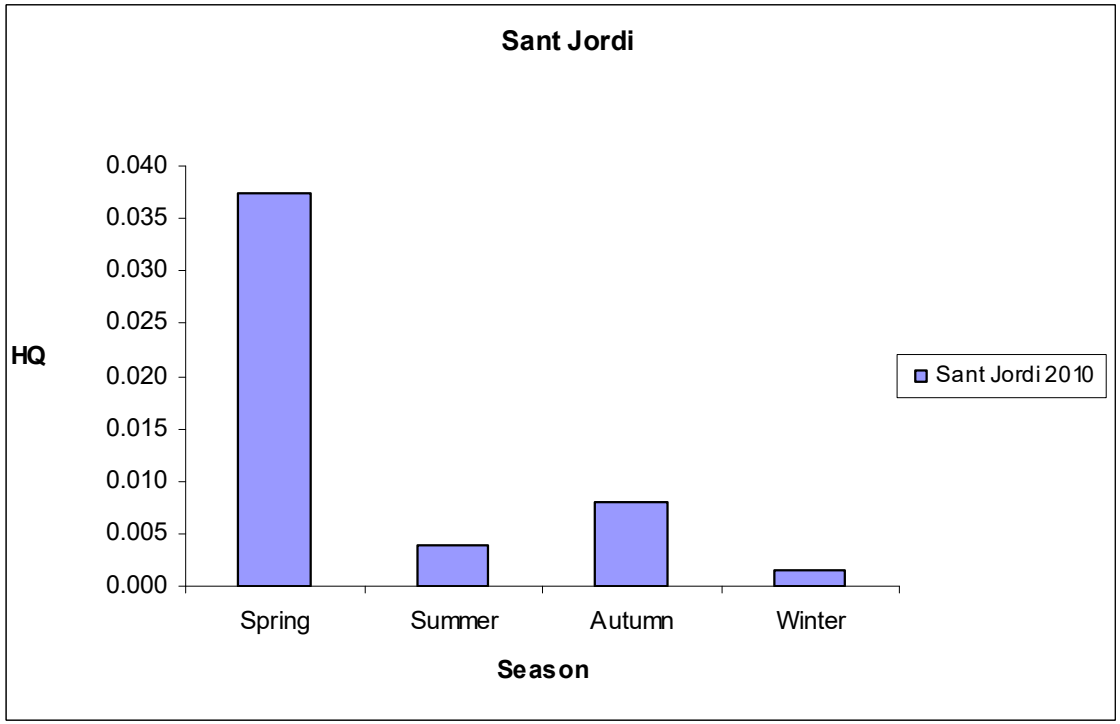


Figure SI-7. Seasonal distribution of HQ in Sant Jordi.

