The Handbook of Environmental Chemistry 92
Series Editors: Damià Barceló · Andrey G. Kostianoy

Ethel Eljarrat *Editor*

Pyrethroid Insecticides

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The Handbook of Environmental Chemistry

Volume 92

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Pyrethroid Insecticides

Volume Editor: Ethel Eljarrat

With contributions by

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Springer

Editor
Ethel Eljarrat
Institute of Environmental Assessment and Water Research (IDAEA-CSIC)
Barcelona, Spain

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Series Editors

Prof. Dr. Damià Barceló

Department of Environmental Chemistry
IDAEA-CSIC
C/Jordi Girona 18–26
08034 Barcelona, Spain
and
Catalan Institute for Water Research (ICRA)
H20 Building
Scientific and Technological Park of the University of Girona
Emili Grahit, 101
17003 Girona, Spain
dbcqam@cid.csic.es

Prof. Dr. Andrey G. Kostianoy

Shirshov Institute of Oceanology
Russian Academy of Sciences
36, Nakhimovsky Pr.
117997 Moscow, Russia
and
S.Yu. Witte Moscow University
Moscow, Russia
kostianoy@gmail.com

Editorial Board Members

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

Preface

The book on "*Pyrethroid Insecticides*" is based on the scientific developments and results achieved along several years of research. Pyrethroid insecticides, introduced in the late 1970s, actually represent 25% of global sales of insecticides. In the last decades, they have increasingly replaced organochlorine pesticides due to their relatively lower mammalian toxicity, selective insecticide activity and lower environmental persistence. They are considered to be "safe" because they are converted to non-toxic metabolites by oxidative metabolism in fish and by hydrolysis in mammals. However, recent studies demonstrated their environmental ubiquity, their bioaccumulation and their toxicity in different aquatic and terrestrial organisms and even in humans.

This book aims to review and compile the main developments and knowledge acquired over many years of study from a multidisciplinary way, including analytical chemistry, environmental, biological and toxicological developments. The book is structured in 12 different chapters, covering the state of the art of analysis, fate and behaviour and toxicity of pyrethroid insecticides. Experts in the field provide an overview of their physico-chemical properties and uses, the advanced chemical analytical methods, the occurrence in environment and biota, the isomeric and enantiomeric behaviour, the toxicological effects and the human exposure. Finally, the last chapter concerns the main conclusions and future trends, being the starting point to be taken in mind for the future studies in the field of pyrethroid insecticides.

We hope the book will be of interest to a broad audience of scientific researchers as well as for authorities and producers. Finally, I would like to thank all the contributing authors of this book for their time and effort in preparing this comprehensive compilation of research papers.

Barcelona, Spain
January 2020

Ethel Eljarrat

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Introduction to Pyrethroid Insecticides: Chemical Structures, Properties, Mode of Action and Use

Ò. Aznar-Alemany and E. Eljarrat

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Abstract During the 1920s, pyrethrin was studied because of its potential as a precursor for synthetic organic pesticides. The first pyrethroid pesticide, allethrin, was identified in 1949. It is a type I pyrethroid because of a carboxylic ester of cyclopropane. Type II was created with the addition of a cyano group in α position. Some phenylacetic 3-phenoxybenzyl esters missing the cyclopropane but with the cyano group are also considered type II. In the 1970s, pyrethroids transitioned from mere household products to pest control agents in agriculture. Later, pyrethroids have replaced organophosphate pesticides in most of their applications the same way the latter had replaced organochlorinated pesticides before. Works on the optimisation of pyrethroids has granted them better photostability without compromising their biodegradability, as well as selective toxicity, metabolic routes of degradation and more effectivity, translating into the use of smaller amounts. Most pyrethroids present different isomers, each with different biological activity and, therefore, different toxicity. Pyrethroids account for a quarter of the pesticides used nowadays. Pyrethroids' relative molecular mass is clearly above 300 g mol^{-1}; they are highly hydrophobic, photosensitive and get easily hydrolysed, with degradation times below 60 days. They are not persistent and mammals can metabolise them.

Ò. Aznar-Alemany (✉) and E. Eljarrat
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain
e-mail: oscar.az.al@gmail.com

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However, pyrethroids have been proven to bioaccumulate in marine mammals and humans. Studies in mammals reported carcinogenic, neurotoxic and immunosuppressive properties and potential for reproductive toxicity mainly. Acceptable daily intake values and no observed adverse effect level values have been established at 0.02–0.07 mg (kg body weight)$^{-1}$ day^{-1} and 1–7 mg (kg body weight)$^{-1}$ day^{-1}.

Keywords Chemical structures, Metabolisation, Pest control, Pesticides, Physicochemical properties, Pyrethroids, Toxicity

Abbreviations

ADI	Acceptable daily intake
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
DDT	Dichlorodiphenyltrichloroethane
DT_{50}	Degradation time for 50% of the substance
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
IC_{50}	Half-maximal inhibitory concentration
K_{ow}	Octanol-water partition coefficient
LOD	Limit of detection
LOEC	Lowest observed effect concentration
M_r	Relative molecular mass
MRL	Maximum residue level
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
POP	Persistent organic pollutant

1 History and Impact

During the 1920s, pyrethrin was studied because of its potential as a precursor for synthetic organic pesticides. Pyrethrin was extracted from pyrethrum, a plant of the family of chrysanthemums [1]. Research on synthetic organic pesticides increased in the 1930s, and in 1939, dichlorodiphenyltrichloroethane (DDT) was synthesised. It proved to be effective for many plagues. DDT was so effective that other organochlorinated compounds were studied with the aim of obtaining cheap and persistent pesticides.

At first, pesticides were not considered to affect health or the environment. However, in 1962 Rachel Carson published *Silent Spring*, where she warned about the effects of pesticides on the environment with the image of dead birds in her garden.

This field observation prompted several research studies about environmental and mesocosm models focused on the assessment of pyrethroids and other pesticides [1]. As a consequence, some regulation agencies came into existence. In 1970, the Environmental Protection Agency (EPA) was founded. From that moment on, the use of organochlorinated compounds was restricted or banned as they were considered toxic and contaminant [2, 3]. Nevertheless, they are still allowed to fight malaria [4–6].

In the 1940s, it was discovered that many organophosphate compounds had unique properties for the protection of plants – and that the most volatile and toxic could be used as chemical weapons. However, not until the 1960s did organophosphate compounds become popular. At the end of the same decade, there was an increasing interest in carbamate pesticides.

Organophosphates and carbamates had simple structures, and it was easy to synthesise analogous derivatives. They also showed some advantages over organochlorinated pesticides [1]. They were selectively toxic with different effects depending on the species; they affected insects more than mammals [7]; the effects on mammals occurred mostly after intense exposition rather than accumulation; they were more biodegradable, therefore, less persistent, and they allowed the creation of compounds that stay inside the plants for a few weeks and protect them. On the other hand, regulations and bans on the use of organophosphates and carbamates emerged as a consequence of new data on their actual toxicity [8]. Toxicology studies are a key element of the development of new pesticides nowadays.

In the 1970s, pyrethroids stopped being mere household products to become pest control agents in agriculture. Moreover, in the last couple of decades, pyrethroids have replaced organophosphate pesticides in most of their applications the same way the latter had replaced organochlorinated pesticides before [9, 10]. Pyrethroids were very effective.

Works on the optimisation of these derivatives from pyrethrin had been going on for decades, and several improvements were achieved [1]. Their photostability was improved without compromising their biodegradability. They achieved a selective toxicity and metabolic routes of degradation – that were different for *cis* and *trans* isomers. They were produced as fumigants as well as soil pesticides. And they were made more powerful so that smaller amounts would need to be used and environmental contamination would be reduced.

The development of pyrethroids included some aspects that helped reduce the impact of pesticides on the environment: higher effectiveness implying smaller amounts of product needed, selective toxicity, concern on the occurrence of pesticides in the environment and replacement of persistent compounds with degradable compounds [1].

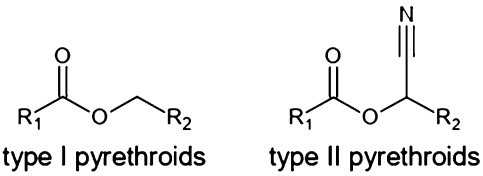

Fig. 1 Pyrethroid types according to their general structure

2 The Compounds

The first pyrethroid pesticide, allethrin, was identified in 1949 [11]. It is a type I pyrethroid because of the carboxylic ester of cyclopropane. Type II was created with the addition of a cyano group in α position, which increased the pesticide effect of pyrethroids (Figs. 1, 2 and 3).

Additionally, pesticide activity was detected in some phenylacetic 3-phenoxybenzyl esters that missed the cyclopropane but had the cyano group [11]. These esters were still considered type II pyrethroids and originated compounds such as fenvalerate.

Due to the cyclopropane and the cyano group, most pyrethroids present different isomers, each with different biological activity and, therefore, different toxicity. Type I pyrethroids have two chirality centres, hence two diastereoisomers or enantiomeric pairs. Type II pyrethroids present three chirality centres, hence four diastereoisomers. The bonds that are responsible for the existence of enantiomeric pairs are represented with winding lines in Figs. 2 and 3. These diastereoisomers present different properties [12]. More detailed information of pyrethroid stereoselectivity is presented in Chapter "Stereoselectivity and Environmental Behaviour of Pyrethroids".

Pyrethroids account for a quarter of the pesticides used nowadays [1, 13]. They were believed to be the ideal pesticides because they are not persistent and were thought to be metabolised and not bioaccumulate [14, 15]. Thus they replaced the previously banned pesticides. Total organic pesticide production in the United States increased from about 15 tons per year in 1945 to over 630 tons per year in 1976 [16]. In 2006 over 433 tons of pesticides were used worldwide, 400 tons in 2007 [17]. Pyrethroids account for about 25% of the pesticide use.

Pyrethroids have applications as pesticides in households, in commercial products and in medicine against scabies and lice (Table 1). In tropical countries, mosquito nets are impregnated with solutions of deltamethrin, cyhalothrin or cypermethrin to control malaria [11].

3 Properties

Pyrethroids present somewhat similar physicochemical properties among them (Table 2). Their relative molecular mass (M_r) is clearly above 300 g mol^{-1}. They are highly hydrophobic, with logarithm of the octanol-water partition coefficient

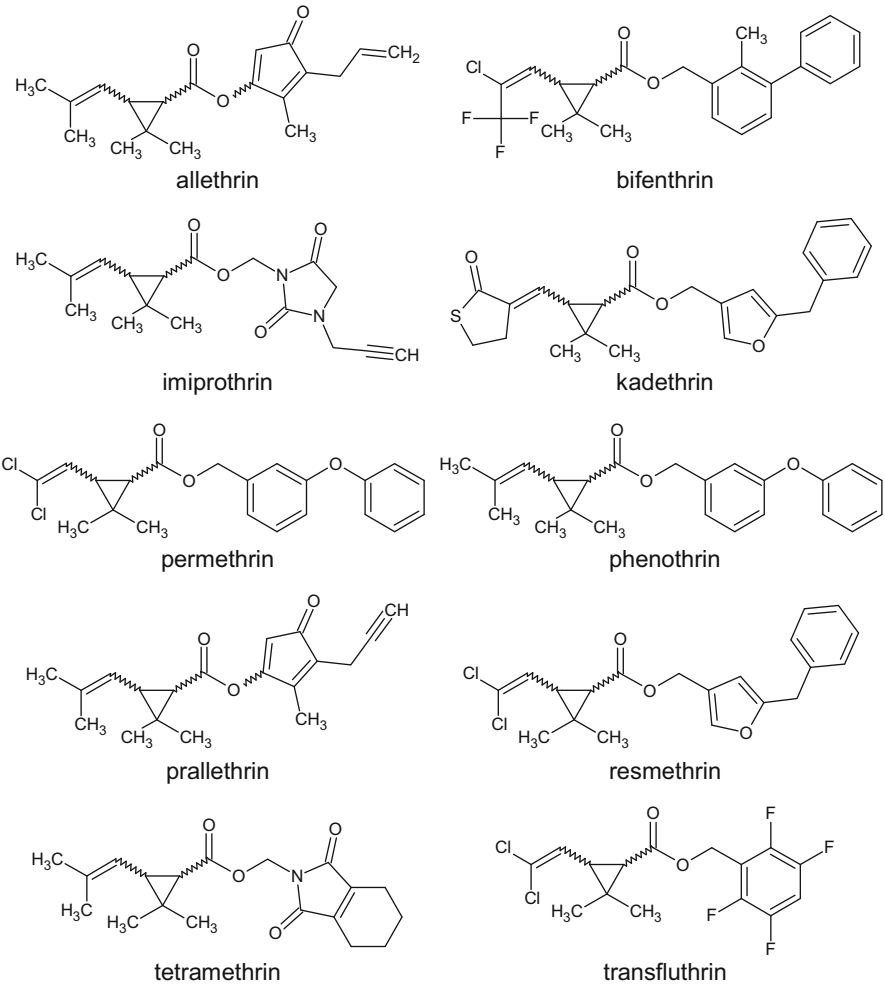

Fig. 2 Type I pyrethroids

(K_{ow}) between 4 and 7, and show low very low solubility in water of a few $\mu g\, L^{-1}$. Pyrethroids are photosensitive and get easily hydrolysed; therefore their degradation time for 50% of the substance (DT_{50}) – indicating persistence – is very low, below 60 days [21].

Organic contaminants include a wide variety of families. Some of them have been considered persistent organic pollutants (POPs). The Stockholm Convention on Persistent Organic Pollutants defined four factors that make a compound dangerous and that qualify it as a POP [22]. These are the requirements a compound needs to meet to be included in the list of the Stockholm Convention:

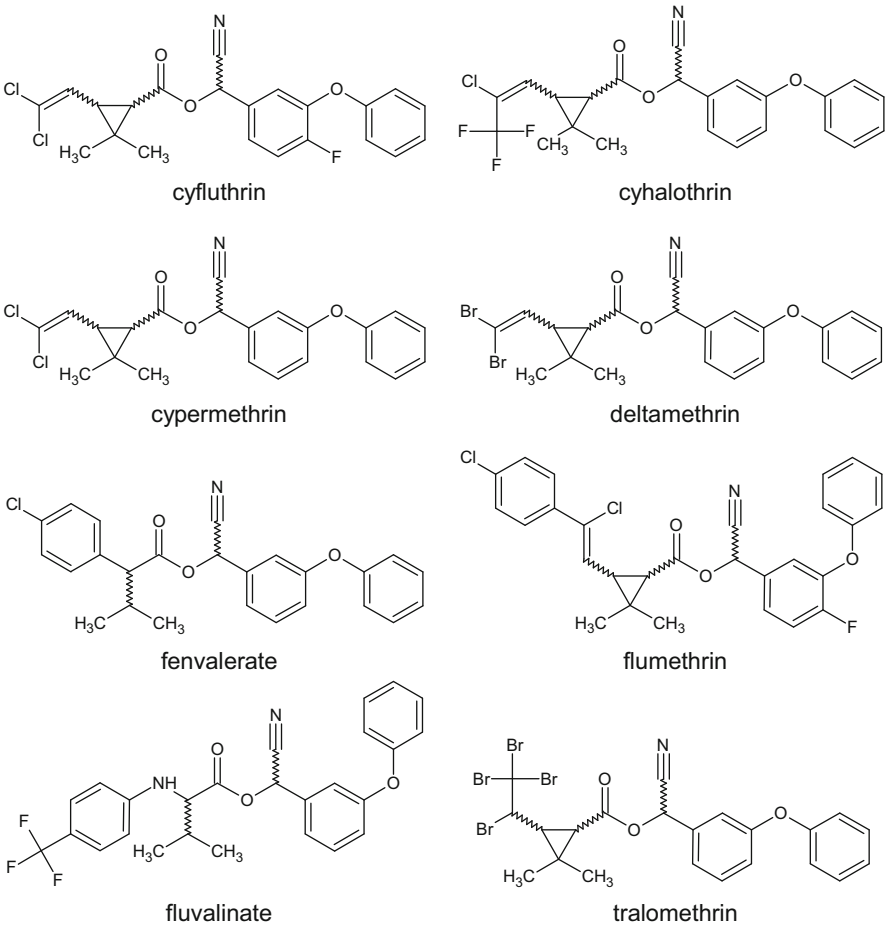

Fig. 3 Type II pyrethroids

Table 1 Pyrethroid applications [18–20]

Insects	Crops	Others
Ants, bedbugs, beetles, caterpillars, cockroaches, flies, greenflies, lice, lobsters, locusts, mites, mosquitoes, moths, termites, wasps, whiteflies	Alfalfa, apple, bean, beetroot, cereal, citrus, coffee, cotton, fig, grape, green bean, lettuce, melon, olive, onion, pea, peach, peanut, pear, potato, rice, seeds, soy, sugarcane, sunflower, tea, tobacco, tomato, walnut, watermelon, wheat	Forests, gardens, grass, greenhouses, households, industries, ornaments, pets, public health, shampoo, shops, warehouses, wood

Table 2 Properties of pyrethroids [21]

Pyrethroid	Type	Molecular formula	M_r ($g\,mol^{-1}$)	log K_{ow}	Water solubility at 20°C ($\mu g\,L^{-1}$)	DT_{50} (days)
Allethrin	I	$C_{19}H_{26}O_3$	302.4	4.96	0.1	–
Bifenthrin	I	$C_{23}H_{22}O_2ClF_3$	422.9	6.6	1	26
Imiprothrin	I	$C_{17}H_{22}N_2O_4$	318.4	2.43	93,500	–
Kadethrin	I	$C_{23}H_{24}O_4S$	396.5	6.29	14	–
Permethrin	I	$C_{21}H_{20}O_3Cl_2$	391.3	6.1	200	13
Phenothrin	I	$C_{23}H_{26}O_3$	350.5	6.01	9.7	–
Prallethrin	I	$C_{19}H_{24}O_3$	300.4	4.49	8,030	–
Resmethrin	I	$C_{22}H_{26}O_3$	338.5	5.43	10	30
Tetramethrin	I	$C_{19}H_{25}NO_4$	331.4	4.6	1,830	3
Transfluthrin	I	$C_{15}H_{12}Cl_2F_4O_2$	371.2	5.46	57	7
Cyfluthrin	II	$C_{22}H_{18}NO_3Cl_2F$	434.3	6	6.6	33
Cyhalothrin	II	$C_{23}H_{19}NO_3ClF_3$	449.9	6.9	4	57
Cypermethrin	II	$C_{22}H_{19}NO_3Cl_2$	416.3	5.3	9	60
Deltamethrin	II	$C_{22}H_{19}NO_3Br_2$	505.2	4.6	0.2	13
Fenvalerate	II	$C_{25}H_{22}NO_3Cl$	419.9	5.01	1	40
Flumethrin	II	$C_{28}H_{22}Cl_2FNO_3$	510.4	–	–	–
Fluvalinate	II	$C_{26}H_{22}N_2O_3ClF_3$	502.9	3.85	2	7
Tralomethrin	II	$C_{22}H_{19}NO_3Br_4$	665.0	5	80	3

1. To be persistent in the environment. POPs have half-lives greater than 2 months in water or greater than 6 months in soil and sediment.
2. To bioaccumulate. POPs have bioconcentration factors (BCFs) or bioaccumulation factors (BAFs) in aquatic species greater than 5,000 or, when unknown, their log K_{ow} is greater than 5.
3. To have potential for long-range transport. POPs are detected far from the emission source; data show they have been transported via air (half-live in air over 2 days), water or migratory species.
4. To have adverse effects. POPs are proved to have adverse effects on human health or on the environment.

The original list of the Stockholm Convention included 12 POPs that were banned or restricted. Eight of them were organochlorinated pesticides: aldrin, endrin, dieldrin, chlordane, DDT, heptachlor, mirex and toxaphene. These pesticides were considered safe when they first entered the market, but data proved them to cause long-term adverse effects on human health and on the environment. New compounds have been added to the list throughout the years.

Some other compounds, like pyrethroids, cannot be classified as POPs, but cause concern in the scientific community due to their properties, sometimes close to those of POPs. Pyrethroids have logarithms of K_{ow} on the limit of POPs and affect organisms by design. However, they are not persistent and thus cannot be transported long distances and mammals can metabolise them [14, 23]. Conversely they have been proved to bioaccumulate in marine mammals and humans [24, 25].

More detailed information of bioaccumulation of pyrethroids in wildlife and humans is presented in Chapter "Bioavailability and Bioaccumulation of Pyrethroid Insecticides in Wildlife and Humans".

The Water Framework Directive (Directive 2000/60/EC) named a group of pesticides that could be toxic, persistent and bioaccumulate. Among them, cypermethrin was listed. Due to their production volume and extensive application, pesticides such as pyrethroids are always present in the environment despite not being persistent and are therefore considered *pseudo*-persistent organic contaminants [26].

4 Metabolisation

The capacity of mammals of metabolising pyrethroids has been regarded as one of the best qualities of these pesticides. The metabolisation route differs with the organism. However, the routes are equivalent for many mammals, and the mechanism in humans will serve as an example.

The liver is the main organ responsible for disintoxication in humans, although other organs and tissues possess the required enzymes to treat xenobiotics. This disintoxication usually proceeds in two steps [27]. The first step consists in increasing the polarity of the xenobiotic molecular through processes like hydroxylation, deamination or the N-oxidation. In the second step, the metabolite – which is more polar than the original molecule – is combined with endogen products of the cell, such as methyl or acetyl groups, monosaccharides or amino acids. This increases the metabolite solubility making it easier for it to be excreted in urine. This is the reason why exposition of humans to pyrethroids is studied through the analysis of their metabolites in urine [28].

The first step of the metabolisation of pyrethroids in humans can occur through two pathways. One is the breakdown of the ester to produce carboxylic acid and the corresponding alcohol by the action of carboxylesterases [29]. Then, alcohol can be oxidised to a benzoic acid (Fig. 4).

The carboxylesterases required for this metabolisation are found in the plasma of mammals at higher concentrations than in fish or birds [30]. This could be a factor in explaining the lower toxicity of pyrethroids in mammals.

On the other hand, carboxylesterases present isoenzymes that can be found in different proportions in each individual depending on factors such as species, age or gender [30]. Each isoenzyme can have a different activity on different isomers of pyrethroids, thus making the capacity of metabolising these compounds change not only among species, but also among individuals of different age and gender [31].

The second pathway for the first step of the metabolisation of pyrethroids in humans is hydroxylation by monooxygenases. The process usually undergoes transformation via both pathways producing secondary products such as 4-hydroxy-3-phenoxybenzoyl and 4-hydroxy-3-phenoxylbenzaldehyde for permethrin. These compounds can be stronger endocrine disruptors than their non-hydroxylated analogues [32].

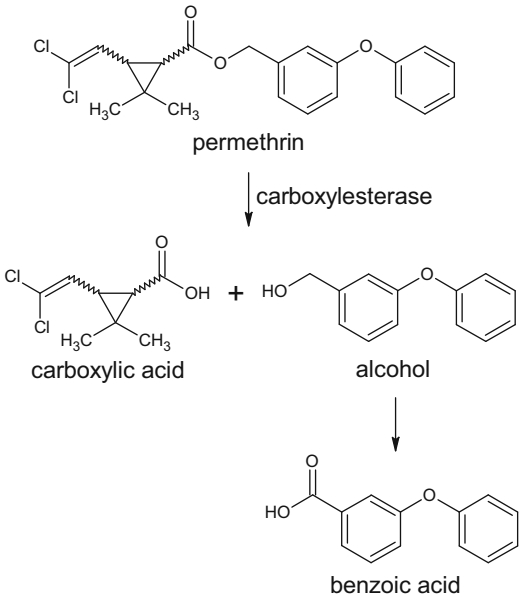

Fig. 4 Metabolisation of permethrin

However, it is important to note that despite the fact that mammals can metabolise pyrethroids, studies have shown that they can also bioaccumulate these pesticides [25, 33].

5 Toxicity

Exposition of organisms to pyrethroids causes concern due to the toxicity of the pesticides [34]. Recent studies in mammals reported carcinogenic, neurotoxic and immunosuppressive properties and potential for reproductive toxicity [12, 35, 36]. Type I pyrethroids cause tremors and reflex hyperexcitability, while type II cause hyperexcitability, salivation, seizures and choreoathetosis [37].

The main action of pyrethroids is on the sodium channels and chloride channels, which drive the ions through the cell membrane [1, 11, 13]. Pyrethroids lower the threshold of the action potential of nerve cells and muscle cells and cause repeated stimulation [7, 38]. At high concentrations, the entrance of sodium can prevent the generation of the action potential, block conduction and cause paralysis. Small amounts are sufficient to affect the sensitivity of nerve cells.

Type II pyrethroids also decrease the flux of chloride through the chloride channels. Additionally, relatively high concentrations of type II pyrethroids can affect the receptors of γ-aminobutyric acid and cause cataleptic attacks, which have been documented in humans [11, 37].

Pyrethroids are about 2,250 times more toxic for insects than mammals. Insects have more sensitive sodium channels, smaller bodies and low body temperatures. Moreover, the absorption through the skin in mammals is weak, and they can metabolise them into non-toxic compounds fast [11].

Human exposition to pyrethroids has been documented studying their metabolites in urine of German children and teenagers [28], in hair and blood of pregnant women and meconium of babies [39], in plasma of pregnant women from rural areas of South Africa [40] and in human milk [25, 33].

A few studies focused on marine organisms including different tissues of Brazilian dolphins [24, 41], Mediterranean dolphins [42] and wild and edible fish from Spanish rivers [43].

Seafood production has experimented a 3.2% yearly growth since 1961 [44]. Aquaculture is responsible for half of the seafood production worldwide, and the world annual fish consumption per capita is about 20 kg. While concern about the application of pyrethroids in fish farms against fish parasites exists, pyrethroid ingestion has been reported to be below the accepted daily intake (ADI) [45]. More detailed information of effect of salmon industry in the marine environment is presented in Chapter "Environmental Risks of Synthetic Pyrethroids Used by the Salmon Industry in Chile".

Most of the professional exposure is due to skin absorption. The main effect of dermal exposition is paresthesia, probably caused by the hyperactivity of cutaneous nerves, especially on the face. Paresthesia increases with stimuli such as heat, sunlight, sweat or contact with water [11]. Paresthesia disappears in 12–24 h and no special treatment is required. However, topical administration of vitamin E can reduce its symptoms.

Ingestion of pyrethroids causes sore throat, nausea, vomit and abdominal pain in a few minutes. Mouth ulcers, increased secretion or dysphagia may occur [11]. Inhalation is less important, but it increases when pyrethroids are used in closed spaces. Systemic effects appear 4–48 h after exposition. The effects usually include dizziness, headache and tiredness. Less frequent effects are palpitations, chest oppression and blurry sight.

Regarding long-term exposition to pyrethroids at low concentrations, a study in humans concluded that chronic toxicity of pyrethroids does not cause any specific symptoms. What could be detected were combinations and correlations of symptoms caused by the accumulative effect of pyrethroids in nerve tissue such as brain dysfunction, polyneuropathy, immunosuppression or motor problems due to multiple sclerosis or Parkinson disease [46, 47]. It was also suggested that chronic toxicity of pyrethroids affect fertility. This hypothesis was proved in rats being administered small doses of permethrin for a maximum time period of 2 months [48].

On the other hand, these results have been criticised [49] because of the experimental design [50], because pyrethroids were not believed to cause irreversible effects according to studies on sodium channels [51] or because it was thought that mammals did not bioaccumulate them [52].

Other studies researched the chronic toxicity of *cis*-bifenthrin in *Daphnia magna* and its cytotoxicity in ovarian cells of Chinese hamster (*Cricetulus griseus*) and in human cervical carcinoma cells [53]. The lowest observed effect concentration

(LOEC) and the no observed effect concentration (NOEC) for daphnia were 0.02 and 0.01 $\mu g\,L^{-1}$, respectively. The chronic value was 0.014 $\mu g\,L^{-1}$. Half-maximal inhibitory concentration (IC_{50}) for hamster ovarian cells and human carcinoma cells were 3.2×10^{-5} and 4.0×10^{-5} $mol\,L^{-1}$, respectively. These data proved the chronic toxicity of *cis*-bifenthrin in both invertebrates and mammals.

Male Wistar rats were administered for a year a mixture of pyrethroids equivalent to a 5th or a 25th of what is in cereals and vegetables consumed by an average Indian adult [54]. Altered oxidant and antioxidant status; severe anatomical damage in the caput, cauda, kidney, liver, lung, prostate and testis; and increased serum glutamate-pyruvate transaminase, serum glutamic oxaloacetic transaminase and alkaline phosphatase activity were clear for all the groups. Decreased levels of 3β- and 17β-hydroxy steroid dehydrogenase activity, litter size and impaired acrosome reaction were detected in all the groups. Exposure to very low levels of pyrethroids for longer periods may cause damage to important tissues and male reproductive physiology [54]. Cypermethrin has been reported to cause adverse effects on the immune system, fertility, the liver metabolism and cardiovascular and enzyme activity in vertebrates, and a recent study suggests that it reduces the ovarian reserve in mice via apoptosis in granulosa cells by mitochondrial-related pathways [55].

An important toxicological parameter for pyrethroids is their enantiomeric composition as different isomers can present different toxicities [56–58].

6 Legislation

No pesticide can be used in the European Union unless it has been proved to be effective against pests and to be safe for the human health and the environment.

The European Union regulates the sustainable use of pesticides in order to regulate their risks and impacts on human health and the environment [59]. Directive 2009/128/EC includes key points about national action plans, education for professional consumers and pesticide distributors, public information and awareness, aerosol regulation, minimisation of use or ban of pesticides, revision of equipment and integral management of pests with limitation of chemical products.

Pesticides leave residues in the treated products. The maximum residue level (MRL) is the highest concentration of a pesticide allowed by the regulation. The European Commission establishes MRLs at concentrations that are safe for the consumers and as low as possible. The MRLs are available at the European Union Pesticides database [60] (Tables 3 and 4).

MRLs have been set for about 1,100 compounds in over 300 fresh products and for the same products after processing in order to take into account dilution or concentration effects. When MRLs for a pesticide are not stated, the accepted default value is 0.01 $\mu g\,g^{-1}$, which usually corresponds to the limit of detection (LOD) [59]. The European Food Safety Authority (EFSA) assesses the safety for every consumer group – adults, kids, vegetarians, etc. – based on the pesticides' toxicity

Table 3 Maximum residue levels for pyrethroids in different products [60]

Product	Limits ($\mu g\ g^{-1}$)
1. Fruits and nuts	0.01–0.5
2. Vegetables	0.01–2
3. Pulses	0.01–1
4. Oilseeds and oilfruits	0.01–1
5. Cereals	0.02–2
6. Tea, coffee, infusions, cocoa and carobs	0.01–5
7. Hops	0.1–30
8. Spices	0.01–1
9. Sugar plants	0.01–0.5
10. Products of terrestrial animals	0.01–3
Tissue	0.02–3
(a) Swine	0.01–3
Fat	0.05–3
Liver	0.01–0.5
(b) Bovine	0.01–3
Fat	0.05–3
Liver	0.01–0.2
(c) Sheep	0.01–3
Fat	0.05–3
Liver	0.01–0.5
(d) Goat	0.01–3
Fat	0.05–3
Liver	0.01–0.5
(e) Equine	0.01–3
Fat	0.05–3
Liver	0.01–0.5
(f) Poultry	0.01–0.2
(g) Others	0.01–3
Fat	0.01–3
Liver	0.01–0.5
Milk	0.02–0.2
Bird eggs	0.01–0.1
Honey	0.01–0.05
Amphibians and reptiles	0.01–0.05
Terrestrial invertebrate animals	0.01–0.05
Wild terrestrial vertebrate animals	0.01–0.05

and the maximum typical concentrations of pesticides in food from the different diets around Europe.

Additionally, acceptable daily intake (ADI) values and no observed adverse effect level (NOAEL) values have been established. ADI values for pyrethroids are between 0.02 and 0.07 $mg\ kg^{-1}\ day^{-1}$ (mg of pyrethroid per kg of consumer's body weight per day), and NOAEL values are set between 1 and 7 $mg\ kg^{-1}\ day^{-1}$ [36] (Table 4).

Table 4 IDA and NOAEL values for pyrethroids [36]

Pyrethroid	IDA (mg kg^{-1} day^{-1})	NOAEL (mg kg^{-1} day^{-1})
Bifenthrin	0.02	1.5
Cyfluthrin	0.02	2
Cyhalothrin	0.002	
Cypermethrin	0.05	1.5
Deltamethrin	0.01	1
Etofenprox	0.03	3.1
Permethrin	0.05	5
D-Fenothrin	0.07	7

Current relevant regulation for pyrethroids is:

- Regulation 283/2013/EU – Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.
- Regulation 284/2013/EU – Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.
- Regulation 1107/2009/EC – Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC (ban on some active substances) and 91/414/EEC (commerce of phytosanitary products).
- Directive 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.

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Analytical Methods for Determining Pyrethroid Insecticides in Environmental and Food Matrices

Maria Luisa Feo

Contents

Abstract In this chapter, an overview of different aspects of current analytical methodologies such as sample preparation, extraction, purification, and instrumental analysis for pyrethroids is discussed. Recent development in sample preparation and extraction is presented. Regarding instrumental analysis, gas chromatography (GC) coupled to electron capture detection or mass spectrometry (MS) including tandem MS is generally preferred for analysis of pyrethroids. Although liquid chromatography has been used as a possible solution to reduce isomerization of pyrethroids that can occur at higher temperature, the advantages and disadvantages of different instrumental techniques are discussed here.

M. L. Feo (✉)
Istituto per lo studio degli impatti Antropici e Sostenibilità in Ambiente Marino-Consiglio Nazionale della Ricerca, Torretta Granitola, Sicily, Italy
e-mail: marialuisa.feo@ias.cnr.it

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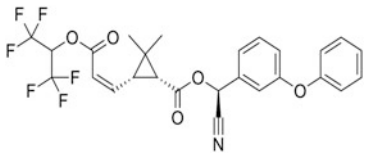
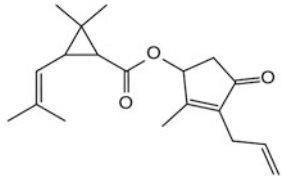
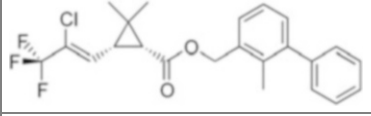
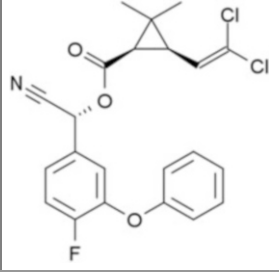
1 Introduction

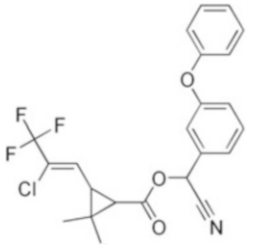
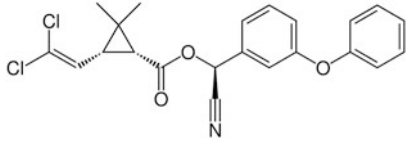
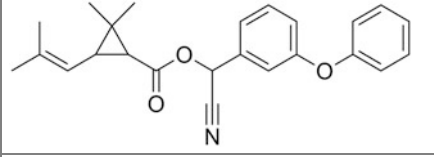
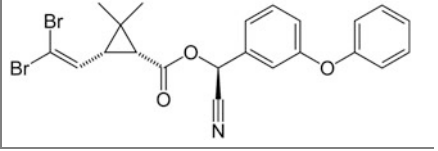
Pyrethroid insecticides were developed to replace organophosphorus pesticides, which were largely used in the past three decades and were demonstrated to have potentially toxic effects on humans [1]. Pyrethroids are the synthetic analogues of pyrethrins which were developed as pesticides from the extracts of dried and powdered flower heads of *Chrysanthemum cinerariaefolium*. Because of the rapidly decomposition of pyrethrins in the presence of light, pyrethroids were developed to increase stability to light and residence time in the environment, maintaining the effective insecticidal activity of the pyrethrins [2]. Pyrethroids are persistent compounds with high hydrophobicity (log Kow 5.7–7.6) [3, 4] and very low water solubility (a few lg/L), so they preferentially adsorbed to solid particles [5]. They can persist in the environment for few months before being degraded [6, 7] and can be bioaccumulated in aquatic organisms [8, 9] and humans [10, 11]. Aquatic organisms such as invertebrates and fish are extremely sensitive to the neurotoxic effect of these insecticides. In fish (e.g., bluegill and lake trout), LC50 values were estimated to be less than 1 g/L [12]. Regarding their effects on humans, reversible symptoms of poisoning and suppressive effects on the immune system have been reported [13]. Moreover, pyrethroids have been included in a list of suspected endocrine-disrupting chemicals [14]. The development of analytical methods for the analysis of pyrethroid insecticides is very important, considering their large usage for domestic and agricultural pest control applications and their presence in the environment and in food and their capacity to be bioaccumulated by organisms. Table 1 shows a list of pyrethroids usually determined in environmental, biological, and food samples. In addition to conventional extraction methods (e.g., liquid-liquid extraction or solid-phase extraction for liquid samples and sonication or pressurized liquid extraction for solid samples), new methods simple and rapid with reduced reagent use have been recently developed for the extraction of pyrethroids from environmental, biological, and food samples. Examples of these are the liquid-liquid microextraction based on solidification of floating organic droplet used for liquid samples or QuEChERS (stands for quick, easy, cheap, effective, rugged, and safe) method applied to solid samples. Following extraction and purification, the detection and quantification of pyrethroids can be performed by gas chromatography (GC) combined with electron capture detection (ECD) or mass spectrometry (MS), as well as by liquid chromatography (LC). This chapter describes the various aspects of sample preparation, extraction, purification, and instrumental analysis of synthetic pyrethroids in different environmental and food matrices mainly focusing on the development made in the last 15 years.

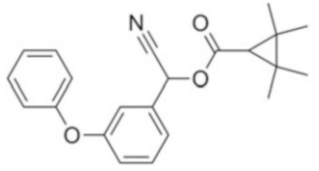
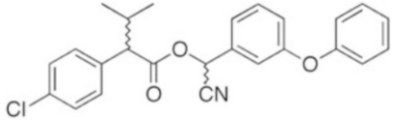
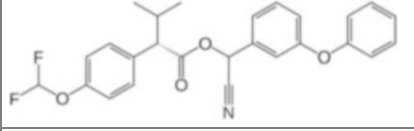
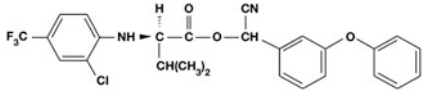
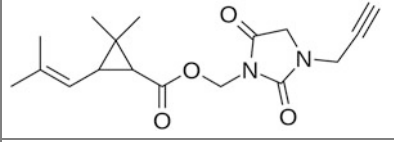
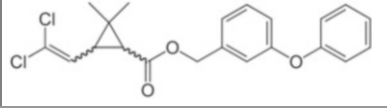
Table 1 List of pyrethroids usually determined in environmental, biological and food samples

Pyrethroid (acronym)	Formula	MW	Molecular structure
Pyrethroid esters			
Acrinathrin (ACRI)	$C_{26}H_{21}F_6NO_5$	541.4	
Allethrin (ALLE) *(bioallethrin)*	$C_{19}H_{26}O_3$	302.4	
Bifenthrin (BIFE)	$C_{23}H_{22}ClF_3O_2$	422.9	
Cyfluthrin (CYFL) *(β-isomer)*	$C_{22}H_{18}Cl_2FNO_3$	453.3	

(continued)

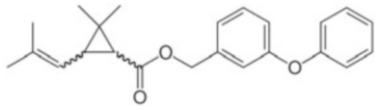
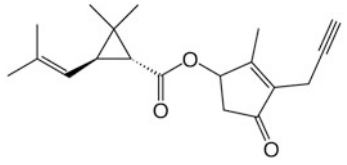
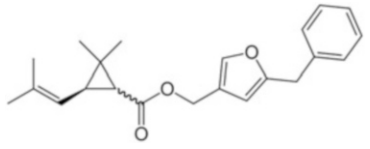
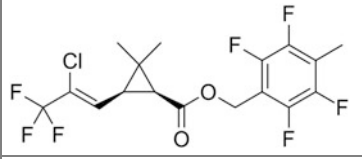
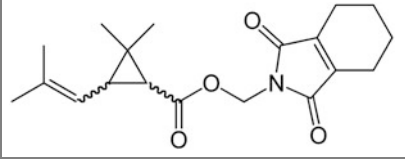
Table 1 (continued)

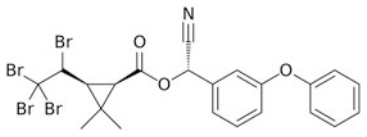
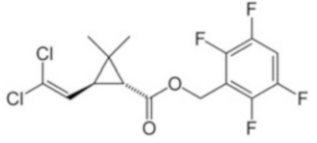
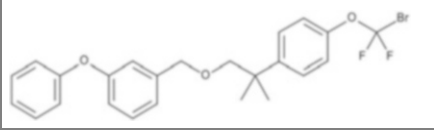
Pyrethroid (acronym)	Formula	MW	Molecular structure
Cyhalothrin (CYHA) *(γ- and λ-isomers)*	$C_{23}H_{19}ClF_3NO_3$	449.9	
Cypermethrin (CYPE) *(α-, β-, θ- and ζ-isomers)*	$C_{22}H_{19}Cl_2NO_3$	416.3	
Cyphenothrin (CYPH)	$C_{24}H_{25}NO_3$	375.5	
Deltamethrin (DELT)	$C_{22}H_{19}Br_2NO_3$	505.2	

Fenpropathrin (FENP)	$C_{22}H_{23}NO_3$	349.4	
Fenvalerate (FENV) *(esfenvalerate)(ESFE)*	$C_{25}H_{22}C1NO_3$	419.9	
Flucythrinate (FLUC)	$C_{26}H_{23}F_2NO_4$	451.4	
Fluvalinate (FLUV) *(τ-isomer)*	$C_{26}H_{22}ClF_3N_2O_3$	502.9	
Imiprothrin (IMIP)	$C_{18}H_{22}N_2O_3$	318.4	
Permethrin (PERM) *(biopermethrin and transpermethrin)*	$C_{21}H_{20}Cl_2O_3$	391.3	

(continued)

Table 1 (continued)

Pyrethroid (acronym)	Formula	MW	Molecular structure
Phenothrin (PHEN)	$C_{23}H_{26}O_3$	350.5	
Prallethrin (PRAL)	$C_{19}H_{24}O_3$	300.4	
Resmethrin (RESM) *(bioresmethrin and cismethrin)*	$C_{22}H_{26}O_3$	338.4	
Tefluthrin (TEFL)	$C_{17}H_{14}ClF_7O_2$	418.7	
Tetramethrin (TETR)	$C_{19}H_{25}NO_4$	331.4	

Tralomethrin (TRAL) *(δ-isomer)*	$C_{22}H_{19}Br_4NO_3$	665.0	
Transfluthrin (TRAN)	$C_{15}H_{12}Cl_2F_4O_2$	371.2	
Pyrethroid ethers			
Halfenprox (HALF)	$C_{24}H_{23}BrF_2O_3$	477.3	

2 Sample Preparation

Table 2 synthesizes the recent analytical techniques in terms of extraction, purification, and instrumental analysis showing recoveries and method limit of detection (MLOD) for determination of pyrethroids in environmental and food matrices.