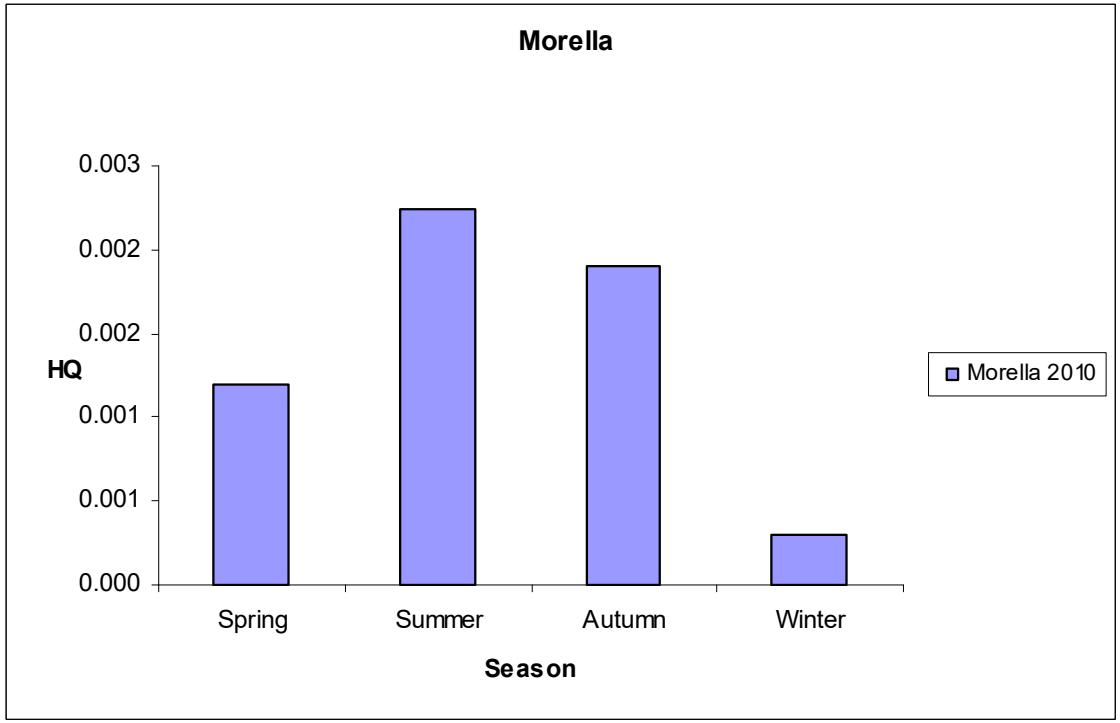


Figure SI-8. Seasonal distribution of HQ in Morella.

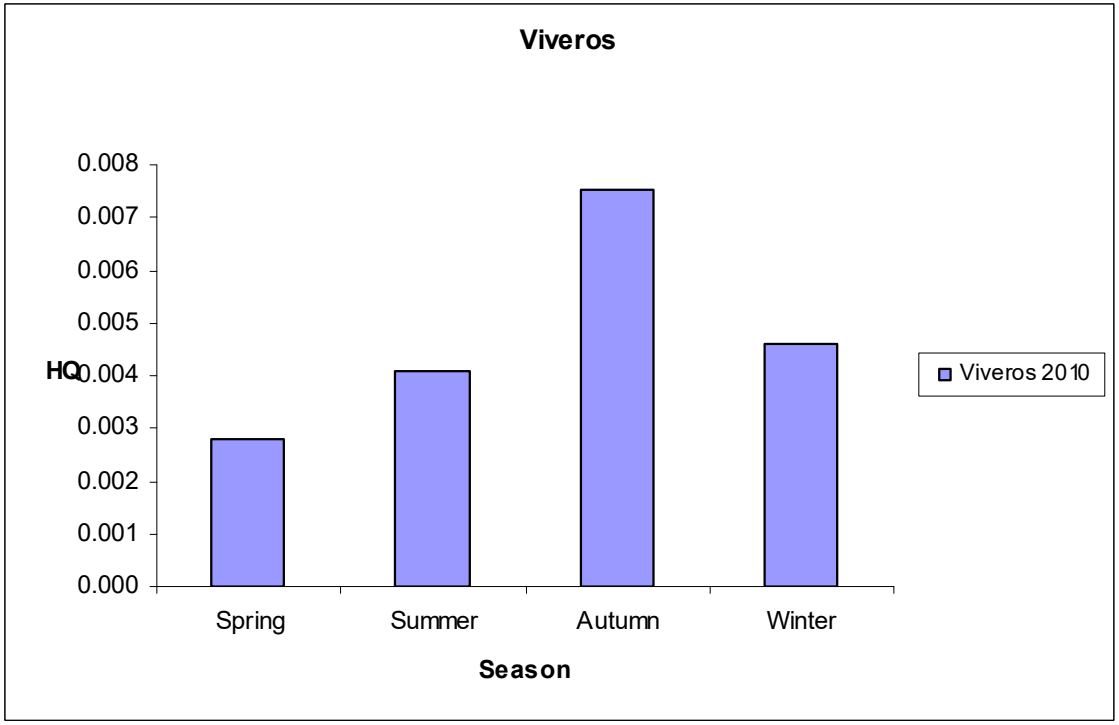


Figure SI-9. Seasonal distribution of HQ in Viveros (Valencia).

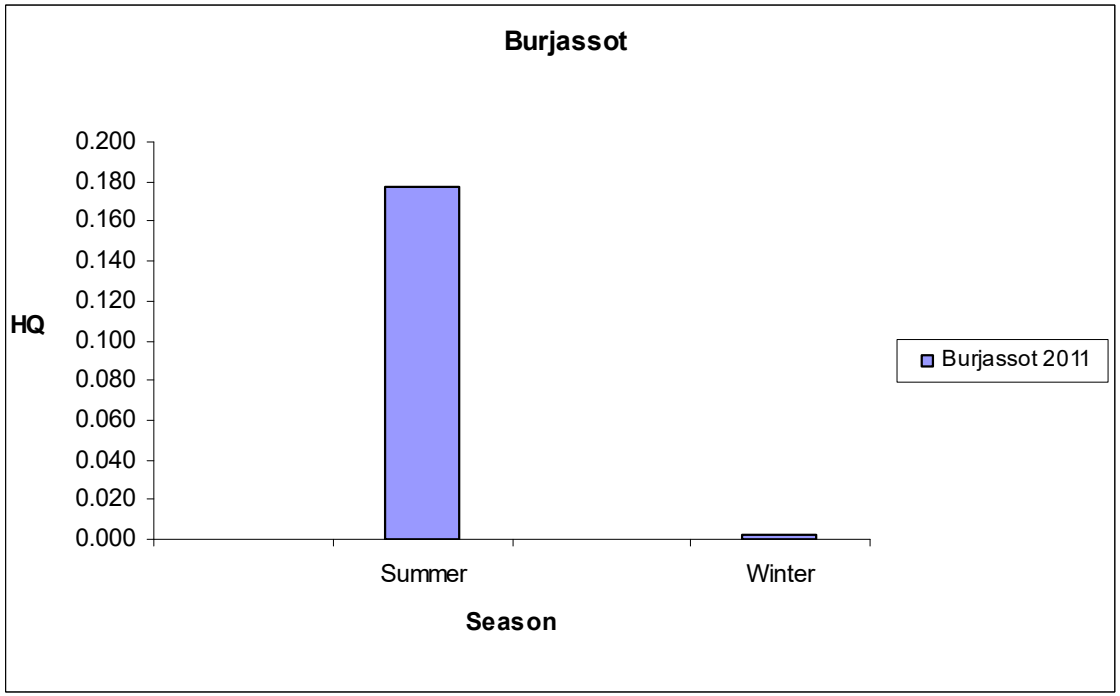


Figure SI-10. Seasonal distribution of HQ in Burjassot.

13.6. Anexo VI. Información suplementaria del artículo científico 6.

Supplementary Data for

Human exposure and risk assessment to airborne pesticides in a rural French community.

Clara Coscollà, Antonio López, Abderrazak Yahyaoui, Patrice Colin, Corine Robin, Quentin Poinsignon, Vicent Yusà.

Table of Contents:

Table SD-1 Selected ions for pesticides in GC-MS

Pesticide	Ions (m/z)
2,4' DDD	235, 237, 165
2,4' DDE	246, 248, 318
Acetochlor	146, 162, 223
Aclonifen	264, 212, 194
α-endosulfan	231, 207, 195
Alachlor	237, 160, 146
Azoxystrobin	388, 372, 345
Captan	79, 149, 77
Chlorothalonil	268, 266, 264
Chlorpyrifos ethyl	316, 314, 286
Chlorpyrifos methyl	286, 288, 125
Cyprodinil	225, 224, 210
Diazinon	304, 179, 152
Dichlobenil	173, 171, 136
Diflufenican	267, 246, 226
Dimethenamid	230, 203, 154
Dimethomorph	389, 387, 301
Diphenylamine	169, 168, 167
Ethofumesate	286, 207, 161
Ethoprophos	200, 158, 139
Ethyl parathion	291, 235, 218
Fenpropidin	273, 145, 117
Fenpropimorph	303, 173, 145
Fludioxonil	248, 182, 154
Folpet	295, 285, 260
γ-HCH	219, 183, 181
Kresoxim-methyl	116, 206, 131
Lambda-cyhalotrin	181, 197, 208
Malathion	285, 256, 173
Metazachlor	277, 209, 134
Methidathion	146, 145, 125
Methyl parathion	263, 233, 125
Metolachlor	162, 238, 240
Oryzalin	317, 275, 301
Oxadiazon	344, 302, 258
Oxyfluorfen	331, 302, 274
Pendimethalin	281, 252, 191
Pirimicarb	166, 72, 238
Procymidone	96, 283, 285
Propachlor	176, 120, 93
Propargite	230, 215, 173
Prosulfocarb	128, 91, 251

Pyrimethanil	198, 199, 200
Spiroxamine	198, 126, 100
Tolylfluanid	346, 238, 181
Trifloxystrobin	116, 131, 222
Trifluralin	306, 264, 290
Vinclozolin	285, 213, 212

Table SD-2 Selected ions for pesticides in LC-MS/MS

Pesticide	Precursor ion (m/z)	Products ions (m/z)	Polarity ESI
Atrazine	216	174, 104	+
Cymoxanil	199	128, 111	+
Difenoconazole	406	251, 337	+
Epoxiconazole	330	121,101	+
Fluazinam	463	416, 398	-
Flufenoxuron	489	158, 141	+
Iprodione	228	141	-
Mecoprop	213	141, 71	-
Phosmet	318	160,133	+
Pyriproxyfen	322	96, 185	+

[1]Chromatographic conditions: column; Gemini-NX3u C18 100A (Phenomenex). Eluents; water and methanol with 5mM of formiate ammonium in a gradient mode. Flow rate; 200 µl/min.