2.1 Extraction from Water Sample

Pyrethroid concentrations in water are generally low, as they are preferentially sorbed to soil or sediment, due to their hydrophobic character. Thus, analytical methods for determination of pyrethroids in water should include extraction and pre-concentration to reach the required limits of detection. Liquid-liquid extraction (LLE) is the most common extraction technique for water samples. Its main drawbacks are the high solvent consumption and the long analysis time. For this reason, alternative extraction methods in which solvent consumption and time of analysis are reduced were introduced. Among these are solid-phase extraction (SPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE). Moreover, recently, liquid-liquid microextraction (LLME) and LLME based on solidification of floating organic droplet (LLME-SFO) have been developed. LLE of pyrethroids from water uses nonpolar solvents such as dichloromethane [15] and hexane [16]. After extraction, the sample is dried and redissolved in a small volume of organic solvent ready to be injected into GC for analysis. Pyrethroid recoveries by LLE were in the range 75–115% for unfiltered river samples with method limit of detection (MLOD) of 1–3 ng/L [15] and 94–105% for aqueous solution [16]. Dispersive liquid-liquid microextraction (DLLME) assisted by ultrasound was developed by Yan et al. as a method for the pre-concentration and determination of six pyrethroids in river water samples [17]. Tetrachloromethane was used as water-immiscible extractant, and acetone was used as water-miscible dispersive solvent. Ultrasonic treatment was performed to make the analytes fully extracted into the fine droplets. The phase separation was performed by a rapid centrifugation. Recoveries were ranging between 86 and 109%. MLODs were 0.1–0.30 μg/L [17]. A novel LLME based on solidification of floating organic droplet (LLME-SFO) has been recently developed by Khalili-Zanjani et al. which was based on the extraction of the analytes by microliter volume of the extraction solvent (floated on the surface of the aqueous sample) from the aqueous sample matrix [53]. In this method, small volume of an organic solvent with a melting point near room temperature (in the range of 10–30°C, such as undecanol and 1-dedecanol) is floated on the surface of aqueous solution. Transferring the sample in an ice bath, the organic solvent microdrop is solidified and ready to be transferred into a conical vial where it melts immediately at room temperature and thus is ready to be injected into a GC for analysis. The advantages of the method are simplicity of operation, small amount of solvent

Table 2 Recent analytical techniques used for pyrethroid determinations in environmental and food matrices

Analytes	Matrix	Sample preparation	Instrumental analysis	MLODs	Recovery (%)	Ref.
BIFE, CYHA, PERM, CYFL, CYPE, ESFENV	River water	LLE	GC-ECD	1–3 ng L^{-1}	75–115	[15]
CYHA, DELT, CYPE, FENV	Aqueous samples	LLE	GC-ECD	Not reported	94–105	[16]
TETR, ALLE, CYPE, PRAL, TRAN, IMIP	River water	Ultrasound LLME	HPLC-UV-Vis	0.1–0.3 μg L^{-1}	86–109	[17]
FENP, CYHA, PERM, CYFL, CYPE, FENV, PERM	Tap water, well water, and river water	LLME-SFO	GC-ECD	2.0–50 ng L^{-1}	79–114	[18]
CYPE, FENV, DELT	Water	SPE	GC-μECD	5.0×10^{-4} to 1.5×10^{-2} ng L^{-1}	70–103	[19]
FENP, CYHA, DELT, FENV, PERM, FLUV, BIFE	Groundwater and marine water	SPE	LC-ESI-MS	0.3–0.7 ng L^{-1} for groundwater sample 0.7–1.5 ng L^{-1} for marine water samples	80–115	[20]
FENP, CYHA, DELT, FENV, PERM, FLUV, BIFE	Groundwater	SPME	LC-PIF-FD	3–9 ng L^{-1}	92–109	[21]
BIFE, FENP, CYHA, PERM, CYFL, CYPE, ESFENV, DELT	Sediment pore water	SPME	GC-ECD	1–7 ng L^{-1}	56–119	[22]
TRANSFU ALLETR, TETRA, CYHA, CYPHE, PERM, CYFL, CYPE, DELT	Tap water, ground-water, river water, runoff, and wastewater	SPME	GC-μECD	0.05–2.18 ng L^{-1}	81–125	[23]
FENP, CYHA, PERM, CYPE FENV, DELT	Water	SPME	GC-ECD	0.12–0.43 ng mL^{-1}	83–112	[24]

(continued)

Table 2 (continued)

Analytes	Matrix	Sample preparation	Instrumental analysis	MLODs	Recovery (%)	Ref.
BIFE, FENP, CYHA, PERM, CYFL, CYPE, FLUV, FENV, DELT	Water and groundwater	MA-HS-SPME	GC-ECD	0.2–2.6 ng/L	88–115	[25]
RESM, BIFE, FENP, CYHA, PERM, CYPE, FENV, DELT	Water	SBSE	GC-MS	0.02–1.4 ng L^{-1}	40–80	[4]
CYFL, DELT, CYPE, FLUCY, ACRINA, FENV, FLUV, CYHA, TEFLU, PERM, HALFE	River water and brewed green tea	Dual-SBSE	TD-LTM-GC-MS	3–100 ng L^{-1}	17–33	[26]
CYHA, CYPE, FENV, FLUC, FLUV, PERM, TEFL	Water	SBSE	TD-GC-MS	>10 ng L^{-1}	82–113	[27]
CYHA, DELT, FENV, FULV, BIFE	Tap water, groundwater, river reservoir water	TILDLME	LC-UV	280–600 ng L^{-1}	76.7–135.6	[28]
CYFL, CYPE, DELT, FENVA, PERM, PHEN, TETR, BIFE, λ-CYHA, ESFE, FEN, τ-FLUV, RESM, and TRAL	River water	UAEE	GC-NCI-MS	0.03–35.8 ng L^{-1}	63–100	[29]
FENV, DELT, CYPE, CYFL, PHEN, BIFE	Aquaculture seawater	MI-SPE	GC-ECD	16.6–37.0 ng/L	86–96	[30]
Solid samples						
BIFE, CYHA, PERM, CYFL, CYPE, ESFENV	Sediments	PFE, GPC	GC-ECD	0.5–4 ng g^{-1}	83.8–108	[15]
CYPE, DELT, FENV	Sediment	Sonication, Florisil	GC-μECD	3.0×10^{-5}–1.5×10^{-3} ng g^{-1}	71–103	[19]

CYFL, CYPE, DELT, FENV, PERM, TETR, BIFE, CYHA, ESFE, FENP, FLUV, RESM	Marine sediment	Sonication Florisil	GC-NCI-MS	2.6–62.4 pg/g	51–105	[6]
ALLE, PRAL, RESM, TETR, BIFE, FENP, PHEN, PERM, CYHA, CYFL, CYPE, FLUC, FENV, DELT	Sediment	Soxhlet, Florisil	HRGC-HRMS	0.16–1.50 ng g^{-1}	89.7–135	[31]
TETR, CYFLU, FLUC, DELT, BIFE, PERM, CYPE, FLUV	Soil	MAE Florisil	GC-NCI-MS	0.3–2 μg/L	97–106	[32]
CYFL, CYPE, FENV	Soil	SFE, C_{18}	GC-ECD	<0.01 mg kg^{-1}	70–97	[33]
ALLE, CYFL, CYHA, CYPE, CYPH, DELT, FENV, FLUC, PERM, TEFL, TETR, TRAN	Soil	HS-SPME	GC-μECD	0.004–1.2 ng g^{-1}	81–122	[34]
BIFE, FENP, CYHA, CYFLU, CYPE, FENV	Soil	PSA, C18	GC-MS-MS	0.3–5 μg/kg	88–96	[35]
BIFE, FENP, CYHA, ACRI, PERM, CYFL, CYPE, FLUCI, FENV, DELT	Urban air	Chromosorb 106 and Tenax TA	GC-MS-MS	0.5–27 ng g^{-1} (LOQ)	67–117 for Chromosorb 106 and 65–115 for Tenax TA	[36]
TRAN, ALLE, TETR, CYHA, CYPH, PERM, CYFL, CYPE, DELT	Indoor dust	MASE, Florisil	GC-μECD	1–7 ng g^{-1}	84–101	[37]
ALLE, CYFL, CYHA, CYPE, DELT, FENP, IMIP, PERM, PHEN, PRAL, RESM, TETR	House dust	Sonification, SPE	GC-MS	1–60 ng g^{-1}	51–101	[38]
CYPH, ALLE, TRAN, CYFL, TETR, PERM, PHEN, CYHA, CYPE	Indoor air	HS-SPME	GC-ECD	0.083–4.6 ng/m^3	77–111	[39]
BIFE, CYHA, PERM, CYFL, CYPE, ESFENV	Biological tissue	PFE, GPC	GC-ECD	1–3 ng g^{-1}	74.3–98.7	[15]

(continued)

Table 2 (continued)

Analytes	Matrix	Sample preparation	Instrumental analysis	MLODs	Recovery (%)	Ref.
DELT	Rat tissue (liver, brain, kidney)	Mix and centrifugation with organic solvent	HPLC-UV-Vis	0.1 μg mL^{-1}	95–114 for liver, 97–108 for kidney, 95–108 for brain	[40]
CYPE, DELT	Porcine tissue (liver, heart, muscle, and kidney)	MSPD	HPLC-UV	Not reported	95.6–90.5–85.8 and 97.8 for CYPE and 92.3, 86.2, 103.8, and 104.1 for DELT in the liver, muscle, heart, and kidney, respectively	[41]
DELT, CYPE	Fish tissue	QuEChERS	GC-MS	0.3 ng/g	35–135	[42]
TETR, FENP, CYHA, CYPE, FENV, DELT	Fish	QuEChERS modified	Fluorescence spectrophotometer	0.008–0.014 μg/mL	76–89	[43]
TETR, PHEN, CYHA, PERM, CYFL, FENP, DELT	Blood	SPE	GC-HRMS	17–93 pg/mL	37–84	[44]
Food samples						
FENP, DELT, PERM, BIFE	Honey	IL-DMME	HPLC-VWD (variable wavelength detector)	0.03–0.05 μg/L	87–92	[45]
FENP, CYHA, PERM, DELT	Oil vegetable	DLLME	GC-FID	0.02–0.16 mg/kg	85–109	[46]
TETR, FENP, CYPE, DELT, FENV, PERM	Fruit juice	DLLME	HPLC-UV	2–5 μg/L	84–94	[47]
CYPE, PERM	Wine, fruit, and vegetables	ELISA	GC-MS	5–10 μg/kg	74–99	[48]
TRAN, ALLE, BIFE, CYHA, PERM, CYFL, CYPE, FENV, DELT	Rice	QuEChERS	GC-MS-MS	1 μg/kg	87–117	[49]

FENP, CYHA, DELT, FENV, PERM, FLUV, BIFE	Cucumber and watermelon	SPME	HPLC-PIF-FD	1.3–5 μg/kg	91–100	[50]
CYPE, DELT	Pasteurized milk	LLE	GC-ECD	7.5 ng/L	84–93	[51]
BIFE, CYHA, PERM, CYFL, CYPE, FENV, DELT, TETR, PHEN, RESM	Human breast milk	Sonication Florisil	GC-NCI-MS-MS	3.1–1,100 pg/g	48–91	[10]
ALLE, TETR, FENP, PERM, CYPE, DELT, FENV, BIFE, CYFL	Tea	SBSE	TDU-GC-ECD	Not reported	93–105	[52]

ACRI acrinathrin, *ALLE* allethrin, *BIFE* bifenthrin, *CYFL* cyfluthrin, *CYHA* cyhalothrin, *CYPE* cypermethrin, *CYPH* cyphenothrin, *DELT* deltamethrin, *FENP* fenpropathrin, *FENV* fenvalerate, *ESFE* esfenvalerate, *FLUC* flucythrinate, *FLUV* fluvalinate, *IMIP* imiprothrin, *PERM* permethrin, *PHEN* phenothrin, *PRAL* prallethrin, *RESM* resmethrin, *TEFL* tefluthrin, *TETR* tetramethrin, *TRAL* tralomethrin, *TRAN* transfluthrin, *HALF* halfenprox

used, good repeatability, low cost, and having very high pre-concentration factors. Chang et al. analyzed eight pyrethroids in tap water, well water, and river water by LLME using 1-dodecanol as extraction solvent. Recoveries were 79–114% and MLOD 2.0–50 ng/L [18]. Ultrasound-assisted emulsification-extraction (UAEE) is another environmentally friendly analytical methodology that can be applied for extraction and pre-concentration of a wide range of pyrethroids prior to GC-MS analysis. Feo et al. used chloroform (1 mL) as immiscible solvent for extraction of pyrethroids from river water samples. Recoveries were of 63–100% and MLODs of 0.03–35.8 ng/L [29]. A novel green enrichment method for pyrethroid pre-concentration was temperature-controlled ion liquid-dispersive liquid-phase microextraction (TILDLME) which was developed by Zhou et al. [28]. An ionic liquid is used as extraction solvent dispersing it in the aqueous solution under the drive of temperature. The analytes will more easily migrate into the ionic liquid phase because of the much larger contact area than that of conventional single drop liquid microextraction. The method was validated on tap water, groundwater, river water, and reservoir water samples filtered through 0.45 μm micropure membrane. Recoveries were 77–136% and MLODs of 280–600 ng/L [28]. Pyrethroid extraction by SPE was realized on an Oasis HLB cartridge with subsequent elution with methanol (MeOH)/acetonitrile (ACN) (50/50 v/v) [19]. Recoveries were of 70–103% for pre-filtered (using 0.45 μm PTFE fiberglass filters) water samples and claim MLODs of 5.0×10^{-4}–1.5×10^{-2} ng/L [19]. C18 cartridge was also applied to pre-concentrate pesticide traces in both unfiltered groundwater and seawater samples adding organic modifiers (methanol or acetonitrile) to water and using hexane as solvent [20]. Recoveries were of 80–115%, and MLODs were of 0.3–0.7 ng/L and 0.7–1.5 ng/L for seawater and groundwater samples, respectively [20]. The major drawback of SPE is large sample volume (e.g., >500 mL) required. For this reason, miniaturized methods (SPME and SBSE) which are simple, solventless techniques were introduced [54, 55]. Parrilla Vazquez et al. developed a procedure for SPME analysis of pyrethroids in unfiltered groundwater, using polydimethylsiloxane/divinylbenzene (PDMS/ DVB 60 μm) as the most appropriate fiber coating [21]. The sample solution was buffered to pH 3 using a phosphate buffer, and the solution was kept at 65 ± 2°C for 30 min. Recoveries were 92–109% with MLODs of 3–9 ng/L [21]. Bondarenko found analyzing sediment pore water recoveries of 56–119% with similar MLODs (30 μm PDMS fiber; 20 min stirring at 600 rpm) [22]. Casas et al. studied the influences (e.g., temperature, fiber coating, salting out effect, and sampling mode) on the efficiency of pyrethroid extraction from unfiltered water samples [23]. The best conditions were found to be using PDMS fibers, direct sampling (D-SPME), at 50°C with an exposure time of only 20 min and without adding salt. The recoveries were 81–125% with MLODs of 0.05–2.18 ng/L [23]. A novel solid-phase microextraction (SPME) fiber coated with multiwalled carbon nanotubes/polypyrrole (MWCNTs/Ppy) was prepared with an electrochemical method and used for the extraction of pyrethroids in natural water samples. The results showed that the MWCNTs/Ppy-coated fiber was more effective and superior to commercial PDMS and PDMS/DVD fibers in extracting pyrethroids in natural water samples. Recoveries were of 83–112%, and MLODs were within the

range 0.12–0.43 ng/mL [24]. A one-step microwave-assisted headspace solid-phase microextraction (MA-HS-SPME) has been applied to be a pretreatment step in the analysis of aqueous pyrethroid residuals by GC analysis [25]. Microwave heating was applied to accelerate the vaporization of pyrethroids into the headspace and then being absorbed directly on a SPME fiber under the controlled conditions. Extraction of pyrethroids from aqueous (at pH 4) was achieved with the use of a 100 m PDMS fiber, microwave irradiation of 157 W, and sampling at 30°C for 10 min. Recoveries were between 88.5 and 115.5%, and MLODs were 0.2–2.6 ng/L [25]. The method was applied to groundwater samples [25]. Van Hoeck et al. developed an SBSE method for the enrichment of pyrethroids from unfiltered water samples [4]. The method consists of adding the stir bar in the water sample (10 mL) together with methanol to minimize wall adsorption. The SBSE method is followed to thermal desorption (TD) in classical GC split/splitless inlet equipped with a flip top inlet sealing system. The extraction was performed at room temperature, with stirring at 900 rpm. Recoveries were of 40–80% and MLODs of 0.02–1.4 ng/L [4]. Sequential SBSE followed by thermal desorption (TD)-low thermal mass (LTM) gas chromatography mass spectrometer (GC-MS) was developed by Ochai et al. [26, 27]. The usage of dual SBE was to provide more uniform enrichment over the entire polarity/volatility range for organic pollutants at ultra-trace levels in water. In a first experiment, two stir bars were added to the unfiltered water, the extraction was performed at room temperature, and then, pyrethroids were desorbed from the two stir bars directly in the glass desorption liner. Recoveries were low (17–33%) and MLODs were 3–100 ng/L [26]. In a second experiment, the authors first added one stir bar to the sample without modifier and then a second stir bar to the same sample after adding 30% NaCl. The first extraction with unmodified sample was mainly to target for solutes with high Kow (log Kow $>$ 4.0); and the second extraction with modified sample solution (containing 30% NaCl) was targeted at solutes with low and medium Kow (log Kow $<$ 4.0). After the extraction, the two bars were placed in a single glass desorption liner and were simultaneously desorbed. Recoveries were 82–113% with low MLODs ($>$10 ng/L) [27]. Molecularly imprinted solid-phase extraction (MI-SPE) based on selective molecularly imprinted polymers (MIPs) has been used for the isolation and cleanup of pyrethroid insecticides in aquaculture seawater [30]. Recoveries were 86–96% and MLODs were 16.6–37.0 ng/L [30].

2.2 Extraction from Soil and Sediment Samples

The interaction between pyrethroids and soil/sediment matrix is much stronger than it is in water due to the hydrophobic character of pyrethroids [5] and to the consequently formation of bound residues in soil/sediment [56]. Thus, more exhaustive extraction procedures are required to liberate pyrethroids from the solid matrix. Conventional methods as Soxhlet extraction have been used for pyrethroid extraction from sediments although the method is time-consuming and requires a large amount of solvents. Dichloromethane was used as solvent and by Florisil for the

cleanup. Recoveries were of 90–135% and MLODs of 0.2–1.5 ng/g [31]. Sonication has been also used for the extraction of pyrethroids from sediment samples. Xue et al. used methanol/acetonitrile (50/50 volume/volume) as extraction solvent and performed the cleanup of the extracts on a Florisil column using dichloromethane/hexane (20/80 v/v) as eluent [19]. They found recoveries of 71–103% and MLODs of 3.0×10^{-5}–1.5×10^{-3} ng/g [19]. However, Feo et al. used hexane/dichloromethane (2:1) as extraction solvent in a sonicator for 15 min at room temperature and performed the cleanup with Florisil cartridge (2 g/15 mL). Ethyl acetate was used as eluent. Recoveries were of 51–105% and MLODs were of 2.6–62.4 pg/g [6]. In the last years, new extraction techniques have been developed for solid samples (such as supercritical fluid extraction, solid-phase microextraction, microwave-assisted extraction, pressurized fluid extraction) with the intent to reduce the volume of the organic solvent used for the extraction and the time of the analysis. Pressurized fluid extraction (PFE), which consists of using organic solvents, pumped into an extraction cell containing the sample and brought to an elevated temperature and pressure [57], has been used for extraction of pyrethroids from sediments [15]. PFE was followed by cleanup with gel permeation (GP) (size exclusion), and dichloromethane was used as eluent. Recoveries were 84–108% with 0.5–4 ng/g MLODs [15]. Supercritical fluid extraction consists of using supercritical fluids (normally water or carbon dioxide), as extraction agents. Supercritical fluids exhibit a liquid-like density, while their viscosity and diffusivity remain between gas-like and liquid-like values. Thus, supercritical fluids have lower viscosity and higher diffusivity compared to organic solvents. The applicability of supercritical fluid extraction (SFE) for multi-residue analysis was studied for soil samples. The best efficiency was achieved at 400 bar using methanol as modifier at 60°C. Cleanup was carried out using C18 cartridge and dichloromethane/hexane (50:50 v/v) as eluent. Recoveries were 70–97% with MLODs <0.01 mg/kg [33]. A simple solvent-free method based on headspace SPME (HS-SPME) was developed in order to determine pyrethroids in agricultural soils [34]. Factors (e.g., extraction temperature, matrix modification by addition of water, salt addition, and fiber coating) were considered in optimizing the procedure. The results showed that temperature and fiber coating were the most significant variables affecting extraction efficiency. Good sensitivity for all investigated compounds was achieved at 100°C by extracting soil samples wetted with 0.5 mL of ultrapure water (0% NaCl) employing a polyacrylate coating fiber. Recoveries were 81–122% with MLODs less than 0.004–1.2 ng/g [34]. Microwave-assisted extraction (MAE) was performed by Esteve et al. for the determination of synthetic pyrethroids in soil using toluene as extraction solvent and an irradiation of 700 W for 9 min [32]. Cleanup was performed with 2 g of Florisil and elution with 20 mL ethyl acetate/hexane 33% (v/v). Recoveries were of 97–106% and MLODs of 0.3–2 μg/L [32]. However, the author observed that different chemical forms of pyrethroids respond differently at low irradiation power (between 350 and 700 W) and irradiation time (between 3 and 12 min). Thus, different extraction conditions are needed to be set for individual pyrethroids during MAE. The stability of pyrethroids under MAE-optimized conditions still needed further studies. QuEChERS method was used for the extraction of pesticides

from different solid matrices including soil. It consists of extracting pesticide with an aqueous-miscible solvent (e.g., acetonitrile) in the presence of high amounts of salts (e.g., sodium chloride and magnesium sulfate) and/or buffering agents (e.g., citrate) to induce liquid phase separation and stabilize acid and base-labile pesticides. Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further cleanup using SPE. Then, the mixture is centrifuged, and the resulting supernatant either can be analyzed directly or can be subjected to minor further treatment before analysis. The method is simple, rapid, and inexpensive with reduced reagent use. Yu et al. developed a multi-residue method for pesticides, including pyrethroids, in soil using QuEChERS sample preparation method. 5 g of soil were extracted with 10 mL acetonitrile with 1% acetic acid. 4 g anhydrous MgSO4 and 1 g sodium acetate (NaOAc) were added, and the mixture was shaked [35]. Then, the supernatant was treated with 900 mg MgSO4, and 150 mg PSA and 150 mg C18 were used as sorbents. Recoveries were of 88–96% with MLODs 0.3–5 μg/kg [35].

2.3 *Extraction from Air Samples*

Pyrethroids were successfully extracted from air samples by SPE method with Chromosorb 106 and Tenax TA as adsorbents and ethyl acetate as eluent [36]. Recoveries were 67% and 117% with both materials [36]. In indoor dust, pyrethroids were extracted by microwave-assisted solvent extraction (MASE) followed by Florisil cleanup. The aqueous phase was 1 M sulfuric acid solution containing ascorbic acid, whereas the nonpolar organic phase was hexane. Recoveries were 84–101% and MLODs 1–7 ng/g [37]. Sonication was also performed for extraction of pyrethroids from house dust samples, followed by cleanup using SPE (C18 cartridge). The recovery range was 51–101% with MLODs of 1–60 ng/g [38]. A method based on the combination of SPE and SPME for the analysis of pyrethroids in indoor air was developed [39]. First, air was pumped through a very small amount of Florisil (60–100 μm mesh) to retain the target analytes. Then the adsorbent, enriched with the target analytes, was transferred to a 10 mL glass vial in the presence of 100 μL of acetone and sealed with a cap. The vial was placed into a water bath at 100°C. The compounds retained by the adsorbent were extracted by exposing an SPME fiber to the HS of the vial (HS-SPME) for a fixed period of time. The fiber was then inserted into the injector port, and pyrethroids were desorbed into the GC for 5 min. Recoveries were of 77–111% with MLODs of 0.083–4.6 ng/m^3 [39].

2.4 *Extraction from Biological Samples*

Deltamethrin was extracted from biological tissue (liver, kidney, and brain) by mixing the tissue sample with acetonitrile, centrifuging, and injecting directly the supernatant onto the LC column [40]. Recoveries from the liver, kidney, and brain

were 95–114% (MLODs, 0.1 g/mL) [40]. Matrix solid-phase dispersion (MSPD) extraction was performed for the determination of cypermethrin and deltamethrin in porcine tissue [41]. Neutral alumina was used as MSPD dispersion adsorbent, and diatomaceous earth was used as cleanup adsorbent, while n-hexane was the eluent solvent (20 mL). For cypermethrin, the recoveries were 96–88%, 90–103%, 86–90%, and 98–94% at spiked levels of 0.5 μg/g and 0.2 μg/g for the liver, muscle, heart, and kidney, respectively [41]. Closely, similar recoveries were found for deltamethrin [41]. Tissue samples were also extracted by PFE with a Dionex ASE 200. Cleanup of extracts was accomplished using automated GP. The GPC column was packed with 65 g Bio-Beads of 200–400 mesh size. The eluent was dichloromethane at a flow rate of 5 mL/min. The sample was a 10 mL dichloromethane extract [15]. Recoveries were 74–98% (MLODs, 1–3 ng/g) [15]. The QuEChERS method was successfully applied for extraction of cypermethrin and deltamethrin from fish product tissues (salmon, arctic char, trout, mussels, oysters, shrimp, tilapia, and crab). Acetonitrile was the extraction solvent, and MgSO4 and acetic acid and sodium acetate were added before centrifugation. Recoveries were of 35–135% and MLODs of 0.3 ng/g [42]. A modified QuEChERS approach was developed for fish sample by Jia et al. replacing the traditional acetonitrile with isopropanol [43]. They found that isopropanol improved the extraction efficiency of the QuEChERS. For the pyrethroids in the protein-matrix samples, the overall recoveries of 76–89% for the modified QuEChERS method are better than those of 69–85% for the original QuEChERS method [43]. MLODs were of 0.008–0.014 μg/mL [43]. Pyrethroids were extracted from heparinized plasma by SPE cartridges. Plasma samples were loaded on the cartridges, and these were washed with 4 mL deionized water followed by 4 mL of 40% methanol in water [44]. Elution was performed with 2 mL of toluene. Samples were reconstituted in 100 μL toluene, ready for GC analysis. Recoveries were of 37–84% and MLODs were of 17–93 pg/mL [44].

2.5 Extraction from Food Samples

Dispersive liquid-liquid microextraction (DLLME) was developed for determination of pyrethroids in fruit juice (apple, red grape, orange, kiwi, passion fruit, pomegranate, and guava juice) samples combined with high-performance liquid chromatography [47]. Methanol was used as dispersive solvent, while chloroform was used as extraction solvent. Recoveries were of 84–94% and MLODs were of 2–5 μg/L [47]. DLLME technique was also employed for the extraction of pyrethroids from vegetable oil after a preliminary liquid-liquid extraction step. Initially, oil samples were partitioned in a dimethylformamide (DMF)-hexane mixture, and then DMF was removed and used as a disperser solvent in the following DLLME procedure in which 1,1,2-trichloroethane was used as an extraction solvent [46]. Recoveries were

of 85–109% and MLODs were of 0.02–0.16 mg/kg [46]. Ionic liquid-linked dual magnetic microextraction (IL-DMME) was developed as novel and facile extraction technique for determination of pyrethroids in honey samples [45]. The method consists of a combination of dispersive liquid-liquid microextraction (DLLME) and dispersive microsolid-phase extraction using an ionic liquid ($[C_6MIM]NTf_2$) and no-modified magnetic nanoparticles (S-BaFe), respectively [45]. Pyrethroids were firstly extracted by the ionic liquid, and then the no-modified magnetic nanoparticle was used to retrieve the ionic liquid containing the pyrethroids. Finally, pyrethroids were extracted from nanoparticles by sonication using acetonitrile as solvents. Recoveries were of 87–92% with MLODs of 0.03–0.05 μg/L [45]. Magnetic nanoparticles (MNPs) exhibit high selectivity and, in small amounts, can provide high recovery of analytes, even from large-volume samples. They also allow easy, rapid isolation of analytes using an external magnetic field. A competitive enzyme-linked immunosorbent assay (ELISA) method was employed for the determination of cypermethrin and permethrin in agricultural products (wine, fruit, and vegetable). No further cleanup was needed. Matrix interferences were minimized by diluting with phosphate-buffered saline containing 40% methanol [48]. Recoveries were 74–99% with MLODs of 5–10 μg/kg [48]. QuEChERS method was employed for extraction of pyrethroid pesticide residue from rice grain [49]. Extraction was performed using acetonitrile, MgSO4, and NaCl. Recoveries were of 87–117% and MLODs were of 1 μg/kg [49]. SPME was employed for extraction of pyrethroids from cucumber and watermelon samples using high-performance liquid chromatography combined with post-column photochemically induced fluorimetry derivatization and fluorescence detector (HPLC-PIF-FD) [50]. The optimum SPME conditions were extraction time 30 min, stirring rate 1,100 rpm, extraction temperature 65°C, sample pH 3, soaking time 7 min, desorption time 5 min, and acetonitrile content 25%. Recoveries were of 91–100% and MLODs 1.3–5 μg/kg [50]. Liquid-liquid extraction of pyrethroids (cypermethrin and deltamethrin) from pasteurized milk was performed using acetonitrile as extraction solvent with cleanup by precipitation at low temperature without additional stages for removal of fat interferences [51]. Recoveries were of 93% for cypermethrin and 84% for deltamethrin with MLODs of 7.5 ng/L [51]. From human breast milk, pyrethroids were extracted by sonication with hexan/dichloromethane 2:1 and cleanup with Florisil cartridge [10]. Eluent was ethyl acetate/dichloromethane 2:1 [10]. Recoveries were of 48–91% and MLODs of 3.1–1,100 pg/g lipid weight (lw) [10]. Stir bar sorptive extraction (SBSE)-thermal desorption (TDU)-gas chromatography (GC) method was employed for the determination of pyrethroid residues in tea. As the tea samples were solid, a preliminary extraction with methanol was implemented, and then the samples of methanol extraction were extracted by stir bar sorptive extraction (SBSE) method [52]. Recoveries were of 93–105% and MLODs were not reported [52].

3 Instrumental Analysis

3.1 GC Methods

The most used capillary columns available for pyrethroid analysis are the nonpolar stationary phase columns {e.g., 5%-phenyl-95% methylpolysiloxane (DB5, HP5%, CP-Sil 8 BC, or similar)} [15, 19]. However, semipolar stationary phases {e.g., 35% diphenyl 65% dimethylpolysiloxane (SPB-608) [16] and methyl 50% phenyl polysiloxane (DB 17 MS, HP-608)} have been also successfully employed [15, 33]. A more polar stationary phase (methyl 7%, cyanopropyl 7%, phenyl polysiloxane, DB17-01) was used for the analysis of sediment pore water samples [22]. Some authors have also proposed the use of short columns in order to reduce analysis time (DB-5, 10 m × 0.18 mm × 0.18 lm) [26]. The chromatogram of synthetic pyrethroids by multiple peaks due to the separation of diastereosimomers (Fig. 1) [29, 56]. Pyrethroids are classified as type I or type II, depending on the alcohol substituent. Type I pyrethroids (resmethrin, phenothrin, tetramethrin, permethrin) have two chiral centers on their cyclopropyl ring; thus, they are resolved in two peaks corresponding to *cis*- and *trans*-isomers. However, type II pyrethroids (cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fluvalinate, fenpropathrin) contain a third asymmetric center, and they are resolved into four peaks. Esfenvalerate is a type II pyrethroid exception: it does not possess a cyclopril ring and has only two diastereoisomers. It is not possible to distinguish esfenvalerate and fenvalerate by GC methods, since esfenvalerate is one of the four isomers found in fenvalerate, and it is the biologically active component of fenvalerate. Undergoing exposure to polar solvent [58], heat [59], and light [58, 60], isomerization of pyrethroids can occur, and additional peaks appear in the chromatogram. This happens, for example, during the GC analysis of lambda-cyhalothrin and deltamethrin. Tralomethrin can be transformed into deltamethrin in the injector port of the GC system [61]. Such pyrethroid transformation can be avoided by using LC-MS instead of GC-MS. With LC-MS, deltamethrin and the two diastereoisomers of tralomethrin were separated and identified by Velverde et al. [61]. Another possible solution to isomerization is reducing the residence time of the sample in the GC inlet where isomerization occurs [62]. Therefore, injection techniques {e.g., pulsed splitless injection [63, 64] and programmed temperature vaporization (PTV)} are recommended to achieve this. Another solution to reduce pyrethroid isomerization used apolar solvent as hexane in presence of an isomer-stabilizing agent (e.g., acetic acid) [62]. GC is generally combined with electron capture detector or a mass spectrometer. Although GC-ECD is robust and highly sensitive for these compounds having halogenated atoms [37] as known, the selectivity of GC-MS is much better than that of GC-ECD. During GC-MS analysis, negative chemical ionization mode (NCI) is preferred to electron ionization (EI) because under EI conditions, pyrethroids give low-mass ions, most of them with the same m/z ratios. Otherwise, NCI reduces fragmentation, which is mainly due to the labile-ester linkage, generating negative molecular ions. Bondarenko et al. found that the instrument response of

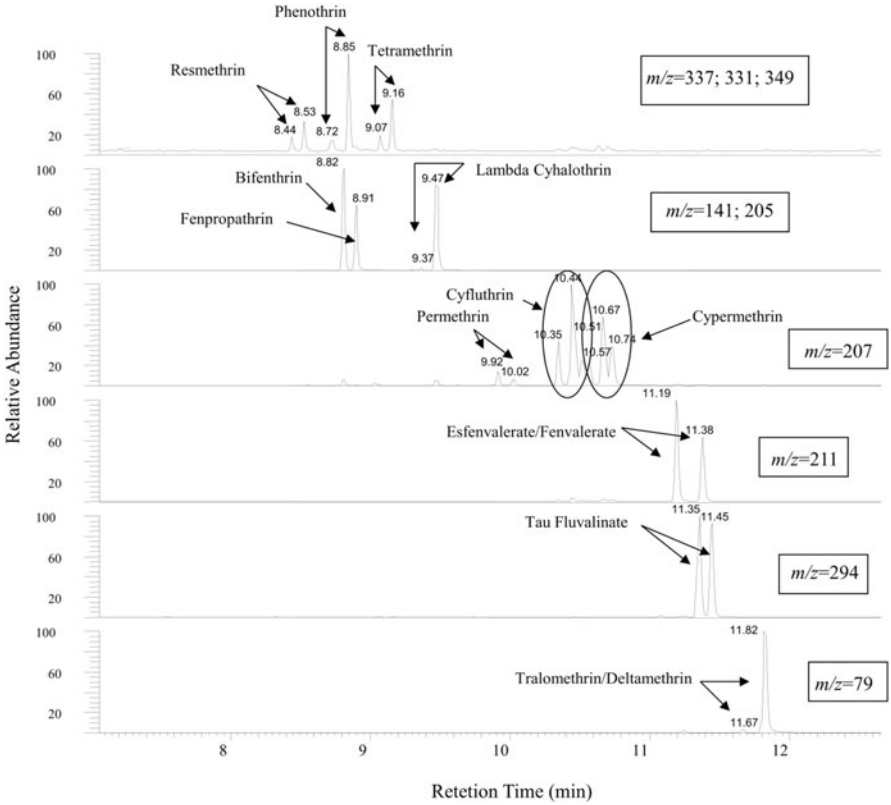


Fig. 1 GC-NCI-MS chromatograms of 14 pyrethroids selected in the study of Feo et al. [29]

GC-MS in the NCI mode was one order of magnitude higher than that of GC-ECD to pyrethroid compounds, largely because of reduced inference from matrix background [22]. Methane was mainly used as moderating gas [27, 43], but Feo et al. also found excellent results (instrumental limit of detection, ILOD, 0.02–1.88 pg injected) when using ammonia as moderating gas [29]. Tandem MS was used for the determination of pyrethroids in chemical ionization (CI) [36, 38, 65]. Sichilingo et al. found ILODs in the range 110–400 pg injected [38] operating with methane as moderating gas, whereas Feo et al. found ILODs ranging between 0.11 and 450 pg injected operating with ammonia as moderating agent [65].

3.2 LC Methods

Recently, liquid chromatography (LC) and high-performance liquid chromatography (HPLC) technique have been increasingly employed for the determination of pyrethroid residues in different matrices [36, 66, 67] with the main advantage of

avoiding pyrethroid isomerization. However, the sensitivity of LC is lower than that of the GC, and this needs to be considered when working at residue levels. Thus, pre-concentration and cleanup procedures are necessary to be applied to comply with tolerance levels. LC separation has been performed on a 250 × 4.6 mm i.d. Water symmetry C18 column (5 μm particle size) coupled by quadrupole MS with an electrospray ionization (ESI) interface [20]. Acetonitrile was the solvent A and ammonium formate 50 mM, 5% of acetonitrile, pH 3.5, the solvent B. ILODs were found in the range 0.3–0.5 μg/L [20]. LC techniques have been used also coupled to photochemically induced fluorimetry (PIF) for derivatization (pyrethroids do not display native fluorescence; thus, they were photolyzed into strongly fluorescent photoproducts) and a fluorescence detector (FD) [21, 50]. The FD is very selective, overcoming matrix interference [68]. LC separation was performed on a 3.5 μm symmetry C18. Acetonitrile water was used as mobile phase [21, 50]. Valverde et al. showed that in LC-ESI-MS (positive ion mode), deltamethrin and the two diastereoisomers of tralomethrin were efficiently separated, whereas, under GC conditions, both insecticides elute at the same retention time and give the same mass spectra [61]. This is probably due to the transformation of the two isomers of tralomethrin into deltamethrin in the GC injector port by elimination of a molecule of bromine. The LC separation was carried out on LiChroCART Superspher 100 RP-18 column using isocrated elution with acetonitrile/water (80:20) as eluent [61]. For HPLC determination, the analytical column employed was C18 stationary phase (150 × 4.6 mmI.D., 5.0 m), and the mobile phase was water-methanol (20:80, v/v). The detection was performed with an UV-Vis detector working at wavelength of 220 nm [17]. Liu et al. employed a Spursil C18 column (5, 4.6, 250 mm) with a Spursil C18 Guard Cartridges (5, 2.1, 10 mm) [45]. The mobile phase was an acetonitrile/water mixture (83/17, v/v). The detector was variable wavelength detector (VWD) with wavelength set at 230 nm [45]. Parilla Vazquez et al. performed liquid separation on a column of 250 × 4.6 mm id packed with 3.5 μm Symmetry C18 [21, 50]. The mobile phase was a programmed gradient with acetonitrile/water. The detector was PIF-FD operating with a programmed excitation and emission wavelengths of 283 and 330 nm [21, 50].

4 Enantioselective Separation

Chiral pollutants as pyrethroids are receiving growing environmental concern due to differential biological activities of their enantiomers. Liu et al. reported enantiomeric separation of *cis*-bifenthrin, permethrin, cypermethrin, and cyfluthrin using LC with variable wavelength UV detection for quantification and a laser polarimetric detection for the identification of the direction of optical rotation of the separated stereoisomers [62, 69]. The separation of the stereoisomers of *cis*-bifenthrin, *cis*-permethrin, and *trans*-permethrin was achieved on a 25 cm Sumichiral OA-2500-I column using hexane/1,2-dichloroethane (500:1, v/v) as eluent, whereas isomer separation for cypermethrin and cyfluthrin was obtained on two 25 cm Chirex

00G3019-DO columns with hexane/1,2-dichloroethane/ethanol (500:10:0.05, v/v/v) as eluent [62, 69]. Xu et al. worked on enantiomeric separation of lambda-cyhalothrin by HPLC using the columns of Chiralpak AD (amylase tris [3,5-dimethylphenyl carbamate]), Chiralpak AS (amylase tris[(S)-1-phenyl carbamate]), Chiralcel OD (cellulose tris[3,5-dimethylphenyl carbamate]), and Chiralcel OJ (cellulose tris[4-methyl benzoate]) with different chiral stationary phases [70]. The enantiomers of lambda-cyhalothrin were separated completely on all the columns tested and detected by circular dichroism at 236 nm. In GC, Corcellas et al. developed a method for simultaneous determination of the different enantiomers of six pyrethroids (bifenthrin, cyhalothrin, cyfluthrin, cypermethrin, permethrin, and tetramethrin) [71] using BGB-172 of 30 m × 0.25 mm and a column with 0.25 μm of film thickness. Previously, the same column was used by Chamberlain et al. for separation of the enantiomers of cypermethrin, cyfluthrin, *cis*-bifenthrin, and permethrin and also by Liu and Gan who showed that this chromatographic column was the best one for the enantiomeric separation of pyrethroids [69, 72]. The chromatography method proposed by Corcellas et al. allowed the separation of all *cis*-enantiomers (two pairs, four peaks), but for *trans*-isomers, the enantiomeric separation was not possible, obtaining two peaks corresponding each one to each pair [71] (Fig. 2).

5 Quantitative Methods

A complication in analyzing pyrethroids is that the concentration of each isomer of an individual pyrethroid in the standard mixture is unknown. Generally, the technical standard mixtures of pyrethroids, which are generally used for quantification, directly provide the sum of the concentrations of the individual isomers for each pyrethroid. Thus, the concentration of each pyrethroid is determined by summing the areas of the observed individual isomers. Moreover, pyrethroid-labeled standards are scarce. Commercially available standards are *trans*-permethrin-d6 [4, 29] and *trans*-cypermethrin-d6 [29] which are generally used as internal standards for an isotope dilution quantification. Other standards used for pyrethroid quantification are PCB-166, PCB-195 [37], and caffeine [36]. Dibromooctafluorobiphenyl has been used as surrogate for aqueous samples and dibutylchlorendate for sediment and biota samples [15].

6 Conclusion

Sample preparation and cleanup methods for pyrethroids are well established for environmental and food samples. Recoveries are high, reproducibility is good, and method limit of detection is adequate for the determination of levels of pyrethroids at environmentally relevant concentrations. Recently, low-solvent consumption and

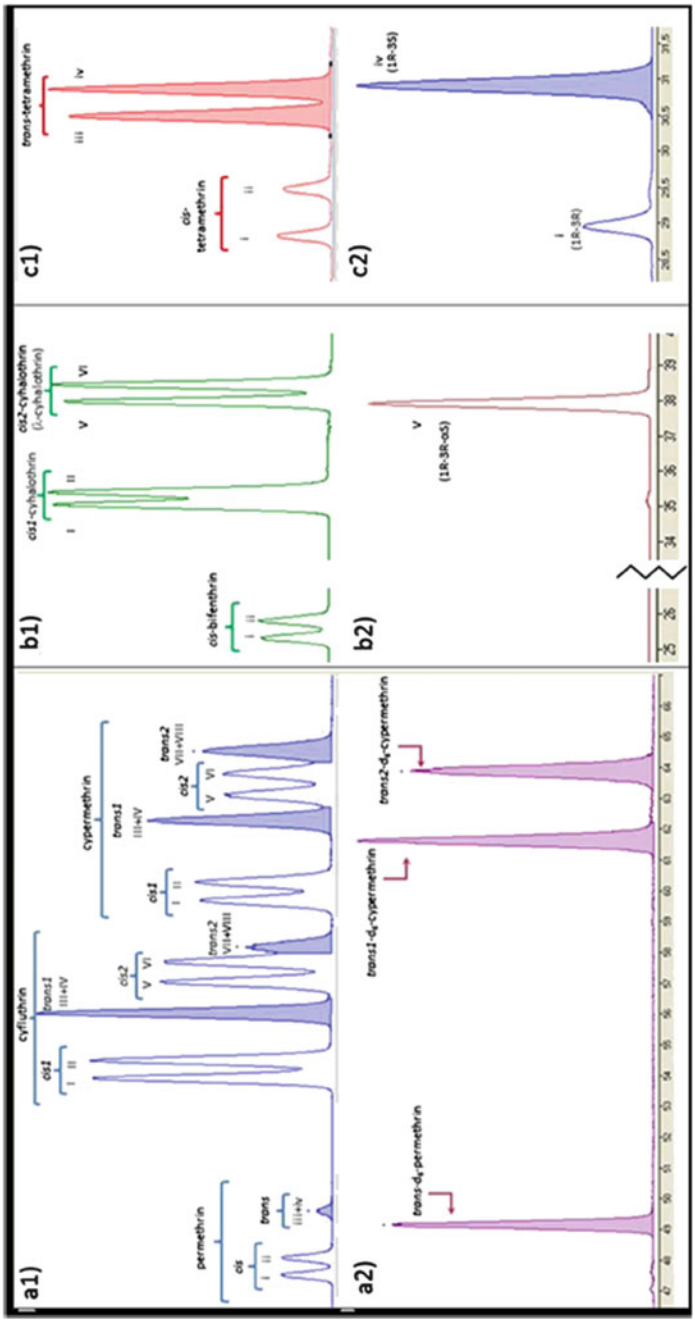


Fig. 2 Enantioselective separation of pyrethroids from the study of Corcellas et al. [71]. Peak assignation: I, 1R-3R-αR; II, 1S-3S-αS; III, 1S-3R-αS; IV, 1R-3S-αR; V, 1R-3R-αS; VI, 1S-3S-αR; VII, 1R-3S-αS; VIII, 1S-3R-αR. i, 1R-3R; ii, 1S-3S; iii, 1S-3R; iv, 1R-3S

time analysis extraction methods have been also successfully applied. The greatest challenges in pyrethroid analysis are the complexity of the mixtures and the lack of standards to enable quantification of individual diastereoisomers and enantiomers. For quantification, several techniques are being applied to the analysis of pyrethroids {e.g., GC-ECD, GC-NCI (methane or ammonia)-MS, and GC · GC-ToF-MS}. GC-NCI-MS provides the highest selectivity and sensitivity. Regarding enantiomeric separation, it is usually performed on a beta-cyclodextrin-based column because of its excellent enantioselectivity. A major drawback of such a column is that the enantiomers from the same *trans* diastereoisomer cannot be separated.

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Analytical Methods for Determination Urinary Metabolites of Synthetic Pyrethroids

Bartosz Wielgomas, Anna Klimowska, and Wojciech Rodzaj

Contents

Abstract Insecticides are natural and synthetic chemicals used to kill unwanted pests. However, humans and insect share similar molecular targets, and thus, insecticides are potentially hazardous to human health. Several health effects might be observed in experimental animals following controlled exposure to insecticides. Synthetic pyrethroids are still a relatively novel group of insecticides widely used not only in agriculture but also in human and veterinary medicine, forestry, and public health and for commercial pest control and residential consumer use. They play a unique role in fighting against malaria in tropical areas, where the WHO recommends pyrethroids among others for indoor residual spraying (IRS) and impregnation of bed nets to prevent mosquito biting.