Table SD-3. NOAEL and relative potency factors for the inhalation exposure

Pesticide	Inhalation	
	NOAEL [a]	RPF [b]
Chlorpyrifos-ethyl	0.1	1
Malathion	121	0.0008
Methyl parathion	0.11	0.91
Phosmet	1.5	0.07

[a] Subchronic of intermediates NOAELs are taken from the re-registration eligible decision for the corresponding chemicals (mg/kg/day)

[b] Inhalation RPFs are calculated based on their NOAELs [262]

Table SD-4. Sources of uncertainty associated with the estimation of exposure to pesticides in the ambient air

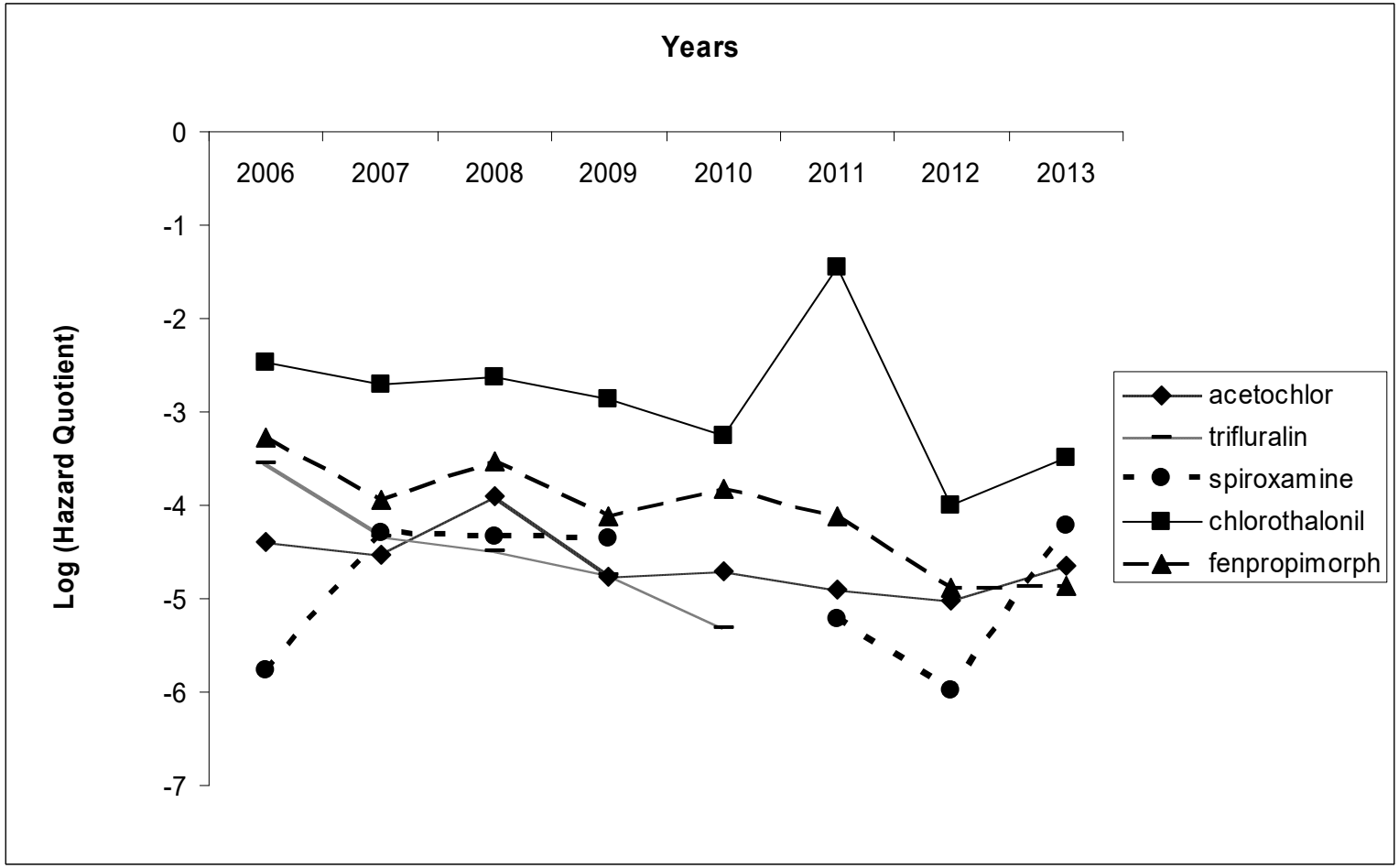
Sources of uncertainty	**Brief description**	**Uncertainty impact**
A. Uncertainties of pesticide concentration in QFF/PUF	Losses of volatile pesticides during sampling	(-)
	Analytical methods: RSD(%) ≤ 20% for all instrumental techniques	(+/-)
B. Exposure assessment	In the equation for DIE calculation we have used the tabulated average of IR_{inh} and body weight. However, these values present a variability inside the population.	(+/-)