B. Wielgomas (✉), A. Klimowska, and W. Rodzaj
Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland
e-mail: bartosz.wielgomas@gumed.edu.pl

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Bearing in mind the widespread use of these substances around the world, one can expect that the exposure of human population is common and may pose a potential health risk. Human biomonitoring (HBM) is a scientific tool that allows to assess the extent of exposure based on the measurement of a given chemical or its metabolites in human body fluids or tissues.

The need to estimate the level of exposure in different populations has led to the development of a methodology based on the measurement of urinary metabolites, as synthetic pyrethroids are rapidly metabolized in humans and excreted mainly in the urine. Human biomonitoring is used commonly in epidemiological studies and provides valuable information on the aggregate exposure.

Numerous analytical methods have been developed for the determination of metabolites of synthetic pyrethroids in human urine capable of detecting both environmental and occupational exposure.

Here, in this chapter, we summarized recent achievements in the analysis of metabolites of synthetic pyrethroids in human urine, with both separation and non-separation methods and methods of sample preparation and some aspects of instrumental analysis.

Keywords Analytical methods, Biomarkers of exposure, Human biomonitoring, Synthetic pyrethroids

1 Human Biomonitoring

HBM is a scientific tool allowing to estimate the extent of exposure to environmental xenobiotics. This assessment is possible based on the results of measurements of the concentrations of substance in biological samples taken from human (e.g., blood, saliva, urine, etc.). HBM is currently recognized as the gold standard in assessing human exposure to chemicals. One of the basic advantages of HBM is that it allows exposure assessment taking into account all exposure sources (e.g., air, water, food, personal care products, etc.) and all exposure routes (e.g., dermal, respiratory, oral). HBM studies conducted on large populations allow to identify particularly vulnerable populations and to assess time trends.

The current state of knowledge does not allow however direct assessment of the health risk resulting from the presence of a chemical in a biological fluid in a specified concentration. However, the results of HBM studies can be a source of valuable data on exposure to a specific chemical in epidemiological studies. To properly conduct exposure assessment with the use of HBM, it is necessary to know the biotransformation pathways of a given substance in humans, its toxicokinetics, and it is necessary to develop analytical methods that allow measuring very low concentrations of substances or their metabolites in very complex biological matrices.

2 Urinary Metabolites as Biomarkers of Exposure

2.1 Metabolism of Synthetic Pyrethroids

Chemical structure of pyrethroids in a large extent determines their biotransformation pathways. As esters they are easily hydrolyzed by human carboxylesterases to form respective alcohol and acidic metabolites. Oxidation by cytochrome P-450 is the second major reaction of pyrethroids in laboratory animals and humans [1]. Both oxidation and hydrolysis are the first-phase reactions which are followed by second-phase reactions – conjugation with endogenous substrates. The last process leads to formation of glucuronides, sulfates, and amino acid conjugates – highly water-soluble metabolites and in some cases lipophilic conjugates with cholesterol, bile acids, and triglyceride. Hydrophilic metabolites of pyrethroids do not show accumulation in human body and are rapidly and almost completely excreted into urine within few days after oral exposure. Although pyrethroids undergo both oxidation and hydrolysis reactions, practically only products of hydrolysis serve as urinary biomarkers of exposure.

Urine as a major route of elimination of pyrethroid metabolites is thus considered the most appropriate matrix for the assessment of aggregate exposure. The plasma half-life for most pyrethroids is shorter than 8 h.

Significant differences occur in respect to cleavage of the ester bond between *trans* and *cis* isomers. *Trans* isomers of pyrethroids possessing chrysanthemic acid moiety are hydrolyzed more efficiently than their corresponding *cis* isomers. Furthermore, *cis* isomers are more susceptible to oxidative metabolism than *trans* isomers [2]. The range of human metabolites identified and used as biomarkers of exposure to pyrethroids is presented in Table 1.

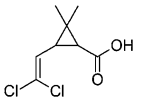
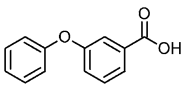
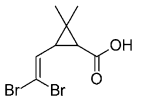
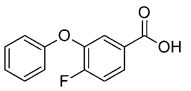
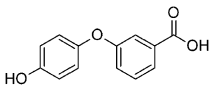
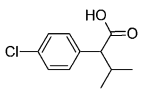
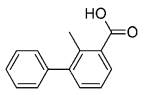
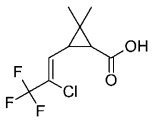
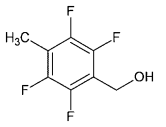
Several urinary metabolites were identified (Table 1) up-to-date, and they can serve as a reliable biomarker of exposure. Besides of that, some biomarkers are more frequently analyzed than others.

The first published methods for the quantitative determination of synthetic metabolites of pyrethroids in human urine included the metabolites of the most commonly used pyrethroids, namely, permethrin, cypermethrin, deltamethrin, and cyfluthrin: *cis* and *trans* DCCA, DBCA, 3PBA, and 4F3PBA [3–7].

Of these, 3PBA is unique, because so far, most research is focused on this biomarker. It is a common metabolite of many pyrethroids, and its concentrations in urine are usually the highest and detectable in the largest number of samples in the population. Finally, the highest availability of analytical methods exists for the determination of this metabolite in the urine; both chromatographic methods and high-throughput immunological methods are described in the literature.

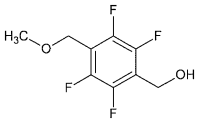
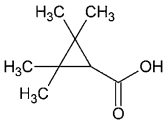
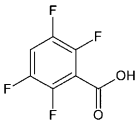
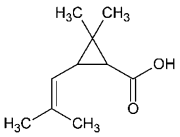
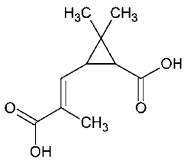
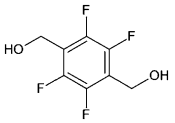
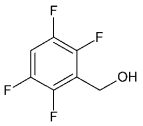
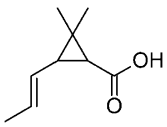
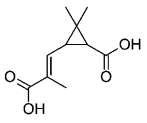
In addition to 3PBA and the aforementioned metabolites, the remaining ones are studied less often, although in recent years, several methods have been published that enable the simultaneous, very sensitive assay of up to eight to nine individual biomarkers in one chromatographic run [8, 9].

Table 1 Human urinary metabolites of synthetic pyrethroids used for the assessment of exposure

Abbreviation	Chemical structure	Chemical name	Parent pesticide
cis, *trans* DCCA		3-(2,2-Dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid	Cyfluthrin, cypermethrin, permethrin
3PBA		3-Phenoxybenzoic acid	Permethrin, cypermethrin, deltamethrin, esfenvalerate, λ-cyhalothrin, fenpropathrin, flucythrinate, fluvalinate, phenothrin
DBCA		3-(2,2-Dibromovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid	Deltamethrin
4F3PBA		4-Fluoro-3-phenoxybenzoic acid	Cyfluthrin, flumethrin
4OH3PBA		4′-Hydroxy-3-phenoxybenzoic acid	Permethrin, cypermethrin, deltamethrin, esfenvalerate, λ-cyhalothrin, fenpropathrin, flucythrinate, fluvalinate, phenothrin
CPBA		4-Chloro-α-isopropyl benzeneacetic acid	Esfenvalerate
MPA		2-Methyl-3-phenylbenzoic acid	Bifenthrin
ClF3CA		3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid	λ-cyhalothrin, bifenthrin
MTFBL		4-Methyl-2,3,5,6-tetrafluorobenzyl alcohol	Profluthrin

(continued)

Table 1 (continued)

Abbreviation	Chemical structure	Chemical name	Parent pesticide
MMTFBL		4-Methoxymethyl-2,3,5,6-tetrafluorobenzyl alcohol	Metofluthrin
TMCA		2,2,3,3-Tetramethylcyclopropane-carboxylic acid	Fenpropathrin
TFBA		2,3,5,6-Tetrafluorobenzoic acid	Transfluthrin
MPCA		2,2-Dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylic acid	Tetramethrin
CXCA		3-(2-Carboxy-prop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid	Imiprothrin
HOCH2-FB-Al		2,3,5,6-Tetrafluoro-1,4-benzenedimethanol	Metofluthrin
FB-Al		2,3,5,6-Tetrafluorobenzyl alcohol	Transfluthrin
MCA		2,2-Dimethyl-3-(1-propenyl)-cyclopropane carboxylic acid	Metofluthrin
CDCA		Chrysanthemum dicarboxylic acid	Imiprothrin, allethrin

Sensitivity is one of the key parameters characterizing the analytical method for the determination of synthetic pyrethroid metabolites in the general population not exposed occupationally, since average concentrations are well below 1 ng/mL. Metabolites other than 3PBA are detected much less frequently and even in lower concentrations. In general, methods based on gas chromatography are more sensitive than methods based on liquid chromatography. Chromatographic methods in combination with mass spectrometry with different types of analyzers are used exclusively in HBM, as only advanced systems allow reliable detection of low concentrations resulting from environmental exposure.

3 Separation Techniques

3.1 Sample Preparation

From a practical point of view, the fewer the stages of sample preparation for the analysis, the lower the probability of making a mistake, but also the smaller workload and consequently the unit cost of the analysis. Very low levels of synthetic pyrethroid metabolites that are found in urine samples from non-occupationally exposed subjects require the use of analytical methods characterized by high sensitivity of the instrument or advanced technique of extraction and purification of the sample before instrumental analysis or the combination of both. Dilute-and-shoot technique which is the simplest way of biological sample preparation for LC-MS/MS was never used for the analysis of synthetic pyrethroid metabolites in human urine.

In general, sample preparation steps include (a) internal standard addition, (b) hydrolysis, (c) sample extraction and cleanup, (d) derivatization (only in GC-MS-based methods), and (e) instrumental analysis.

The better sensitivity of GC-MS over LC-MS mentioned earlier is associated however with a much greater effort at the stage of sample preparation for analysis, whereas in the case of LC-MS, the hydrolyzed sample is only subjected to extraction and possibly enrichment (solvent evaporation) before instrumental analysis. In the case of GC-MS, the extracts are practically always subjected to additional cleanup, concentration, and derivatization.

The vast majority of the immunological methods described do not require advanced sample preparation for analysis. Usually, dilution of the urine sample or simple SPE extraction is sufficient.

3.1.1 Hydrolysis

As mentioned earlier, all of the end products of pyrethroid metabolism when excreted in urine are present as conjugates. No analytical method that was published up to date dealt with determination of conjugated forms or only free form, but in all

cases, metabolites were released from conjugates using different deconjugation procedures before extraction from the matrix. Glucuronides and sulfates consist over 90% of conjugates found in human urine. Considering this, measurement of total concentration of metabolites has to be preceded by hydrolysis. Both acidic and enzymatic hydrolyses can be performed for quantitative release of metabolites before their isolation from urine.

The most significant disadvantage of enzymatic hydrolysis is time consumption since it is usually performed overnight. On the other hand, this process does not need personnel engagement; therefore, it is virtually costless. Enzymatic hydrolysis is considered as a mild process because strong acids used for acidic hydrolysis might destroy labile analytes. For example, it was shown that a common metabolite of metofluthrin and profluthrin, i.e., 2,2-dimethyl-3-(1-propenyl)-cyclopropane carboxylic acid (MCA), was significantly degraded during HCl hydrolysis [8].

Acidic hydrolysis is typically performed with concentrated hydrochloric acid added at an average ratio of 0.2 mL per each mL of urine. Sample is then heated at 90–100°C for 60–120 min [4, 6, 9–14].

Toshima et al. [15] observed some discrepancies between determined concentrations of 3PBA from two laboratories during cross-validation study. Authors observed significantly lower concentrations of 3PBA following enzymatic deconjugation in some of the urine samples. The results suggested the presence of other conjugated species of 3PBA than glucuronide and sulfate in human urine. Although the overall agreement between the values obtained by the deconjugation methods was fair, it appears that urine samples should be pretreated by acidic deconjugation for the analysis in biological monitoring of pyrethroid exposure.

Different enzymes, such as β-glucuronidase type HP-1 from *Helix pomatia* [16], type HP-2 [14, 15, 17, 18], glucuronidase arylsulfatase enzyme [19] and sulfatase from *Helix pomatia*, type H-1, lyophilized powder [20, 21], were used for enzymatic hydrolysis. Incubation time with enzyme in 0.2 M acetate buffer (pH 4.5–5.0) varied between 5 and 17 h (overnight) at 37°C.

3.1.2 Extraction

Liquid-Liquid Extraction

Liquid-liquid extraction is the simplest extraction technique commonly used for isolation of pyrethroid metabolites from human urine. After acidic hydrolysis of urine, no pH adjustment is needed before extraction. In contrary, when enzymatic hydrolysis is performed, the sample should be acidified before extraction. Analytes are usually extracted to *n*-hexane [3, 6, 9, 11, 14, 22–24], dichloromethane [5, 25], isopropanol-hexane (5:95) [26], tert-butyl-methyl-ether (MTBE) [8, 10], chloroform [27], or toluene [20]. Due to the acidic character of metabolites, re-extraction from organic solvent to alkaline solution might be later performed for sample cleanup. Usually NaOH solution is utilized for this purpose [3, 9, 11, 14, 24, 27]. Liquid-liquid extraction is considered as difficult to automate; however, Ueda et al. [8] used

robotic system Extrahera™ (Biotage, Uppsala, Sweden) for automation of liquid-liquid extraction on 24-well plates with MTBE as extraction solvent. Same authors observed that conditions of evaporation of MTBE extract are essential for optimal derivatization efficiency. Due to high volatility of fluorinated alcoholic metabolites, significant loses were observed during evaporation at 40°C. Finally, satisfying recoveries were obtained while vacuum evaporation at 4°C was employed. On the other hand, acidic metabolites are not sensitive to overdrying even at 40°C.

The liquid-liquid extraction, however, has several disadvantages. First of all, it is characterized by a very high consumption of organic solvents; in one case the use is even over 50 mL per one sample [3]. In these methods, moreover, the solvents are evaporated, resulting in a significant environmental burden.

The principles of green chemistry aimed at limiting the use of toxic and environmentally harmful organic solvents have found application in two microextraction methods. In both cases, a microporous membrane impregnated with 1-octanol (8 μL) or dihexyl ether, respectively, was used as the extraction device. In the first case, a microsyringe pre-filled with derivatizing agents and syringe needle connected to solvent-impregnated hollow-fiber segment was used as LPME probe. Pyrethroid metabolites were extracted and enriched simultaneously. After sampling, the in-syringe derivatization (ISD) was performed, and the extract was subjected to GC-ECD analysis [28]. In turn, Bartosz et al. [12] used polypropylene hollow-fiber membrane tightly fitted onto Nylon rod and impregnated with dihexyl ether for 3PBA and 4OH3PBA extraction from human and rat urine. This disposable device was first placed in acid-hydrolyzed urine for 120 min and then transferred into 0.1 M NaOH for 120-min desorption. This extract was further analyzed by HPLC-DAD. Limits of detection for 3PBA (15 ng/mL) and 4OH3PBA (15 ng/mL) were too high to measure environmental exposure. Nevertheless, the general concept may be used with more sensitive LC-MS/MS method to increase sample preparation throughput [12].

Solid-Phase Extraction

Solid-phase extraction is devoid of certain disadvantages of liquid-liquid extraction. It allows for smaller consumption of organic solvents and can be easily automated to reduce human costs and improve reproducibility. In the case of biomonitoring studies conducted on large populations, where the number of samples for analysis reaches hundreds or even thousands, the unit cost of sample preparation plays a significant role.

Different formats of SPE are available nowadays, and some of them were used for isolation of pyrethroid metabolites from human urine. Standard SPE cartridges are most commonly used, but 96-well plates were also successfully employed [16] as well as microextraction by packed sorbent (MEPS) or SPE columns for online sample preparation in combination with liquid chromatography.

Oasis HLB being the polymeric sorbent with a hydrophilic-hydrophobic balance is used usually in the form of cartridges [21, 29–31] as well as 96-well plate format [16]. However, C18 sorbents are also suitable [6, 7, 32, 33].

Miniaturized format of SPE, named microextraction by packed sorbent (MEPS), was used by Klimowska and Wielgomas to extract five metabolites from only 0.4 mL of human urine [17]. Extraction was carried out using a semiautomatic syringe equipped with a needle with a bin filled with a small amount of C18 sorbent (4 mg) – BIN (barrel insert and needle). The advantage of this technique is that the sample flows through the bed twice, once when the sample is drawn into syringe and the second time when the sample is dispensed. This technique is based on the SPE principles, but thanks to miniaturization, it allows the extraction of very small sample volumes and elution of analytes with microliter volume of solvent directly to the injector. The authors, thanks to the use of large-volume injection (40 μL) and GC-MS (LVI-GC-MS), could achieve limits of quantification in the range of 0.06–0.08 ng/mL [17].

3.1.3 Derivatization

Analytes while released during enzymatic or acidic hydrolysis and following extraction and cleanup might be directly analyzed by liquid chromatography or have to be converted to more volatile and thermally stable products suitable for gas chromatography. Hexafluoroisopropanol (HFIP) combined with diisocarboxyldiimide (DIIC) is the most often used derivatization reagent. The major advantage of this reagent is that the reaction is completed at room temperature in minutes, usually, residue after organic solvent evaporation if treated with a mixture of HFIP/DIIC in the presence of acetonitrile or isooctane. After a few minutes, the reaction mixture is washed with $NaHCO_3$ to remove excess of reagents.

Furthermore, Klimowska and Wielgomas [17] documented that hexafluoroisopropyl esters of acidic pyrethroid metabolites are formed on the solid support (C18) during elution with hexane containing HFIP and DIIC. No byproducts, which are harmful to GC injection liner, column, or MS detector, are formed.

Much less frequently, analytes were methylated to methyl esters by incubation with a mixture of methanol and sulfuric acid [3, 6, 23].

Alcohol metabolites are less frequently analyzed in urine samples. Ueda et al. [8] developed the GC-MS/MS method for determination of alcoholic metabolites (HOCH2-FB-Al, CH3-FB-Al, CH3OCH2-FB-Al, and FB-Al) of fluorinated pyrethroids: metofluthrin, profluthrin, tefluthrin, and transfluthrin. Unfortunately, metabolites mentioned above could not be derivatized sufficiently by the HFIP/DIIC reagent even with any modification of reaction temperature and time. On the other hand, these metabolites were derivatized by the reagents for trimethylsilylation such as TMSI, TMSI-TMCS, MTBSTFA, BSTFA, and BSTFA-TMCS. Of these, only BSTFA-TMCS (99:1) showed reactivity with all hydroxyl metabolites [8].

Schettgen et al. [9, 11] and Guo et al. [26] derivatized acidic metabolites with MTBSTFA before GC-MS/MS analysis.

Aprea et al. [5] used pentafluorobenzyl bromide (PFBBr) for transformation of 3PBA into pentafluorobenzyl ester, which was further determined by gas chromatography with an intermediate polarity capillary column and an electron-capture detector. PFBBr as a strong lachrymator should be handled with special care.

Yoshida et al. used *N*-trimethylsilylimidazole (TMSI) with trimethylchlorosilane (TMCS) for derivatization of hydroxylated alcohols and *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) for efficient derivatization of carboxylic metabolites of several synthetic pyrethroids [20].

Recently, Schettgen et al. [9] modified and widened the scope of their original method [11] by adding new metabolites, namely: ClF3CA (BIF), CPBA (4-chloro-α-isopropyl benzene acetic acid), and MPB (2-methyl-3-phenylbenzoic acid). Effective sample cleanup was achieved by extraction to hexane and re-extraction to 0.1 M NaOH. Gas chromatography with tandem mass spectrometry was used for separation and quantitative analysis. The limit of quantification for all metabolites was 0.01 ng/mL when 10 mL of urine was processed.

3.2 Gas Chromatography

Gas chromatography (GC) methods were the first developed for determination of synthetic pyrethroid metabolites in human urine. Up to now this technique dominates over others for this group of analytes. Although acidic metabolites need to be derivatized before gas chromatography separation, GC-MS remains the method of choice when considering the determination of pyrethroid metabolites in urine. Due to the high separation power, equipment availability, reasonable purchase, and maintenance cost, GC-MS serves as a reliable method.

Typical nonpolar capillary columns, such as DB-5 ms (5%-phenyl-95%-dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 μm) [7, 20, 26], HP-5 ms (60 m × 0.25 mm × 0.25 μm) [6, 9], VF-5 ms low-bleed column (30 m × 0.25 mm × 0.25 μm) [24], XLB column (60 m × 0.25 mm × 0.25 μm film thickness), [22, 25] as well as medium polarity HP-35 (cross-linked 35% diphenyl-dimethylpolysiloxane, 60 m × 0.25 mm × 0.25 μm) [11], DB-608 (30 m × 0.25 mm × 0.25 μm) [28], and relatively polar column Rtx 65 (cross-linked 65%-phenyl-35%-dimethylpolysiloxane 30 m × 0.25 mm × 1 μm) [10], were used for separation of respective derivatives of synthetic pyrethroid metabolites.

In two published methods, electron-capture detector (ECD) was used [5, 28]. High sensitivity of ECD toward halogen-containing molecules allows for detection of hexafluoroisopropyl esters and pentafluorobenzyl esters of pyrethroid metabolites. Despite the high sensitivity of the detector, these methods were not used further in biomonitoring studies possibly due to the lack of specificity in comparison to MS detection. Both quadrupole and ion-trap mass spectrometers operated in single-ion mode (SIM), as well as multiple reaction monitoring (MRM), offered sufficient sensitivity.

3.3 Liquid Chromatography

The biggest advantage of LC is the ability to analyze metabolites without the need to derivatize them. Unlike GC, there is no need for additional extract cleanup before instrumental analysis. As in the case of gas chromatography, only highly specific and sensitive methods, i.e., using mass spectrometry, are useful in biomonitoring studies. The sample preparation process is simplified, but it comes at a price. LC-MS methods are susceptible to ion suppression phenomenon which can strongly affect both sensitivity and repeatability [14, 29].

Only two published methods used HPLC with spectrophotometric detection for the determination of synthetic pyrethroid metabolites. Smith et al. [27] developed a HPLC-UV method for the determination of 3PBA and MPA – a metabolite of bifenthrin in the urine of people professionally exposed to this insecticide. Bartosz et al. [12] in turn developed HF-LPME-HPLC-DAD method for determination of 3PBA and 4OH3PBA in rat and human urine. Both methods, due to high LOD and LOQ values, are not suitable for the determination of metabolites in the urine of non-occupationally exposed subjects.

Separation of analytes is carried out using HPLC, UPLC, and UHPLC coupled with mass spectrometers with various types of analyzers: triple quadrupole ESI [14, 16, 21, 29, 31, 34], turbo ion spray (TIS) [30], Q-TOF (ESI) [32, 35, 36], and high-resolution Orbitrap [19, 37].

Sample preparation for LC-MS analysis included offline solid-phase extraction [16, 21, 29–32, 34–36], liquid-liquid extraction [14], online SPE [37], and the QuEChERS [19].

The popularity of the QuEChERS methodology stems from its unique simplicity and applicability to almost any type of matrix. Therefore, an attempt was made to apply this methodology to the preparation of a biological sample in order to quantify the concentration of pesticide metabolites in human urine.

5 mL of urine was hydrolyzed enzymatically (1 mL of 0.2 M acetic buffer and 10 μL of β-glucuronidase aryl sulfatase) and then subjected to simplified QuEChERS procedure by addition of 10 mL of acetonitrile and QuEChERS salt packet. Acetonitrile layer was then evaporated at 37°C under a stream of nitrogen and reconstituted in 200 μL of methanol/water (10:90, v:v) containing 0.1% of acetic acid. Extract was analyzed with the use of UHPLC-HRMS system. Five pyrethroid metabolites were monitored: cis-DCCA, *trans*-DCCA, DBCA, 3PBA, and 4F3PBA. Additionally, Plackett-Burman design was used to optimize the parameters affecting the analytical response [19]. Unfortunately, LOQs were in the range of 2–10 ng/mL.

López-García et al. [37] developed a method for simultaneous quantification of selected organophosphate and pyrethroid metabolites in human urine and compared three independent sample preparation protocols including offline SPE, TurboFlow™, and online SPE. For TurboFlow™ and online SPE protocols, raw urine sample (without hydrolysis) was filtered through a 0.2 μm nylon filter, and 0.5 mL was subjected to online extraction. The best peak shapes and recoveries were obtained with TurboFlow™ methodology. This technique was the only one enabling detection of *cis*-/*trans*-DCCA, since no signal was produced when offline

or online SPE was performed. TurboFlow™ is recommended for matrices rich in macromolecules like proteins. Using described method, LOQs for *cis*-/*trans*-DCCA, 3PBA, and 4F3PBA were 10, 5, and 1 ng/mL, respectively (Tables 2 and 3).

4 Non-separational Techniques

4.1 Immunoassays

Non-chromatographic methods could be a good alternative to expensive and time-consuming chromatographic methods. A small sample volume, high throughput, and sensitivity as well as simple detection systems are the advantages of immunoassays. A number of immunoassay methods have been developed for the determination of 3PBA, *cis*-/*trans*-DCCA-glycine conjugate, and 3PBA-glycine conjugate in various formats. Most methods are indirect competitive ELISA [38–45]; others are luminescent paramagnetic particle-based immunoassay [46], direct competitive fluorescence enzyme immunoassay [47], noncompetitive magnetic bead-based PHAIA (polyclonal antibody-based noncompetitive immunoassay) [48], noncompetitive PHAIA real-time PCR [49], and quenching fluoroimmunoassay [50].

Depending on the method, 0.001–10 mL of the urine sample is required for one assay. These methods are characterized by high sensitivity, since the limit of quantification in the buffer is in the range of 0.01–0.25 ng/mL and 0.1–2.5 ng/mL in the urine. Practically, the method with the limit quantification of 0.1 ng/mL can be used to study exposure in the general population. Currently, however, no immunoassay for pyrethroid metabolites is commercially available. The main weakness of immunoassays is cross-reactivity with other compounds with similar structure or properties.

4.2 Other Methods

Recently, Pandey et al. [51] published a method for optical sensing 3PBA in urine samples by surface imprinting polymer capped on manganese-doped zinc sulfide quantum dots (QD). Developed sensor is highly stable and does not require any sample pretreatment. However, quantitative analysis is not affordable with this system (Table 4).

5 Quality Control

5.1 Intra- and Interlaboratory Quality Control

One of the key challenges of the HBM research methodology is the highest quality of quantitative results. It is worth noting that the concentrations of synthetic

Table 2 Gas chromatography methods for determination of synthetic pyrethroid metabolites in human urine

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Derivatization	Notice	Ref.
cis-DCCA (0.2; 0.5); DF, nd *trans*-DCCA (0.2; 0.5); DF, nd DBCA (0.2; 0.5); DF, nd 3PBA (0.3; 1.0); DF, nd 4F3PBA (0.3; 1.0); DF, nd	V: 5 mL H: acidic (1 mL HCl; 1 h, 90°C) E: LLE C: LLE I: GC-MS (quadrupole, SIM)	LLE: • 2 × 4 mL hexane • Evaporation	• 10% H_2SO_4 in MeOH (v/v) – 1 h • Dilution with 10 mL water and 15 mL 1 M NaOH • Re-extraction: 3 × 10 mL hexane • Drying with 2 g anhydr. Na_2SO_4 • Evaporation – gentle stream of nitrogen • Reconstitution: 0.5 mL iso-octane	51.5 mL of organic solvents per sample	[3]
NCI-detection *trans*-CDCA (0.05; nd); DF, 91% *cis*-DCCA (0.02; nd); DF, 69% *trans*-DCCA (0.02; nd); DF, 90% DBCA (0.02; nd); DF, nd 3PBA (0.01; nd); DF, 97% 4F3PBA (0.005; nd); DF, nd *EI+ detection* *trans*-CDCA (0.05;	V: 2 mL H: acidic E: LLE C: No I: GC-HRMS (NCI- or EI+ mode; SIM)	LLE: • 4 mL MTBE • Drying of organic layer – gentle stream of nitrogen • Reconstitution: 250 μL ACN	• 30 μL HFIP, 20 μL DIC – 10 min, RT • Wash: 1 mL 1 M $NaHCO_3$ • Re-extraction to 250 μL iso-octane (EI+ mode) or 2 mL iso-octane (NCI- mode)	External Quality Control: yes Population: 30 persons, who applied commercially available vaporizer plates or sprays containing pyrethrum (Germany)	[10]

(continued)

Table 2 (continued)

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Derivatization	Notice	Ref.
nd); DF, nd *cis*-DCCA (0.05; nd); DF, nd *trans*-DCCA (0.05; nd); DF, nd DBCA (0.10; nd); DF, nd 3PBA (0.02; nd); DF, nd 4F3PBA (0.02; nd); DF – nd					
3PBA (0.5; nd); DF, nd	V: 5 mL H: acidic (1 mL H_2SO_4; 2 h, 100°C) E: LLE C: SPE (silica) I: GC-ECD	LLE: • Extraction: 2 × 5 mL DCM • Dehydration: anhydr. Na_2SO_4 • Evaporation (rotary vacuum) Derivatization Cleanup: • Dilution with 5 mL H_2O + 2 × 2 mL *n*-hexane • Dehydration: anhydr. Na_2SO_4 • Evaporation (rotary vacuum) • Reconstitution: 1 mL hexane SPE • Conditioning: 2 mL toluene, 4 mL hexane	200 µL of acetone solution of PFBBr (dilution 1:100) + 15 µL K_2CO_3 (60% w/v in water) + 4 mL acetone ->overnight at RT	PFBBr – strong lachrymator	[5]

		• Washing: 5 mL hexane and 2 mL hexane-toluene (70:30) • Elution: 8 mL hexane-toluene (60:40) • Evaporation – gentle stream of nitrogen • Reconstitution: 0.5 mL hexane			
CDCA (0.0088; 0.029); DF, 52.6–69.4% *cis*-DCCA (0.0072; 0.024); DF, 95.6–95.9% *trans*-DCCA (0.0096; 0.032); DF, 94.7–100% DBCA (0.0060; 0.020); DF, 5.3–12.2% 3PBA (0.013; 0.043); DF, 91.8–94.7% 4F3PBA (0.0053; 0.018); DF, 32.5–55.1%	V: 5 mL H: enzymatic E: LLE I: GC-MS (quadrupole, SIM, negative mode)	LLE: • Extraction: 2 × 5 mL DCM • Evaporation	• 250 μL ACN, 30 μL HFIP, 20 μL DIIC, 10 min, RT • Wash and extraction: 1 mL 1 M $NaHCO_3$ + 250 μL hexane	• External Quality Control: G-EQUAS • Population: rural population of the Montérégie area, Quebec (adults $n = 114$, children, $n = 49$), Canada	[25]
cis-DCCA (0.007; nd); DF, 99% *trans*-DCCA (0.01; nd); DF, 99% DBCA (0.006; nd); DF, 77% 3PBA (0.01; nd); DF, 100% 4F3PBA (0.008; nd); DF, 71%	V: 5 mL H: enzymatic E: LLE C: No I: GC-MS (quadrupole, negative mode, SIM)	LLE: • Extraction: 9 mL *n*-hexane • Evaporation to dryness	• 30 μL HFIP, 20 μL DIIC, 2 mL of iso-octane/hexane (2:98) • Evaporation • Reconstitution: hexane	• External quality control: (G-EQUAS) • Population: pregnant women residing in ten English-speaking Caribbean countries ($n = 295$)	[22]

(continued)

Table 2 (continued)

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Derivatization	Notice	Ref.
DBCA (0.833; 2.5); DF, nd 3PBA (0.008; 0.025); DF, 30–35% 4F3PBA (0.017; 0.05); DF, 20–25%	V: 10 mL H: acidic E: LLE I: GC-MS/MS (triple quadrupole)	LLE • Extraction: 20 mL isopropanol-hexane (5:95) • Evaporation to dryness	• 0.5 mL toluene + 20 μL MTBSTFA (75°C, 45 min)	• Population: 20 adults and 20 children without occupational exposure (China)	[26]
cis-DCCA (0.1; nd); DF, 95.3% *trans*-DCCA (0.1; nd); DF, 98.3% 3PBA (0.1; nd); DF, 98.8%	V: 5 mL H: acidic (1 mL HCl; 1 h, 90°C) E: LLE C: LLE I: GC-MS (quadrupole, SIM)	LLE: • Extraction: 2 × 4 mL hexane • Evaporation (gentle stream of nitrogen) • Derivatization: 3 mL 10% H_2SO_4 in MeOH (75°C, 1 h) Cleanup LLE: • Re-extraction: 3 mL hexane +3 mL saturated NaCl solution • Evaporation (gentle stream of nitrogen) • Reconstitution: 100 μL of toluene	• 3 mL 10% H_2SO_4 in MeOH (75°C, 1 h)	• Modification of Kühn et al. [3] • Population: pregnant women in an agricultural area of the Province of Jiangsu (n = 1,149), China	[23]
cis-DCCA (nd; 0.01); DF, 100% *trans*-DCCA (nd; 0.01); DF, 100% *cis*-DBCA (nd; 0.01); DF, 80% 4F3PBA (nd; 0.01); DF, 5%	V: 5 mL H: acidic (1 mL HCl; 1 h, 90°C) E: LLE C: LLE I: GC-MS/MS (quadrupole, EI)	LLE: • Extraction: 2 × 5 mL hexane • Cleanup: 2 mL 0.1 N NaOH • Re-extraction: +0.1 mL conc. HCl + 1.8 mL hexane • Evaporation under nitrogen to 50 μL	MTBSTFA (10 μL) • 80°C, 1 h	• External quality control: G-EQUAS • Population: general with no known exposure to synthetic pyrethroids (n = 38), Germany	[9]

3PBA (nd; 0.01); DF, 100% ClF_3CA (nd; 0.01); DF, 90% CPBA (nd; 0.01); DF, 40% MPA (nd; 0,01); DF, 5%					
cis-DCCA (0.05 μg L^{-1}); DF, 52% *trans*-DCCA (0.05 μg L^{-1}); DF, 72% DBCA (0.05 μg L^{-1}); DF, 13% 3PBA (0.05 μg L^{-1}); DF, 70% 4F3PBA (0.05 μg L^{-1}); DF, 4%	V: 10 mL H: acidic (1 mL HCl; 1 h, 90°C) E: LLE C: LLE I: GC-MS (quadrupole, EI, SIM)	LLE • Extraction: 2 × 5 mL hexane • Organic phase + 2 mL 0.1 N NaOH • Aqueous phase + 0.1 mL conc. HCl + 2 mL hexane • Drying organic phase (vacuum centrifuge) • Dissolution: 50 μL toluene	10 μL MTBSTFA • 70°C, 45 min	• External quality control: yes • Population: group without known exposure to pyrethroids (n = 26 females, 20 males) Germany	[11]
cis-DCCA (0.006; 0.020); DF, nd *trans*-DCCA (0.005; 0.017); DF, nd DBCA (0.019; 0.062); DF, nd 3PBA (0.003; 0.010); DF, nd CPBA (0.002; 0.007); DF, nd	V: 3 mL H: acidic (0.5 mL H_2SO_4; 1 h, 90°C) E: SPE (C18 Sep-Pak, 500 mg, waters) C: LLE I: GC-MS/MS (ion trap)	SPE: • Conditioning: 7.5 mL MeOH, 5 mL water • Sample • 6 mL water • Drying • Elution: 10 mL acetone • Evaporation Cleanup: • Residue dissolution in 2 mL	• 10 μL HFIP, 15 μL DIIC – 3 min, RT • Wash: 1 mL 5% K_2CO_3	• High usage of organic solvents: about 35 mL • Population: six occupationally exposed, two non-exposed occupationally, Spain	[7]

(continued)

Table 2 (continued)

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Derivatization	Notice	Ref.
		of saturated NaCl + 2 × 5 mL *n*-hexane • Evaporation to dryness (gentle stream of nitrogen) • Reconstitution: 1 mL hexane			
cis-DCCA (0.5; nd); DF, 100% *trans*-DCCA (0.5; nd); DF, 100% DBCA (0.3; nd); DF, nd 3PBA (0.5; nd); DF, 100% 4F3PBA (0.5; nd); DF, nd	V: 10 mL H: acidic (2 mL H_2SO_4; 1 h, 90°C) E: SPE (C18, 500 mg, Bakerbond) I: GC-MS (quadrupole, SIM)	SPE: • Conditioning: 3 mL EtAc, 3 mL *n*-hexane, 6 mL MeOH, 9 mL H_2O • Sample • Washing: 6 mL water • Drying Elution: 3 mL MeOH	• 1 mL of 98% H_2SO_4, 1 h, 75°C	• External quality control: yes • Population: occupational exposure ($n = 8$), Germany	[6]
ClF3CA (LLOQ 0.06); DF, nd *cis*-DCCA (LLOQ 0.08); DF, nd *trans*-DCCA (LLOQ 0.08); DF, nd DBCA (LLOQ 0.06); DF, nd 3PBA (LLOQ 0.06); DF, nd	V: 0.4 mL H: Enzymatic E: MEPS (C18, SGE) I: LVI-GC-MS (ion trap, μSIS)	SPE (MEPS): • Conditioning: 4 × 50 μL MeOH, 3 × 20 μL 2% HCOOH • Sample: 5 × 100 μL • Washing: 3 × 50 μL 30% MeOH • Drying under vacuum • Elution: 2 × 40 μL 1%HFIP and 2%DIIC in hexane	• 1% HFIP, 2% DIIC in hexane	• External quality control: G-EQUAS	[17]

cis-DCCA (nd; 0.1); DF, 8% *trans*-DCCA (nd; 0.1); DF, 7% DBCA (nd; 0.1); DF, 11% 3PBA (nd; 0.1); DF, 80%	V: 3 mL H: acidic (0.6 mL HCl; 90 min, 95°C) E: LLE C: LLE I: GC-MS (ion trap, SIS)	LLE • Extraction: 2 × 4 mL hexane • Re-extraction to 0.5 mL 0.1 M NaOH • Re-extraction (0.1 mL HCl + 2 mL hexane) • Evaporation – under stream of nitrogen	• 10 μL HFIP, 15 μL DIC, 250 μL hexane – 10 min, RT • Wash: 1 mL 5% K_2CO_3	• External quality control: ClinCal Urine Calibrator	[24]
HOCH2-FB-Al (0.01; 0.1); DF, 82% MTFBL (0.05; 0.31); DF, 54% MMTFBL (0.05; 0.31); DF, 56% FB-Al (0.02, 0.31); DF, 84% *cis*-DCCA (0.03; 0.10); DF, 88% *trans*-DCCA (0.02; 0.10); DF, 100% 3PBA (0.01; 0.05); DF, 100% *trans*-CDCA (0.06, 0.31); DF, 68% CH3-FB-Ac (0.01; 0.31); DF, 38%	V: 2.5 mL H: acidic (0.6 mL, 6 mol/L HCl; 2 h, 106°C) E: LLE (24-well plate; Extrahera) C: No I: GC-MS/MS (triple quadrupole, EI)	LLE • Extraction: 3 mL and 2.5 mL MTBE • Organic phase divided into two parts • Evaporation under vacuum (4°C in ice block)	Part I: 100 μL acetone + 50 μL BSTFA: TMCS (99:1) 30 min, room temperature • Wash with 1 mL $NaHCO_3$ (1 mol/L) + 100 μL iso-octane Part II: 100 μL of ACN + 15 μL HFIP + 10 μL DIC 30 min, room temperature • Wash with 0.5 mL $NaHCO_3$ (1 mol/L) + 100 μL iso-octane	• Population: 3-year-old children ($n = 50$), Japan	[8]
MTFBL (0.01; 0.04); DF, nd MMTFBL (0.02; 0.07); DF, nd TMCA (0.04; 0.12); DF, 0%	V: 25 mL H: enzymatic E: LLE C: No I: GC-MS (EI, SIM)	LLE • Extraction: 2 × 2 mL toluene • Dehydration: 1 g Na_2SO_4 • +1 mL toluene (washing Na_2SO_4)	• Alcoholic group (MTFBL, MMTFBL): 50 μL TMSI (30 min, 70°C) • Carboxylic acid: 30 μL MTBSTFA (30 min, 70°C)	• Population: six subjects without occupational exposure, Japan	[20]

(continued)

Table 2 (continued)

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Derivatization	Notice	Ref.
TFBA (0.06; 0.20); DF, nd ClF3CA (0.03; 0.09); DF, nd MPCA (0.04; 0.14); DF, nd DCCA (0.08; 0.26); DF, nd CXCA (0.12; 0.41); DF, nd FPBA (0.07; 0.24); DF, 0% 3PBA (0.09; 0.30); DF, nd MPA (0.10; 0.33); DF, nd		• Concentration to 1 mL – under stream of nitrogen			
cis-DCCA (12, nd); DF, nd *trans*-DCCA (9, nd); DF, nd DBCA (7, nd); DF, nd 3PBA (1.8, nd); DF, nd 4F3PBA (17, nd); DF, nd	V: 1 mL H: acidic (0.1 mL HCl; 10 min, 70°C) E: HF-LPME C: No I: GC-ECD	HF-LPME: • Extraction to Accurel Q3/2 polypropylene hollow-fiber membrane (600-µm ID, 800-µm OD, pore size 0.2 µm) impregnated with 8 µL 1-octanol)	• 1 µL HFIP, 2 µL DIC – derivatization in the syringe barrel	• In-syringe derivatization	[28]

DCCA (mixture of isomers) (0.02; nd); DF, 100% DBCA (0.04; nd); DF, 50% 3PBA (0.06; nd); DF, 100% 4F3PBA (0.02; nd); DF, 100% MPA (0.04; nd); DF, 0% CPBA (0.04; nd); DF,70% ClF3CA (0.08, nd); DF, 80%	V: 2.5 mL H: enzymatic E: automatic SPE (C18 Agilent SampliQ, 200 mg) C: No I: GC-MS (quadrupole, EI, SIM)	SPE: • Conditioning: 6 mL MeOH, 3 mL ACN, 3 mL 2% HCOOH • Sample • Washing: 1.5 mL 20% MeOH 2% HCOOH in water • Drying • Elution: 3.5 mL ACN • Concentration to 200 μL (TurboVap Evaporator) • Derivatization • LLE: 200 μL isooctane	• 20 μL DIC, 30 μL HFIP (vortexing 10 min) • Wash: 1 mL 1 M $NaHCO_3$	• SPE (C18) – RapidTrace auto-SPE WorkStation; • Population: ten children living in Boston, MA (USA)	[33]

Table 3 Liquid chromatography methods for determination of synthetic pyrethroid metabolites in human urine

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Notice	Ref.
cis-DCCA (0.5; nd); DF, 36% *trans*-DCCA (0.5; nd); DF, 50% DBCA (0.2; nd); DF, 3.2% 3PBA (0.1; nd); DF, 64% 4F3PBA (0.3; nd); DF, 8.6%	V: 5 mL H: enzymatic E: SPE (waters Oasis HLB 6 cc) I: HPLC-MS/MS (triple quadrupole, negative mode)	SPE: • Conditioning: 4 mL MeOH, 4 mL water • Sample • Washing: 2 mL 5% MeOH • Elution: 2 mL MeOH • Evaporation (TurboVap) • Reconstitution: 50 µL ACN • Evaporation • Reconstitution: 7 µL ACN	• Population: half the cohort was suspected to have residential exposure ($n = 217$), USA	[29]
3PBA (15; 50); DF, nd 4OH3PBA (15; 50); DF, nd	V: 1 mL H: acidic (0.2 mL HCl; 90 min, 95°C) E: HF-LPME I: HPLC-DAD	HF-LPME • +100 mg NaCl • Extraction (solvent dihexyl ether) 120 min • Desorption: 0.1 mL 0.1 M NaOH 120 min	• Hollow-fiber microextraction	[12]
cis-DCCA (0.4; nd); DF, 14.5% *trans*-DCCA (0.4; nd); DF, 16.4% DBCA (0.4; nd); DF, nd 3PBA (0.03; nd); DF, 52.7% 4F3PBA (0.03; nd); DF, 5.5%	V: 1 mL H: enzymatic E: automated SPE (OASIS HLB 96-well 30 mg, Waters) I: HPLC-MS/MS (quadrupole, negative mode)	SPE: • Conditioning: 0.5 mL acetone, 0.5 mL 1% acetic acid • Sample • Washing: 25% MeOH in 1% acetic acid • Drying (TurboVap under stream of nitrogen) • Elution: 2 × 325 µL acetone • Evaporation • Reconstitution: 10 µL internal standard (3-chloro-2-phenoxybenzoic acid) and 110 µL 25% MeOH in water	• 96-well plate SPE • External quality control: G-EQUAS • Population: diverse group of male and female adult volunteers with no documented occupational exposure to the target pesticides or their precursors ($n = 55$), USA	[16]

cis-DCCA (0.015; 0.020); DF, 97% *trans*-DCCA (0.015; 0.030); DF, 100% DBCA (0.015; 0.025); DF, 97% 3PBA (0.015; 0.025); DF, 100% 4F3PBA (0.015; 0.025); DF, 10%	V: 5 mL H: Enzymatic E: LLE C: LLE I: LC-MS/MS (triple quadrupole; negative mode)	LLE: • Extraction: 2 × 6 mL hexane • Re-extraction to 3 mL 0.1 M NaOH • Re-extraction to 0.2 mL conc. HCl + 6 mL hexane • Evaporation – gentle stream of nitrogen • Reconstitution in 80 μL 0.1% formic acid in water/methanol mixture (70/30, v/v)	• Extraction similar to Schettgen et al. [11] • Population: 39 adults – 23 women and 16 men – aged between 24 and 62 years old Limousin Region, France	[14]
4F3PBA (0.019; nd); DF, 54% 3PBA (0.018; nd); DF, 82%	V: 1 mL H: enzymatic E: SPE (Oasis HLB 3 cc, waters) C: No I: UPLC-MS/MS (triple quadrupole, ESI-)	SPE: • Conditioning: 1 mL MeOH/acetone (25:75); 1 mL 1% CH_3COOH in water • Sample • Washing: 0.5 mL 1% CH_3COOH in water • Cartridge drying (20 min under vacuum) • Elution: 1.5 mL MeOH/acetone (25:75) • Evaporation – gentle stream of nitrogen • Reconstitution in 120 μL MeOH: H_2O (25:75)	• External quality control: G-EQUAS • Population: human urine samples from two adult Spanish populations in Catalonia and Galicia ($n = 125$), Spain	[21]
cis-DBCA (0.1; nd); DF, nd *cis*-DCCA (0.2; nd); DF, nd *trans*-DCCA (0.4; nd); DF, nd 4F3PBA (0.2; nd);	V: 2 mL H: enzymatic E: SPE (Oasis HLB 3 cc, waters) C: No I: HPLC-TIS-MS/MS (triple quadrupole, negative mode)	SPE: • Conditioning: 1 mL MeOH, 1 mL 1% CH_3COOH in water • Sample • Washing: 5% MeOH in 1% acetic acid solution • Cartridge drying (30 s – vacuum)	• Population: 115 samples	[30]

(continued)

Table 3 (continued)

Analytes (LOD ng/mL; LOQ ng/mL); DF	Sample volume (V), hydrolysis (H), extraction (E), cleanup (C), instrument (I)	Extraction and cleanup details	Notice	Ref.
DF, nd 3PBA (0.1; nd); DF, nd	TIS – turbo ion spray atmospheric pressure ionization	• Elution: 1.5 mL MeOH + 2 mL ACN • Evaporation to dryness • Reconstitution in 50 µL ACN (reconstitution)		
cis-DCCA (14 fmol injected; nd); DF, nd *trans*-DCCA (18 fmol injected; nd); DF, nd 3PBA (31 fmol injected; nd); DF, nd LODs range: 0.1–0.3 ng/mL	V: 5 mL H: enzymatic E: SPE (Sep-Pak C18 cartridges; waters) C: No I: UHPLC- MS Q-TOF (ESI-)	SPE: • Conditioning: 4 mL MeOH, 8 mL water • Sample • Washing: 8 mL water • Elution: 8 mL MeOH • Evaporation – gentle stream of nitrogen • Reconstitution in 1 mL MeOH	None	[32, 35, 36]
MPA (2.5 ng/mL); DF, nd 3PBA (2.5 ng/mL); DF, nd	V: 10 mL H: acidic (HCl, conditions not specified) E: LLE C: LLE I: HPLC-UV	LLE: • 10 mL of urine, 0.1 mL HCl, 2 mL chloroform • Organic phase, 0.1 mL 4 M NaOH, 1 mL water • 0.5 mL aqueous phase, 0.5 mL HPLC mobile phase (ACN:1% H_2SO_4; 1:1)	• Large sample volume • Not applicable for the measurement of environmental exposure levels	[27]
3PBA (0.02; nd); DF, 97.8%	V: 1 mL H: acidic (6 M HCl; 2 h, 100°C) E: SPE (OASIS HLB 150 mg/6 cc, waters) C: No I: HPLC-MS/MS	SPE: • Conditioning: MeOH, water • Sample • Washing: 5% MeOH • Drying • Elution: MeOH • Evaporation • Reconstitution in 100 µL ACN	• Based on Baker et al. [29] • Population: pregnant women in the first trimester of gestation ($n = 231$), Japan	[31]

cis-DCCA (nd; 10); DF, nd *trans*-DCCA (nd; 10); DF, nd 3PBA (nd; 5); DF, nd 4F3PBA (nd; 5); DF, nd	V: 0.5 mL H: none reported E: online TurboFlow™ C: no I: UHPLC-Orbitrap-MS	Online SPE: • Raw urine filtration through a 0.2 μm nylon filter • 0.5 mL of sample was injected onto the online extraction procedure	• Population: women and men living near the agricultural areas of Almeria ($n = 37$), Spain	[37]
cis-DCCA (nd; 3.2); DF, nd *trans*-DCCA (nd; 3.2); DF, nd DBCA (nd, 10); DF, nd 3PBA (nd; 10); DF, 10% 4F3PBA (nd; 2); DF, nd	V: 5 mL H: enzymatic E: QuEChERS EN C: no I: UHPLC-Orbitrap-MS	QuEChERS: • 10 mL of acetonitrile • Pouch of QuEChERS EN extraction salt packet • Evaporation to dryness under a stream of nitrogen • Dissolution • Analysis	• Population: children from Valencia Region ($n = 20$), Spain	[19]
cis-DCCA (nd; 0.4); DF, 10% *trans*-DCCA (nd; 0.4); DF, 26% DBCA (nd, 0.8); DF, 23% 3PBA (nd; 0.8); DF, 23% 4F3PBA (nd; 0.2); DF, 0%	V: 5 mL H: enzymatic E: SPE, (Strata-X, 500 mg/3 mL, Phenomenex) C: no I: LC–MS/MS	Generic method to Olsson et al. [30]	• Population: children from Valencia Region ($n = 125$), Spain	[34]

Table 4 Non-chromatographic methods for determination of synthetic pyrethroid metabolites in human urine

Format	Analytes	Sample volume (mL)	Sample preparation	Antibodies	Competitive hapten	Cross-reactivity[a]	Detection	Recovery (urine, %)	IC_{50} (ng/mL)	Sensitivity (ng/mL)	Ref.
Immunoassays											
Indirect competitive ELISA	*cis*-/*trans*-DCCA-glycine	0.5	SPE (C18), elution with EtAc	Primary: pAb (rabbit) Secondary: GAR-HRP	*cis*-hapten 5[b]-BSA	*cis*-DCCA-glycine 28%, *trans*-DCCA 0.44%, permethrin 0.04%, glycine <0.01%, 3PBAG <0.01%[c]	UV-VIS (450–650 nm)	65–123	1.24 (buffer)	LOQ: 1 (urine)	[38]
Luminescent paramagnetic particle-based immunoassay	3PBA	0.5	Mixed-mode SPE (C8 + SAX), elution with 1% acetic acid in 70:30 Hex/EtAc	Primary: pAb (rabbit) Secondary: GAR-HRP (on a paramagnetic particle)	Acridinium ester-labeled 3PBA-BSA	4OH3PBA 126%, FPBA 72%,3PBAG 2.4%, 3PBAlc 0.8%, 3PBAlc-Gluc 0.2%	Luminometric	77–121	0.1 (buffer)	LOD: 0.01 (IC_{10}, buffer)	[52]
Indirect competitive ELISA	3PBA	0.5	Mixed-mode SPE (C8 + SAX), elution with 1% acetic acid in 70:30 Hex/EtAc	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBA-BSA	4OH3PBA 103%, 3-phenoxybenzaldehyde 75%, FPBA 72%	UV-VIS (450–650 nm)	93.7–136.5	0.77 (buffer)	LOQ: 2 (IC_{20}, urine)	[39]
Indirect competitive ELISA	3PBA	10	LLE (DCM)	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBA-BSA	FPBA 72%	UV-VIS (450 nm)	70–117	1.5 (urine)	LOD: 0.1 (urine)	[40]
Direct competitive fluorescence enzyme immunoassay	3PBA	0.05	Dilution with buffer (20-fold)	Anti-analyte: Nb-AP fusion protein	3PBA-BSA	3-phenoxybenzaldehyde 22.6%, 3PBAlc <0.01%, permethrin <0.01%, cypermethrin <0.01%, deltamethrin <0.01%, fenpropathrin <0.01%, phenothrin <0.01%	Fluorometric (440/550 nm)	84–109	0.082 (buffer)	LOD: 0.011 (buffer)	[47]
Indirect competitive ELISA	3PBAlc-Gluc	0.05	Dilution	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBAlc-Gluc-BSA	3PBAG 0.21%, 3PBA 0.16%, 4-hydroxybenzoic acid <0.06%, *p*-nitrophenyl glucuronide <0.06%, *cis*-	UV-VIS (450–650 nm)	>86	0.5 (urine)	LOD: 0.1 (urine)	[41]

						DCCA <0.06%, *trans*-DCCA <0.06%, *trans*-DCCA-glycine <0.06%, cypermethrin <0.06%, permethrin <0.06%, esfenvalerate <0.06%, cyfluthrin <0.06%					
Noncompetitive magnetic bead-based PHAIA	3PBA	0.5	Direct dilution with buffer (25-fold) or mixed-mode SPE (C8 + SAX) eluted with 1% acetic acid in 70:30 Hex/EtAc	Anti-analyte: protein A-purified pAb (immobilized on magnetic beads) Anti-phage: MAb-HRP	NA	NR	UV-VIS (450 nm)	87–109	SC_{50}: 0.2–0.4	NR	[48]
Noncompetitive PHAIA real-time PCR	3PBA	0.05	Dilution	Anti-analyte: protein A-purified pAb Anti-phage: MAb-HRP	NA	NR	Fluorometric	80–115	NA	LOD: 0.02 (buffer)	[53]
Indirect competitive VELISA	3PBA	0.002	Dilution with buffer (25-fold)	Primary: soluble VHH (alpaca) Secondary: goat anti-HA tag PAb-HRP	3PBA-BSA	4OH3PBA 150%, 3-phenoxybenzaldehyde 10%[d]	UV-VIS (450–650 nm)	80–110	1.4 (buffer)	LOD: 0.1 (buffer)	[42]
Indirect competitive PELISA				Primary: phage-displayed VHH Secondary: goat anti-M13 phage MAb-HRP		NR			0.1 (buffer)	LOD: 0.01 (buffer)	

(continued)

Table 4 (continued)

Format	Analytes	Sample volume (mL)	Sample preparation	Antibodies	Competitive hapten	Cross-reactivity[a]	Detection	Recovery (urine, %)	IC_{50} (ng/mL)	Sensitivity (ng/mL)	Ref.
Quenching fluoroimmunoassay	3PBAG	0.002	Dilution (1,000-fold)	Primary: pAb (rabbit)	F3PBAG	3PBAlc 6.2%, esfenvalerate 1.4%, 3PBA 0.35%, *N*-[(*S*)-4-chloro-α-(1-methylethyl) benzene-acetyl]glycine <0.02%, permethrin <0.02%	Fluorometric (490/512 nm)	85–111	1.2 (buffer)	DL: 0.25 (buffer)	[50]
Indirect competitive ELISA	3PBAG	10	SPE (C18), elution with methanol	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBA-BSA	Fluvalinate 0.07%, esfenvalerate 0.04%, methyl 3-phenoxybenzoate 0.03%, 3PBA 0.02%, 3PBAlc 0.01%, benzamidoacetic acid <0.01%, sFAG <0.01%, CPBA <0.01%, methyl (2*S*)-2-(4-chlorophenyl)-3-methylbutanoate <0.01%, {[(2*R*)-2-(4-chlorophenyl)-3-methylbutanoyl]amino} acetic acid <0.01%, DCCA <0.01%, methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate <0.01%, cypermethrin <0.01%	UV-VIS (450–650 nm)	98–109	0.42 (buffer)	LOQ: 1 (urine)	[43]
	sFAG			Primary: pAb (rabbit) Secondary: GAR-HRP	Hapten 3[e]-BSA	{[(2*R*)-2-(4-chlorophenyl)-3-methylbutanoyl]amino} acetic acid 2.8%, methyl (2*S*)-2-(4-chlorophenyl)-3-methylbutanoate 0.3%, (2S)-2-(4-chlorophenyl)-3-methylbutanoic acid 0.08%, fluvalinate 0.02%, esfenvalerate 0.02%, 3PBAG 0.01%, methyl 3-phenoxybenzoate <0.01%, 3PBA <0.01%, 3PBAlc		95–115	0.40 (buffer)		

						<0.01%, benzamidoacetic acid <0.01%, DCCA <0.01%, methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate <0.01%, cypermethrin <0.01%					
Indirect competitive ELISA	3PBA	0.001	Dilution (50-fold)	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBA-BSA	FPBA 72%, PBAG 0%, FPBA-glycine 0%, 3PBAlc 0%, 4-hydroxybenzoic acid 0%, permethrin 0%, cypermethrin 0%, esfenvalerate 0%, deltamethrin 0%, cyfluthrin 0%	UV-VIS (450–650 nm)	>86	1.65 (urine)	LOQ: 0.1 (urine)	[44]
Indirect competitive ELISA	3PBA	0.5	Mixed-mode SPE (C8 + SAX)	Primary: pAb (rabbit) Secondary: GAR-HRP	3PBA-BSA	4OH3PBA 103%, 3-phenoxybenzaldehyde 75%, FPBA 72%	UV-VIS (450 nm)	87.3–98	15.3 (urine)	LOQ: 2.5 (urine)	[45]
Other methods											
Optical sensing	3PBA	0.05	Dilution with water (50-fold)	Optical sensors: Mn-doped ZnS quantum dots anchored to molecularly imprinted polymer			Fluorometric (330/590 nm)	80.18–90.02	LOQ: 25.0[f] (urine)		[51]

Abbreviations: *BSA* bovine serum albumin, *DCCA* 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *DCM* dichloromethane, *DL* detection limit, *ELISA* enzyme-linked immunosorbent assay, *EtAc* ethyl acetate, *FPBA* 4-fluoro-3-phenoxybenzoic acid, *F3PBAG* fluorescin-labeled glycine conjugate of 3-phenoxybenzoic acid, *GAR-HRP* goat antirabbit IgG-horseradish peroxidase conjugate, *Hex* n-hexane, *IC10, 20, 50* inhibition of 10, 20, or 50% by the analyte, *IgG* immunoglobulin G, *LLE* liquid-liquid extraction, *LOD* limit of detection, *LOQ* limit of quantification, *MAb-HRP* mouse anti-M13 phage monoclonal antibody-horseradish peroxidase conjugate, *Nb-AP* nanobody-alkaline phosphatase, *NA* not applicable, *NR* not reported, *4OH3PBA* 4-hydroxy-3-phenoxybenzoic acid, *pAb* polyclonal antibody, *3PBA* 3-phenoxybenzoic acid, *3PBAG* glycine conjugate of 3-phenoxybenzoic acid, *3PBAlc* 3-phenoxybenzyl alcohol, *3PBAlc-Gluc* 3-Phenoxylbenzyl β-D-Glucuronide, *PBS* phosphate buffer saline, *PCR* polymerase chain reaction, *PELISA* isolated phage-VHH ELISA, *PHAIA* phage anti-immunocomplex assay, *SAX* strong anion exchange, *SC50* 50% of a saturating concentration, *sFAG* glycine conjugate of S-fenvalerate acid, *SPE* solid phase extraction, *VELISA* soluble VHH ELISA, *VHH* single domain antibody

[a]Relative to analyte (100%)

[b]Hapten 5—N-(*cis*/*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carbonyl)-4-amino-L-phenylalanine

[c]Cross-reactivities of *cis*/*trans*-DCCA-glycine mixtures were omitted. All values relative to trans-DCCA-glycine

[d]Cross-reactivities of other compounds (3PBAG, 3PBAlc, permethrin, cypermethrin, esfenvalerate, deltamethrin, cyfluthrin) were described as 'negligible'

[e]Hapten 3—3-[Cyano[(S)-2-(4-chlorophenyl)-3-methyl-1-oxobutanoxy]methyl]phenoxyacetic acid

[f]Calculated from molar concentration

pyrethroid metabolites in general populations, not occupationally exposed, are low, usually below 1 ng/mL, and therefore advanced methods of sample preparation for analysis and proper and sensitive apparatus are required. However, even the most modern and advanced equipment does not ensure reliable results. The laboratory must follow the principles of good laboratory practice, and the quality assurance system must necessarily work. Quality control procedure is a prerequisite for obtaining credible results. Both internal and external quality controls should be carried out in all laboratories specializing in HBM.

Internal quality control is realized by the purchase or in-house preparation of appropriate quality control (QC) materials. The QC material should be human biological material prepared at least at two different levels. The concentration levels should be adjusted in respect to the expected levels of exposure in studied population. At least one sample of each concentration level should be analyzed in each analytical batch and the QC results interpreted with the use of quality control charts.

Usually in-house quality control (QC) material is prepared according to generic procedure: urine is collected from multiple anonymous donors, combined, diluted with water (1:1 v/v) to reduce endogenous concentrations of the analytes of interest, and carefully mixed. Urine is filtered and divided into three pools. The first quality control (QC) pool (low concentration) is spiked with the native standards to yield low-concentration quality control (LQC) material. The second pool is spiked with higher amount of native standards to yield the so-called high quality control (HQC) material. The third pool is not spiked (blank urine) and is used later as a matrix material for calibration standards and blanks. It is recommended to characterize each pool by producing a minimum of 20 analytical runs over a period of 20 days. Obtained results are used then to determine the 95 and 99% control limits by which the QC sample results in each batch will be evaluated.

External quality control is carried out by analyzing samples obtained from external laboratories within the intercomparison program. The most well-known program that has been offering the assessment of the quality of methods for determination synthetic pyrethroid metabolites for many years is the German external quality control scheme (G-EQUAS,) organized and managed by the Institute and the Outpatient Clinic for Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg (Erlangen, Germany). Scheme, evaluation, and certification are based on the German Federal Medical Council (http://www.g-equas.de/). As part of this program, it is possible to verify the suitability of the method for the determination of five synthetic metabolites of pyrethroids: DBCA, *cis*-/*trans*-DCCA, 3PBA, and 4F3PBA. The rounds of this program take place two times a year.

6 Conclusions and Further Research

It seems that at the present time, analytical methods are available covering a fairly broad spectrum of metabolites with sufficient sensitivity to assess environmental exposure in global populations. Despite the much simpler sample preparation

procedure for LC-MS analysis and the use of very advanced mass spectrometers, GC-MS-based methods are still the most sensitive methods available.

Despite the availability of numerous modern and miniaturized techniques of extraction and purification of samples before instrumental analysis, in principle the only valid techniques remain classical extraction techniques: liquid-liquid and solid-phase extraction. The latter can be performed automatically by robotic systems both in the format of cartridges and 96-well plates. Automation increases the precision of determinations but also significantly reduces the labor cost, which is of great importance in population studies with hundreds or thousands of samples.

Analytical methods are also being developed for the metabolite determination of new pyrethroids and those less frequently used or hitherto not covered by biomonitoring. The problem is the commercial availability of reference substances and relevant isotopically labeled internal standards.

A very important tool that facilitates the achievement of reliable results by analytical laboratories is the availability of interlaboratory comparison programs. This type of harmonization of analytical methods makes it possible to compare the results of human biomonitoring studies carried out in different countries by various laboratories.

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Fate of Pyrethroids in Freshwater and Marine Environments

Laurence Méjanelle, Bibiana Jara, and Jordi Dachs

Contents

Abstract As a consequence of their increasing use, pyrethroid insecticides are recognized as a threat for nontarget species and ecosystem health. The present chapter gives a state-of-art overview of individual pyrethroid occurrence in waters and sediments worldwide, together with recent reports of their quantification in the atmospheric gas and aerosol phases. Degradation rates, transport processes, and partitioning of pyrethroids between environmental phases are reviewed. River flow efficiently transports pyrethroids to river mouths and estuaries, while pyrethroid

L. Méjanelle (✉)
Sorbonne Université/CNRS, UMR 8222 Laboratory of Ecogeochemistry of Benthic Environments, Observatoire Océanologique de Banyuls, Banyuls-sur-Mer, France
e-mail: laurence.mejanelle@upmc.fr

B. Jara
Programa de Postgrado en Oceanografía, Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile

Facultad de Ciencias, Universidad de Magallanes, Punta Arenas, Chile

J. Dachs
Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain

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impact on the marine environment remains difficult to appraise due to lack of comprehensive studies. Nevertheless, aquaculture arises as an important but poorly understood environmental burden. Owing to their large organic carbon pool, sediments may act as a sink for pyrethroids and impair nontarget aquatic species. Partitioning potential of pyrethroids is compared to that of other well-known legacy pollutants in the light of their position in the phase space defined by key physicochemical properties (K_{OW} and H'). The transport and partition of pyrethroids away from their source are strongly dependent on their half-life, but their quasi constant emissions in urban and agricultural area may compensate for their degradation, therefore sustaining the occurrence and behavior of some individual pyrethroids as "quasi persistent organic pollutants."

Keywords Air, Freshwater, Marine, Partition, Pyrethroids, Sediment, Transport, Water

1 Introduction

A major change in the use of pesticides over the last 20 years has been the gradual replacement of organophosphate and organochlorine pesticides by synthetic pyrethroids. The regulation and the ban of formerly used active agents have been followed by an increased use of a wide variety of current-use pesticides such as pyrethroids in agriculture and aquaculture [1]. Pyrethroids are also extensively used in urban and industrial areas and livestock farms to control pests such as mosquitoes, lice, and wood-destroying dwellers. In addition, synthetic pyrethroids have the advantage of low cost, low mammalian toxicity, and shorter persistence in the environment than other classes of pesticides [2].

The exposure mechanism leading to acute neuronal toxicity to insects and crustaceans is through dissolved water in the water column and through pore water in the sediments [3]. Other impacts have been reported and are related to trophic transfer in food webs. Even though pyrethroids are degraded faster than other pesticides, they have been shown to occur in water bodies, allowing their transfer to the aquatic food webs [4]. Pyrethroids have hydrophobicities in the same range as legacy organochlorine pesticides (log K_{OW} from 4.8 to 7.0) and thus tend to sorb on organic particles and sediments. Insecticides sorbed in particles may be consumed by filter feeders and be transferred to higher trophic levels, or alternatively, particles may consist in a reservoir for these pollutants, probably reducing their biodegradability in natural waters. As a result of biomagnification at high trophic levels, negative impact of pyrethroids has been suggested causing immunity and estrogenic disruption to mammalians [4].

The impact of pyrethroids is the result of both the exposure to dissolved pyrethroids and to particle-associated ones. A comprehensive understanding of pyrethroid impact to nontarget species starts with the understanding of pyrethroid occurrence in the various environmental phases: dissolved water phase, particles, and sediments. This chapter reviews the current knowledge on the occurrence of

pyrethroids in water, particles and sediments of freshwater and marine environments, and the underlying partition and transport processes between those phases. Pyrethroids are often applied to water bodies, and after introduction to the dissolved phase, they partition between the different environmental compartments, being subjected to a number of sinks, particularly degradation. The elucidation of the occurrence, partition, and sinks of pyrethroids will allow to identify research lines that would help to better constraint the environmental risk associated to pyrethroids and to orientate protection measures.

2 Pyrethroid Sources and Emissions in Surface and Marine Water Bodies

Because of their wide spectrum of targets, pyrethroids are used in a variety of applications; agriculture and urban householding pest control compose two of the major market shares. Accurate estimates of their use are made difficult because nonprofessional uses are often not reported and by off the counter sales. The use of pyrethroids by aquaculture activities leads to important amounts of pyrethroids directly released to the marine environment, which can be important in specific marine areas [5, 6]. Overall, pyrethroids represent more than one third of the insecticide market, with a worldwide annual use of active ingredients around 7,000 tons per year between 1990 and 2013 (with peaks above 12,000 tons in 1997 and 2012) [7].

Structural and householding usages constitute an important part of the pyrethroid market. Several studies report that these compounds are not completely eliminated in conventional wastewater treatment plants (WWTPs) [8, 9], and thus they can be introduced into the environment through WWTPs effluents. Pyrethroids from urban sources were identified as the cause of toxicity in 80% of river sediments in the vicinity of the city of Salinas in Southern California [10].

3 Occurrence and Composition of Various Pyrethroids in Water Ecosystems

In order to estimate the potential impact of pyrethroids on aquatic environments, research projects and monitoring programs have surveyed pyrethroid occurrence mostly in the vicinity of agricultural and urban areas concerned by pyrethroid primary use. California is the world location from which more data are available as a result of numerous monitoring programs setup at the municipal to state level [11]. As a result of their affinity for organic matter, pyrethroids have been detected both in the water phase and in the sediments. Table 1 reviews water concentrations of pyrethroid in the current literature, and Table 2 reports their levels in sediments.

Table 1 Non-exhaustive selection of concentrations ranges, in ng L^{-1}, of individual pyrethroids in water from different locations worldwide

	Year	Sample type	Individual pyrethroids								References
			Bifenthrin	Fenpropathrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin	
Freshwater											
Northern California											
American River, flood events	2009–2010	WS	nd–106	nd	nd	nd–111	nd–26.6	nd–9.4	nd	nd	[12]
San Francisco Bay, drains sampled after storm	2014	WS	nd–9.9								[13]
Central California											
Creeks and drains in the San Joaquin watershed	2007	Diss	nd–15.8	nd–2.6	nd–19.8	nd–1.1	nd–2.9	nd–5.7	nd–5.1		[14]
Creeks and drains in the San Joaquin watershed	2007	P	nd–9.6	nd	nd–11.1	nd–1.1	nd	nd	nd–5.1		[14]
Puerto Creek channel into San Joaquim Rivers	2007	WS				nq–93					[15]
Wadworth channel into Sacramento River	2003	WS				nd–94					[15]
Sacramento River	2008–2009	Diss	nd–24	nd–8.5	nd	nd	nd	nd	nd	nd	[16]
Del Puerto and Oreshimba creek	2007–2008	WS?	nd–5	nd	nd–16	nd	nd–21	nd	nd	nd–6.28	[17]
Salinas River and Monterey Bay, storm events	2008–2009	P	0–21.6		0–7.6	0–36.0		0–23.4	0–35.6	0–1.8	[18]
Creeks and drain on the Salinas and Santa Maria River watershed	2014–2015	WS?	nd–11.4		nd–447	nd–17.1	nd	nd	nd–39.7		[19]
Southern California											
Los Angeles and San Gabriel Rivers low flow conditions	2011	WS	nd–9	nd	nd	nd–18	nd	nd	nd	nd	[20]
San Diego River during storm events	2017	Diss	1–20.4	nd–24.9	nd–30.3	nd–55.9	nd–50.2	nd–55.4	nd–102	nd–62.2	[21]
San Diego River during storm events	2017	P	1–347	nd–63.2	nd–96.9	x–367	nd–205	nd–492	nd–56.9	nd–253	[21]

Asia											
Leyte island, Philippine rice agriculture	2010	Diss									[22]
Rivers passsing through large Vietnamese cities	2011–2012	WS				x–4,390					[23]
Beijing GuanTin Reservoir, China	2003–2004	Diss						nd–1.89		nd–6.28	[24]
Urban stream, Guangzhou, China		Diss	0.28 ± 0.25[a]		0.52 ± 0.59[a]	3.7 ± 3.1[a]		5.0 ± 3.3[a]			[25]
Urban stream, Guangzhou, China		P	0.84 ± 0.48[a]		4.3 ± 4.4[a]	9.0 ± 7.2[a]		20.0 ± 14.5[a]			[25]
Chenab River, Pakistan		Diss	nd–92			nd–103		nd–97		nd–108	[26]
Europe											
Rivers in the Humber catchment, UK	1996–1997	P				nd–3,500					[27]
Ebro River delta, Spain	2008–2009	Diss	nd	nd	nd	nd	nd	0.73–57.2	nd	2–58.8	[28]
Ebro River delta, Spain	2008	Diss						5–30			[29]
Valencia paddy field, surface water and groundwater, Spain		Diss									[30]
Streams, Central Germany	2009	Diss			nd–55			nd–86	nd		[31]
Streams, Central Germany	2009	P			nd–88			nd–180	nd		[31]
Seawater											
Estuarine catchment sites, NE Australia	2016–2017	WS?	nd–20.6 mg L^{-1}			nd		nd			[32]
NW Portugal Coast	2016–2017	Diss	nd		nd	nd	nd	nd–31	nd	nd	[33]
South African estuary	2002–2003	P						0.33–2.78	0.03–0.79		[34]
Pearl River estuary, urban creek at Guanzhou, China	–	Diss	0.3 ± 0.3		0.5 ± 0.6	4 ± 3		5 ± 3			[25]
Pearl River estuary, urban creek at Guanzhou, China	–	P	0.8 ± 0.6		4 ± 4	9 ± 7		20 ± 14			[25]

(continued)

Table 1 (continued)

	Year	Sample type	Individual pyrethroids								References
			Bifenthrin	Fenpropathrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin	
In seawater concerned by salmon aquaculture											
Close to salmon cages, Southern Chile	–	Diss						4.4 ± 0.7			[35]
Close to the shore, Southern Chile	–	Diss						2.1 ± 0.8			[35]
1–2 weeks after treatment, Norway	2014	Diss						nd		nd	[36]
Near aquaculture centers, New Brunswick, Canada	2010	Diss								nd–40	[5]
Near aquaculture centers, New Brunswick, Canada	2010	P								nd–400	[5]

The first part of the table reviews concentration from freshwater bodies, and the second part reviews data from marine environments. Sample type is referred to as follows: *WS* refers to Whole Samples (dissolved phase + particles), *Diss* stands for dissolved phase analyzed after prefiltration, *P* stands for for particles suspended in the water and collected on a filter. When the method description does not describe in detail if the water is prefiltred before extraction, its is assumed that the data concerns whole sample, and the sample type is indicated as WP?
nd not detected, *nq* below quantification limits, *x* minimum value not reported
[a]When the concentration range is not available in the reference, the average and standard deviation is reported instead

Table 2 Non-exhaustive selection of individual pyrethroid levels, in ng g^{-1}, in sediments from different locations worldwide

	Year	Sample type	Individual pyrethroids														References
			Allethrin	Resmethrin	Bifenthrin	Fenpropathrin	Tetramethryn	Ametryn	Promethryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Denvalerate	Fenvalerate	Deltamethrin	
Freshwater sediments																	
Northern California																	
Del Puerto Creek	2005–2006	SED			0–286					43.3	23.1	0–21			[a]1.5–2.2		[37]
Central California																	
Orcutt Creek, Santa Maria River watershed	2002–2003	SED								nd–59.4	nd–107				nd–32.6		[38]
Lower Santa Maria River	2003	SED									1.54				0.56		[39]
Creeks and drains in the San Joaquin watershed,	2007	SED		nd–nq	nd–15.8	nd–2.6				nd–19.8	nd–14.5	nd–6.9	nd–5.7		nd–2.9		[14]
Southern California																	
Orange county		SED			21–487					nd–79.7	nd–165.2	nd–66.7	nd–34.4		nd–4.7	nd–23.1	[40]
Alamo River and New River, Salton Sea	2010	SED			nd–1.90	nd				nd	nd–17.56	nd	nd		nd	nd	[41]
Salton Lake sediments Southern California	–												nd–183				[42]
San Diego River during storm events	2017																[21]
Other sites from the USA																	
Metropolitan streams, nationwise survey in the USA	–			nd–38.3	nd–11.2					nd–3.0	nd–9.3		nd–8.9				[43]
Minnesota stormwater ponds	2009			nd	nd–37.2		nd			nd	nd–41.9	nd	nd		nd	nd	[44]
Argentina																	
Carnaval creek, Buenos Aires, Argentina	2015–2016				nd					1.8–649	nd		4.2–14.8			nd	[45]
Australia																	
Queensland rivers and drains	1998				0–29			0–130	0–45								[46]
Asia																	
Vietnamise Rivers	2011										nd–7,850					nd–59,700	[47]
Rice cultivation in Leyte island, Philippines	2010							59		nd–29			nd–1,400			nd–43	[48]

(continued)

Table 2 (continued)

	Year	Sample type	Individual pyrethroids														References
			Allethrin	Resmethrin	Bifenthrin	Fenpropathrin	Tetramethryn	Ametryn	Promethryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Denvalerate	Fenvalerate	Deltamethrin	
Pearl River sediments, South China	–				0.38–6.54	0.37–1.49				0.35–1.87	0.88–35.4	nd	0.22–20.4		nd	nd–1.29	[49]
Wetland, Beijing, China	2004–2006												nd–0.008		nd–0.047	nd–0.448	[50]
Beijing GuanTin Reservoir, China	2003–2004	Diss											nd–8.87	nd–26.3	nd–54.2		[24]
Beijing GuanTin Reservoir, China	2003–2004	SED											nd–0.00877	0.0454–0.158	0.0786–0.301		[24]
Urban creek in Guangzhou, South China	–	SED			6 ± 1					11 ± 8	40 ± 56		68 ± 67				[25]
Liaohe River, northeastern China	2014	SED	0.6–29		nd–0.33	nd–23	nd–1.7			nd–4.4			1.6–33		nd–4.6	nd–4.7	[51]
Chenab river, Pakistan	2015–2016	SED			nd–325						nd–291		nd–343			114–411	[26]
Europe																	
River United Kingdom, river sediments	1996–1997	SED									50–300						[27]
Ebro River delta sediments	Jun 2009	SED		nd	nd	nd	nd			nd	nd	nd	8.27–71.9		nd	nd	[28]
Ebro River delta sediments	Oct 2009	SED											0.13–2.92				[29]
Marine sediments																	
Southern California																	
Creeks and estuary, Ballona creek,	2007–2008	SED			3–80[b]					nd–15[b]	5–150[b]	nd–25[b]	1–190[b]		nd–2[b]		[52]
Southern California Bight	2008	SED			nd–64.8						nd–132						[53]
Ports and bays, Monterey Bay	2008–2009	SED			2.80 ± 3.31[a]												[18]
Europe																	
North Western Portugal Coast	2016–2017	SED			nd		nd		nd	nd	nd	nd	nd		nd	nd	[33]
China																	
Heibei creek, Guangzhou, Southern China	–	SED			nd–18.8	nd–54.5				nd–32	nd–128	nd–2.5	nd–179		nd–5.4		[54]
Pearl River estuary, China	2012	SED			5 ng g												[55]

The first part of the table documents freshwater sediments and the second part reviews results obtained from marine sediments

Sample type is referred to as follows: *SED* refers to the solid phase of the sediment; *Diss* stands for pore water dissolved phase

nd not detected, *nq* non quantified, compound identified in concentrations below the limits of the calibration curve

[a]When the concentration range is not available in the reference, the average and standard deviation is reported instead

[b]For [53], numbers were graphically read on Fig. 4 in [35]

Many studies reported pyrethroid concentrations in total water samples: the water collected is directly adsorbed on a SPE cartridge or is directly solvent-extracted, without previous filtration [12, 17, 22, 30]. Therefore, in these reports, both dissolved and particle-bound pyrethroids are jointly extracted and reported. A filtration step before pre-concentration was the preferred approach in some studies [26, 28, 29, 31, 33, 48], and the concentrations reported herein are that of dissolved pyrethroids, which includes the truly dissolved form and the colloidal-associated pyrethroids as part of the dissolved organic carbon pool. Pollutants associated to dissolved organic carbon are also retained in the adsorbents designed for sampling truly dissolved pollutants, together with pollutants associated to colloids, as known to occur for other hydrophobic chemicals [56]. Distinguishing concentrations of dissolved active compounds from those of particulate ones is important because both modes of occurrence are affected by distinct processes of transport and degradation rates (see later), in turn shaping differently the ultimate fate of pesticides. A strong recommendation for futures studies is to analyze separately the dissolved and particulate phases [21], and in any case, to state clearly which phase is characterized. The first part of Table 1 reviews dissolved and particle-bound pyrethroid concentration ranges. Whereas dissolved pesticides are bioavailable, it is not clear if the sorbed pyrethroids are toxic through feeding intake or as a transient repository, being desorbed later on and supporting the dissolved phase levels [31].

Pyrethroids dissolved in fresh and marine waters have been measured in a number of studies worldwide with the objective to check whether their concentrations were below thresholds of water quality guidelines. The dissolved form of pesticides is the form that is bioavailable and represents a threat for arthropods and fish. Dissolved pyrethroids were detected in agricultural drains, creeks, streams, and also in their collecting large rivers downstream agricultural land (Table 1). For example, in seven counties of California, 65–153 metric tons of pyrethroids were sold for licensed use between 1999 and 2008 [52], and 422 tons for the whole California state in 2010 [18].

The occurrence of individual pyrethroids varies geographically and seasonally as a response to agricultural use [19], and the consequent emission to the water, but probably also to different seasonal and site degradation potential. In Hospital Creek, a tributary of the San Joaquin River (Central California), bifenthrin was responsible for the greatest part of the toxicity of particles, whereas cyhalothrin was the prominent toxicant of particles in Ingram Creek, another tributary located less than 50 km away from the former [14]. Esfenvalerate and permethrin occurred in some water samples of tributaries of the Sacramento River after storm events in 2003 [15]. In tributaries of the San Joaquin River, cyfluthrin and cyhalothrin were the most frequent pyrethroids detected after winter storms, whereas bifenthrin and cyhalothrin were only identified in samples collected in March [17]. In central California, several surveys also reported bifenthrin as the main pyrethroid detected, its occurrence being related to storm events [13, 14, 16], while cyhalothrin and esfenvalerate dominated in the San Joaquin watershed [16]. Another study in Southern California sampled San Diego River during storm events and showed that six pyrethroids were present for 80% of the particle samples: bifenthrin, λ-cyhalothrin, permethrin, deltamethrin,

cypermethrin, and cyfluthrin [21]. Even though the same compounds were also detected in the dissolved phase, their relative abundance differed from that of the particles. Comparison of the suspended/dissolved concentration ratio to the soil-water partition constant showed that bifenthrin was not at equilibrium and in excess in the particles [21]. In contrast, dissolved+particulate samples collected in two others rivers of Southern California during low flow period showed much lower concentrations, and only bifenthrin and permethrin were detected [20].

Generally, the past and on-going water survey programs setup in California have yielded an important and valuable amount of data on the occurrence of pyrethroids. These studies demonstrated that one or two pyrethroids were frequently present in whole water samples, and that the dominant active compound differed in space and time (both years and seasons), reflecting the distinct agricultural targets, shifts in usages, and emissions from urban pest control [11, 19]. A metadata analysis gave the integrated view that cyhalothrin and bifenthrin were the compounds most frequently exceeding Regulatory Threshold Levels in surface freshwater of the USA and reached higher maxima in concentration [2].

In developing countries, the impact of current-use pesticides on freshwater quality is a growing concern, and an increasing literature documents pyrethroids in Asian water bodies, whereas reports on Africa are still too scarce [34]. Together with hundreds of other micro-pollutants, two pyrethroids were monitored in rivers and canals flowing through Vietnamese large cities and showed occasionally very high permethrin concentrations [23]. Cypermethrin and permethrin also dominated in the dissolved phase and in suspended particles of an urban creek, close to Guangzhou (Southern China, [25]). In GuanTin reservoir close to Beijing, deltamethrin was the more frequently detected pyrethroid insecticide in spring [24]. In streams and rivers of a rice cultivation area in the Philippines, cyhalothrin, cypermethrin, and deltamethrin were frequently detected, at concentrations exceeding water quality thresholds in half of the samples [48]. In Pakistan, deltamethrin and permethrin were close to water quality threshold in winter samples [26].

In European Rivers, permethrin was detected in the UK [27], cyhalothrin and cypermethrin in dissolved water and suspended particles of seven streams of Central Germany, especially after rain events [31]. Cypermethrin was the most frequently detected pyrethroid in the dissolved phase of the Ebro Delta (Spain), where rice is cultivated [28, 29]. Cypermethrin and deltamethrin concentrations varied in space and time, with peaks in concentration at the end of May followed by an apparent removal within 3 weeks [28, 29]. This finding demonstrated, by in situ observations, the fast degradation of pyrethroids in freshwater. In another Spanish rice paddy area, cypermethrin, bifenthrin, esfenvalerate, and cyhalothrin were present in most surface and groundwater total water samples analyzed [30], with the number of pyrethroids detected and their concentrations exceeding those measured in the Ebro Delta. In addition to broadcast on paddy fields, urban emissions through waste water treatment plant emissaries were likely responsible for this contamination. Despite a more restricted literature on European waters than for American ones, pyrethroid residues occur in agricultural freshwater environments and their concentrations may exceed

threshold values especially in suspended particles after rain events (in 80% of the samples in Germany [31]).

Because pyrethroid pesticides have been quite often detected in streams, creeks, and receiving rivers, they should also reach marine coastal waters. However, research addressing the occurrence of pyrethroids in estuarine and marine environments is limited. Due to the dilution of river water into the sea, pesticides often fall below detection limits. For instance, in seawater off Portugal, only two of the nine targeted pyrethroids could be detected, and only one could be quantified, whereas five were present in oysters [33]. Analytical difficulties may be a reason for the scarcity of published data in seawater (Table 1).

A specific risk for the marine environment is associated with aquaculture treatment of salmon against ectoparasites [5, 57]. Formulations used in aquaculture contain deltamethrin or cypermethrin together with emulsifiers for bath treatment of caged fish. Once the treatment is over, the bath water is released into the seawater, where pyrethroids are diluted by currents. In a case study in Canada, the deltamethrin plume could be detected up to 5.5 h after emission and the plume extended a few km away from the cages [5]. In this study, deltamethrin was emitted as a dissolved pesticide, and it was monitored both in the dissolved phase and in the suspended particles. Interestingly, deltamethrin concentration in the particle phase was approximately three to four times greater than in the aqueous phase, which demonstrates the quick partition of pyrethroids to organic carbon in seawater and, thus, their affinity for particles [5]. Variable responses of natural marine microbial communities to the input of anti-lice pesticides have been evidenced in Southern Chile [58]. At some locations and season, deltamethrin inputs resulted in an increase of carbon fixation by photosynthesis, likely resulting from a decrease in arthropod grazing pressure; however increase in carbon fixation was also observed at other sites and seasons. The diverse responses observed evidenced complex relationships between environmental factors (nutrient levels, zooplankton abundance, etc.) and pesticide impacts. These responses of marine organisms, distinct from toxicity alone, need further research to understand the overall impact of aquaculture and, more generally, of pyrethroid emissions, on marine ecosystems. More detailed information on the effect of salmon industry in the marine environment is presented elsewhere [6].

However difficult it is to detect pyrethroids in the marine environment, this task should not be overlooked because marine crustaceans and fish have been reported to be more susceptible to pyrethroids than freshwater ones [29, 34, 48].

4 Occurrence and Composition of Pyrethroids in Sediments

Table 2 documents pyrethroid occurrence in sediments. The solid phase of sediments acts as a sorbent for pesticides and likely integrates over time water pyrethroid concentrations in the overflowing water and also the accumulation of sinking particles in sea and river beds. Because of their quick association to river sediment, pyrethroid contamination of riverbed sediment has emerged as an important environmental threat to benthic organisms, and the literature reporting sediment toxicity

of pyrethroids has developed in the recent decade. Sediment toxicities toward the benthic amphipod *Hyalella azteca*, toward the cladoceran *Ceriodaphnia dubia*, and toward the midge of the Diptera *Chironus dilutus* are common tools to survey environmental quality of freshwater sediments. When pesticides are also measured, it allows to identify which toxicant causes the observed impairment [11, 38, 49, 59].

Recent monitoring studies document the occurrence of several pyrethroids in riverbed sediments (Table 2) and have been reviewed at the global scale by Stehle and Schulz [60]. Their residual occurrence in sediments is presently recognized as a threat to diversity of sediment-dwelling invertebrates and also as the cause of a decrease of diversity in aquatic environments at a global scale. Table 2 reports sediment pyrethroid concentrations at sites covering several continents. In some studies, sediment pore water concentrations are also given together with solid phase sediment concentrations. The occurrence of pyrethroids in sediments evidences clearly the propensity of pyrethroids to sorb onto and into particles. Owing to the large organic carbon pool comprised in sediments, sediments have the potential to act as a sink for pyrethroids. Organic carbon content, silt, and clay fractions are sediment bulk characteristics that usually correlate with pesticide levels [11, 24].