RSD: Relative Standard Deviation
(+): could increase the final exposure and risk
(-): could decrease the final exposure and risk

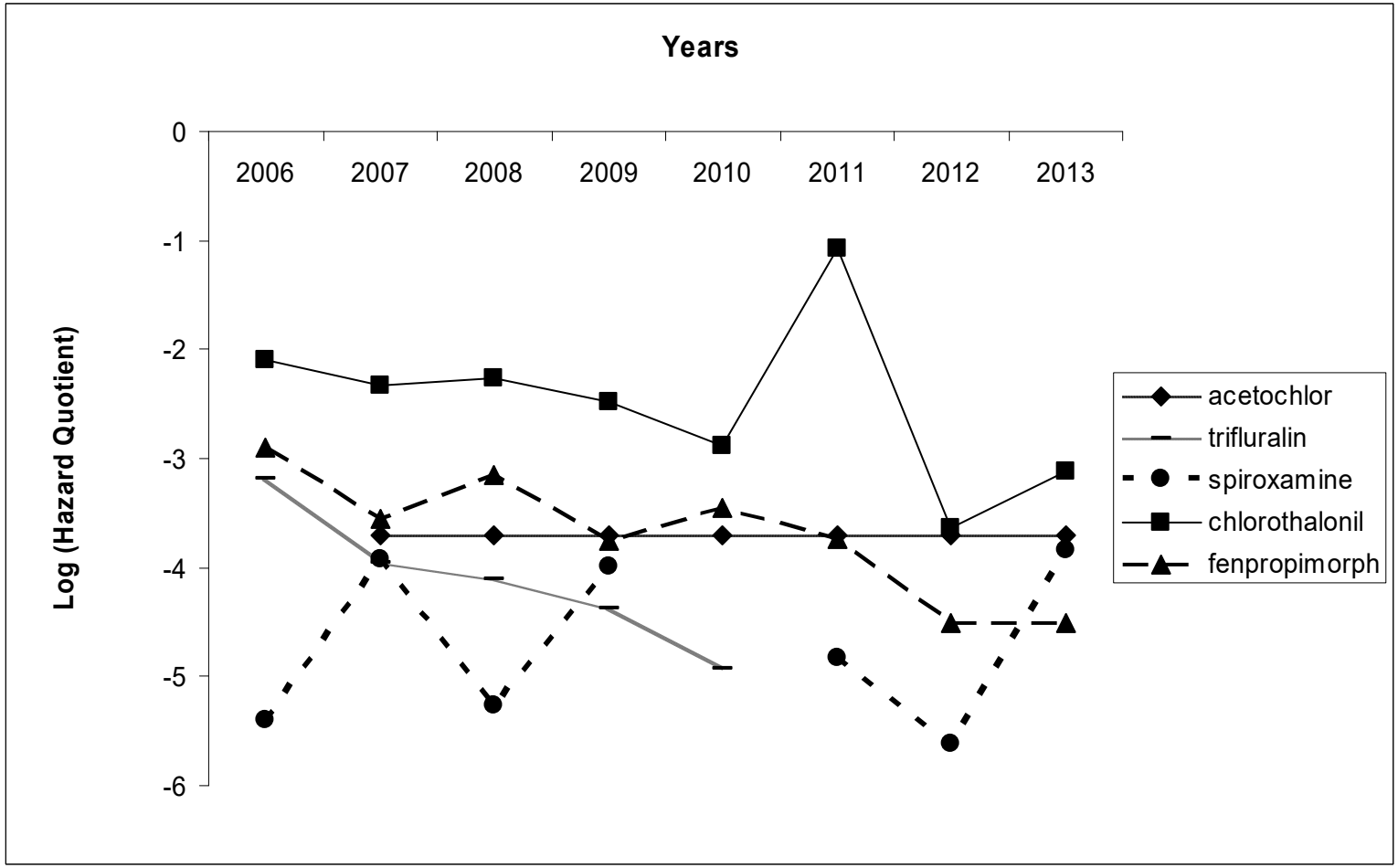
Table SD-5. Cumulative exposure for organophosphates in adults, children and infants (mg/kg/day)											
2006			2007			2008			2009		
Adults	Children	Infants	Adults	Children	Infants	Adults	Children	Infants	Adults	Children	Infants
1.08 E-07	2.55 E-07	3.01 E-07	7.87 E-08	1.86 E-07	2.51 E-07	1.83 E-10	4.33 E-10	5.11 E-10	1.18 E-10	2.78 E-10	3.28 E-10
2010			2011			2012			2013		
Adults	Children	Infants	Adults	Children	Infants	Adults	Children	Infants	Adults	Children	Infants
-	-	-	4.55 E-08	1.08 E-07	1.27 E-07	-	-	-	3.94 E-07	9.30 E-07	1.10 E-06