The concern about pyrethroid sorption to sediments in Californian streams exposed to agricultural and urban emissions led to the development of monitoring programs addressing the benthic environment in addition to water-based surveys. The considerable amount of data generated by those programs points to bifenthrin being the most commonly found residues in the sediments (Table 2). In Del Puerto Creek, a northern California stream flowing through agricultural land, it was the main contributor to sediment toxicity, with a smaller contribution of cyhalothrin, esfenvalerate, and cyfluthrin [37]. In sediments from the Santa Maria River (central California), the pesticide chlorpyrifos was the main contributor to the toxicity to the benthic amphipod *Hyalella azteca*, while cyhalothrin and permethrin also contributed to sediment toxicity in some locations in June 2002, but not in May 2003 [38]. In sediments collected in California from 2008 to 2012, the most frequent pyrethroid detected was bifenthrin; the other active compounds cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate/fenvalerate, fenpropathrin, or permethrin, occurred in one fifth to one third of the samples [11]. Bifenthrin was also the main pyrethroid in sediments of rivers alimenting Salton Sea in southern California [41]. In an urban estuary of southern California (Ballona Creek, Los Angeles), permethrin dominated over bifenthrin, while cypermethrin and cyfluthrin were next in abundances [52]. In Minnesota, permethrin and bifenthrin were at the top of pyrethroid sales, permethrin for animal care, structural applications, home and garden holding, while bifenthrin was mostly used as crop chemical [44]. In this state, 33% of sediments of stormwater ponds contained permethrin and 20% bifenthrin; this pattern was in line with results from other urban locations statewide as reviewed by Crane [44]. Another nationwide study addressed metropolitan streams in the USA and found bifenthrin detected in 47% of the bed sediments followed by cyhalothrin, while permethrin, resmethrin, and cypermethrin occurred with much lower frequency [43]. Recent observations in 99 streams across Midwest USA also found

bifenthrin responsible for most of the toxicity in half of sediments and also attributed urbanization rather than agriculture as responsible for its emission [59].

In Southern America (Argentina), cyhalothrin was the dominant pyrethroid in sediments of rivers flowing through large monocultural horticultural fields [45]. The percentage of detected herbicides and pesticides varied seasonally according to their application, while pyrethroid residues were consistently detected in sediments, attesting for an environmental risk for the benthic biota.

An increasing body of literature evidences pyrethroid occurrence in Asian riverbed sediments and shows the prevalence of cypermethrin at many sites (Table 2). In large cities of Vietnam, permethrin was the dominant pyrethroid, and its geographical repartition brings evidences that it is sourced by structural and householding uses and disease vector controls rather than agricultural spraying [47]. Deltamethrin was only detected once in this study but at very high levels from an undetermined source. In Southern China, cypermethrin, cyhalothrin, permethrin, and deltamethrin dominate over other pyrethroids in sediments of the Pearl River; their concentrations may reach notably high values in small creek sediments collected upstream in the river [49]. Cypermethrin and permethrin also dominate in sediments from an urban creek, close to Guangzhou (Southern China, [25, 61]). In Beijing GuanTin reservoir, fenvalerate and deltamethrin were the dominant pyrethroids [24]. In Pakistan deltamethrin and permethrin were the dominant pyrethroids, with deltamethrin present in all samples and reaching concentrations above environmental quality thresholds (namely, NOEC of *Hyalella azteca* [26]).

Australia's state Queensland has a low population and sugarcane and cotton cultivation dominate its agricultural activities. Ametryn and prometryn were the most frequent pyrethroids detected in sediments from irrigation drains and channels, reaching high concentration levels, while bifenthrin occurred in only one cotton production area [46].

In Europe, cyhalothrin and cypermethrin are ubiquitous at large river mouths, whereas riverbed sediment also showed frequent amounts of bifenthrin and tefluthrin, together with cypermethrin and cyfluthrin in some rivers of Italy and France [62]. In sediments of the Ebro Delta (Spain), cypermethrin was detected in some sediments, whereas deltamethrin, detected in the water, was below detection limits in the sediments [28]. In contrast, cypermethrin, cyfluthrin, and esfenvalerate were abundant in the paddy fields of Albufera de Valencia [30]. These paddy fields are filled with water coming from a lake receiving agricultural and urban effluents, and both surface water and groundwater contained high levels of dissolved phase pyrethroids.

Similarly to the reports of seawater concentrations, pyrethroid abundances in marine sediments are evaluated by a limited number of comprehensive studies. In an intensely urbanized estuary in Southern California, bifenthrin and cyfluthrin were the most frequently detected pyrethroids with their highest concentrations at 132 and 65 ng/g, respectively, at sites located near sources of runoff emissions from urban watersheds. They accounted for a part of the toxicity of the sediments to a standard amphipod *Eohaustorius estuarius*; however they were not the major toxicant at all the studied stations [52]. Samples with the highest concentrations of pyrethroids

were located in close proximity to river mouths and cities, whereas samples located more offshore showed lower concentrations, or pyrethroids were below detection limits. This distribution supports urban pyrethroid emissions. In another area of Southern California, sediments from the Monterey continental shelf were analyzed together with suspended solids in the three rivers flowing into this marine region. Whereas pyrethroids were found in almost all rivers particles (sampled after rain events), with bifenthrin and permethrin as the dominant pyrethroids, they could not be detected in the estuary nor in the deeper sediments of the Monterey canyons (from 100 to 300 m depth). A similar situation was observed in marine coastal waters off Portugal, whereas no pyrethroid could be detected in sediments, while cypermethrin was detected in the dissolved phase and tetramethrin, bifenthrin, cyhalothrin, fenvalerate, and permethrin occurred at low concentrations in some samples of oysters collected in the same area [33]. In marine sediments, contaminated river particles are diluted by the autochthonous marine particles and by older riverine particles in which pyrethroids have had the time to be degraded. As a consequence of dilution, pyrethroids are often below detection limits in marine sediments (Table 2).

A recent review documented the occurrence of pyrethroids in sediments worldwide and showed significant correlations between pyrethroid occurrence and sediment toxicity [7]. The good correlations obtained proved that pyrethroids were the main cause of toxicity and strongly suggested potential ecological risk to nontarget aquatic species. Nevertheless, at some locations, such as in sediments from the Pearl River Delta (China), other pollutants than pyrethroids likely contributed to the overall toxicity of sediments. The authors concluded that the frequent occurrence at high concentrations of pyrethroids in sediments from agricultural and residential areas constitute a threat to freshwater ecosystems [7].

5 Pyrethroid Degradation

A characteristic feature of pyrethroid contamination in water and benthic ecosystems is that a few compounds of the pyrethroid family may be present but not all the series, in concentrations generally under the 100 ng/L range for water samples or under the 100 ng/g range for sediments. Pyrethroid occurrence is highly variable in time and space, so that samples from a given area may show detectable amounts of one or several pyrethroids while others do not or comprise other active compounds. This feature is much different from other ubiquitous pesticides classes and is a consequence of their higher lability. The routes of degradation of pyrethroids may be abiotic (hydrolysis, photolysis, and oxidation) or mediated by bacteria and fungi. Pyrethroids degradation by microorganisms and fungi have been studied in soils [63, 64]. Various carboxylesterases may induce the degradation of pyrethroids; generally one gene exists in one pyrethroid-degrading microorganisms, with the exception of *Ochrobactrum anthropi*, that possesses two pyrethroids degrading genes [63]. Optimal conditions of pyrethroid biodegradation are between 30 and 35°C. Organic matter and clay content are also important parameters controlling

pyrethroid bioavailability to microorganisms. Half-lives of bifenthrin, cypermethrin, and permethrin in soils were 12–1,410, 14–106, and 5–55 days respectively, under temperature conditions between 25 and 30°C (Table 2 in [63]). The biodegradation rates in freshwater sediments have been seldom determined, and they are longer than in soils [18]. Depending on conditions, long persistence was observed for bifenthrin and permethrin. Under both aerobic and anaerobic conditions, and the half-life of bifenthrin in sediment of drainage channels ranged from 8 to 17 months at 20°C, while that of *cis* and *trans* permethrin varied between 2 to 13 months [65]. In liquid media, bacteria (*Bacillus*, *Brevibacillus*, *Ochrobactrum*, *Pseudomonas*, *Serratia*, and *Sphingobium*) and fungi (*Cladosporium*, *Candida*) degrade efficiently pyrethroids. At temperatures ranging from 27 to 38°C, most strains degraded pyrethroids within 5 days, with the fastest degradation observed for permethrin in 3 days [63]. However, the experimental conditions at which the experiments were carried out were not the same as natural field conditions, where lower temperatures and lower bacteria or fungi abundance can be expected to increase half-life of pyrethroids.

6 Pyrethroid Occurrence in the Atmosphere

Because of their relatively low vapor pressure, pyrethroids are assumed to have low tendency to volatilize during application, as well to revolatilize from soils or water bodies [7]. During application, 20–30% of the applied doses can be emitted as aerosols and drift away from their source by atmospheric transport [66]. Post-application emissions have also been reported to occur via volatilization [67]. For deltamethrin, having one of the lowest Henry's law constant values among pyrethroids, it was experimentally demonstrated that 70% of deltamethrin sprayed on the surface of the water was quickly emitted as aerosols [68]. Taken as a whole, these evidences point to likely atmospheric emissions of pyrethroids, at least during and shortly after application by spray broadcasting.

The widespread occurrence of pyrethroids in some areas also questions whether their volatilization to the gas phase is possible, ensuing a likely atmospheric transport to proximate or remote ecosystems (see Sect. 7). A few reports have recently evidenced that pyrethroids were present in the atmosphere, both as aerosols and as vapors in the gas phase. The particle-bound fraction is susceptible to be atmospherically deposited or to be washed out by rain or snow whereas gas-phase pyrethroids will be removed by photodegradation or air-soil, air-vegetation, or air-water diffusive exchange, probably resulting in longer atmospheric residence times [69]. Table 3 reviews the concentrations of pyrethroid insecticides bounds to aerosols or as vapors. The first report of pyrethroids in the gas phase of Brazilian alpine reserves showed that cypermethrin was the second pesticide in abundance, whereas gas phase concentrations of legacy pollutants, such as chlordane, chlorinated cyclodienes and hexachlorobenzene, were around background levels [70]. In aerosols and in the gas phase of Guangzhou (south China), eight pyrethroids were detected, and

cypermethrin was the dominant one [71]. Concentrations of aerosol-bound cypermethrin were comparable to those measured in a horticulture area in Malaysia [72]. Li et al. measured allethrin and tetramethrin in higher proportions in the gas phase whereas bifenthrin, cyhalothrin, permethrin, cyfluthrin, and cypermethrin were predominantly associated with the aerosols [71]. Bifenthrin was also detected in almost all samples of fine aerosols in Northern Brazil [73].

The recent recognition of pyrethroid occurrence in aerosols and in the gas phase opens a challenging view of their biogeochemical cycle and prompts further research to assess the relevance of atmospheric transport and occurrence of pyrethroid insecticides.

7 Key Physicochemical Properties of Pyrethroids, Transport Processes, and Modelling

Legacy pollutants like polychlorinated biphenyls (PCBs), chlorinated pesticides such as *p*,*p*′-dichlorodiphenyltrichloroethane (DDT), lindane, and organophosphate pesticides persist long enough in the environment to be transported by advective and diffusive processes and undergo long-range transport far away from their primary emission regions. Diffusive transport of pesticides results in an environmental partitioning of these pollutants among the different environmental matrices, such as water, particles, air, soils, biota, and sediments. For instance, water-particle partitioning is the result of a net quantity of pesticides transferred from the dissolved water phase to the organic part of the particles. Meanwhile the quantities of water, of particles, and of organic carbon do not change concurrently when pesticides partition among these phases. A change of any of these quantities would induce a re-partitioning of the chemical. Other relevant diffusive processes are air-water exchange, water-sediment partitioning, gas-aerosol partitioning, bioconcentration in organisms at different trophic levels, etc. Organic carbon occurrence in water stretches from truly dissolved organic carbon to particulate organic carbon, with a continuum in particle sizes. The division of dissolved and particle phase is operational, usually the dissolved phase refers to the pesticides passing through the filter cut-off size (e.g., 0.7 μm for a GF/F filter), but this dissolved phase can also include the colloidal phase. In Fig. 1 relevant diffusive (partitioning) processes for pyrethroids are represented by the wide gray arrows. Diffusive partitioning is always driven by a fugacity gradient among the two phases and is always a bidirectional process. In contrast to diffusive processes, an advective transport consists in the movement or flux of the phase itself, transporting the pesticides which it contains. Advective transport processes of pyrethroids in aquatic environments are represented by the thin black arrows in Fig. 1. For example, the transfer of atmospheric pesticides to soils or aquatic ecosystems can be by air-water exchange (partitioning) or by wet and dry deposition, which are advection transport processes. In dry deposition there is a settling of aerosol-bound pesticides, while in wet

Table 3 Selection of individual pyrethroid concentration in the atmospheric gas phase, in ng m^{-3}, and in aerosols in ng g^{-1} from different locations worldwide

	Year	Sample type	Individual pyrethroids							
			Allethrin	Bifenthrin	Tetramethryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	References
Gas in pg m^{-3}										
Brazilian alpine mountains, nationa parks	2013–2015	G					nd–40		nd–881	[70]
Guangzhou, urban area, South China	2011–2012	G	nd–66	nd–48	nd–8	nd–nq	nd–37	nd–nq	nd–16	[71]
Floriculture region Malaysia	2004	G							142–2,740	[72]
Aerosols in pg m^{-3}										
Guangzhou, urban area, South China	2011–2012	A	nd–139	nd–54	nd–28	nd–51	nd–88	nq–17	17.1–1,380	[71]
Todos os Santos, Northern Brazil	2010	A		14–72			62–945			[73]

nd not detected, *nq* under quantification limits, *G* atmospheric gas phase, *A* atmospheric aerosols

deposition by rain or snow, there is a scavenging of gas and aerosol phase pesticides by the rain drops or snowflakes. In terms of primary sources, after pesticide application on agriculture fields (rice, cotton, wineyard, etc.) by spraying, pyrethroids may reach surface aquatic environments through edge of field runoff, which is an advective soil to water input of irrigation water or rain water, entraining dissolved pyrethroids and also pesticides bound to particles or that have re-partitioned to the run-off water. Storm events after pesticide treatment have been shown to release high amount of pyrethroids into freshwater streams in the vicinity of fields [37]. Despite degradation and dilution processes, pyrethroids sorbed to river suspensions are effectively transported to the lower stretches of rivers [18, 63]. Particle vertical settling and sediment resuspension are advective processes transporting pyrethroids between water and sediment, which transport chemicals in parallel to the water-sediment diffusive partitioning. Nevertheless, the latter may only be effective for sediment pore water and benthic waters, while settling of organic carbon-bound pyrethroids is an advective flux affecting all the water column. Soils may act as transient repositories for pyrethroids that may gradually be desorbed into irrigation or rain water by leaching. In addition, sorption to soils, particle, and sediment may lower their degradability and thus increase their persistence in the environment [65]. Similarly to diffusive sediment-water exchange, particle-water exchange (or partitioning) continuously occurs, with a distribution of the chemical between organic carbon and the dissolved phase depending on temperature and quality of the organic matter.

The key condition for pyrethroids to be transported away from their source is that they persist long enough in the environment before being degraded. Their potential for being transported is also dictated by their physicochemical properties. The octanol-water partitioning coefficient, K_{OW}, characterize the potential of compounds for being absorbed into organic matter, either in sediments or in suspended particles. Even though, conceptually, it does not take into account surface adsorption, it is a common practice to use K_{OW} as a surrogate for adsorption/absorption, as experimentally it is very difficult to discern organic pollutants adsorbed or absorbed to particulate organic carbon. Henry's law constant (H) or the dimensionless Henry's law constant ($H' = K_{AW}$=H/RT) of a given pollutant characterizes its air-water diffusive partitioning and thus its potential to accumulate in water or being volatilized to the atmosphere facilitating their long-range transport. Each pyrethroid has specific values for these physicochemical constants. Figure 2 shows the phase space for organic chemicals and compares the values of both constants for pyrethroids to the values of these partitioning constants for other pollutant classes which behavior in the environment is better studied and understood. The phase space shown in Fig. 2 provides a simplified view of environmental partitioning and transport potential. Compounds in the upper area of the plot space have a higher potential to partition to the gas phase relatively to water than compounds on the bottom area of the plot. Similarly, compounds plotted on the right area of the plot have a greater potential to partition to organic carbon relatively to water than those plotted on the left side. Permethrin is plotted very close to PCB 101, thus have the similar partition characteristics than PCB101 and bifenthrin have an even higher K_{AW}. Therefore, both

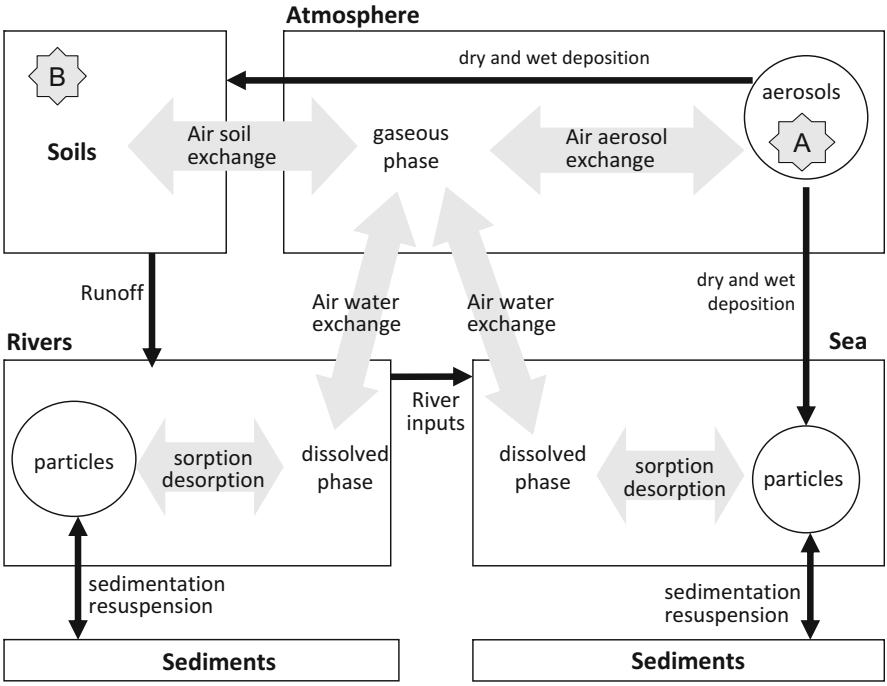

Fig. 1 Scheme of the geochemical cycle of pyrethroids in the environment. Boxes represent the environmental phases. The soil box represents both the solid phase of soils (plants and soil particles) and the soil porous water. Arrows represent the fluxes between phases, thin black arrows stands for fluxes of key transport (advective) processes and large gray arrow show key partition (diffusive) fluxes. Gray stars symbolize pyrethroid direct emissions to the environment; A is the emission that remains as aerosol during spray application, mostly to cropland; B is the emission that is deposited on soils and plant during spray application. See text in Sect. 7 for more explanation

compounds have a potential for long-range transport through grasshopping, that is, successive volatilization and deposition steps. In the case of pyrethroids, the potential for long range transport is limited by their potential degradation in the environment. It has to be underlined that in the case of cold environments with snow deposition events, even chemicals with high K_{AW} partition coefficients can be deposited due to the high sorption capacity of snow [74]. More importantly, the physicochemical characteristics of the other pyrethroids are similar to that of high molecular weight polycyclic aromatic hydrocarbons (PAHs), DDT and its degradation products (DDE and DDD), and hexachlorobenzene; therefore pyrethroids can be expected to have the same environmental behavior. In contrast, organophosphosphate pesticides have a greater solubility in water (lower K_{AW}) and will behave more as "swimmers," tending less to sorb on particles and with limited atmospheric transport [75].

In the case of legacy persistent organic pollutants (POPs), their important emissions combined to analytical progresses made it possible to quantify their

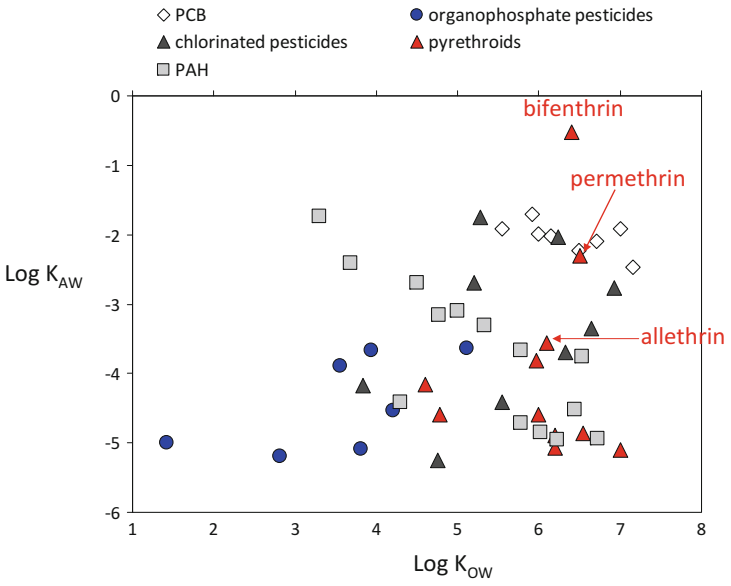

Fig. 2 Comparison of the partition behavior of current-use pyretroid insecticides and of other legacy pollutants. K_{AW} is the air-water partition coefficient, and K_{OW} is the octanol-water partition coefficient

abundances in water, suspended particles, sediments, atmospheric gas, and aerosols phases from regional to a global scales. Scientific efforts addressing pollutant detection in several environmental compartments brought quantitative appraisals and understanding of transport fluxes between air, seawater, soils, etc. This holds true for PCBs [76] and PAHs [77] but also for pesticides like lindane [78]. In contrast to legacy pollutants, pyrethroids are current-use pesticides, and they have been used and emitted to the environment for only the last few decades, and scientists have been able to quantify pyrethroids at environmental levels only for a decade [79]. As a consequence, the occurrence of pyrethroids in environmental phases relevant to the understanding of their biogeochemical cycle is still incompletely understood.

A comprehensive assessment of pyrethroid cycle in an urban area of Southern China used a fugacity-based model coupled to concentrations measured in different environment phases to calculate the diffusive and advective fluxes [25]. Sinking of suspended particles accounted for the higher fluxes, and resulted in water bed sediments fluxes 1 or 2 order of magnitude higher than air-water diffusive exchange. The higher fugacity of pyrethroid in water than in the gaseous atmosphere drove volatilization fluxes from the water to the air, permethrin, and cypermethrin having the higher fluxes. Despite this work, pyrethroids have received less attention in terms of their fate, transport, and biogeochemistry, and how these processes ought to be modelled. The comparison with other families of POPs with similar properties provide clues of their environmental fate and point to potential research efforts to be carried out in the future. Unless pyrethroids are efficiently degraded in the

atmosphere, some of them have the potential for long range transport as pentachorinated PCBs, 4–5 rings PAHs and DDD (Fig. 2). In comparison to those legacy pollutants and hydrocarbons, current-use pesticides such as pyrethroids are often reported in one environmental phase, chiefly dissolved freshwater phase or riverine sediments. Both dissolved phase and suspended particles [31] or suspended particles and sediments [14] or dissolved water phase and sediments [24, 27, 28, 33] are considered jointly in order to assess combined risks for the water ecosystem and for the benthic ecosystem. Future research efforts should address their multiphase partitioning, including the atmosphere, to elucidate their capacity to affect proximate or distant ecosystems from their primary sources. The advective transport of pyrethroids has been largely addressed only in relation to their dispersion by river flow notably during storm events. However, the partition between dissolved pyrethroids and particles is specifically addressed by one study, showing that for this particular site, a diffusive flux of bifenthrin existed from the particles toward the dissolved water [21].

8 Future Research Integration

Because of their rapid decay, pyrethroids are reported above detection levels in areas and at times closed to their point sources, and a global appraisal is still missing. It can be foreseen that pyrethroids might threaten biodiversity in some geographical areas where data is still lacking to date. Most croplands are indeed not studied for pyrethroids (Africa, Brasil, etc., see review [62]). In African market, esfenvalerate was the highest pesticide residue in fruits and vegetables, and allethrin was also detected, attesting for their use [80–83]. Ukraine, Pakistan, Turkey, Paraguay, and India registered the larger pyrethroid use while environmental informations on pyrethroid occurrence are mainly lacking for those countries [7, 26].

Pyrethroids are degraded in the environment so that they are not conspicuously detected, with the exception of some agricultural or urban areas. Their high degradation rates with respect to legacy pollutants support the belief that they are unlikely to persist in the environment. However, extension of cropland and of urbanized space will likely result into an increase in pyrethroid uses and emissions, because better alternatives to control pests are still lacking. In the case where the rate of inputs of pyrethroids would compensate for their degradation, pyrethroid occurrence may become more continuous and their behavior may then be assimilated to that of “quasi persistent organic pollutants”, with secondary transport evading them away from their application area. In California, past and current monitorings have demonstrated that there is a persistent threat to aquatic ecosystems because of current-use pesticides, with an increasing share by pyrethroids [19].

In conclusion, the shift to current-use pesticides demands a better understanding of the occurrence of pyrethroids in developing countries where the market shares are the highest. The partition, transport, and degradation fluxes of pyrethroids need to be

better appraised locally, regionally, and globally, taking into account the so far underestimated importance of atmospheric transport.

River flow efficiently transports pyrethroids to river mouths and estuaries. It is difficult to detect pyrethroids in the marine environment because of dilution. However aquaculture is a locally direct source that likely constitutes an important environmental burden for seawater, which it is very poorly surveyed and comprehensively understood.

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The Ecological and Evolutionary Implications of Pyrethroid Exposure: A New Perspective on Aquatic Ecotoxicity

Kaley M. Major and Susanne M. Brander

Contents

Abstract Pyrethroids are one of the most heavily used insecticide classes globally because they have low mammalian toxicity. However, they are highly toxic to arthropods. Pyrethroids are ubiquitous in the aquatic environment as a result of urban (landscaping, structural pest control, home, and garden) and agricultural runoff and spray drift, often at levels that exceed water quality benchmarks established for the protection of aquatic life. Pyrethroids also enter the aquatic compartment through direct application to treat crustacean parasites in commercial fisheries. Here, we briefly review the acute and sublethal toxicities of pyrethroids with a focus on aquatic invertebrates. Our primary focus is on evidence of the evolution of adaptive pyrethroid resistance in aquatic invertebrates (sea lice (*Lepeophtheirus salmonis*), mosquitoes (*Anopheles gambiae* and *A. coluzzi*) black flies (*Simulium* spp.), and amphipods (*Hyalella azteca*)) driven by target and nontarget applications of pyrethroids in the aquatic environment. We explore the human health, evolutionary, ecological, and risk assessment implications of the evolution of

K. M. Major (✉) and S. M. Brander
Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA
e-mail: kaley.major@gmail.com; susanne.brander@oregonstate.edu

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pyrethroid resistance and suggest using resistance in the model invertebrate *H. azteca* to further our understanding of evolutionary toxicology in wild populations.

Keywords Adaptation, Evolutionary toxicology, *Hyalella azteca*, Mosquito, Pyrethroid resistance

Definitions

Adaptation: Any heritable, genetically based tolerance mechanism. Adaptation occurs at the level of the population.

Chemotherapeutant: Chemical agents or drugs that are selectively toxic to the causative agent of a disease or infection.

Cytochrome P450: Genes that code for enzymes that are involved in the formation (synthesis) and breakdown (metabolism) of various molecules and chemicals within cells.

DNA Methylation: The addition of a methyl group to DNA, sometimes resulting in the alteration of gene expression.

EC_{50}: The concentration that produces the designated effect in 50% of the population over a given period. Here, it is used primarily to describe an effect of immobilization on the organism of interest (moribund + dead) as a response to pyrethroid toxicity unless otherwise noted.

ET_{50}: Median effective time, e.g., the time required until impaired swimming and/or attachment behavior becomes apparent in 50% of the population.

Epigenetics: The study of mechanisms that facilitate phenotypic variation through genotype-environment interactions.

Epimutation: Epigenetic alterations that are specific and heritable.

Evolutionary Toxicology: The study of the effects of pollution on the genetics of wild populations.

Fitness: A measurement of the ability of an organism to survive, grow, and reproduce in its environment.

KDT_{50}: The time it takes to a produce a knockdown phenotype in 50% of the population.

"Knockdown" Phenotype: Paralysis caused from acute pyrethroid exposure in sensitive animals.

Kdr: A knockdown resistance mutation, attributed to a change in the target site that reduces pyrethroid binding affinity, thereby conferring resistance to the typical mechanism of action for pyrethroids.

LC_{50}: The concentration that produces mortality in 50% of the population over a given period.

Log K_{ow}: The logarithm of the octanol-water partition coefficient; the higher the Log K_{ow}, the greater the potential of a chemical to partition into sediment, soil, and organic matter.

Maternal Effects: The influences of maternal environment, phenotype, and/or genotype on offspring phenotypes, independently of offspring genotype.
Maternal Inheritance: When the inherited traits of the offspring are passed down through extranuclear (i.e., mitochondrial) DNA in the egg.
Metabolic Resistance: Insecticide resistance conferred through modifications of the systems responsible for xenobiotic metabolism.
Convergent Evolution: The independent evolution of the same features in different groups.
Physiological Acclimation: Any coping mechanism that is governed by physiological processes that are nonheritable. Acclimation occurs at the level of the organism.
Polygenic: Characterized by influence from multiple genes, particularly in the context of a phenotype.
Resistance: A decrease in chemical sensitivity caused by an adaptive, genetic change.
Selective Force: Anything that favors certain genotypes or phenotypes over others.
Single Nucleotide Polymorphism (SNP): A change to the DNA sequence caused by a single base pair substitution.
Target Site Insensitivity: Insecticide resistance conferred through a modification of the target site, leaving the insecticide unable to bind and elicit primary toxic action.
Tolerance: A decrease in chemical sensitivity from acclimatory mechanisms; reversible, nonheritable.
Transgenerational Epigenetic Inheritance: Refers to the transmission of specific epigenetic marks/processes across generations, via the germline.
Voltage-Gated Sodium Channel: The primary target site for pyrethroids, *vgsc* is the abbreviation for the gene that codes for the channel; Vgsc is the abbreviation for the protein channel.

1 Background

Pyrethroid pesticides, which are chemically enhanced derivatives of the natural pyrethrin compounds produced by common flowers (*Chrysanthemum cinerariaefolium*), are the fourth most prevalent insecticide class in use globally [1, 2]. The first synthetic pyrethroid pesticides were developed in the late 1940s [3] but were not stable or persistent enough for widespread agricultural use until the 1970s [4]. With modifications to increase their potency and persistence [5], the use of pyrethroid pesticides has increased by an order of magnitude over the past 20 years, as organophosphate pesticides that are acutely neurotoxic to mammals have been phased out [6]. Pyrethroids have several advantages over other classes of pesticides including the organochlorines, organophosphates, and carbamates because of their greater field stability, rapid metabolism and elimination in mammals, and high insecticidal potency requiring lower inputs [7]. At present, pyrethroids are important globally for food security and disease vector control [8].

As neurotoxicants, pyrethroids elicit their primary toxic mode of action in insects by acting on the *voltage-gated sodium channel* (Vgsc). In the central and peripheral nervous system, pyrethroids prevent the Vgsc from closing, causing repeated firing of the neurons, leading eventually to paralysis, known as the *"knockdown" phenotype*, and death. To a lesser extent, pyrethroids also interact with a variety of other sites including voltage-sensitive calcium and chloride channels [9–13]. Based on chemical structure and mammalian (rat, mouse) toxicity phenotypes, pyrethroids are broadly classified into two types: Type I or Type II [14]. Type II pyrethroids (deltamethrin, cismethrin, esfenvalerate, λ-cyhalothrin, cyfluthrin, fenpropathrin) have an α-cyano-3-phenoxybenzyl moiety, while Type I pyrethroids (*S*-bioallethrin, cypermethrin permethrin, tefluthrin, bifenthrin) do not [15]. In general, Type I pyrethroids tend to be reserved for urban use, while Type II pyrethroids are used in agriculture [2]. Type II pyrethroids also produce a distinctive convulsive phenotype in invertebrates [16] and cause prolonged channel opening compared to Type I pyrethroids [17].

In soils, most pyrethroids have half-lives ranging between 30 and 100 days, and their hydrolysis in the aquatic compartment occurs on the order of days to weeks [18]. Pyrethroids have high n-octanol-water partition coefficients (K_{ow}), with $log\ K_{ow}$ values ranging from roughly 4 to 7.54, indicating that these chemicals are much more likely to partition into the sediment and sorb to particulate organic matter than to remain in the water column [19]. Despite being highly lipophilic, pyrethroids may remain in the water column for days to weeks after introduction [20, 21] and are soluble enough to produce biological and toxic effects at low dissolved concentrations [11, 22]. Because they are lipophilic, pyrethroids bioaccumulate in both fishes and marine mammals. A recent study conducted in Spain found pyrethroids in 100% of tissue samples collected from riverine fish [23, 24]. These insecticides also adsorb to and persist in sediments [25] and associate with other environmental compartments such as algae [26].

Although they are still detected less frequently in the environment worldwide than organochlorine- and organophosphate-based products [27], pyrethroids are prevalent in aquatic ecosystems and are often found at levels sufficient to cause toxicity to aquatic invertebrates [2, 6, 25, 28–31]. Pyrethroids are used ubiquitously in agricultural and residential areas, primarily entering as runoff into the aquatic compartment, but also through spray drift as well. Pyrethroids are also ubiquitous in treated wastewater effluent, mostly due to high urban use for pest control in homes [32, 33]. Historically, some pyrethroids were added to water directly as mosquito and black fly larvicides [34–36], but their toxicity, hydrophobicity, and sediment persistence have since been restricted their direct use in aquatic environments. However, in aquaculture, pyrethroids are still added directly to the water as chemotherapeutants to remove parasites from farmed fish [37] and shrimp [38].

While the relatively low mammalian toxicity of pyrethroids has fueled their popularity and increased usage over the past few decades, pyrethroids are highly toxic to fish and aquatic invertebrates at low part per billion or parts per trillion concentrations. Toxicity to aquatic organisms is particularly problematic following storm events, which transport residentially applied pyrethroids into local streams and

other waterways, severely impairing invertebrate assemblages as well as causing sublethal and sometimes lethal toxicity to fishes [1, 28, 39, 40]. Newer pyrethroids (e.g., cypermethrin) are generally more toxic than older formulations, especially to aquatic invertebrates that are physiologically most similar to the insects which these chemicals are designed to target [6, 26, 41–43]. Cypermethrin, for example, hydrolyzes more slowly than Type I pyrethroids such as permethrin, resulting in a toxic potency up to 20-fold greater [41]. In fact, pyrethroids have often been implicated in causing sediment toxicity to the amphipod *Hyalella azteca* commonly used for bioassessments in urban and/or agricultural areas [44–48]. And while pyrethroids used in agriculture still contribute to aquatic impairment, urban pyrethroid inputs have been cited as a major source of pyrethroid contamination in the environment. Bifenthrin, cyfluthrin, and cypermethrin cause the most concern in waterways surrounded by residential and urban areas [49, 50]. Bifenthrin applied by homeowners and structural pest control professionals has reached levels in the water column during storm events that are sufficient to cause acute invertebrate toxicity [25, 42]. In fact, for the period 2009–2015, bifenthrin has shown one of the highest risk quotients in inland surface waters in the European Union [51].

For these numerous reasons, pyrethroids are ubiquitous in the aquatic environment. They have long been implicated as a strong selective pressure in the pest species they are meant to control [52–55], and accumulating evidence now shows they are capable of driving resistance in nontarget aquatic organisms exposed to pyrethroids unintentionally [56–63]. In the present work, we briefly review the acute and sublethal effects of pyrethroid exposure for invertebrates and insects in aquatic ecosystems. We then focus on the evidence of increased tolerance to pyrethroids that has been documented in pests that inhabit pyrethroid-laden aquatic environments such as sea lice, as well as nontarget life stages of mosquitoes and black flies, and nontarget aquatic invertebrates (cladocerans, amphipods), with an emphasis on adaptive resistance (Fig. 1). In doing so, we describe the influence of pyrethroid use in the environment in the context of evolutionary toxicology. Finally, we explore the ecological and evolutionary implications of pyrethroids as a strong selective pressure driving resistance in the aquatic environment and discuss impacts on evolutionary processes, ecosystems, and risk assessment.

2 Acute Toxicity

A wealth of literature exists concerning the acute toxicity of pyrethroids to aquatic organisms, and this topic is extensively reviewed elsewhere [6, 19, 64–67]. Acute mortality has been documented far below the 1 μg L^{-1} range for fish, crustaceans, and insects [6] with the amphipod *H. azteca* being among the most sensitive (Fig. 2), having a 96 h LC_{50} (median lethal concentration) in the low ng L^{-1} range [6, 57, 58, 68]. Acute toxicity has even been documented at levels below 1 ng L^{-1} [43]. A review by Mian and Milla [66] illustrated that that many nontarget aquatic insects (Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, Trichoptera) and

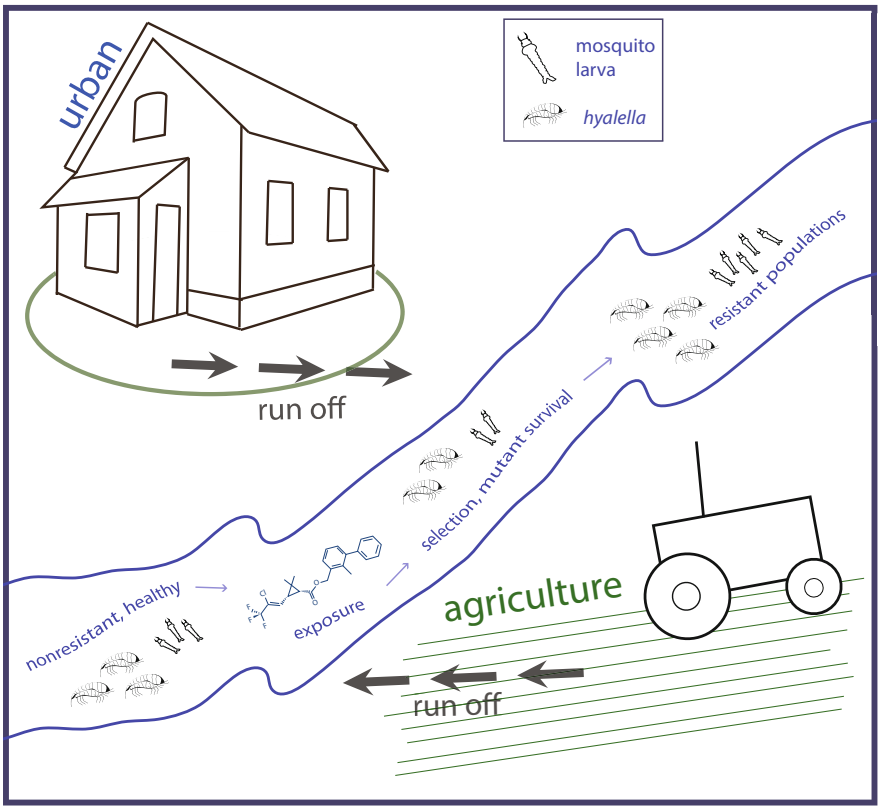

Fig. 1 Selection for adaptive pyrethroid resistance in nontarget aquatic populations is driven by pyrethroid exposures from both urban and agricultural runoff. Sensitive individuals are removed from populations by strong selective pressures, leading to adapted, pyrethroid-resistant populations instead. Nontarget pyrethroid exposure drives selection in populations of the amphipod *Hyalella azteca* as well as in larval mosquitoes and black flies

crustaceans (Cladocera, Ostracoda, Copepoda, Amphipoda, Isopoda, and Decapoda) were more acutely sensitive to pyrethroids than other invertebrate groups (bivalves, mollusks). Further, these sensitive groups had pyrethroid sensitivities in the range of some pest species including midge, fly, and mosquito larvae, suggesting that aquatic pesticide applications intended to eliminate these pest insects could be lethal to other aquatic invertebrates [66]. At lethal doses, pyrethroid binding to the Vgsc target site elicits a response which includes altered swimming behaviors, convulsions, and eventually paralysis and death, and immobilization phenotypes are often irreversible [69]. Acute toxicity is exacerbated by increased salinity and decreased temperatures [70]. At higher salinities, pyrethroids are less soluble in water, rendering them more likely to adsorb to the sediment or more prone to partitioning into lipid (within biota) [70, 71].

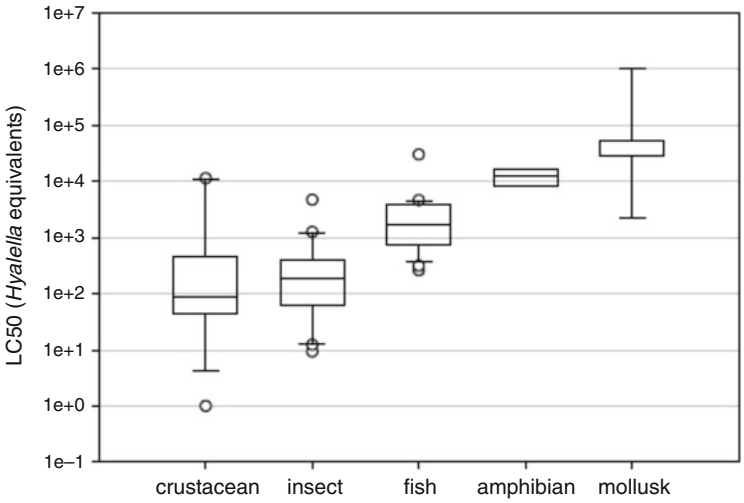

Fig. 2 The relative sensitivity (*Hyalella* equivalent LC_{50}s) of crustaceans, insects, fish, amphibians, and mollusks to pyrethroids, using data from tests with measured concentrations. Horizontal lines in boxes indicate 25th, 50th (median), and 75th percentiles; vertical bars indicate 10th and 90th percentiles (where data were sufficient to calculate); individual points are values above the 90th percentile or below the 10th percentile. Data are normalized to *Hyalella* because they are the most sensitive to pyrethroids. Reprinted with permission from Giddings et al. [68]

3 Sublethal Effects

In addition to being acutely toxic at environmentally relevant concentrations, pyrethroids cause a myriad of sublethal impacts in nontarget aquatic invertebrates [6, 72]. Effects on invertebrate behavior are widely documented, including impaired movement, resulting in the inability to respond to a simulated predator by swimming away or by taking shelter [26, 43, 73]. Increased predation risk caused by sublethal pyrethroid exposure can affect entire food webs or assemblages of lower trophic level organisms integral to the diet of fishes and birds [28, 31]. Other commonly observed effects include changes in the rate of development and growth, or effects on reproduction [74–76], indicating that pyrethroids act as endocrine disruptors in invertebrates. For example, midges (*Chironomus riparius*) exposed to cypermethrin developed more slowly than controls, and the effect on male development was more severe [75]. The aquatic oligochaete *Lumbriculus variegatus* had lowered reproductive output following exposure to part per billion concentrations of esfenvalerate [77]. Pyrethroids act as immunotoxicants in invertebrates, such as mollusks [78], and cause oxidative stress in a wide variety of species including crayfish, tiger shrimp, and the model invertebrate *Daphnia magna* [79–81]. More sensitive sublethal endpoints, such as swimming performance, are exacerbated by alterations in salinity and temperature [70]. The magnitude of fluctuations in these abiotic parameters is expected to increase in magnitude as global climate change

progresses, potentially worsening sublethal responses to pesticide exposure [82, 83]. In combination, the acute and chronic (sublethal) effects of pyrethroid pesticides are reshaping aquatic ecosystems, altering the makeup of communities and likely reducing biodiversity as less sensitive species and taxa are favored to thrive and survive.

4 Resistance to Pyrethroid Pesticides

Toxic levels of chemicals such as pyrethroids in the environment leave organisms with few options: move, die, or acclimate. To date, pesticide resistance has been described in more than 500 arthropod species (https://www.pesticideresistance.org/, [84]). Measurable evolved resistance to a new pesticide class is considered a certainty within 10 years, and resistance has even been observed within the span of a single year [85]. If the *selective force* is strong enough to cause mortality or other *fitness* (survival, growth, and reproduction) costs, adaptation can occur at the population level in response to pyrethroid presence. The distinction between acclimation and adaptation is important in the discussion of decreased chemical sensitivity, largely because these two processes occur by different mechanisms that carry with them different implications for affected populations. *Physiological acclimation* refers to any coping mechanism that is governed by physiological processes that are nonheritable. These mechanisms can include upregulation of detoxifying or sequestering enzymes and are characterized by an increased tolerance that is temporary based on environmental conditions – when the stressor is removed, the tolerance disappears over time. *Adaptation* refers to any heritable, genetically based tolerance mechanism [86], such as the rise in frequency of a mutation conferring pyrethroid target site insensitivity. Adaptive changes have the potential to be permanent and to cause long lasting changes in populations [87]. The terms tolerance and resistance have been used interchangeably in the literature to describe decreased chemical sensitivity based on acclimation and/or adaptation. For clarity, we define *tolerance* as a decreased sensitivity that is acclimatory or temporary in nature, occurring at the organismal level, while *resistance* is a permanent change in sensitivity, conferred through an adaptive mechanism (Fig. 3). Further we focus specifically on adaptive responses to pyrethroid presence in the environment, and to be conservative, we refer to decreased sensitivity caused by any mechanism (known or unknown) other than an adaptive change as tolerance.