Table SD-6. Lifetime cancer risk for infants

Chlorothalonil	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Cancer risk	8.55E-06	4.90E-06	5.89E-06	3.51E-06	1.39E-06	8.94E-05	2.46E-07	8.09E-07
Maximum	8.55E-05	4.90E-05	5.89E-05	3.51E-05	1.39E-05	8.94E-04	2.46E-06	8.09E-06
Metolachlor	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Cancer risk	6.95E-08	5.93E-08	1.58E-07	6.15E-08	6.02E-08	2.77E-08	1.43E-08	3.48E-08
Maximum	6.95E-07	5.93E-07	1.58E-06	6.15E-07	6.02E-07	2.77E-07	1.43E-07	3.48E-07
Trifluralin	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Cancer risk	2.04E-06	3.34E-07	2.38E-07	1.31E-07	3.56E-08			
Maximum	2.04E-05	3.34E-06	2.38E-06	1.31E-06	3.56E-07	-	-	-
Pendimethalin	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Cancer risk	9.29E-06	1.38E-07	2.88E-07	3.95E-07	1.92E-07	1.15E-07	3.80E-08	3.15E-07
Maximum	9.29E-05	1.38E-06	2.88E-06	3.95E-06	1.92E-06	1.15E-06	3.80E-07	3.15E-06

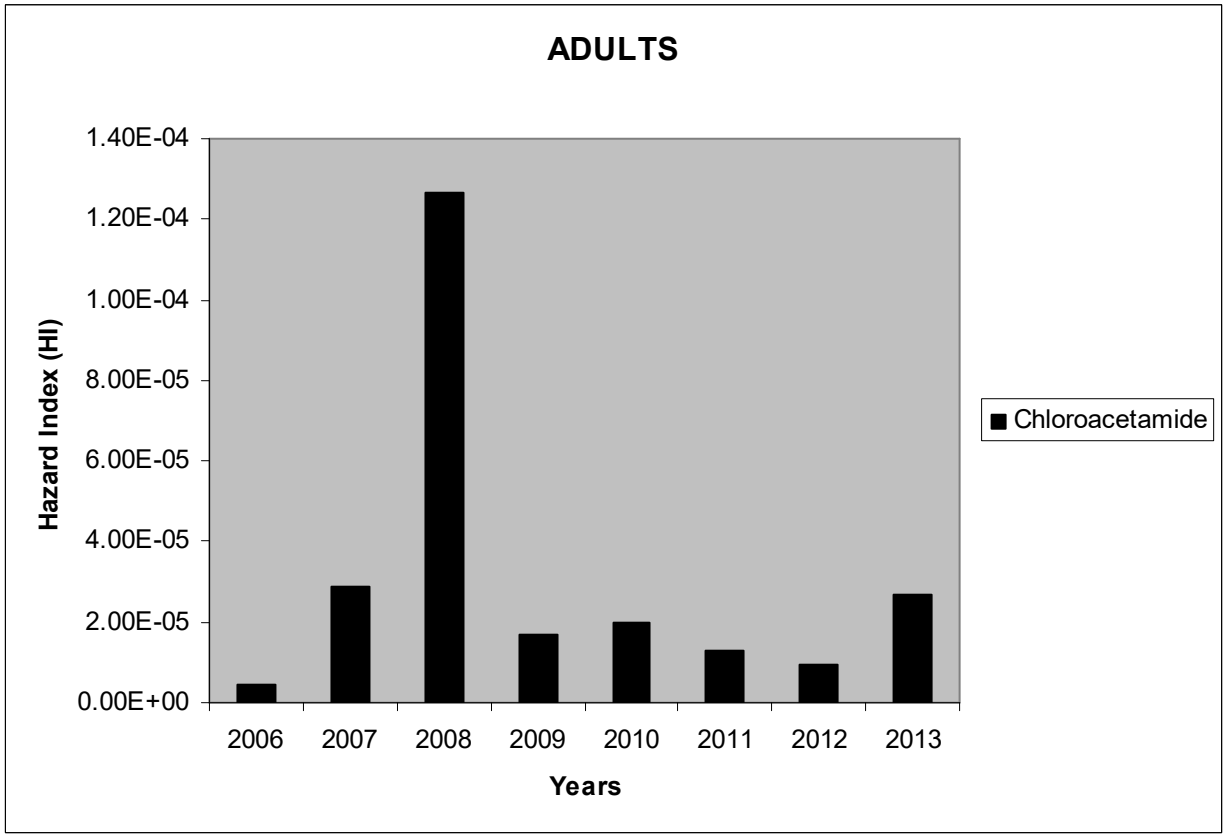

Years
2006
2007
2008
2009
2010
2011
2012
2013
0
-1
-2
-3
-4
-5
-6
-7
Log (Hazard Quotient)
acetochlor
trifluralin
spiroxamine
chlorothalonil
fenpropimorph

a)

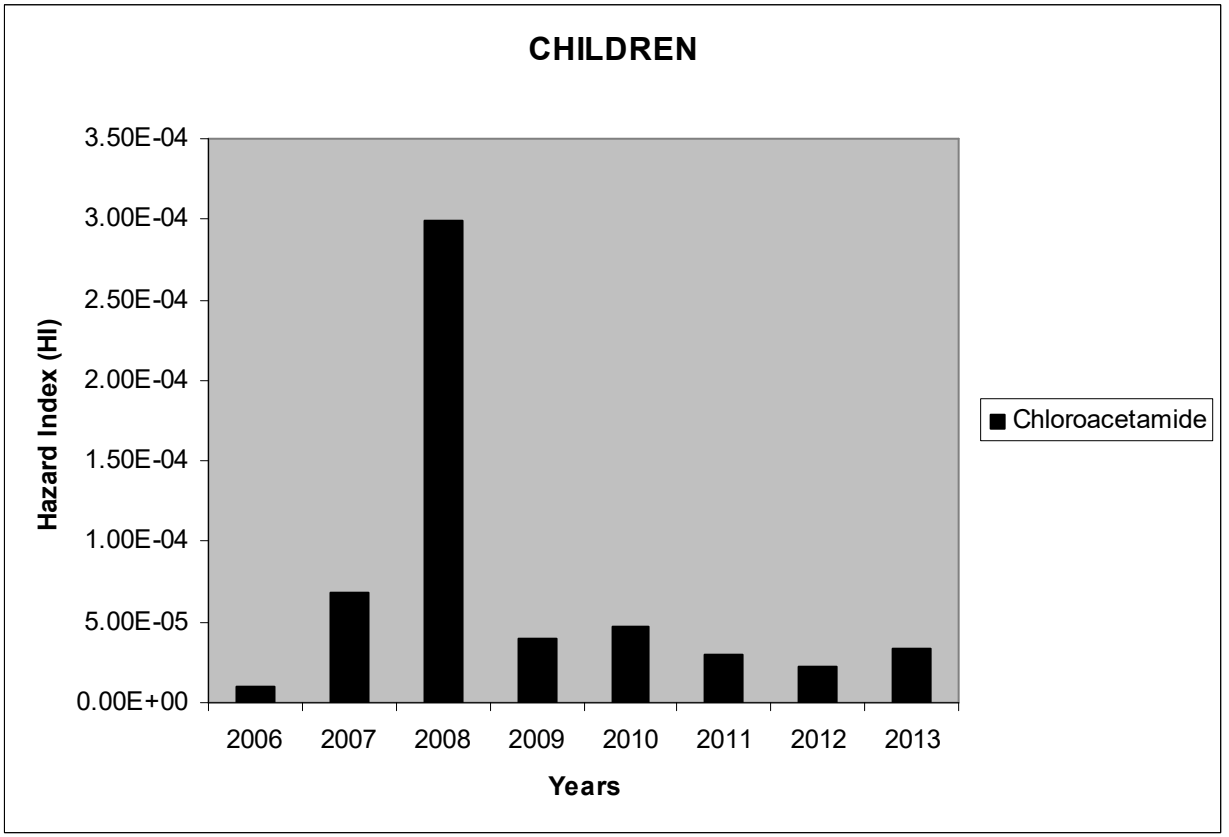


b)