In general, acclimation and adaptation are sufficient to describe many ways that the animals or populations respond to environmental conditions. Even in cases where the phenotype of the offspring is determined by the genotype or environment provided by the mother (*maternal effects*) [88], our understanding of individual or population responses to the environment holds. Maternal effects caused by RNA or protein transfer to the egg will fade in subsequent generations when the environment of the mother is no longer relevant [89], qualifying these effects as a specific subgroup of acclimation. *Epigenetics* can be broadly defined as the study of

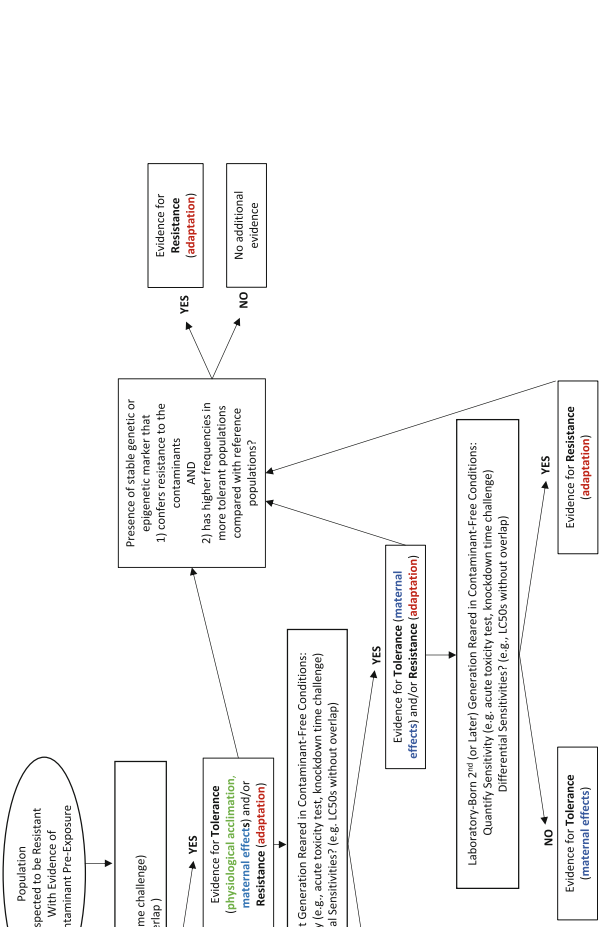

Fig. 3 Decision tree to aid in identifying the mechanism behind differential chemical sensitives in wild populations, adapted from Amiard-Triquet et al. [86] and updated to include the use of known adaptive molecular markers to support evidence of evolved, adaptive resistance. This approach can be particularly useful if the selective force applied by the contaminant is strong, and if the target site of chemical is known, as is the case with pyrethroids. Physiological acclimation and maternal effects can contribute to tolerance which we define as a decrease in chemical sensitivity from acclimatory mechanisms that is reversible and nonheritable. Conversely, we define resistance as increased tolerance based on an adaptive mechanism, which is caused by the rise in frequency of stable (genetic or epigenetic) and heritable traits within a population. In a population with expected adaptive resistance, chemical sensitivity can be quantified via acute toxicity tests and compared to that of reference populations. If differential sensitivities are maintained through the second generation (or later) of populations reared in controlled, contaminant-free conditions, physiological acclimation and maternal effects can be ruled out at mechanisms of decreased chemical sensitivity, and the likely mechanism is adaptive resistance. However, if molecular markers that are known to confer resistance in other animals (e.g., pest insects and pyrethroids) are measured at increased frequencies in resistant but not sensitive populations, then the molecular marker also provides evidence of adaptive resistance. Including molecular markers may allow for the quicker detection of adaptive resistance in wild populations

mechanisms that facilitate phenotypic variation through genotype-environment interactions. Some environment-genotype interactions may create heritable changes that can be passed down through germline cells (sperm or egg) referred to as *transgenerational epigenetic inheritance* [90]. Epigenetic modifications (also sometimes termed *epimutations*) involve changes to the DNA structure that are not reflected in the actual code itself. Epigenetic inheritance mechanisms include methylation, DNA or histone acetylation, self-perpetuating loops, noncoding RNAs, and structural inheritance [90]. The effects and diversity of epigenetic changes are complex and still being explored. *DNA methylation*, for example, in the promoter region, can decrease gene expression but, in the gene body, may instead cause increased expression or an increase in splice variants. DNA methylation can also suppress transposable elements [91]. Epigenetic changes that occur in response to environmental exposures produce alterations in the gene expression that would generally qualify them as acclimatory responses, except for the evidence that is building indicating these changes may sometimes persist transgenerationally in subsequent unexposed generations [92, 93], suggesting that generations of animals distantly removed from the environmental conditions that created a given epigenetic change may be expressing a phenotype based on those changes. In fact, epigenetic changes such as DNA methylation may provide a direct link between acclimation and adaptation, since *epimutations* (methylation at specific locations) may in some cases increase the likelihood for mutations to occur in a methylated region of DNA [94]. A growing body of research is investigating whether and how epimutations may contribute to acclimation and/or adaptation [95].

The emerging field of *evolutionary toxicology* focuses on the genetic impacts of pollution on populations. Its relatively recent rise into focus can be attributed largely to the refinement and expansion of genetic methods that make the study of pollution effects on population genetics more accessible to researchers [96, 97]. Further, although epigenetic mechanisms have not explicitly been included in the definition of evolutionary toxicology, an epigenetic change that persists transgenerationally would be considered to have an evolutionary significance and as such could fall within the definition of an adaptive trait [98]. The existence of pollutant-adapted populations in the wild has implications for human and animal health, evolution, ecological processes, and risk assessment (see discussion below). However, evolved pollution responses in wild populations have been historically difficult to characterize, especially in the face of complex mixtures acting on often unknown target sites [99]. In contrast, as we will show below, pyrethroids' potency, ubiquitous presence in the environment, and known mode of action provide an opportunity to more easily identify and study adaptive responses in populations in comparison to many other chemical toxicants in the environment.

4.1 Resistance in Target Populations

The most prominent examples of resistance to pyrethroids come from the arthropod populations these chemicals are designed to eliminate. As with all other classes of

insecticides, widespread use of pyrethroids to target arthropod pests has resulted in significant evolved resistance, increasingly rendering these chemicals ineffective as treatments against pests that affect public health and food security. What is known from the study of resistance in pest insects can be used to inform our understanding of effects in nontarget invertebrates.

4.1.1 Pest Insects

A detailed review of pyrethroid resistance in pest insects is beyond the scope of the present work but has been reviewed extensively elsewhere [52–55]. The strength of the pesticide selective pressure is a function of dose and potency [100]. In general, low level, sublethal pesticide exposures can drive a *polygenic* adaptation, potentially involving adaptation in many genes of small effect to create a resistant phenotype. Acute, lethal exposures instead drive adaptive responses outside the phenotypic response range distribution of the population, much more likely to result in the selection of small changes in genes of large effect (e.g., a *single nucleotide polymorphism* (*SNP*) leading to an amino acid base pair substitution that prevents binding in the target site) [see Ffrench-Constant et al. [101] for a discussion]. It is clear that pyrethroids are capable of acting as strong selective forces that drive evolution in pests over short timescales. A variety of mechanisms underlie pesticide resistance phenotypes, again related to dose and potency, but they can generally be classified into two main groups: those that reduce the amount of the pesticide able to reach the target site and those that modify the target site to reduce its sensitivity to the pesticide [102]. Some of the most frequently described adaptive changes include *metabolic resistance* (e.g., gene duplications, *cis* or *trans* gene mutations leading to constitutive up- or downregulation of genes responsible for pesticide metabolism) and *target site insensitivity* (mutations that prevent or reduce pyrethroid binding affinity at the target site) [55]. Mutations that lead to *target site insensitivity* are also sometimes referred to knockdown resistance (*kdr*) mutations, because they prevent the "knockdown" phenotype by reducing target site binding affinity. Given that the primary target site for pyrethroids (the Vgsc) is essential for arthropod nervous systems, its functional constraints limit the non-synonymous base pair substitutions that produce a sufficiently functional target protein while conferring resistance. Thus it is even common to see the same target site mutations arise across many arthropod species independently, providing examples of *convergent evolution* [103]. It is also not uncommon to observe some adaptive mechanisms of resistance that confer cross-resistance to a several different classes of insecticide at once. These types of resistance are typically modulated by metabolic resistance mechanisms such as cytochrome P450s, esterases, and glutathione S-transferases [104, 105]; target site insensitivity can also confer cross-resistance if pesticide classes have the same target sites (e.g., pyrethroids and DDT, organophosphates and carbamates) [106].

Recently, epigenetic changes in resistant insects have also been increasingly suggested as players in adaptive resistance [91]. Epigenetic control of a trait affecting fitness may even allow for adaptation to occur at a quicker rate (see Oppold and

Muller [107] and references therein). In the peach potato aphid (*Myzus persicae*), resistance to organophosphates and carbamates is mediated via a genetically based increase in copy number of carboxylesterases. DNA methylation controls whether or not esterase copies are expressed, thereby providing a heritable epigenetic mechanism that can silence esterase production in the absence of insecticide in the environment [108, 109], potentially ameliorating fitness costs associated with energy-intensive esterase production [110]. Altered global DNA methylation patterns have been correlated with insecticide sensitivity in mosquitoes through the F2 generation [111]. For pyrethroids specifically, decreased global methylation was apparent in pyrethroid-resistant mites, suggesting that epigenetic control mechanisms may play a part in pyrethroid resistance [112], although the extent to which those methylation changes are heritable has not been addressed. The evidence of adaptive (genetic) and potentially adaptive (transgenerational epigenetic) features associated with pesticide resistance is both abundant and rapidly expanding.

4.1.2 Sea Lice

In the aquatic environment, salmon fisheries have been employing pyrethroids, specifically deltamethrin and cypermethrin, as delousing agents (*chemotherapeutants*) for commercially raised fish since the 1990s [113, 114]. Sea lice are copepod ectoparasites in the family Caligidae that feed on the mucous, blood, and tissue of host fish [115] to the detriment of the fish, causing outcomes including decreased size/weight, suppressed immune function, and increased morbidity and/or mortality [116]. *Lepeophtheirus salmonis* is the most frequently reported parasite for salmonids, while those in the genus *Caligus* are more generalist sea lice, with *C. elongatus* as one of the most frequently cited pests in the Northern Hemisphere [117]. In the Southern Hemisphere, *C. rogercresseyi* is the primary species that infects salmonids in Chile [118]. The most common method for delousing fish is a bath treatment that involves enclosing submerged fish cages with a tarpaulin, applying the pesticide at a recommended dose for a specific duration of time (on the order of 30 min to 1 h) and then removing the tarpaulin, allowing the pesticide to disperse in the surrounding water [119]. An appropriate dose is high enough to be toxic to the sea lice without eliciting toxicity to the fish. Pyrethroids have been administered as bath treatments to kill sea lice in Canada, Chile, the Faroe Islands, Ireland, Norway, and Scotland with treatment failures reported beginning in the early 2000s [120]. Resistance of sea lice to pyrethroid (and other chemical) treatments has negatively impacted the aquaculture industry and has generally required increased pyrethroid use over time [121], which in turn may negatively impact host fish. To overcome treatment failures from pyrethroid resistance, pyrethroids are even sometimes combined with other classes of pesticides such as avermectins (added to fish feed), organophosphates, and/or hydrogen peroxide (both as bath treatments).

By the early 2000s, it was clear that pyrethroid treatments were becoming less effective among some sea louse populations from regions where bath treatments were common. Decreased pyrethroid sensitivity has been documented

in both *L. salmonis* (up to 140-fold) [122] and *C. rogercresseyi* (up to 13-fold) [118]. Several studies have endeavored to quantify resistance levels among *L. salmonis* as well as to identify genetic adaptive changes that are mechanistically responsible for decreased pyrethroid sensitivity. In general, experiments to discern resistance phenotypes from sensitive ones involve a time-to-impairment measurement (ET_{50}) with a discriminating dose of pyrethroid or the derivation of a concentration (EC_{50}) that elicits the desired effect (e.g., immobility, detachment from fish) based on a dose-response [123]. In at least some populations, resistance has been maintained stably for at least 3–4 years after bringing populations into a pyrethroid-free laboratory setting, suggesting an adaptive response. The median effective concentration (EC_{50}) of deltamethrin needed to treat resistant and susceptible strains of *L. salmonis* from Scotland sometimes differed by over 140-fold [124]. The high magnitude of differential sensitivity between strains suggests an adaptive mechanism (see Ffrench-Constant et al. [101]). To date, no single mechanism has been identified to explain the stark differences in sensitivity among resistant and sensitive strains of *L. salmonis* although several studies have identified nuclear [115, 125] and mitochondrial [124, 126] markers that have been correlated with resistance, and a resistant phenotype seems to result from a combination of both nuclear and mitochondrial changes. Fallang et al. [125] identified a novel point mutation in the domain II (S5) region of the *para*-type Vgsc ($LsN_v1.1$) that produced a glutamine-to-arginine amino acid substitution at position 945 (Q945R, *Musca domestica* numbering) that was prominent in *L. salmonis* populations with documented control failures and absent from sensitive populations. However, that amino acid substitution was not documented in other resistant populations [115] nor had it been previously documented in resistant pest insects, leaving its functional role in target site insensitivity tenuous. Carmona-Antonanzas et al. [115] searched for potential *kdr*-type mutations in three different *L. salmonis* sodium channel homologues. The authors identified several non-synonymous base pair substitutions in one ($LsN_v1.3$) of the sodium channel homologues among two resistant sea lice populations, sometimes at high frequencies (0.80). One mutation, an isoleucine-to-valine substitution at position 936 (I936V; *M. domestica* numbering), was absent in two sensitive populations of *L. salmonis*, supporting its role in conferring resistance (Fig. 4) [115]. While evidence for the I936V playing a role in pyrethroid resistance is limited, this mutation has been previously associated with pyrethroid resistance in the corn earworm [127] and has shown a capacity to decrease pyrethroid binding when mutant channels from *Drosophila melanogaster* were cloned into *Xenopus* oocytes and subjected to voltage clamp analysis [128]. Interestingly, isoleucine at this position is usually present in arthropods, while valine is typically present in vertebrates, potentially providing evidence for lineage-specific differences in sensitivity [129].

Two studies have demonstrated that pyrethroid resistance in *L. salmonis* has a strong maternal component, potentially mediated through some form of mitochondrial-based inheritance. Carmona-Antonanzas et al. [124] crossed the resistant (140-fold) and sensitive strains of *L. salmonis* from Scotland and reared offspring out to the third filial (F1 to F3) generation. When F2 organisms came from

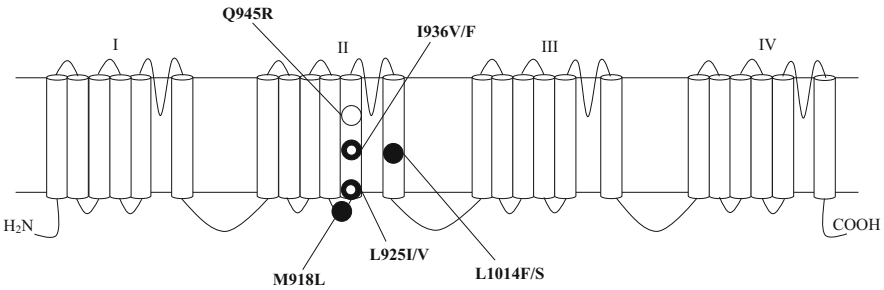

Fig. 4 Location of pyrethroid-associated resistance mutations in the target site for pyrethroids, the voltage-gated sodium channel (Vgsc) identified in aquatic invertebrates resulting from either target or nontarget applications of pyrethroids. The first letter represents the wild-type (sensitive) amino acid, while the letter to the right of the position number represents the amino acid coded by the resistance mutation. Filled circles represent those mutations that have been confirmed to reduce pyrethroid sensitivity. Open circles are those that have not been confirmed but have been reported in resistant aquatic populations. Filled circles with open circles at the center indicate that the primary amino acid mutation has been verified to reduce pyrethroid sensitivity, but the secondary amino acid has only been associated with resistant populations. The Vgsc has four repeat domains (I–IV) each with six transmembrane segments (represented by cylinders). Position numbering is according to *Musca domestica* para sodium channel

resistant dam and sensitive sire parentage, over 98% of animals were resistant when acutely exposed to deltamethrin, and a resistance phenotype (34-fold) persisted into the F3 generation. Conversely, resistant sire and sensitive dam parentage produced only 16% of F2 animals that were resistant, and F3 animals had no increase in resistance. It is important to note, however, that *maternal inheritance* (the inheritance of mitochondrial DNA) did not fully explain resistance phenotype, suggesting that a combination of nuclear and mitochondrial changes contribute to a resistance [115]. In a similar breeding study, Bakke et al. [126] crossed a deltamethrin-resistant strain of *L. salmonis* from Norway with a sensitive strain and found that resistant F2 progeny was only produced when the female of the parental generation was deltamethrin-resistant. Both Bakke et al. [126] and Carmona-Antonanzas et al. [124] found evidence of the same four amino acid substitutions in mitochondrial proteins ((NADH dehydrogenase I (glycine-to-serine at position 251), NADH dehydrogenase 5 (leucine-to-serine at position 411), cytochrome C oxidase subunit 1 (leucine-to-serine at position 107), and cytochrome C oxidase subunit 3 (glycine-to-glutamic acid at position 33) (numbering according to GenBank AY625897.1) from Scottish and Norwegian resistant populations and not in sensitive populations. The presence of the same SNPs in geographically isolated-resistant populations provides evidence for their role in pyrethroid resistance. Carmona-Antonanzas et al. [124] measured the ATP depletion in deltamethrin-exposed sea lice and only found that ATP was depleted in sensitive animals. Bakke et al. [126] found that deltamethrin-resistant sea lice experienced lower levels of skeletal muscle apoptosis than their sensitive counterparts after deltamethrin treatment. Both studies showed that deltamethrin resistance is maternally inherited, and they suggest that in

sea lice, pyrethroids may have an additional target site encoded by the mitochondrial genome. Maternally inherited, mitochondrially associated pesticide resistance mechanisms have been infrequently documented, although they do exist [130] and more work is required to fully understand the role of the mitochondrial encoded genes in pyrethroid resistance conferral for *L. salmonis*.

It is important to note that maternal effects may play a role in resistance conferral, via RNA or protein transferred from the mother to the eggs [126]. This suggestion also leaves room for the possibility that transgenerational, environmentally induced epigenetic changes may be contributing to resistance in salmon lice. One recent study found that non-synonymous base pair substitutions were present in mitochondrial DNA in some pyrethroid-resistant honeybee mites (*Varroa destructor*) compared with sensitive mites. Further, resistant mites had lower overall levels of DNA methylation compared with sensitive animals, suggesting that pyrethroid resistance in these populations may have epigenetic and mitochondrial components [112].

4.2 Resistance in Nontarget Populations

Pyrethroids are not specifically selective for pest insects – they remain toxic to nontarget arthropods through the same mode of action (as reviewed by Palmquist et al. [65]). Given that exposure to pyrethroids is the driver selecting for resistance in a population, it follows that resistance could occur in other arthropods under selective pressure from these chemicals in their environment. However, pyrethroid resistance in nontarget populations is a phenomenon that remains more difficult to quantify than in target populations for several reasons. With the ubiquitous use of pyrethroid pesticides, it can be difficult to find appropriate control populations against which to compare those that are suspected to be resistant. It can be difficult to quantify pyrethroid exposure from terrestrial inputs that move into the aquatic compartment through agricultural and urban runoff and spray drift. Populations suspected of being resistant must be screened for phenotypic resistance in a controlled setting, and a genetic marker or other adaptive mechanism of resistance must also be documented to reasonably conclude that resistance is indeed adaptive rather than acclimatory or due to maternal effects (Fig. 3). For example, one study induced a pyrethroid-tolerant phenotype in the cladoceran *D. magna* by exposing 12 generations to acutely toxic levels of the pyrethroid cyfluthrin and monitoring sensitivity for an additional 12 generations in the absence of the pyrethroid. *D. magna* developed a measurable decrease in sensitivity after only four generations (up to 4.8-fold), which was then lost in 6–10 generations with the absence of exposure [131]. Tolerant phenotypes were likely conferred via cytochrome P450 activity based on the loss of resistance with the addition of the P450 inhibitor piperonyl butoxide (PBO). The authors suggested that the gain and subsequent loss of tolerance were adaptive, but without more research to determine the mechanistic basis of that tolerance conferral, the gain and loss of tolerance in *D. magna* may actually have been caused by acclimatory and/or maternal effects of cyfluthrin exposure mediated via P450

detoxification. Finally it is worth noting that pyrethroid resistance in nontarget organisms may be difficult to identify because not all populations will evolve due to the genetic and functional constraints of variation within the population in question, and further, not all populations will evolve in the same way [58]. Some of the most pronounced evolved resistance results when insecticides select for otherwise rare genotypes that confer resistance. Without the genetic background to allow for adaptation, acutely affected populations may be likely to move or experience local extinction.

4.2.1 Mosquitoes

Evidence that pyrethroid application is capable of driving resistance in aquatic environments comes somewhat surprisingly from studying resistance in pest species. In Africa, where malaria is a prominent public health threat, decreased sensitivity to pyrethroids in *Anopheles gambiae* s.l., the primary malaria vector, has been attributed to, in part, agricultural or urban pyrethroid applications not specifically targeting mosquitoes. This is of particular concern because the World Health Organization (WHO) relies heavily on the use of pyrethroid-treated bed nets to reduce malaria transmission in humans, and if mosquitoes are becoming resistant from nontarget exposures, it may render these bed nets less effective for protecting human health. While it can be difficult to determine the relative contributions of resistance drivers for pests that are targeted with pyrethroids for human health [132], mosquito larvae taken from aquatic breeding grounds near agricultural fields and some urban areas then reared and challenged with pyrethroids and other insecticides in a controlled setting consistently exhibit increased pyrethroid resistance compared to those from reference sites [56, 59, 63]. Most of these studies rely on the methods of resistance screening recommended by the WHO. These methods involve challenging adult mosquitoes with insecticide-impregnated paper treated with a prescribed amount of pesticide (e.g., 1% permethrin) and monitoring the time to impairment ("knockdown"/immobilization) [133]. As a result, the resistance phenotypes for mosquitoes are often reported in a time-to-knockdown phenotype (KDT_{50}) for 50% of the population or in mosquito survival or mortality after a 1 h insecticide-impregnated filter paper exposure and subsequent 24 h recovery period.

The challenge in determining whether pyrethroids are responsible for adaptive resistance in some populations of less sensitive mosquitoes is rooted in the use of another pesticide, the organochlorine DDT, that also targets the Vgsc to elicit its toxic action. Historically, DDT has been used in urban and agricultural settings in much of Africa. However, its use has been restricted to necessary public health uses when other insecticides are not available following a resolution by the United Nations Stockholm Convention in 2001 [134]. Still, DDT presence or use in the environment could potentially select for prominent adaptive resistance mechanisms in pyrethroids, such as the leucine-to-phenylalanine amino acid substitution at position 1014 (L1014F, *M. domestica* numbering) *kdr* mutation, located in the S6 transmembrane segment of the domain II of the *para* sodium channel (Fig. 4). This

mutation has been associated with pyrethroid and DDT resistance in houseflies [135] and was later identified in pyrethroid- resistant mosquitoes [136]. In addition to recommending resistance screening with insecticide-impregnated papers, the WHO also recommends screening for the L1014 resistance *kdr* mutations to aid in data collection for documenting the extent of insecticide resistance in malaria vectors. As a result, a majority of the studies that provide evidence of adaptive resistance to pyrethroids (as we have defined it) cannot, with certainty, attribute that resistance development to pyrethroid selective pressures alone, because these populations harbor a resistance mutation that is common in DDT-resistant populations, and would also result from selection pressures exerted by DDT in the environment. Still, the evidence of adaptive pyrethroid resistance in larval mosquitoes receiving nontarget insecticide input is discussed below.

Diabate and colleagues [56] collected *An. gambiae* s.l. as larvae from four different types of field sites in Burkina Faso including near cotton-growing regions where pyrethroids are common agrochemicals, near an urban area where pyrethroid use is common, and reference sites where pyrethroid use is uncommon. The authors kept the larvae in a laboratory setting until the emergence of adults, at which time they were challenged with filter paper containing 1% permethrin, 0.05% deltamethrin, or 4% DDT as recommended by WHO protocols and animals, was also monitored for common *kdr* mutations. These collections and tests were performed over 2 years (1999 and 2000) in both dry and rainy seasons to elucidate temporal trends. The authors found an increase in resistance to permethrin KDT_{50} (threefold to fourfold) in cotton-growing and urban areas compared with reference sites. In addition to pyrethroid resistance, DDT resistance (4- to 40-fold) was also noted in cotton-growing and urban areas compared with reference sites. These resistance phenotypes were associated with a marked increase in the leucine-to-phenylalanine (L1014F; *M. domestica*) *kdr* allele frequencies in the *vgsc* (cotton-growing = 0.896, urban = 0.956, control = 0.18). Resistance in urban areas was attributed to coil and bomb use, while the intensive agrochemical use in cotton areas explained the resistance increase in cotton areas. Further, in the dry season when fewer pesticides are used, *An. gambiae* populations from cotton-growing areas were more sensitive than during the wet season, when selective pressures are greater.

In a study in Northern Benin, Yadouleton et al. [59] collected *An. gambiae* larvae from cotton production areas with different pest control regimes: two that involved pesticide use and a third that only involved biological control measures (e.g., *Bacillus thuringiensis*). Larvae were sampled and then reared to adulthood for sensitivity screening with 0.75% permethrin, 0.05% deltamethrin, or 4% DDT insecticide-impregnated papers. Animals from cotton-growing agricultural regions that used insecticides had increased KDT_{50}s (up to 3.2-fold) for permethrin compared to those from cotton-growing regions with only biological control and the reference laboratory population. A similar trend was noted with DDT (up to 2.5-fold resistance), with elevated KDT_{50}s from animals in sites with agricultural insecticide use compared with biological control sites and control laboratory reference populations. L1014F mutation frequencies were the highest among populations from conventional pesticide use areas (0.51–0.78) and lowest (0.32–0.35) in

populations from biological control cotton-growing sites. While DDT cannot be ruled out as a selective pressure, the authors suggested that pyrethroids are likely to be causing selection for pyrethroid and DDT-resistant *An. gambiae* populations given that pyrethroids, not DDT, were the recommended insecticides for cotton farming in West Africa. A recent structured survey of farmers in North-East Benin confirmed that the most reported insecticides used were pyrethroids and organophosphates [137].

Two studies have provided more evidence of pyrethroids as likely drivers of resistance in larval mosquitoes by collecting and analyzing environmental media (water, sediments) for pesticides in addition to tracking pyrethroid-resistant phenotypes and *kdr* mutation frequencies in *Anopheles* mosquitoes. Hien et al. [63] collected water and soil samples in pesticide-intensive cotton-growing agricultural sites and biological control (or organic) cotton-growing sites in Burkina Faso. They also collected larval mosquitoes from the same sites and subjected them to control (spring water), biological cotton, or conventional cotton water samples to document mortality at the larval stage. Larval mortality was the highest in conventional cotton site waters (66.5%) and biological site waters (49.75%) and low in spring water control (3%), indicating that agricultural site waters were toxic to larval mosquitoes. Treatment with insecticide-impregnated filter papers (0.05% deltamethrin) for 1 h followed by a 24 h recovery period showed that emergent adults were nominally more resistant to deltamethrin at conventional cotton sites compared with biological cotton sites (52.04% and 75.96% mortality, respectively), although that result was not statistically significant. Importantly, the authors also documented that allele frequencies of the L1014 *kdr* mutations were high ($F = 0.95$, $S = 0.4$) in resistant populations. The L1014S mutation confers DDT and permethrin (Type I) resistance based on voltage clamp analysis with modified *Drosophila* para Vgsc expressed in *Xenopus* oocytes [138]. Soil samples taken at sites before seasonal pesticide treatments revealed trace amounts of compounds including diuron, benzoyprop-ethyl, and fungicides chloroneb, pyridate, allethrin, and bromacil, mostly at low concentrations. Water samples taken after pesticide application but before harvest at conventional cotton sites revealed deltamethrin and lambda cyhalothrin at high levels (0.0147 μg L^{-1} and 1.49 μg L^{-1}, respectively), documenting a direct link between agricultural pyrethroid use and selective pressure on larvae [63]. Notably, the authors did not detect DDT in soil or water samples, suggesting that pyrethroids are the primary drivers of resistance in these populations.

In a second study of larval mosquitoes, resistance mutations, and environmental media, Kudom et al. [139] surveyed urban residential mosquito breeding sites in Ghana and collected larval mosquitoes and water samples. Larval mosquitoes were reared to adulthood and then challenged with pyrethroid-impregnated filter papers containing either 0.05% deltamethrin, 0.75% permethrin, 0.15% cyfluthrin, or 0.5% etofenprox for 1 h and allowed to recover for 24 h after which time mortality was scored to determine resistance phenotype. Most mosquitoes were classified as *Anopheles coluzzii*, with a minority being *An. gambiae*, and all resistant animals were genotyped for L1014 resistance mutations. While water samples revealed that pyrethroids, organochlorines, and organophosphates were present in most samples,

pyrethroids (especially permethrin at 1.283 μg L^{-1} and deltamethrin at 0.370 μg L^{-1}) were present at high levels above regulatory threshold levels for surface waters [140]. Conversely, most organochlorine and organophosphates were found at lower levels, considered to be within regulatory thresholds. This was true for the organochlorines DDT and methoxychlor, which also target the Vgsc [106]. Further, mosquitoes sampled from urban sites were highly resistant across all four pyrethroids (0–16.7% mortality) and harbored high frequencies of the L1014F *kdr* mutation (0.935). While these studies do not definitively demonstrate that pyrethroid exposure alone is driving resistance in mosquitoes, they provide evidence that pyrethroids are prevalent at toxic levels in urban and agricultural larval breeding grounds, which suggests that pyrethroids play a role in driving adaptive resistance.

Low frequencies of the L1014F *kdr* mutation of some less sensitive *An. gambiae* mosquitoes collected adjacent to conventional agricultural activity as larvae in the field and then screened for pyrethroid resistance suggest that metabolic resistance also exists in some areas [141]. In fact, many of the studies discussed above fail to test for other mechanisms that may be contributing to resistance or tolerance. Further, most studies that have documented pyrethroid resistance in mosquitoes near agricultural and urban areas rely on the collection and testing of mosquitoes coming directly from the field, which means that differences in sensitivity between resistant and sensitive populations may reflect mechanisms including physiological acclimation, maternal affects, and/or adaptive resistance (Fig. 2). However, the studies discussed herein also screen for the *kdr* target site mutation at locus L1014 in the Vgsc because it has been implicated in the conferral of pyrethroid resistance elsewhere [135, 142]. It is important to realize that these studies provide key data that nontarget pyrethroid exposure drives adaptive resistance in *Anopheles*. They document (1) a pyrethroid-tolerant phenotype, (2) evidence of increased frequency of well-documented resistance mutations in these populations near agricultural and urban areas, and (3) a pyrethroid presence in acutely toxic levels in associated environmental media.

4.2.2 Black Flies

Black flies (*Simulium* spp.) are another human health and livestock disease vector and pest worldwide that have demonstrated decreased pyrethroid sensitivity attributed to nontarget exposure to agricultural spray drift and runoff. Larvae from fruit production agricultural irrigation channels in Northern Patagonia (Argentina) have demonstrated up to 400-fold decreased sensitivity to deltamethrin and fenvalerate relative to field-reference larvae during controlled laboratory exposures [60, 62]. The source of that decreased sensitivity has been suggested to be target site insensitivity in the form of a *kdr* resistance mutation [62, 63] and/or increased esterase and monooxygenase activity [60, 61]. In the first study, larval black flies were taken from agricultural and reference areas and then subjected to 24-h water-only toxicity challenges with organophosphates, carbamates, pyrethroids (cypermethrin,

deltamethrin, or fenvalerate), or an organochlorine (DDT). Larvae from agricultural areas were significantly more tolerant to fenvalerate (88.2-fold), deltamethrin (90.0-fold), cypermethrin (22.9-fold), and DDT (59.2-fold) compared with reference site larvae. Given the high levels of DDT and pyrethroid tolerance in larvae from agricultural sites, the authors concluded that a *kdr*- type mutation was likely. Further, the lack of tolerance to organophosphates and carbamates indicated a limited contribution of detoxifying enzymes toward resistance phenotypes. The authors concluded that tolerance was likely to be driven by pyrethroid exposure given that pyrethroids were heavily used in that agricultural region at the time of the study, while DDT had not been utilized for two decades in the same region [62]. In a subsequent study, Montagna et al. [61] showed that the basis for increased *Simulium* spp. tolerance to DDT and fenvalerate in some populations from an agricultural area was likely to be more complex than a *kdr* mutation alone could explain. In larval toxicity challenges with fenvalerate or DDT in the presence of synergists PBO (which inhibits monooxygenases) and tribufos (which inhibits esterases), the authors found reduced tolerance to both DDT and fenvalerate with pre-treatment with PBO, indicating that tolerance likely involved monooxygenase activity. Pre-treatment with tribufos only marginally reduced the resistance phenotype to fenvalerate, but esterase activity in the tolerant population was nearly threefold higher than in the sensitive population, indicating that esterase activity also played a role in the tolerant phenotype. Despite the implication of metabolic enzymes in the tolerant phenotype, a *kdr*-type mutation was still presumed to confer a portion of the tolerance, although that mutation remained uncharacterized [61]. A third study on *Simulium* spp. documented both pyrethroid (deltamethrin, 130–250-fold) and organophosphate (azinphos methyl, 1.7–4.6-fold) tolerance in an agricultural population. Given that pyrethroids had recently been replaced by organophosphates after nearly two decades of consistent agricultural use, the authors highlighted the role of increased esterases in the tolerant population as a mechanism of metabolic resistance that confers resistance to both pyrethroids and organophosphates [60]. While the mechanism of increased tolerance to pyrethroids and DDT in black flies appears complex, the high magnitude of resistance between agricultural and reference animals and the inability of the metabolic enzymes to fully explain that tolerance suggests a *kdr* mutation may be responsible for the partial loss in sensitivity. Further, the primary use of pyrethroids in the agricultural region that harbors tolerant animals suggests that pyrethroids have been responsible for driving that tolerance in some black fly populations given that DDT had not been used in that region for two decades at the time that tolerance was first documented. This suggests that DDT would have played a minimal role in selecting for and then maintaining resistance in black flies. Given that larval animals were taken directly from the field and challenged with toxicants, their increased tolerance phenotypes could reflect a mixture of physiological acclimation, maternal effects, and adaptive resistance, which is supported by the complex metabolic and potential *kdr* mutation tolerance mechanisms proposed to play a role in resistance phenotypes [60–62]. While this marked decreased in *Simulium* spp. sensitivity as a result of agricultural pesticide use cannot technically be termed "resistance" by our strict definition, we conclude that increased tolerance in black

flies is likely to involve adaptive resistance, given the lines of evidence listed previously. Additional work would contribute more evidence toward resolving the tolerance/resistance classification, including target site genotyping, testing with multiple generations of laboratory reared animals, and environmental media measurements (to relate pyrethroid concentrations to tolerance).

4.2.3 Amphipods

In the Central Valley of California, pyrethroid resistance has been documented in the nontarget amphipod *H. azteca*. Unlike mosquitoes and black flies, members of the *H. azteca* species complex have no history as pests and instead act as important indicators of water quality in bioassessments as well as model laboratory organisms in ecotoxicological studies. *H. azteca* have also been documented as a food source for fish [143] and birds [144] in North America, confirming their role in aquatic food webs. This species complex has been documented as one of the most sensitive arthropods to pyrethroid pesticides [68], with LC_{50}s consistently under 5 ng L^{-1} in cyfluthrin 96 h water only exposures [57, 58]. By exposing field-collected and laboratory populations of *H. azteca* to the pyrethroid pesticides cyfluthrin and bifenthrin in 96 h acute toxicity tests, the authors found up to 550-fold resistance in some populations of *H. azteca* from waterways surrounded by agricultural and urban land use. Although the populations screened for pyrethroid sensitivity spanned six different species groups, laboratory-reared populations and wild populations in waterways without pyrethroid pesticide inputs remained similarly sensitive to pyrethroids, indicating that pyrethroid pre-exposure from nearby land use was responsible for the changes in sensitivity, rather than species group composition. Analysis of sediment samples for commonly used pyrethroids (bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, permethrin, and tefluthrin) consistently showed levels of pyrethroids in agricultural and urban sites that were sufficient to be acutely toxic to sensitive *H. azteca* during 10 d acute exposures, while reference sites without predicted pyrethroid use did not have sediments with acutely toxic levels of pyrethroids [57, 58]. Point mutations leading to single amino acid substitutions (L925I or L925V and M918L, *M. domestica* nomenclature) in the Vgsc were identified only in resistant (by tenfold or greater) populations, at high frequencies (>0.8), and sometimes appearing to be fixed within the population [58]. These mutations have been previously associated with resistance in target pest species [145–147]. Further, given that multiple species of *H. azteca* harbored resistance alleles, the phylogenetic structure of the species complex revealed that pyrethroid-resistant alleles in *H. azteca* evolved independently a minimum of six separate times, suggesting that pyrethroid selective pressures in urban and agricultural waterways are sufficient enough to repeatedly lead to genetic convergent evolution in impacted *H. azteca* spp. [58]. Interestingly, some pyrethroid-resistant *H. azteca* also harbored a nonsynonymous base pair substitution at the same Vgsc I936 locus as pyrethroid-resistant sea lice, although in *H. azteca*, the mutation was documented as a change from isoleucine to phenylalanine (I936F instead of I936V).

The I936F mutation was identified at low levels in a survey of genetic resistance markers of the pyrethroid-resistant bed bugs (*Cimex lectularius*) in Israel [148]. I936F has also been associated with d-allethrin-resistant populations of bedbugs in Australia, although the authors noted that its function in pyrethroid resistance is tentative and requires further investigation [149]. In addition to the identification of target site mutations in *H. azteca*, Weston et al. [57] used a microarray to detect gene expression differences between sensitive laboratory and a highly resistant pyrethroid- resistant population (Grayson Creek) at each population's no observable effects concentration (NOEC = 0.4 ng L^{-1} and 170 ng L^{-1}, respectively). Differentially expressed genes in the sensitive laboratory population were consistent with the mechanism of action of the pyrethroids – these animals had differentially expressed genes related to neural function, while Grayson Creek animals instead expressed stress response genes related to oxidation/reduction (cytochrome P450s, glutathione S-transferases, other oxidases), heat shock proteins, and metabolic enzymes. These results are consistent with a differential mode of toxic action in sensitive versus resistant populations which can be explained by differential Vgsc amino acid sequences.

In contrast with the other cases of potential nontarget pyrethroid resistance in aquatic invertebrates previously mentioned, *H. azteca* that have demonstrated pyrethroid resistance and high frequencies of the L925I mutation appear to be more sensitive to toxicant challenges with DDT. Regardless, there is no indication that pyrethroid-resistant animals confer any resistance to DDT [150, 151]. DDT has been banned in the United States since the early 1970s and therefore would have been unlikely to contribute to the selection and maintenance of resistance alleles measured in *H. azteca* nearly four decades later. Because field-collected *H. azteca* were used to screen for pyrethroid sensitivity, it is possible that some of the decreased sensitivity to pyrethroids observed can be attributed to physiological acclimation and/or maternal effects instead of exclusively adaptive resistance. However, three populations of resistant *H. azteca* have been maintained in a pyrethroid-free laboratory setting between 9 and 16 months, with a maximum of a 35% loss in tolerance to cyfluthrin [151]. A decrease in tolerance during that time could be attributed to nonadaptive resistance mechanisms that have not been explored in *H. azteca*, but the high frequencies and substantial (62-fold) increase in tolerance compared to sensitive populations still support the presence of an adaptive resistance mutation (L925I). Another study showed a 50% decrease in pyrethroid resistance between field-collected and laboratory-reared F1 animals in the absence of pyrethroids, but again, that population still maintained a 40-fold greater tolerance than sensitive *H. azteca* [150], supporting the existence of genetic, adaptive target site mutations in the conferral of resistance in *H. azteca*.

4.3 *Implications of Pyrethroid Resistance in the Aquatic Environment*

Pyrethroid resistance in the aquatic environment can have far-reaching implications that are important from a variety of different perspectives (human and animal health, evolutionary, ecological, and risk assessment). Below we expand on the consequences of pesticide resistance in aquatic ecosystems, with a particular focus on the effects resulting from pyrethroid resistance driven by nontarget exposures.

4.3.1 Human and Animal Health Implications

Sea lice, mosquitoes, and black flies are disease vectors. Sea lice transfer salmon anemia (ISA) between fish, apart from contributing to weakened fish immune function so that infections are more likely [152]. ISA can cause extreme mortality in heavily affected populations. One analysis estimated the cost of sea lice infestations on global salmon fisheries to be nearly US $335 million per year [153]. Increased resistance to pyrethroids among sea lice populations may call for an increased dosage of pyrethroids during bath treatments, potentially to the detriment of the fish on a sublethal level [154, 155].

Mosquitos and black flies transfer disease to humans. In 2016, 445,000 human mortalities were documented from malaria, mostly in sub-Saharan Africa [156]. *An. gambiae* is a primary vector for *Plasmodium* parasites that transmit malaria to humans and livestock in Africa [157]. Burkina Faso, Ghana, and Benin, the same countries in which nontarget pyrethroid exposures are contributing to resistance [56, 59, 63, 139], are at high risk for malaria, even in urban regions. Thus, nontarget, aquatic exposures of larval mosquitoes in urban and agricultural areas pose a great challenge to the WHO, which relies heavily on pyrethroid-treated bed nets for the prevention of malaria [158]. Bed net failures have already been attributed to pyrethroid resistance in Benin [159]. Further, selection for L1014F *kdr* mutations from pyrethroid overuse also confers DDT resistance, decreasing the efficacy of emergency DDT applications to fight malaria. Urban and agricultural overuse of pyrethroids accelerates the development of pyrethroid (and DDT) resistance, which in turn may increase the risk of contracting malaria.

Black flies act as disease vectors for *Onchocerca volvulus* – a nematode that causes onchocerciasis (river blindness) in Africa and Central and South America [36]. Nearly 1 million people currently suffer from blindness or visual impairment due to this parasite [160], and resistance gained from agricultural spray drift and runoff exposures of pyrethroids can potentially render recommended protective measures, such as permethrin-treated clothing [156], far less protective. Increased resistance to pyrethroids means that the prevalence of river blindness may increase.

4.3.2 Evolutionary Implications

In pyrethroid-laden environments, pyrethroid resistance genotypes confer a fitness advantage. In the absence of a pyrethroid selective pressure, classical theory predicts that resistance genotypes come at a cost [161]. However, the fitness costs associated with resistance are related to the specific mechanism underlying the resistance [162], and fitness costs have only sometimes been measured in cases of adaptive resistance (see Ffrench-Constant and Bass [163] for a discussion). Fitness costs associated with resistance mutations of large effect can be ameliorated by subsequent mutations in other genes (modifiers) [162], so that even resistance driven by an apparently simple mutation can actually be the result of a complex genetic profile [99]. Still, pyrethroid resistance has often been documented in other insects to come at an overall fitness cost. Boivin et al. [164] documented fitness costs including decreased fecundity and fertility, slower development, lower weight, and shorter lifespans in deltamethrin-resistant codling moths (*Cydia pomonella*) compared with sensitive strains. Konopka et al. [165] showed cost of fitness through developmental and reproductive life history traits with a population of pyrethroid-resistant *C. pomonella*. One study monitored the allele frequency of a *kdr* mutation in houseflies (*M. domestica*) in pyrethroid-free environment for 15 generations, and found a significant decrease in frequency over time, suggesting a strong cost of having the mutation in the absence of pyrethroids [166]. In mosquitoes (*Culex quinquefasciatus*), *kdr* resistance mutations were associated with a decreased chance of surviving to adulthood [167]. Reduced overall fitness noted with some pyrethroid resistance mutations in the Vgsc may be caused by reduced efficiency in mutant Vgsc or related metabolic costs [168]. Fitness costs have been documented in homozygous recessive L1014F mutant *An. gambiae* females [169]. In *H. azteca*, the L925I resistance mutation is more common than the M918L mutation, suggesting that L925I is preferred, potentially because of lower fitness costs [58, 129]. Several populations of *H. azteca* appear to be functionally fixed for a resistance mutation at the L925 locus, including the population studied for tolerance to other chemicals and fitness costs [58, 150]. One population fixed for L925I showed lower reproductive capacity, lower thermal tolerance, and trends toward increased sensitivity to other chemicals including DDT, copper (II) sulfate, and sodium chloride, potentially indicating some fitness costs associated with the L925I allele, although more work will be necessary to determine that definitively [150].

If the selective pressure is sufficiently strong, population size can be reduced to leave only a select group of founder genotypes to continue that population, potentially leading to "genetic erosion" or a loss of genetic diversity [170]. Unlike physiological acclimation, changes to the genetic structure of populations including loss of sensitive genotypes and reductions in genetic diversity are permanent alterations to the population in question [87]. Losses in genetic diversity also increase vulnerability to extinction [171]. It is noteworthy that the populations that have evolved resistance to pyrethroids are often also harboring evolved resistance to other pesticides. For example, many populations of *H. azteca* that are resistant to pyrethroids are also resistant to organophosphates through analogous target site

mutations [40, 151, 172]. Salmon lice from Norway harbor organophosphate and carbamate resistance alleles [173, 174]. Other populations of sea lice show a marked reduction in sensitivity to emamectin benzoate, an avermectin [123]. *An. gambiae* have demonstrated adaptive resistance to pyrethroids and organophosphates [175]. Some populations of black flies (*Simulium* spp.) that are resistant to pyrethroids are less sensitive to organophosphates [60]. These examples of evolution to multiple classes of pesticides serve as evidence that these populations are under potentially strong selective pressures from multiple chemicals. Concurrent, strong selective pressures may leave populations even more vulnerable to extinction or losses in genetic diversity. While concerns regarding the genetic diversity of pest species (e.g., sea lice, mosquitoes, black flies, and sea lice) are rarely expressed, these concerns are markedly more prominent when considering nontarget species (like *H. azteca*) that are not disease vectors or pests. Evidence of decreased genetic diversity caused by insecticide applications are suggested in the literature for insects. Allelic richness was negatively correlated with deltamethrin resistance in mosquitoes (*Aedes aegypti*) harboring a Vgsc *kdr* mutation, potentially due to founder effects from genetic bottlenecks caused by insecticide selective pressures [176]. However, if gene flow is high, losses in genetic diversity are not always apparent in pyrethroid-resistant insect populations [177]. *H. azteca* is a poor disperser relative to the flying insects [178]. Thus, if selection for pyrethroid resistance *kdr* alleles is capable of driving genetic bottlenecks in *H. azteca*, and if gene flow is not sufficient to compensate for decreases in genetic diversity, resistant *H. azteca* populations with *kdr* mutations at high frequencies may be particularly prone to having low genetic diversity or being at a greater risk for genetic drift. The functional fixation of resistance alleles at the L925 locus in six different populations of *H. azteca* also suggests that genetic diversity may be reduced in those populations. As mentioned previously, one L925I-fixed population of pyrethroid-resistant *H. azteca* have already demonstrated a reduced tolerance to other stressors and increased fitness costs compared to sensitive populations [150], potentially due to fitness costs associated with the resistance mutation, or possibly from a loss in genetic diversity associated with a past founder effect. Explicit studies of genetic diversity in resistant populations of *H. azteca* have yet to be performed, but are essential to building our understanding of the way that selection for *kdr* mutations is affecting populations and their resilience to other environmental changes and stressors. These studies may also serve to move the field of evolutionary toxicology forward as we gain a better understanding of the evolutionary impact of strong selective pressures on nontargets.

4.3.3 Ecological Implications

Adaptive pyrethroid resistance from target (sea lice) and nontarget (mosquitoes, black flies, and amphipods) pyrethroid exposures may signal ecosystem-level pesticide stress. If pyrethroids are present at levels sufficient to drive selection of target site mutations of large effect in these populations, then they are likely causing acute

and sublethal toxicity to other organisms in these environments. In the case of sea lice, the pyrethroids from fish bath treatments are typically released into the surrounding area after the prescribed therapeutic duration [179]. Several reviews have considered the potential impacts of sea lice pesticide treatments on nontarget aquatic biota [37, 180]. One study estimated that the concentration of deltamethrin at approximately 100–350 m from the treatment area was sufficient to immobilize the benthic marine amphipod *Eohaustorius estuarius* in as little as 1 h. Given its tendency to sorb to particles instead of remaining in the water column, deltamethrin release from sea lice treatments is likely to impact sediment dwelling organisms more strongly than those in the water column [181]. A previous study using a similar approach to track cypermethrin during a simulated bath treatment found cypermethrin in the surrounding water between 2 and 5.5 h after tarp release, at distances ranging from 900 to 3,000 m away from the pen at low ng L^{-1} concentrations – the same range of concentrations causing irreversible immobilization in the *E. estuarius* population after 48 h of exposure [69]. Burridge et al. [179] tested the acute, short-term toxicity of Alphamax® (active ingredient deltamethrin) on several nontarget marine organisms including American lobsters (*Homarus americanus*) at a variety of different life stages and shrimp (*Crangon septemspinosa* and *Mysid* spp.) to determine toxicity over short- term exposures (1, 24 h) that may realistically follow bath treatment chemical release. Deltamethrin concentrations ranging from 3.4 to 18.8 ng L^{-1} caused lethality in 50% of animals after only 1 h, with lobsters being the most sensitive. Concentrations ranging from 0.8 to 27 ng L^{-1} were sufficient to cause the same effects after only 24 h of exposure, with the earliest life stages of lobsters being the most sensitive [179]. These findings are important because they demonstrate that nontarget animals near fish pens being treated for sea lice come in contact with pyrethroids at concentrations that cause acute toxicity on ecologically relevant timescales. Further, they highlight that other important fisheries such as the American lobster, sometimes located near salmon fisheries [179], are likely to be impacted by sea lice treatments. It is likely that invertebrate assemblages near sea lice treatment pens are experiencing toxicity from pyrethroids released after treatment. Given the evidence of acute toxicity in some marine organisms at low pyrethroid concentrations, it follows that these same assemblages may be experiencing strong selective pressures from these nontarget pyrethroid exposures, potentially contributing to mortality or the development of resistance in some populations. These effects could also extend to other fisheries, such as shrimp farming in Central Asia, which sometimes use pyrethroids to treat pests [38], but for which treatment regimes and other exposure data are severely lacking. As the doses of pyrethroids in sea lice treatments are increased to compensate for the development of resistance in sea lice populations [121], effects on nontarget animals near salmon fisheries are only likely to become more severe.

The arthropod taxa and life stages for which nontarget, adaptive resistance to pyrethroids has been documented are among the most sensitive to pyrethroids in comparison to other members of the aquatic community. The selection for and rise in frequency of resistance mutations of large effect (Vgsc L1014F/S, M918L, L925I/V) in these sensitive groups are consistent with exposure to acutely toxic concentrations

of pyrethroids in the environment. However, other less sensitive taxa may still be under substantial selective pressures from pyrethroids. Environmentally relevant measurements of pyrethroids in water and sediment often exceed regulatory recommendations [2]. A variety of other freshwater and marine crustaceans (*Menippe mercenaria*, *Gammarus lacustris*, *Crangonyx pseudogracilis*, *Gammarus pseudolimnaeus*, *Americamysis bahia, Chaoborus* sp.) have similar pyrethroid sensitivities (2.6- to 9.3-fold lower) to *H. azteca* [68]. Potential impairment for other important prey for fish including caddisfly (*Hydropsyche* spp.) have been documented at environmentally relevant levels of bifenthrin [182]. The abundance of sensitive invertebrate taxa, % Ephemeroptera-Plecoptera-Trichoptera (EPT), and some mayfly taxa has been negatively correlated with bifenthrin sediment concentrations [28]. Further, a mesocosm experiment with bifenthrin-laden sediments has documented reduced larval macroinvertebrate abundance, richness, and biomass at concentrations 2.5 times lower than the recorded 10 d sediment LC_{50} for *H. azteca* [31]. The same authors also predict altered emergence dynamics and trophic cascades in some stream scenarios. Another mesocosm experiment showed impairment of the majority of examined macroinvertebrate and zooplankton taxa in response to a tertiary mixture of environmentally relevant concentrations of two pyrethroids and an organophosphate. *H. azteca* and *D. magna* showed acute toxic responses, while snails (*Radix* sp.) and copepods displayed chronic, sublethal responses [183]. Thus, it is possible that other taxa are under substantial acutely toxic selective pressures from pyrethroids, and at minimum, they are experiencing sublethal fitness costs from pyrethroid presence. Even sublethal fitness costs incurred by aquatic populations under pyrethroid stress may drive resistance to pyrethroids in affected populations, although that adaptive resistance would most likely occur through complex phenotypes caused by polygenic selection, which would be likely to carry with them their own set of fitness costs [99]. In populations without sufficient standing genetic variation on which evolution can act, or in taxa that have longer life cycles, evolution may not be a feasible response to environmental stress. For example, *H. azteca* are obligate aquatic invertebrates and have a generation time of 1 month under standardized laboratory conditions [184]. In contrast, some mayflies, for example, remain nymphs for up to multiple years before emergence [185], and a longer generation time may allow pyrethroids to impact population densities via acute or sublethal toxicity to an extent that prevents evolved resistance to pyrethroids and instead contributes to local extinctions.

Aside from the loss of sensitive taxa from ecosystems with toxic levels of pyrethroids, potential fitness costs and decreased resilience to other environmental stressors in some resistant *H. azteca* populations [150] may contribute to present or future declines in densities, which could also impact the fish and other predators that rely on them for food. In addition, pyrethroid-resistant *H. azteca* harbor higher levels of these pesticides capable of causing sublethal toxicity to forage fishes and potentially increasing the risk of bioaccumulation in piscivores or birds which may reach farther up the aquatic food web [186]. The mosquito and blackfly populations that are resistant to pyrethroids may also pose a higher risk for bioaccumulation in predators (birds, fish, frogs, and other insects) that rely on these larval and adult insects as a food source, although those studies have yet to be performed.

4.3.4 Risk Assessment Implications

If the pyrethroid presence is strong enough, some populations of sensitive taxa may evolve. However, relying on populations to have the genetic background population size to evolve to resist pyrethroids is not a sufficient protective strategy for aquatic ecosystems. Even when evolution is possible, it may not happen quickly enough in wild populations and can come with fitness trade-offs [99]. In itself, the measurement of genetic, adaptive resistance is an indicator that pyrethroids' selective pressures have removed sensitive individuals from the population. It may also signal acute and/or sublethal toxicity for other members of the aquatic ecosystem, potentially leading to the loss of other sensitive taxa. Regarding sea lice treatment with pyrethroids, risk assessments should be undertaken in a fish farm site-specific manner to prevent undue harm from pesticide treatments on nontarget life [180]. The evolution of resistance in aquatic invertebrate nontargets in urban and agricultural environments on a global scale suggests that pyrethroids are not being adequately regulated to prevent undue harm in the aquatic environment. In the United States, agricultural pyrethroid use is monitored, but urban use is not [58]. In African countries, such as Ghana, pyrethroid use in general is poorly regulated [139]. Without closer regulation of pyrethroid use, disease vectors and other invertebrates will continue to experience strong selective pressures from pyrethroids, perpetuating the human health, evolutionary, and ecological effects described above.

Adequate protection for wild populations may not be achieved by utilizing adapted populations to make risk assessment decisions [86], largely because sensitive organisms have been removed from resistant populations. However, the genetic adaptive pyrethroid resistance in some wild populations of *H. azteca* presents a unique model system to incorporate the field of evolutionary toxicology directly into risk assessment decisions. *H. azteca* are both sensitive to pyrethroids and amenable to laboratory culture. As such, they are ideal candidates for bioassessment and biomonitoring programs and an ideal laboratory surrogate for determining thresholds for the protection of aquatic life. The stable pyrethroid resistance mutations in one population of *H. azteca* have already been used as a type of biological toxicity identification evaluation (TIE) tool to identify the source of toxicity in environmental samples [151]. The repeated, convergent evolution of the same resistance mutations across different species groups within the *H. azteca* species complex suggests that screening new populations for genetic changes in the target site (*vgsc*) may provide evidence of pyrethroid impairment in new locations. Further, given that other crustaceans are often similarly sensitive to pyrethroids [69], phenotype assays and genetic screening for pyrethroid resistance could be developed for taxa that are often used in regulatory decisions outside of the United States (e.g., *Gammarus*). These methods may be able to refine which areas or regions are at the greatest risk for impairment from pyrethroids.

5 Conclusion

Pyrethroids are present in aquatic environments globally, from river, estuarine, and marine sediments to irrigation channels, lakes, rural, and suburban waterways. They have been identified in sediments in the United States, Great Britain, Spain, Vietnam, Norway, Thailand, Australia, Pakistan, Argentina, Paraguay, Brazil, and Nigeria (see Tang et al. [2] and references therein), often at levels that exceed regulatory thresholds [140]. These compounds are widely implicated in causing acute and sublethal effects in aquatic organisms at low, environmentally relevant concentrations in water and sediment. Both target and nontarget applications of pyrethroids drive adaptive pyrethroid resistance in a number of invertebrate taxa. We present evidence that pyrethroids drive the evolution of resistance in nontarget aquatic organisms on three continents [57, 58, 60–63]. Both urban and agricultural pyrethroid use are responsible for the selection of genetic adaptive resistance in vector (mosquitoes, black flies) and nonvector (*H. azteca*) populations. Resistance in disease vectors threatens public health, while resistance in other nontarget invertebrates serves as an indicator of pyrethroid impairment in aquatic environments. Further exploration of the evolutionary implications of pyrethroid resistance in aquatic organisms is highly warranted. Taking full advantage of model systems such as *H. azteca* and as well as incorporating the repeated evolution of genetic resistance into risk assessment decisions will greatly expand our understanding of the evolutionary processes that occur due to the presence of pyrethroids and other chemical stressors in the environment.

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Stereoselectivity and Environmental Behaviour of Pyrethroids

Cláudio Ernesto Taveira Parente, Olaf Malm, and Francisco Radler de Aquino Neto

Contents

Abstract Pyrethroids are chiral insecticides due to the occurrence of up to three asymmetric carbons. Each stereogenic centre generates two possible spatial configurations (*R*- or *S*-enantiomers), which are non-superimposable mirrored forms. Two chiral carbons on the cyclopropane ring generate four enantiomers on Type I pyrethroids, while a third chiral centre on Type II pyrethroids generates eight enantiomers. The chiral nature of enzymatic sites favours specific insecticidal activity only for some enantiomers in commercial formulations. On the other hand, there is an overabundance of enantiomers with no desired activity or even undesired side effects. In this sense, in addition to the previously described toxicity of insecticide enantiomers to nontarget organisms, adverse effects, such as endocrine

C. E. T. Parente (✉) and O. Malm
Laboratório de Radioisótopos Eduardo Penna Franca, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
e-mail: cparente@biof.ufrj.br; claudioetparente@gmail.com

F. R. de Aquino Neto
Laboratório de Apoio ao Desenvolvimento Tecnológico – LADETEC, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

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disruption, have been reported for enantiomers with low or no insecticidal action. In addition, the different metabolic pathways of pyrethroid enantiomers have consequences for their persistence and bioaccumulation profiles in biological systems. Therefore, a stereochemical approach is required to better understand the undesired impacts of pyrethroids on the environment and on human health, since the studies point to patterns of toxicity and persistence at enantiomeric levels. The occurrence of degradation/persistence patterns in environmental samples may be useful for understanding enantiomeric fate, contributing to more accurate risk assessments aimed at preventing or mitigating the impacts of continuous pyrethroid release into the environment.

Keywords Chirality, Cypermethrin, Enantiomers, Environment, Isomerism, Permethrin

1 Introduction

Chiral compounds are characterized by the presence of at least one asymmetric molecular centre. Currently, approximately 30% of commercialized pesticides present chirality [1, 2]. The need to increase the efficiency and economic viability of new pesticides, as well as the evolution of knowledge about molecular interactions in biological systems, favoured the development of more specific chiral active ingredients [2]. In this context, the development of chiral molecules is aligned with the strategy of achieving efficient and environmentally sustainable pesticides [3]. Among the various molecular structures of pesticides, asymmetric centres can occur on carbon, sulphur, nitrogen and phosphorus atoms [4]. In pyrethroids, chirality is due to the presence of one to three stereogenic tetrahedral carbons. The occurrence of a chiral centre (e.g. fenpropathrin) gives this structure optical isomerism with two possible spatial configurations, which are non-superimposable mirrored forms of the same compound (*R*- and *S*-enantiomers). On typical Type I pyrethroids (e.g. permethrin), the presence of two chiral carbons generates four diastereomers, resulting in one pair of *cis*- and *trans*-enantiomers. On Type II pyrethroids (e.g. cypermethrin), the inclusion of a third chiral carbon (*alpha*-cyano) generates eight diastereomers, resulting in a second pair of each *cis*- and *trans*-enantiomer.

Due to chirality at enzymatic sites, pyrethroid enantiomers may be related to different toxicities and preferential metabolic pathways in biological systems [5]. Furthermore, variation in the biochemical transformation patterns of these compounds directly influences the persistence and preferential bioaccumulation of stereoisomers [6]. Enantioselectivity is a determining factor for the occurrence of isomeric patterns in the environment, including the different rates of bioaccumulation observed in species living in the same ecosystem [7].

Among commercial products containing pyrethroids, there is a predominance of racemic formulations (equal proportions of enantiomers) and enriched isomers [8]. Although single isomers (e.g. *gamma*-cyhalothrin and bioresmethrin) are more efficient and environmentally safe due to their specific effect on target receptors, their industrial-scale production is often limited by cost-efficient technologies [2, 4]. On the other hand, only a few enantiomers in racemic formulations have the desired action. For example, only one enantiomer of each pair of diastereomers of permethrin (1*R-cis* and 1*R-trans*) and cypermethrin (1*R-cis-αS* and 1*R-trans-αS*) has strong insecticidal activity. The remaining two enantiomers of permethrin and six stereoisomers of cypermethrin are not as efficient or have no specific activity [9]. Considering racemic permethrin, if only 50% of enantiomeric molecules are efficient as insecticides, a greater environmental burden is expected due to the expense of material resources and the need for greater volume of application. In addition to the increased risk of contamination of urban and agricultural environments, possible impacts on nontarget organisms are expected for all permethrin enantiomers, since toxic effects were reported for some insecticidal enantiomers, and endocrine disruption and immunotoxicity are related to others [10–12].

Considering the widespread use of pyrethroids and their chemical complexity, it is essential to consider their stereoisomerism to more accurately assess the persistence, risk of bioaccumulation and possible undesired impacts of pyrethroids on nontarget organisms. In this sense, an achiral analytical approach in environmental and toxicological studies is able to only partially assess the potential adverse effects of pyrethroids in biological systems [3].

Therefore, this chapter presents data with the aim of discussing the stereochemical behaviour of pyrethroids in the environment. Relevant studies on the consequences of pyrethroid toxicity to nontarget organisms, the potential bioaccumulation of pyrethroids and their fate at isomeric levels and the use of isomeric profiles as markers of environmental origin will be discussed.

2 Pyrethroid Structure Configuration

The synthesis of pyrethroids was modelled upon esters (pyrethrins) that constitute approximately 25–50% of pyrethrum, a natural extract of *Chrysanthemum* spp. flowers used for centuries as insecticide [13]. Among the six isolated esters of pyrethrum responsible for its insecticidal activity, there are two related groups: three esters similar to cyclopropanecarboxylic acid, also named chrysanthemic acid, and three esters related to pyrethric acid [14]. Both acids occur esterified with three alcohols (cinerolone, jasmolone and pyrethrolone), known generically as rethrolones (Fig. 1a). The esterification of the chrysanthemic acid with each rethrolone generates pyrethrins I, while the esterification of pyrethric acid with rethrolones forms pyrethrins II [14].

Among these main structures found in pyrethrum extract, chrysanthemic acid served as a model for the synthesis of pyrethroids. Chrysanthemic acid has two

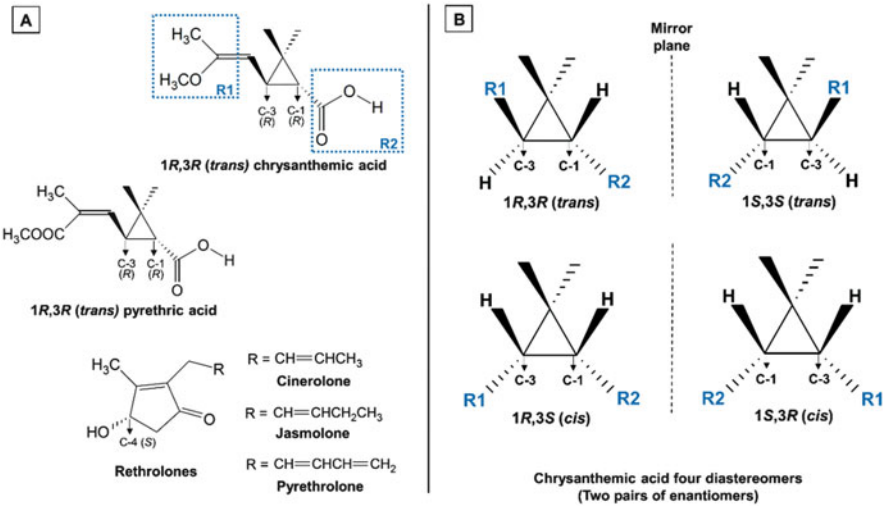

Fig. 1 (**a**) Chrysanthemic acid, pyrethric acid and basic structure of rethrolones. Chiral carbons (C-1, C-3 and C-4) are presented with their natural configuration; (**b**) possible spatial configurations of chrysanthemic acid based on chiral carbons (C-1 and C-3) of the cyclopropane ring. Radicals R1 and R2 of the chrysanthemic acid are represented in the dashed frames of (**a**)

asymmetric carbons, resulting in four enantiomers. Pyrethric acid differs by a change of the methoxy group for a carbomethoxy group at the double bond, and rethrolones have a chiral carbon (C-4) and geometric isomerism due to their side-chain double bond (Fig. 1a). Chiral carbons (C-1 and C-3) of chrysanthemic and pyrethric acid occur only in the 1*R*,3*R*-configuration (Fig. 1b) [15].

The instability of exposure to light and heat of chrysanthemic acid was solved with the inclusion of halogen atoms (at first chlorine) in substitution of the terminal group at the double bond, giving rise to permethric acid (Fig. 2) [14]. The synthesis of the current pyrethroids was completed with the esterification of the benzylic alcohol (*m*-phenoxybenzyl alcohol) by the permethric acid giving rise to permethrin – the first pyrethroid with photostability suitable for agricultural application (Fig. 2) [13, 15]. Subsequently, another compound, cypermethrin, was synthesized, with the esterification of racemic cyanohydrin (hydroxy group of m-phenoxybenzyl cyanohydrin) with permethric acid giving rise to Type II pyrethroids (Fig. 2).

Compared to Type I pyrethroids, Type II compounds have higher photostability, higher insecticidal activity and a further asymmetric centre on *alpha*-cyano-3-phenoxybenzyl alcohol (Fig. 2) [16, 17]. Aiming to further improve these features, new molecules were synthesized with the inclusion of other halogen atoms (bromine and fluorine), as well as changes in the number of carbons. Among Type I pyrethroids, we can highlight bifenthrin, resmethrin and tefluthrin. Common examples of Type II pyrethroids are cyfluthrin, cyhalothrin, fenvalerate (an acyclic compound) and the single isomer deltamethrin (Fig. 3). The number of asymmetric carbons (*n*) is

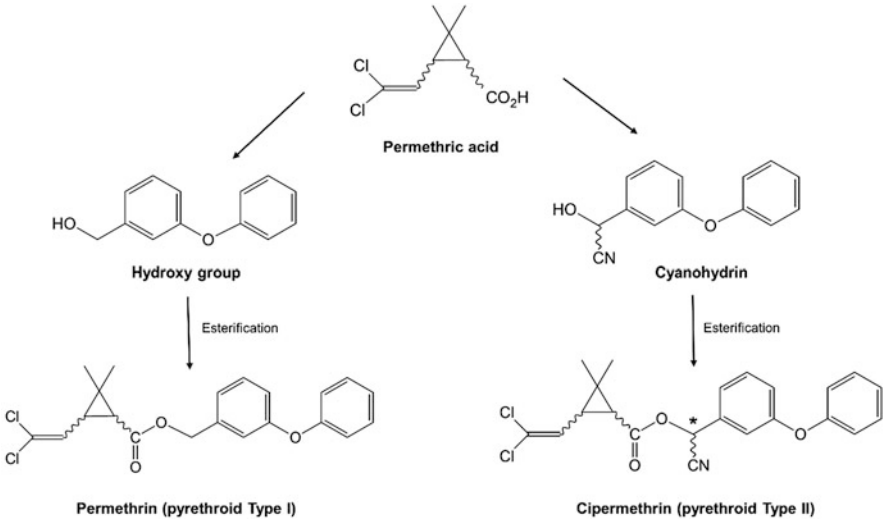

Fig. 2 Synthesis of permethrin (Type I pyrethroid) and cypermethrin (Type II pyrethroid) based on esterification of permethric acid with hydroxy groups. ∗New chiral centre introduced upon esterification

related to the number of possible isomers in Type I ($2^2 = 4$ diastereomers) and Type II pyrethroids ($2^3 = 8$ diastereomers).

The nomenclature of chiral carbons *R* (rectus) and *S* (sinister) based on the Cahn-Ingold-Prelog (CIP) system [18, 19] is related to the priority (e.g. highest atomic number and other rules) of bonded groups to an asymmetric atom or chiral centre. According to this system, the least priority substituent is positioned at the greatest distance from the observer, counting the three remaining substituents in descending order, which can be clockwise (*R*) or counterclockwise (*S*) (Fig. 4).

Another written representation related to optical isomerism considers the molecular property to divert the plane of polarized light to the right, *dextro*rotary (+), or to the left, *levo*rotary (−). There is no necessary correlation between the designation (*R*) and (*S*), which is directly related to the molecular tridimensional structure and the direction of rotation (±) of plane-polarized light, which is experimentally determined [20].

Different ways of writing chiral carbon configurations can be found in the literature [7, 21–23]. According to the International Union of Pure and Applied Chemistry (IUPAC) recommendations, *cis*-enantiomers of Type I pyrethroids should be written as 1*R*,3*R* and 1*S*,3*S*, and *trans*-enantiomers should be written as 1*R*,3*S* and 1*S*,3*R*. In Type II, *cis*-enantiomers should be written as 1*R*,3*R*,*αR*; 1*S*,3*S*,*α S*; 1*R*,3*R*,*αS*; and 1*S*,3*S*,*αR*, and *trans*-enantiomers should be written as 1*R*,3*S*,*αR*; 1*S*,3*R*,*αS*; 1*S*,3*R*,*αR*; and 1*R*,3*S*,*αS*. However, as a way of shortening the nomenclature, some authors fix the C-1 chiral configuration and state the geometric isomerism, thereby establishing the chirality of the other. For example, Type I pyrethroids *cis*-enantiomers are named 1*R*-*cis* and 1*S*-*cis* instead of 1*R*,3*R* and 1*S*,3*S*,

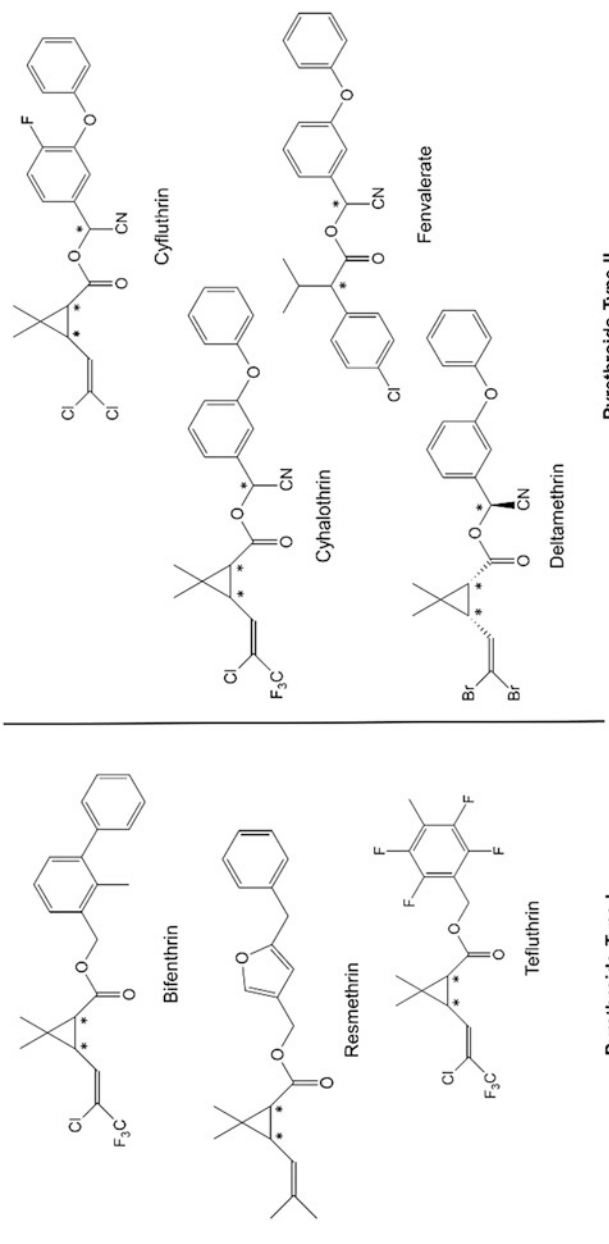

Fig. 3 Type I and II pyrethroids. Asterisks highlight chiral carbons, and the single isomer deltamethrin is presented in its configuration 1*R-cis-αS*

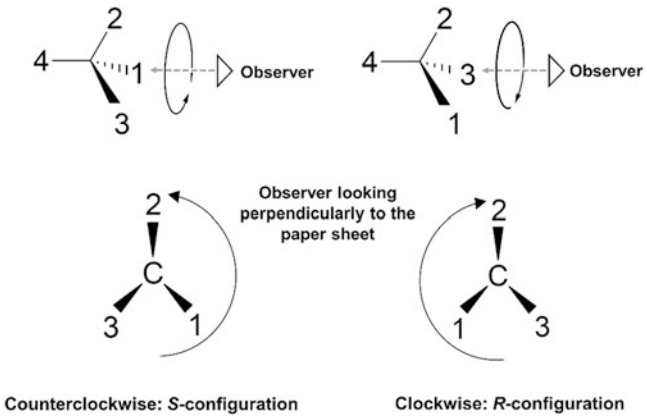

Fig. 4 Nomenclature of *R*- and *S*-configuration based on the Cahn-Ingold-Prelog (CIP) system related to the priority of bonded groups to chiral centres. In both spatial arrangements, with *R*- or *S*-configuration, the order of priority is 1, 2, 3 and 4

respectively [22, 23]. This approach is useful, since the *cis*/*trans* nomenclature is immediately associated with the spatial position of the substituents on the cyclopropane ring. Thus, in this instance, we represent the carbon configuration with the abbreviated nomenclature to simplify the writing and the tridimensional understanding of beginners in this field.

Chiral *R*- and *S*-configurations of widely used pyrethroids and their isomeric ratios in commercial formulations are presented in Table 1. Bold enantiomers have higher insecticidal activity than other enantiomers.

3 Metabolic Pathways and Toxicity in Nontarget Organisms

In biological systems, pyrethroids act with receptor-ligand interactions related to molecular tridimensional arrangements. Stereoselectivity at enzymatic sites directly influences binding to specific enantiomers with consequences on biotransformation reactions, such as hydrolysis, reduction, oxidation and conjugation [20]. Therefore, different responses to toxicity, bioaccumulation, biodegradability and adverse effects of enantiomers are expected. Although commercial pyrethroid formulations are a complex mixture of stereoisomers, only a few enantiomers have insecticidal activity. Considering the stereoisomeric configuration, only the *R*-configuration of C-1 chiral carbons (1*R*-*cis* and 1*R*-*trans*-isomers) presents the desired activity (Fig. 5), whereas with a chiral carbon in the cyanohydrin group (Type II pyrethroids), only the *S*-configuration at *alpha*-cyano-3-phenoxybenzyl ester presents high insecticidal activity [15, 31].

Table 1 Pyrethroid enantiomers and their isomer ratios on commercial formulations

Pyrethroids	Type	Chiral carbons	*cis*-Isomers[a]	*trans*-Isomers[a]	Isomer ratio (*cis*/*trans*)	Total isomers	References
Permethrin	I	2	**1*R*-*cis***; 1*S*-*cis*	**1*R*-*trans***; 1*S*-*trans*	80:20; 40:60; 25:75[b]	4	[24–26]
cis-Bifenthrin	I	2	**1*R*-*cis***; 1*S*-*cis*	–	*cis* ≥97%	2	[8, 17]
Resmethrin	I	2	**1*R*-*cis***; 1*S*-*cis*	**1*R*-*trans***; 1*S*-*trans*	20–30:70–80[c]	4	[3, 24]
Bioresmethrin	I	2	–	**1*R*-*trans***	Isomer ≥90%	1	[8, 24]
Phenothrin	I	2	**1*R*-*cis***; 1*S*-*cis*	**1*R*-*trans***; 1*S*-*trans*	50:50	4	[8]
Cypermethrin	II	3	1*R*-*cis*-*αR*; 1*S*-*cis*-*αS*; **1*R*-*cis*-*αS***; 1*S*-*cis*-*αR*	1*R*-*trans*-*αR*; 1*S*-*trans*-*αS*; 1*S*-*trans*-*αR*; **1*R*-*trans*-*αS***	45:55	8	[24, 27]
alpha-Cypermethrin	II	3	**1*R*-*cis*-*αS***; 1*S*-*cis*-*αR*	–	–	2	[8]
beta-Cypermethrin	II	3	**1*R*-*cis*-*αS***; 1*S*-*cis*-*αR*	1*S*-*trans*-*αR*; **1*R*-*trans*-*αS***	40:60	4	[8]
theta-Cypermethrin	II	3	–	1*S*-*trans*-*αR*; **1*R*-*trans*-*αS***	50:50	2	[8]
zeta-Cypermethrin	II	3	1*S*-*cis*-*αS*; **1*R*-*cis*-*αS***	1*S*-*trans*-*αS*; **1*R*-*trans*-*αS***	45–55:55–45[c]	4	[8, 27]
Deltamethrin	II	3	**1*R*-*cis*-*αS***	–	Isomer ≥98%	1	[8]
Fenvalerate	II	2	**2*S*-*αS***; 2*R*-*αR*	2*R*-*αS*; 2*S*-*αR*	50:50	4	[28]
Esfenvalerate	II	2	**2*S*-*αS***	–	Isomer ≥75%	1	[8, 28]
lambda-Cyhalothrin	II	3	**1*R*-*cis*-*αS***; 1*S*-*cis*-*αR*	–	–	2	[8, 17]
gamma-Cyhalothrin	II	3	**1*R*-*cis*-*αS***	–	Isomer ≥98%	1	[29]
Cyfluthrin	II	3	1*R*-*cis*-*αR*; 1*S*-*cis*-*αS*; **1*R*-*cis*-*αS***; **1*S*-*cis*-*αR***	1*R*-*trans*-*αR*; 1*S*-*trans*-*αS*; **1*S*-*trans*-*αR***; **1*R*-*trans*-*αS***	45–55:55–45[c]	8	[27, 30]
beta-Cyfluthrin	II	3	**1*R*-*cis*-*αS***; **1*S*-*cis*-*αR***	**1*S*-*trans*-*αR***; **1*R*-*trans*-*αS***	33:66	4	[8]

Bold enantiomers have higher insecticidal activity than the other enantiomers

[a]*cis*- and *trans*-isomerism from pyrethroids are related to their *R*- or *S*-configurations of chiral carbons (C-1 and C-3) on the cyclopropane ring

[b]Different permethrin isomeric ratios are related to the manufacturing processes; however, technical formulation with predominance of *trans*-isomers, e.g. 40:60 and 25:75, is more common [25]

[c]Expected variation during the manufacturing process

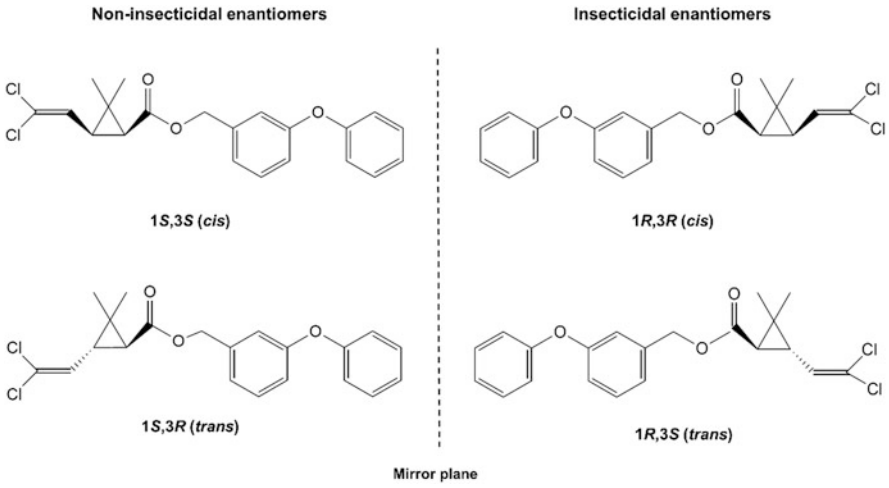

Fig. 5 Permethrin enantiomers with noninsecticidal and insecticidal activity

In recent decades, the wide occurrence of pyrethroids in different environmental matrices, such as soil, surface waters and sediments, has driven further investigations that characterize the major metabolic pathways in nontarget organisms [10, 32, 33]. In addition, stereoselective toxicity has stimulated studies to assess adverse effects on diverse model organisms and mammalian cells, including human cell lines. Table 2 presents in vivo and in vitro assays with nontarget organisms after exposure to pyrethroid enantiomers.

3.1 Soil Organisms

Enantioselective degradation is expected in soils due to the presence of enzymes capable of metabolizing pyrethroids in soil microbiota [43]. Among degradation pathways in soils, the main routes occur through oxidation of the alkyl portion and aromatic rings, as well as the cleavage of the ester linkage by hydrolysis [43, 44].

Previous studies addressing diastereomeric degradation in soils reported greater persistence of *cis*-isomers [43–45]. At the enantiomeric level, the 1*R-cis-αS* enantiomers of cypermethrin were less persistent in soils compared to their epimer (1*S-cis-αR*) [46, 47]. These results are in agreement with the long half-life observed for 1*S-cis-αR* enantiomers compared to 1*R-cis-αS* after application of *alpha*-cypermethrin in edible plants (cabbage, cucumber, rape, tomato and pepper) [48].

Selective degradation was also observed for *trans*-enantiomers: 1*S-trans*-permethrin and 1*R-trans-αS* enantiomers of cypermethrin and cyfluthrin [49]. The authors emphasized that 1*R-trans-αS* enantiomers were least persistent in alkaline and acid soils, although they have high insecticidal activity. Faster mineralization

Table 2 Enantioselective toxicity and oestrogenic effects on nontarget organisms based on in vivo and in vitro assays

Organisms	Species/cells	Pyrethroids	Tests – endpoints	Enantiomers	Comments
Soil earthworms	*Eisenia fetida*	*alpha*-Cypermethrin	Filter paper contact toxicity – LC_{50}	(+)-1*R-cis-αS*	Among 3- and 33-fold more toxic than other isomers [34]
	Eisenia fetida	Esfenvalerate	Filter paper contact toxicity – LC_{50}	2*S-αS*[a]	Approximately fourfold more toxic than fenvalerate [28]
	Eisenia fetida	Esfenvalerate	Artificial soil – LC_{50} (7 days)	2*S-αS*[a]	Approximately threefold more toxic than fenvalerate [28]
	Eisenia andrei	Deltamethrin	Filter paper contact toxicity – LC_{50}	1*R-cis-αS*[a]	Increased toxicity LC_{50} 0.72 (1 h) to 0.55 μg cm^{-2} (48 h) [35]
	Lumbricus rubellus	Deltamethrin	Filter paper contact toxicity – LC_{50}	1*R-cis-αS*[a]	Fivefold more susceptible than *Eisenia andrei* (48 h) [35]
Aquatic microcrustaceans and shrimp	*Daphnia magna*	*cis*-Bifenthrin	LOEC[b] – survival and fecundity	1*R-cis*	LOECs in 14 days were 80-fold lower (more toxic) than 1*S-cis* [36]
	Daphnia magna	*cis*-Bifenthrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis*	Approximately 22-fold more toxic than 1*S-cis* [37]
	Daphnia magna	*cis*-Permethrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis*	Above 15-fold more toxic than 1*S-cis* [37]
	Daphnia magna	*trans*-Permethrin	Acute toxicity assay 96 h – LC_{50}	1*R-trans*	Above 19-fold more toxic than 1*S-trans* [37]
	Ceriodaphnia dubia	*cis*-Bifenthrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis*	Approximately 18-fold more toxic than 1*S-cis* [37]
	Ceriodaphnia dubia	*cis*-Permethrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis*	Above 38-fold more toxic than 1*S-cis* [37]
	Ceriodaphnia dubia	*trans*-Permethrin	Acute toxicity assay 96 h – LC_{50}	1*R-trans*	Above 30-fold more toxic than 1*S-trans* [37]
	Ceriodaphnia dubia	Cypermethrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis-αS*; 1*R-trans-αS*	Above 10- and 7.5-fold more toxic than other 6 isomers [37]
	Ceriodaphnia dubia	Cyfluthrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis-αS*; 1*R-trans-αS*	Above 96- and 47-fold more toxic than other 6 isomers [37]
	Macrobrachium nipponense	*lambda*-Cyhalothrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis-αS*; 1*S-cis-αR*	Sevenfold more toxic than *gamma*-cyhalothrin (1*R-cis-αS*) [9]
Fishes	*Danio rerio* (zebrafish)	*beta*-Cypermethrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis-αS*	Mean eightfold more toxic than 1*R-trans-αS* [38]
	Oryzias latipes (Japanese medaka)	*cis*-Bifenthrin	VTG[c] (liver) by ELISA[d] – oestrogenicity	1*S-cis*	123-fold greater oestrogenic effect compared to 1*R-cis* [39]
	Oryzias latipes (Japanese medaka)	Permethrin	VTG[c] (liver) by ELISA[d] – oestrogenicity	1*S-cis*; 1*S-trans*	2.5- and 1.3-fold greater oestrogenic effect compared to their epimers [10]

	Danio rerio (zebrafish)	Permethrin	Expression of VTG[c] 1 and 2 mRNA – Oestrogenicity	(−)-*trans*	2.6- and 1.8-fold greater than (+)-*trans* based on VTG1 and VTG 2 mRNA induction [11]
	Oncorhynchus mykiss (rainbow trout)	Permethrin	VTG[c]-mRNA expression in primary hepatocytes – oestrogenicity	1*S-cis*	Twofold higher expression compared to 1*R-cis* [10]
Anuran amphibian (tadpoles)	*Rana nigromaculata*	*alpha*-Cypermethrin	Acute toxicity assay 96 h – LC_{50}	1*R-cis-αS*	Approximately 29-fold more toxic than 1*S-cis-αR* [40]
Mammals	*Mus musculus* – ICR mice	*cis*-Bifenthrin	Expression of genes biomarkers of endocrine disruption – at mRNA, protein and enzymes levels	1*S-cis*	Maternal exposure during pregnancy resulted in significant endocrine disruption (male offspring) compared to 1*R-cis* [41]
	In vitro – rat adrenal pheochromocytoma (PC12) cells	Permethrin	Growth-inhibition effect – cytotoxicity	1*R-trans*	Approximately 1.6-fold higher compared to 1*S-cis* [42]
	In vitro – human breast carcinoma cell line MCF-7	*cis*-Bifenthrin	Oestrogen response gene expression (pS2, ER*α*)[e]	1*S-cis*	2.2-fold oestrogenic activity compared to 1*R-cis* [12]
	In vitro – macrophage cell line RAW264.7	*cis*-Bifenthrin	Macrophage apoptosis – immunotoxicity	1*S-cis*	Apoptosis was 13% more compared to 1*R-cis* [12]

[a]Single isomer
[b]Lowest observed effective concentration
[c]Vitellogenin
[d]Enzyme-linked immunosorbent assay
[e]Biomarkers of oestrogen exposure in MCF-7 cell lines

was also described for 1*R*-enantiomers of *cis*-/*trans*-permethrin, fenpropathrin and *lambda*-cyhalothrin after soil incubation and by using a bacterial consortium isolated from Brazilian savannah [50–52].

Considering the different groups of soil organisms, earthworms play an important role in the dynamics of organic matter and in the maintenance of soil structure in addition to ecological and environmental functions [17, 34]. Among the widely occurring pyrethroids in soils, cypermethrin has been predominant [33]. In an acute toxicity assay (filter paper contact) with earthworms *Eisenia fetida* exposed to *alpha*-cypermethrin (1*R*-*cis*-*αS* and 1*S*-*cis*-*αR* enantiomers), high toxicity of 1*R*-*cis*-*αS* enantiomer was observed with LC_{50} = 49.5 ng cm^{-2} [34]. The toxicity was approximately threefold higher compared to racemic *alpha*-cypermethrin and 33-fold higher compared to the 1*S*-*cis*-*αR* enantiomer (Table 2).