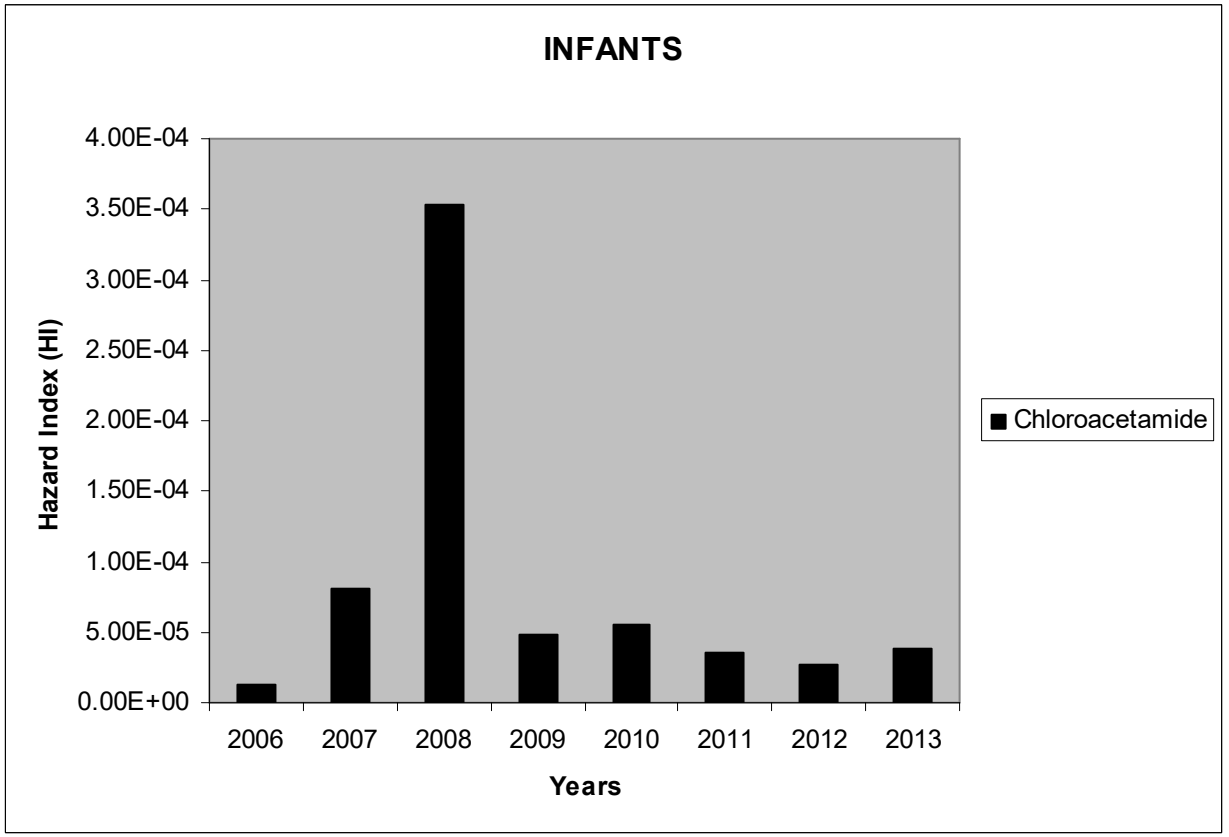
Figure SD-1 Temporal evolution of Hazard Quotient (HQ) for adults (a) and children (b) population.


ADULTS
1.40E-04
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
Hazard Index (HI)
2006
2007
2008
2009
2010
2011
2012
2013
Years
Chloroacetamide

a)


CHILDREN
3.50E-04
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
Hazard Index (HI)
2006
2007
2008
2009
2010
2011
2012
2013
Years
Chloroacetamide

b)



c)

Figure SD-2 Temporal evolution of Hazard Index (HI) for adults a), children b) and infants c) population.