Although *E. fetida* and *E. andrei* are earthworm species widely used in toxicological assessments, the preferential use of *Eisenia* spp. may underestimate impacts to other worm species on the environment. In a study comparing the response of enzymatic biomarkers with *E. andrei* and *Lumbricus rubellus* exposed to deltamethrin (1*R*-*cis*-*αS*), a greater susceptibility of *L. rubellus* was observed [35]. In addition, LC_{50} (48 h) = 0.11 μg cm^{-2} to *L. rubellus* was fivefold lower than observed in tests with *E. andrei* (Table 2). According to these studies, *cis*-isomers with the same configuration (1*R*-*cis*-*αS*) were toxic to earthworms in a concentration range between nanograms and micrograms per cm^{-2}. Additionally, the single isomer with *cis*-configuration esfenvalerate (2*S*-*αS*), which has a different molecular structure (there is no cyclopropane ring) with a chiral centre on C-2, was approximately fourfold more toxic to *E. fetida* than racemic fenvalerate (Table 2) [28]. In the specific case of fenvalerate, its insecticidal activity is related to the 2-*S* configuration, which is structurally compatible with the 1-*R* configuration of the cyclopropane ring that also presents high insecticidal action [15, 53].

In addition to the differences between compounds, including their chemical structures and their spatial arrangements, the soil matrix presents great variation related to such physicochemical characteristics as pH, redox potential, soil moisture, soil texture and organic matter content [44]. Among soil parameters, organic matter content plays an important role in pyrethroid sorption on soils, which directly affects their bioavailability and environmental fate [54]. Soil characteristics also influence the diversity and abundance of soil microbiota, including its catabolic activity related to important functions, such as nutrient cycling and pyrethroid biodegradation [55, 56].

Although there is some progress in studies approaching enantioselectivity by soil microbiota and earthworms, to the best of our knowledge, there is a lack of studies considering other soil organisms, such as the enchytraeids *Enchytraeus albidus* and *Enchytraeus crypticus*, and soil arthropods, such as the collembolans *Folsomia candida* and *Folsomia fimetaria*, and the soil mite *Hypoaspis aculeifer* [57].

Considering the impacts on organisms of different trophic levels, such as detritivore species (e.g. earthworms) and predators (e.g. *Hypoaspis aculeifer*), an enantioselective approach will be an important step for more precise risk assessments, aiming to protect and maintain soil functions.

3.2 Aquatic Environments

Reports on pyrethroid occurrence in river sediments around the world point out a relevant contribution of these compounds to contamination of aquatic ecosystems [33, 58, 59]. Pyrethroids have high sorption potential in soils and can reach aquatic environments mainly through spray drift and consequent via atmospheric deposition, as well as rainfall and runoff events [43, 60–62].

Considering the effect of technical formulations, without an approach on single stereoisomers, previous studies have noted the high toxicity of pyrethroids, mainly to fish and aquatic arthropods [63]. On the other hand, chiral studies were performed with *Daphnia magna*, a zooplanktonic crustacean with an important ecological role as a food web base in freshwater aquatic environments [32]. In a chiral approach with bifenthrin (10 μg L^{-1}), *D. magna* presented a low capacity for metabolism of *cis*-isomers [36]. Among stereoisomers, 1*R*-*cis*-bifenthrin presented a high bioaccumulation ratio and higher toxic effects on fecundity and survival compared to 1*S*-*cis* enantiomers (Table 2). High toxicity was also reported in tests with *Ceriodaphnia dubia* (a microcrustacean) and *Daphnia magna* exposed to 1*R*-*cis* enantiomers of bifenthrin and permethrin, confirming the stereoselectivity on metabolism, bioaccumulation and toxicity in these aquatic organisms [22].

In a study with adult zebrafish (*Danio rerio*), significant oxidative stress was observed in liver and brain tissues due to exposure to *beta*-cypermethrin racemic formulation and single isomers: 1*R*-*cis*-*αS* and 1*R*-*trans*-*αS* [38]. These same enantiomers were more lethal in the acute toxicity test than their epimers 1*S*-*cis*-*αR* and 1*S*-*trans*-*αR* (Table 2).

The enantiomeric results of these studies are in agreement with the reported high toxicity of 1*R*-*cis* (bifenthrin and permethrin), 1*R*-*cis*-*αS* and 1*R*-*trans*-*αS* enantiomers of cyhalothrin and cypermethrin in assays with species of different trophic levels, such as microcrustaceans (*Ceriodaphnia dubia* and *Daphnia magna*) [22, 36], shrimp [9], zebrafish [38], and tadpoles of anuran amphibian [40].

On the other hand, studies have shown that the *S*-configuration of C-1 at the cyclopropane ring is associated with endocrine disruption in fishes (Table 2). The enantiomer 1*S*-*cis*-bifenthrin (10 ng mL^{-1}) induced 123-fold greater oestrogenicity compared to the 1*R*-enantiomers in Japanese medaka *Oryzias latipes* [39]. Additionally, 1*S*-*cis* enantiomers of permethrin induced significantly higher oestrogenic activity compared to its epimer (1*R*-*cis*), as determined through assays performed in vivo with Japanese medaka and in vitro with primary rainbow trout hepatocyte [10]. Another study reported enantioselective oestrogenic effects in tests with zebrafish exposed to 500 ng L^{-1} of permethrin. Levorotary (−)-*trans*-enantiomers induced the greatest oestrogenic activity compared to other permethrin enantiomers [11]. According to the authors, (−)-*trans*-permethrin induced an oestrogenic effect fourfold higher than the oestrogen 17-*beta*-estradiol at 50 ng L^{-1}. The greatest oestrogenic effects of levorotary (−)-*trans*-permethrin are comparable to the greater oestrogenic effects of the 1*S* configuration of bifenthrin and permethrin [10, 39], suggesting that both nomenclatures are related to the same configuration.

These previous studies with aquatic organisms demonstrated toxic effects derived from insecticidal enantiomers 1*R-cis*/*trans* (Type I pyrethroids) and 1*R-cis*/*trans*-*αS* (Type II pyrethroids), while endocrine disruption was induced by enantiomers with low or no insecticidal activity (1*S-cis*/*trans*). In addition, the adverse effects shown with zebrafish assays point to the need for further studies on the potential toxicity of specific enantiomers in humans, since this organism has been used as a model of human cellular metabolism [64, 65].

3.3 Mammals: In Vivo and In Vitro Tests

Among the *cis*-enantiomers of Type I pyrethroids, 1*R-cis* are more stable and present toxicity to mammals, whereas among *trans*-enantiomers, 1*R-trans* does not present toxicity in acute assays [8, 15, 66]. Exceptions to this rule occur with 1*R-cis*-phenothrin that do not present toxic effects to mammals and 1*R-trans*-ethanomethrin, which present high neurotoxicity [53]. In Type II pyrethroids, *alpha*-cyano carbon with the *S*-configuration presents greater neurotoxicity to mammals than its epimer [15].

In mammals, the main route of detoxification of *trans*-isomers is through hydrolysis, while the major metabolic process of *cis*-isomers is oxidation [67]. In relation to the acid group, ester hydrolysis greatly depends on the spatial configuration of C-1 and C-3 chiral carbons, with 1*R-trans* and 1*S-trans* enantiomers undergoing high metabolization rates compared to 1*R-cis* and 1*S-cis* enantiomers. In the alcohol portion of the molecule, hydrolysis of esters of primary alcohols (Type I pyrethroids) is faster than esters of secondary alcohols (Type II pyrethroids) [68]. Hepatic enzymes, such as carboxylesterases, are important in pyrethroid metabolism. Selective metabolization of *trans*-permethrin through human pyrethroid-hydrolysing carboxylesterases (hCE-1 and hCE-2) was observed compared to the hydrolysis rate of *cis*-permethrin [31].

In humans, permethrin oxidation occurs through metabolization by cytochrome P450 enzymes (CYP450) and by alcohol and aldehyde dehydrogenases [69]. The main metabolites from hydrolysis and oxidation reactions are *cis-trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis-trans*-DCCA), 3-phenoxybenzyl alcohol (3-PBAlc), 3-phenoxybenzyl aldehyde (3-PBAld) and 3-phenoxybenzoic acid (3-PBA) [66, 69].

Pyrethroids have been associated with a wide range of toxicological effects upon the reproductive function of mammals [70–72]. In assays with adult male mice exposed by oral administration to permethrin, only the *cis*-isomers resulted in wide reproductive adverse effects with reduction of epididymal sperm count, sperm motility and testosterone levels in testes [73]. On the other hand, no adverse effects on reproductive function were observed after *trans*-permethrin administration. In addition, the presence of the urinary metabolite 3-PBA in *trans*-permethrin treatment was up to sevenfold higher compared to a treatment by its isomeric pairs (*cis*-isomers). Additionally, the hepatic microsomal hydrolase activity for the

trans-permethrin in vitro assay was approximately 62-fold higher than with *cis*-permethrin exposure.

In a study that assessed adverse effects on physiology, histopathology and gene expression levels (T synthesis), *cis*-permethrin induced the greatest endocrine disruption effects, resulting in reproductive toxicity in male mice during puberty age [70]. In female mammals, adverse effects on reproductive function have been reported after exposure to *beta*-cypermethrin, permethrin, fenvalerate and deltamethrin, which include decreased fertility and inhibition of hormones affecting the endocrine system [71, 72]. However, considering a chiral approach, studies on potential adverse effects on the reproductive function of female mammals (e.g. mice) are still scarce. This fact has relevance, since studies note possible adverse effects, such as endocrine disruption, through the mother-foetus system (Table 2) [41, 74].

At the enantiomeric level, C-1 in the *S*-configuration of bifenthrin resulted in greater effects as endocrine disruptors in assays performed in vivo and in vitro with mammals [41, 74, 75]. For example, 1*S*-*cis*-bifenthrin induced 2.2-fold oestrogenic activity compared to 1*R*-*cis*-enantiomers measured through the expression of biomarker genes in a breast cancer cell line (MCF-7) [12]. The authors also observed enantioselective cytotoxicity in macrophage cells by 1*S*-*cis*-bifenthrin, indicating possible adverse effects on the immunological system (Table 2). In another study with bifenthrin, 1*S*-*cis* enantiomers also induced adverse effects with significant accumulation of cellular triglycerides in human hepatoma cells (HepG2) compared to their epimers [76]. Other in vitro studies reported that permethrin modifies lipid metabolism, affecting the intracellular functions of adipocytes and glucose homeostasis through the reduction of glucose uptake in myotubes [77, 78]. In addition, epidemiological and in vivo studies contribute to evidence between exposure to insecticides and the development of obesity and type 2 diabetes [79].

Pyrethroids undergo selective diastereomeric metabolization in mammals, being more persistent *cis*-diastereomers [31, 73]. This fact deserves attention regarding the possible impacts on human health, since *cis*-permethrin induced the greatest adverse effects on the reproductive system, as well as endocrine disruption, as shown in previous assays with mammals [70, 73].

In addition, endocrine disruption in mammalian assays by the 1*S*-configuration of bifenthrin [41, 74, 75] was also observed in aquatic organisms by the same configuration of bifenthrin and permethrin [10, 39], suggesting a broad potential to affect organisms from different environmental compartments, including humans.

3.4 Abiotic and Laboratory-Based Epimerization

In addition to the selectivity in biological systems, epimerization can occur by photolytic isomerization in sunlight, during sample preparation and analysis with polar solvents, and by heat [27, 51, 80]. Stereoisomer epimerization can decrease the insecticidal activity of commercial formulations. This effect may lead to erroneous analytical interpretation and may influence the results of bioassays, since polar

solvents are used in these tests. Regarding photolytical isomerization, approximately 20–30% of single enantiomers of permethrin (1*R-trans*), cypermethrin and cyfluthrin (1*R-trans-αS*) were epimerized to other enantiomers after 7 days of sunlight irradiation [27]. However, epimerization occurred only in C-1 and C-3 carbons, while *alpha*-carbons of cypermethrin and cyfluthrin remained in the *S*-configuration. On the cyclopropane ring, the recombination of biradicals on carbon bonds occurs after internal rotation, resulting in chiral carbons C-1 and C-3 epimerization [81]. Photo-induced isomerization at diastereomeric or enantiomeric levels was also observed for deltamethrin (including on *alpha*-carbon), tralomethrin and tralocythrin [81].

Epimerization induced during analysis procedures is only expected in the *alpha*-cyano carbon present in Type II pyrethroids. This chiral carbon is unstable under high temperature and protic solvents, such as primary alcohols [3, 82]. For example, methanol, ethanol, *n*-propanol, 2-methyl-1-propanol and *n*-butanol induced *alpha*-carbon epimerization of cypermethrin enantiomers 1*R-cis-αR* to 1*R-cis-αS* and 1*R-trans-αR* to 1*R-trans-αS* [83]. On the other hand, no epimerization was observed on C-1 and C-3 during stability tests with sterile water and aprotic solvents (acetone, *n*-hexane, ethyl acetate and dichloromethane) [81, 82].

In addition, the heated injector in gas chromatography analysis (GC) induced some epimerization on the *alpha*-carbon of cypermethrin and cyfluthrin [80]. In acidic solution (0.1% acetic acid) with *n*-hexane, chiral centres of pyrethroids remained stable during GC analysis, and an almost twofold increase of peak intensity was observed compared to non-acidified solvent [84].

In light of the above findings, it should be considered that stereoisomeric profiles found in environmental samples are the result of several transformations, both biotic and abiotic, on the commercial formulations used. In addition, it is crucial to avoid analytical procedures that induce changes in chiral carbon configurations in studies addressing pyrethroid stereoisomerism.

4 Stereoisomeric Profile and Environmental Dynamics of Chiral Pollutants

Initially, the stereoselective behaviour of chiral pesticides in the environment, such as organochlorines (e.g. *cis*- and *trans*-chlordane and *alpha*-hexachlorocyclohexane – *α*-HCH), allowed the use of their degradation pattern as a tracer of sources of contamination [85]. This approach is employed because enantiomers present the same physicochemical characteristics (e.g. solubility in water, vapour pressure, octanol-water partition coefficient). However, upon entering the environment, the chiral compounds undergo selective enantiomeric degradation in biological systems that may alter their initial isomeric pattern [86]. In this context, the differentiation of a racemic profile of atmospheric contamination (primary emissions from applied products) compared to a nonracemic contamination profile, for example, from the revolatilization (secondary emission) of pesticides from

contaminated soils, brought an important discussion about global transport dynamics of persistent organic pollutants (POPs) [85–87]. This approach was proposed around the 1990s in a period of increasing banning of organochlorine pesticides in industrialized countries but with their continued use in tropical and subtropical countries [85, 88]. The use of degradation profiles of chiral POPs as tracers of their sources is still required for monitoring global contamination. There is evidence of increased secondary emissions of POPs into the atmosphere, including industrial pollutants, such as polychlorinated biphenyls (PCBs), due to rising temperatures and melting in Arctic regions in the face of global climate change [86, 89].

As described for some organochlorines, pyrethroid stereoselectivity is potentially suitable for environmental signature interpretation. Considering the greater toxicity of specific enantiomers, the finding of contamination profiles in different environmental compartments can be a fundamental tool for more accurate risk assessments.

4.1 Pyrethroid Stereoisomerism on Environmental Samples

Over the last several decades, pyrethroids have been increasingly used as an alternative to more toxic and persistent pesticides, such as organochlorines, organophosphates and carbamates. However, reports on pyrethroid contamination in aquatic mammals and atmospheric air from mountains of biosphere reserve of the Atlantic Forest demonstrate their persistence in the environment and their long range of contamination [90, 91].

Therefore, a more extensive investigation is required considering the fate and the possible impacts of these compounds in the environment.

Pyrethroid stereoisomerism on environmental samples must be interpreted considering some relevant points: (1) the current limitation on the number of published works, since in many studies, the results are presented only with the sum of isomers; (2) the occurrence of different commercial formulations must be checked to avoid misunderstandings regarding the profile found in the environment; and (3) the multiple chiral centres in pyrethroids generate up to four peaks in an achiral stationary phase and up to eight peaks in a chiral phase, which require an adequate peak resolution during the analytical procedures for the subsequent profile comparison.

Some previous studies have presented the enantiomeric factor (EF) as a means to discuss the environmental dynamics of pyrethroids, which includes their degradation/persistence pattern in environmental samples [90, 92, 93]. Depending on the analysis, EF can be calculated to compare enantiomeric pairs (*cis*- and *trans*-diastereomers) or single enantiomers (*R*- and *S*-enantiomers). EF is calculated through the equation ($EF = A_{sp}/A_T$), where A_{sp} is a specific stereoisomer chromatographic peak area and A_T is the sum of peak areas of all structurally related stereoisomers present in the sample [90, 93]. In equal proportion, each diastereomer of Type I pyrethroids is expressed as EF = 0.5 or 50%. In Type II pyrethroids, an equal proportion of each diastereomer is expressed as EF = 0.25 or 25% due to the

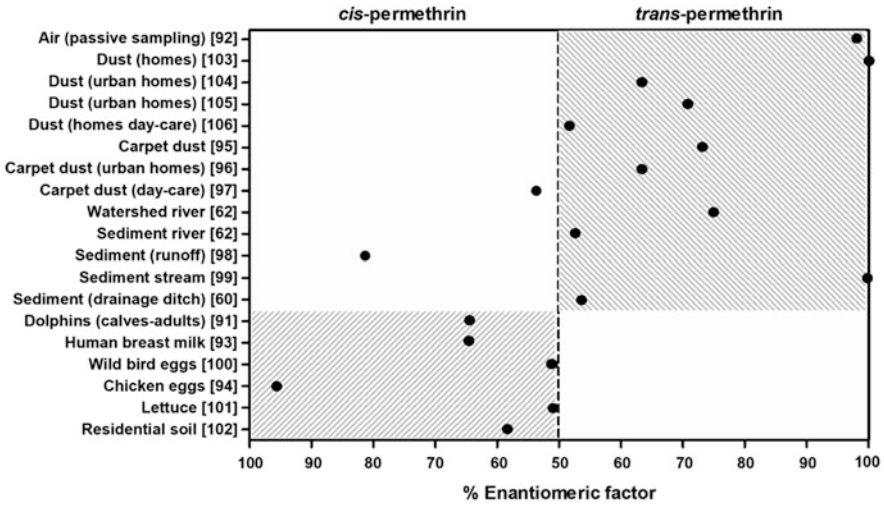

Fig. 6 Enantiomeric factor percentage (%EF) of permethrin in environmental samples; *y*-axis: sample types (reference numbers in square brackets); *x*-axis: percentage of contribution (50–100%) of *cis* or *trans*-permethrin calculated through equation $\%EF_{trans} = (trans/cis + trans) \times 100$

presence of four chromatographic peaks on an achiral separation analysis. Figure 6 presents the EF of permethrin in environmental samples from previous studies [60, 62, 90–105]. This figure is based on measures of central tendency (means or medians) of *cis*- and *trans*-isomer concentrations, since in most publications, the area of the chromatographic peaks was not available.

The compiled data (Fig. 6) show a clear pattern of diastereomeric selectivity between *cis*- and *trans*-permethrin in environmental samples. According to the figure, there is a trend of higher contribution of *trans*-permethrin in atmospheric air and indoor dust samples. Permethrin technical formulations have several *cis*/*trans* isomer ratios (Table 1). However, formulations with a higher proportion of *trans*-isomers are more common in the market [25, 26, 104]. Therefore, enriched *trans*-permethrin in atmospheric air and indoor dust samples matches commercial formulations, suggesting the maintenance of the original distribution of isomers in these samples. These results are in line with the earlier assertion that indoor dust may not only reflect the amount of insecticides applied by residents but may also maintain the same profile for the components of commercial formulations [104].

As observed with permethrin, atmospheric air samples presented a similar profile of cypermethrin commercial products [93] with the following diastereomer proportions: *cis*1 (26%), *cis*2 (21%), *trans*1 (28%) and *trans*2 (25%) [91]. The results show that cypermethrin measured in mountains (2,200 m a.s.l.) from a biosphere reserve of the Atlantic Forest (Brazil) matches the commercial formulation, which suggests a possible source of primary emission. Indeed, insecticide application is common for urban vector control and agricultural production in regions surrounding the protected area [91].

As observed in air and dust samples, a high contribution of *trans*-permethrin in watershed river and sediment samples was observed after rains during the dry season compared to the wet season [62]. According to the authors, extreme concentrations of *cis*- and *trans*-permethrin (4,800 and 13,000 ng L^{-1}, respectively) may be related to application drift or product misuse.

Although sediment samples have a similar profile with a predominance of *trans*-permethrin, Fig. 6 shows an atypical result (outlier) with a high contribution of *cis*-permethrin in sediment samples carried with surface runoff from a commercial nursery of plants [97]. According to the authors, permethrin is applied with the planting mix material before seeding, and the required intensive irrigation results in a heavy runoff. Therefore, the source of this sediment is from a contaminated soil, which may explain the greater contribution of *cis*-permethrin in these sediment samples, since a predominance of *cis*-isomers in soil samples has been described [45, 96, 101]. Among sediment samples, *trans*-permethrin had the highest predominance ($\%EF_{trans} \cong 100$) in samples from Aiba Stream, Nigeria [98]. Although this stream's drainage basin is highly impacted by agricultural activities, according to the authors, additional sources of pyrethroids may occur through their urban vector control and domestic use and through untreated sewage discharge.

According to Fig. 6, *cis*-permethrin was predominant in biological samples, such as human breast milk, dolphin tissues and commercial chicken eggs [90, 92, 93]. The results are in agreement with the reported high degradation rate of *trans*-permethrin in biological systems [15, 66]. Furthermore, a comparative study showed an increase in the *cis*-cypermethrin epimers (1*R*-3*R*-*αS* and 1*S*-3*S*-*αR*) in human breast milk samples compared to profiles found in commercial formulations [21]. Regarding the above-mentioned findings, the main concern is the reported toxicity to mammals related to the *cis*-enantiomers 1*R*-3*R*-permethrin and 1*R*-3*R*-*αS*-cypermethrin [15].

In wildlife, a great predominance of *cis*-isomers was also observed, such as in bird egg samples (permethrin and cypermethrin) [99] and in river fish samples (cypermethrin and cyfluthrin) [7]. However, for the specific compound tetramethrin, commercial formulations have a much higher predominance (80:20 ratio) of 1*R*-*trans*-enantiomers over 1*R*-*cis*-enantiomers [8]. According to the authors, it is possible that the higher proportion of *trans*-tetramethrin in commercial formulations has influenced the observed results [7, 99].

In a study of pyrethroid contamination in commercial chicken eggs [93], the difference between the cypermethrin diastereomeric profile of a product applied topically in chickens and that observed in egg samples from the same farm was observed (Fig. 7a, b).

A higher percentage of *cis*-cypermethrin contribution was determined in the egg sample (Fig. 7b) compared to the racemic formulation (Fig. 7a). The proportion of the first *cis*-isomer (49%) compared to the total cypermethrin measured in the egg sample is almost two times the proportion of the same isomer in the commercial formulation (27%). Additionally, in a wide variety of contaminated food samples (fish, beef, chicken and milk), a predominance of *cis*-cypermethrin [106] was verified. However, the reference values for food safety – maximum residue limit (MRL) and acceptable daily intake (ADI) – consider the sum of isomers

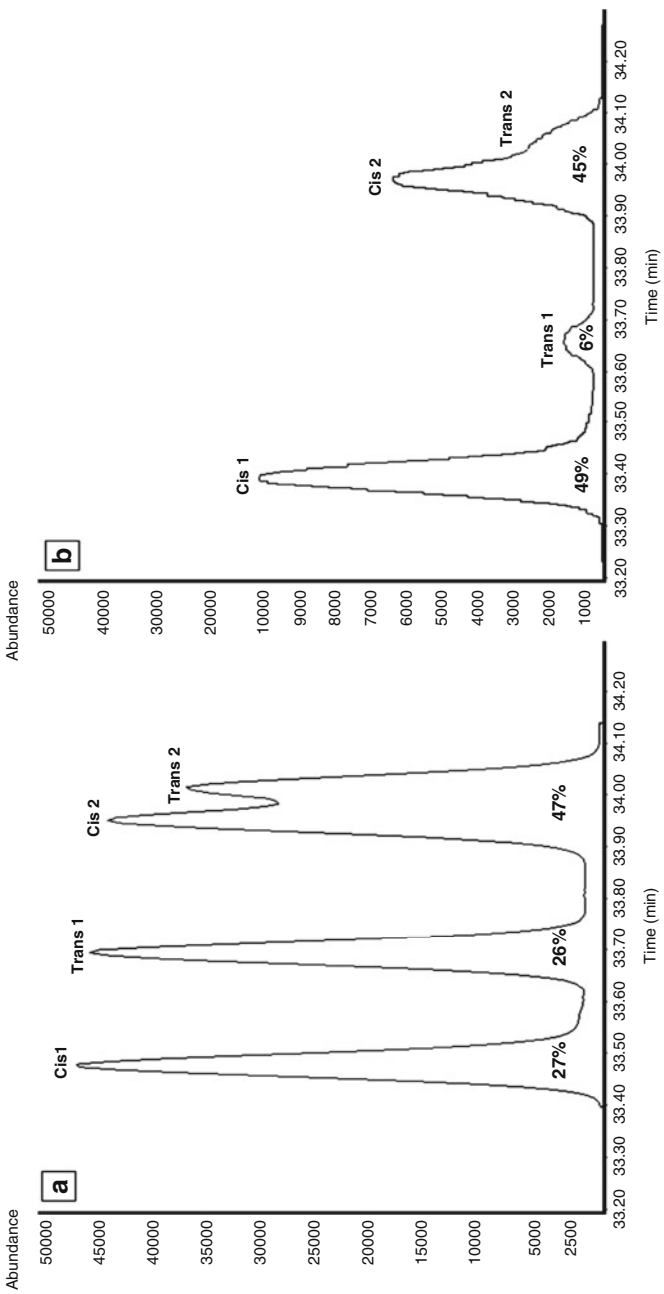

Fig. 7 (**a**) Cypermethrin isomeric profile and percentage found in a commercial racemic formulation; (**b**) profile and isomeric percentage in an egg sample after topical application of the formulation in hens. Analysis by gas chromatography–mass spectrometry in negative chemical ionization mode [93]

[107]. Therefore, food samples that present different profiles of stereoselectivity, which may include the most toxic and persistent isomers, should not be compared to reference values based on racemic formulations, since they are not equivalent. In this case, variation at enantiomeric levels found in food samples can result in an imprecise comparison between their contamination profile and the established limits for quality and food safety.

5 Conclusions and Trends

The desired effects attributed to pyrethroids are related to specific enantiomers. In this sense, studies have shown higher acute toxicity of active insecticide enantiomers to nontarget organisms, such as earthworms, zooplankton, fish and tadpoles. However, adverse effects, such as endocrine disruption and cytotoxicity, determined through in vivo and in vitro assays with fish and mammals have been reported in studies considering enantiomers with low or no insecticidal action.

Although previous studies point to a higher occurrence of *cis*-isomers in biological systems, considerable research remains to be performed on the persistence of pyrethroid enantiomers, their effects on sensitive organisms and the possible impacts on complex environmental functions, such as the degradation of pollutants in soils and at the base of the food chain in aquatic environments.

A possible action for minimizing environmental enantiomer overloads and the expected impacts on environmental and human health was proposed in Regulation (EC) No 1107/2009 from the European Community, which suggests the substitution of agrochemicals applied to crops containing a significant proportion of non-specific isomers [99]. In this direction, future policies aimed at sustainable innovation should be considered for companies that propose enantiomerically pure, safe and less persistent commercial formulations.

Although the persistence of pyrethroids is important for the maintenance of their insecticidal action for an extended period, which can vary widely (from hours to months) according to the compounds and the environmental conditions [8], the use of less persistent formulations in domestic environments should also be considered. However, studies are required to assess potential acute and chronic toxicity in a scenario of increased human exposure to pyrethroid metabolites and their degradation products.

With regard to feeding safety, studies are required to address the stereoselective behaviour of pyrethroids in food samples and the consequent dietary exposure to more persistent and more toxic isomers. This approach should include the established limits for food quality, as well as the behaviour and stability of these chiral compounds during the preparation and cooking steps up to the industrial food processing.

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Environmental Risks of Synthetic Pyrethroids Used by the Salmon Industry in Chile

Felipe Tucca and Ricardo Barra

Contents

Abstract Synthetic pyrethroids such as cypermethrin and deltamethrin have been widely used in Chile to treat sea lice on salmon since 2007. The environmental risks of aquaculture practices are evaluated through the use of several tools such as fugacity-based models for predicting environmental dynamics and the fate of pyrethroids after their release into the marine environment and the determination of

F. Tucca (✉)
Norwegian Institute for Water Research (NIVA) Chile, Puerto Varas, Chile

Departamento de Ecología y Biodiversidad, Facultad Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile
e-mail: f.tucca@gmail.com

R. Barra (✉)
Department of Aquatic Systems, Faculty of Environmental Sciences and EULA-Chile Centre, University of Concepcion, Concepcion, Chile
e-mail: ricbarra@udec.cl

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pyrethroid occurrence in environmental samples (i.e., water and sediment). For seawater, passive sampling devices (PSDs) are proposed as a good alternative for field monitoring. Finally, by means of ecotoxicological bioassays, the effects of pyrethroids on native biota were assessed. The results show that the application of pyrethroids may trigger some unintended risks to nontarget organisms, particularly copepods, since modeled and observed concentrations in water (dissolved phase) are in the range of fractions of ng L^{-1}, but higher cypermethrin and deltamethrin concentrations in sediment in the range of 1,323 and 1,020 ng g^{-1}, respectively, have been observed. These measured concentrations were in the range of concentrations toxic to native invertebrate species in Chile. We conclude that a stricter process should be followed when pyrethroids, particularly cypermethrin, are recommended for use in combating sea lice in the Chilean salmon farming industry. Risk assessment procedures and the establishment of stricter regulations on matters such as the maximum allowable concentrations around the cages when these pesticides are applied and recommended.

Keywords Aquaculture, Patagonia, Pyrethroids, Sea lice, Toxicity

1 Introduction

The salmon industry has become the driving force of aquaculture development in Chile. The high volume of salmonids produced by Chilean aquaculture has positioned the industry as an important exporter in the international market. However, salmon productivity in southern areas has been vulnerable to salmon lice infections and other environmental issues [1]. The occurrence of ectoparasitic diseases caused by sea lice called *Caligus rogercresseyi* [2] has forced to the industry to use chemotherapeutic alternatives that contribute to the control and prevention of salmon infections. In the 1990s, emamectin benzoate (Slice®) became the exclusive means of treatment for salmonids; however, studies evidenced a loss of sensitivity in sea lice [3–5]. Therefore, veterinary medicines have been required by the salmon industry [6–8]. Currently, the synthetic pyrethroids cypermethrin (Betamax®) and deltamethrin (AMX® and Deltafav®) are alternatives for treating ectoparasites of salmon. Nevertheless, it has been suggested that these pyrethroids have adverse consequences for marine biota that ought to be of concern (e.g., [9–13]). This chapter presents an overview of the occurrence, behavior, and potential environmental risks of pyrethroids currently used on salmon farms located in the northern Chilean Patagonia.

1.1 The Salmon Industry in Chile: An Overview

Due to the growing demand for protein for human consumption, aquaculture is recognized as an important food source for the global population. Fish farming accounts for the greatest share of aquaculture production, with Norway and Chile considered the biggest farmed salmon producers in the world [14].

Salmon farming started on an experimental level in the 1960s and became an industry in Norway in the 1980s, while in Chile it started in the 1990s. The emergence of salmon farming since the 1970s has changed the rules of the sea-farming sector, and Norway and Chile have been the main producers and exporters since 1997.

Aquaculture in Chile has grown exponentially since the early 1990s. Farmed salmon is the dominant species in terms of both harvest volume and export values. Salmon production reached 842,700 tons in 2018, with Atlantic salmon (*Salmo salar*) the most harvested species in the salmon industry, accounting for 75.1% of the total, followed by coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*), at 16.3% and 8.6%, respectively [15]. Salmon industry activity takes place mainly in the southern Patagonia regions of Chile, namely, Los Lagos and Aysén. Salmon farming is projected to expand into the most austral areas of the Magallanes Region (Fig. 1), in which only rainbow trout harvests are proposed. The Chilean National Fishery and Aquaculture Service (SERNAPESCA) and Undersecretariat for Fisheries and Aquaculture (SUBPESCA) are the agencies under the Ministry of Economy that establish the basis for regulating aquaculture activity, but the veterinary medicine market is controlled by the Ministry of Agriculture, specifically the Agricultural and Livestock Service (SAG), and the Ministry of Defense through the General Directorate of the Maritime Territory and Merchant Marine (DIRECTEMAR). The main function of DIRECTEMAR is to establish aquatic pollution control regulations.

1.2 Sanitary Consequences: The Sea Lice Issue

The exponential growth of aquaculture has been socially and economically impacted by the increased presence of sea lice on farmed salmon [16–19], which has required the use of chemicals to control and mitigate adverse consequences for fish. The action of ectoparasites on farms and wild fish has been widely described [20]. During parasitic stages in marine environments, sea lice may cause visible skin damage, hemorrhages, vulnerability to secondary infections, and stress-inducing mortality of host species.

In Chile, it has been reported that there is a high infestation pressure of the sea lice *C. rogercresseyi* [2] on production of the most harvested species, namely, *S. salar* and *O. mykiss*. Meanwhile, the species *O. kisutch* has been described as less susceptible to infection by ectoparasites [21–23]. Until 2007, emamectin benzoate

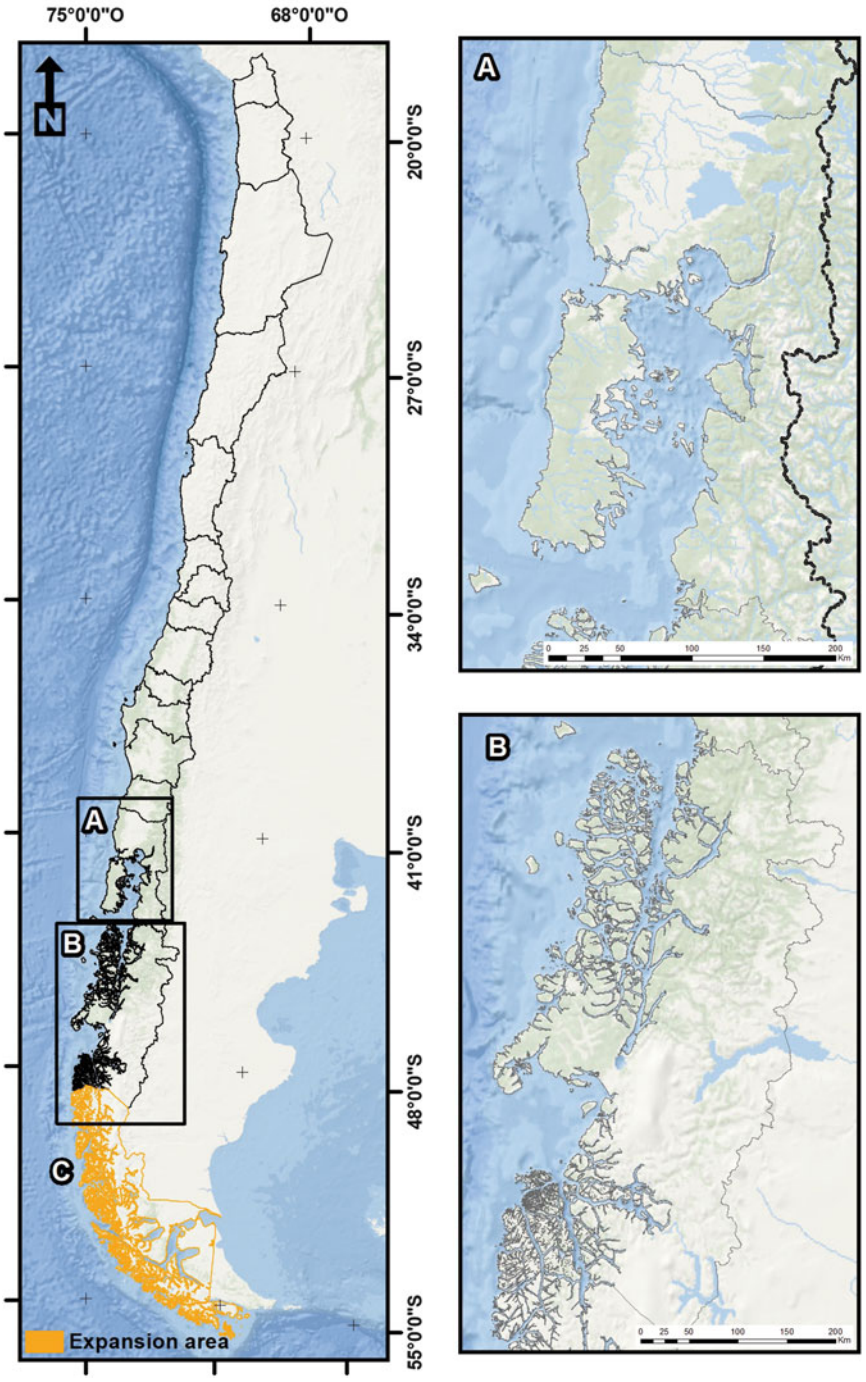


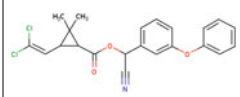
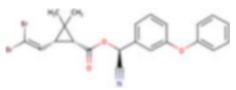
Fig. 1 Salmon farm expansion from Los Lagos (**a**) and Aysén (**b**) regions toward Magallanes (**c**) region, Chilean Patagonia

was an effective alternative authorized in Chile for the treatment of salmonids, but its effects were decreasing against sea lice. This brought Chilean authorities to choose other chemical alternatives and implement strategic periods of coordinated treatments using synthetic pyrethroids as the most effective means to prevent and control sea lice [23].

1.2.1 Addressing the *Caligus* Problem: Use of Pyrethroid Pesticides in the Salmon Industry

Synthetic pyrethroids are a group of antiparasitic drugs that are characterized as being highly hydrophobic (log $K_{OW} > 5$) and having low solubility in water (0.002–0.004 mg L^{-1}) and low volatility (Table 1). Their hydrophobic properties allow pyrethroids to be absorbed into the organic matter available in the water column and reach bottom sediment. In Chile, since 2007 cypermethrin and deltamethrin have typically been applied to treat sea lice infections on salmon farms, but these treatments were approved by authorities only in 2010 (SAG), due to increased resistance to other antiparasitic chemicals [3, 4]. However, over the years severe problems of ectoparasite resistance to pyrethroid treatments in Southern Chile have been reported [25, 26], even during synchronized sea lice treatments (i.e., bath treatments coordinated among neighboring farms), with lower adult lice levels, but juvenile stages less affected [27, 28]. There have been similar reports in Norway, where increased sea lice resistance has triggered pyrethroid use by the salmon farming industry [8, 29, 30].

Table 1 Physical–chemical properties of anti-sea lice pyrethroids

Properties[a]	Pyrethroids	
	Cypermethrin	Deltamethrin
Chemical structure		
CAS number	52315-07-8	52918-63-5
Chemical formula	$C_{23}H_{19}Cl_2NO_3$	$C_{22}H_{19}Br_2NO_3$
Molecular weight (g mol^{-1})	416.297	505.199
Water solubility at 25°C (g m^{-3})	0.004–0.041	< 0.0002
Vapor pressure at 25°C (Pa)	1.9×10^{-7}–2.75×10^{-6}	2.0×10^{-06}
Henry's law constant (Pa m^3 mol^{-1})	0.0195–0.080	12.60
Log K_{OW}	4.47–6.60	4.60–6.20
Log K_{OC}	2.36–5.54	3.66–4.21

[a]Data from *Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals* [24]

1.3 Synthetic Pyrethroids: Mode of Use

Currently, pyrethroids are applied through bath treatments using commercial product doses of 0.3 mL m^{-3} (active principle dose of 15 μg L^{-1}) for cypermethrin (Betamax®) and between 0.2 mL m^{-3} (active principle dose of 2 μg L^{-1}) (AMX®) and 0.3 mL m^{-3} (active principle dose of 3 μg L^{-1}) (Deltafav®) for deltamethrin in water. Suspended tarpaulins are used for these bath treatments, in which the fishnet is raised to a depth of no more than 4 m to subsequently apply the doses indicated above. The salmon exposure time to cypermethrin (Betamax®) is 30 min, while for deltamethrin it is between 30 and 40 min [31]. Once the treatments are released, their main mechanism of action on organisms involves interference in the central nervous system, generating an interruption in the transmission of nerve impulses between cells [32, 33].

The recommended treatment regime for sea lice using pyrethroids consists of a "relatively high concentration at the levels of micrograms per liter–short duration bath exposure" within skirted net pens, after which treatment water is released to disperse into the surrounding marine environment [34, 35]. While pyrethroids such as deltamethrin are highly effective treatments for ectoparasites such as sea lice, the implications for nontarget species such as migratory salmonids and other commercial species that traverse multiple aquaculture areas are currently unclear.

2 Exposure Assessment: Approaches to Assess the Risk of Pyrethroids in the Marine Ecosystem

The environmental fate of chemicals is determined by a combination of factors, of which the most important are those related to the nature of the compound and the environment. Physical and chemical properties define potential mobility and reactivity, while environmental variables determine the extent to which these potentials are manifested [36]. Under field conditions, environmental variables (e.g., temperature, pH value, wavelength and radiation intensity, air–water exchange, turbulence, organisms) are very complex to analyze and can produce significant changes in the environmental behavior of chemicals. Therefore, the use of physical–mathematical models is difficult in complex environmental chemistry, especially when a considerable number of details are required to successfully simulate the environment.

An alternative approach consists of developing simple, appraisable models that simulate environments, in which the environmental variables are standardized and reduced to their essentials (evaluative models). Initially, evaluative models were developed as a means to interpret and understand the trends that govern the movement of chemical substances in the environment. Over time, this approach has proved to be extremely reliable and versatile, to the extent that it has become applicable not only in theoretical scenarios, but in local situations as well, providing credible predictions at the actual-environment level. They tend to be very simple

models that require only a reduced set of input data. Additionally, they are based on conceptual outlines and easily understood, solvable algorithms, which produce results that are easily handled and of simple, practical use, to the point that they have been proposed for official procedures to evaluate chemical risks. Among the different approaches that constitute the theoretical foundations of the multimedia partition models, that derived from the fugacity concept has proved to be one of the most effective. The use of multimedia fugacity-based models is an approach that allows the estimation of the dynamics and fate of single pollutants in the environment [37–39]. Fugacity is defined as the chemical activity of a gas and expresses the tendency to escape from one compartment to another. In these thermodynamic models, the different behaviors of various chemical agents principally depend on their physical–chemical (intrinsic) properties, contributing to the development of a better interpretation and understanding of the fate, transport, and degradation of pollutants released into the ecosystem. Moreover, the use of this tool can be an important approach for conducting risk assessment and improving chemical management [40–42], in which predicting concentrations through multimedia fugacity-based models has proved to be effective according to measured environmental concentrations (e.g., [43–45]).

On the other hand, one of the issues of a risk assessment of synthetic pyrethroids in the marine environment is that they are used at very low concentrations that with dilution in the marine environment reach very low levels, on the order of ng L^{-1} units, which are actually very difficult to measure using traditional sampling methods (i.e., grab sampling). In this chapter a method based on passive sampling is introduced as a cost-effective way to address the analytical challenge of detecting hydrophobic chemicals in the aquatic environment for risk assessment purposes. More details are presented below (see Sect. 2.2.1).

2.1 Salmon Farm Models

The most prominent route of entry of veterinary medicines into the environment is direct discharge of aquaculture chemicals. Surprisingly, little attention has been paid in the open literature to chemical-based modeling efforts for aquaculture chemicals. Most physical-based models have been developed as tools to predict the distribution of particulate waste from fish farm cages to the seabed. These predictive particulate waste distribution models, through Geographic Information Systems (GIS), have enabled temporal deposition zones and salmon cage impacts on benthic ecosystems to be visualized [46, 47]. More sophisticated fish farm models such as the DEPOMOD model have independently described particle tracking and resuspension, benthic responses, and fish growth and biomass to assess the impact of salmon cages on marine environments [48–52]. Additionally, the DEPOMOD model has been validated to assess the deposition footprint of antiparasitic drugs added to feed after treatment of fish. In Scotland, the Scottish Environmental Protection Agency (SEPA) uses a regulatory DEPOMOD-based model (AutoDEPOMOD) to predict the concentration of in-feed antiparasitic medicine

residues in the sediment beneath fish farm zones [53]. In addition, this regulatory agency has developed a dispersion model in order to simulate the dispersing plumes of cypermethrin pyrethroid after multiple releases during bath treatments (BathAuto v5) [54]. However, for aquaculture there are not yet models that provide a comprehensive representation of diffusive and non-diffusive fluxes and fates of organic chemicals in multiple environmental compartments. Under field conditions, the sampling and analysis of chemicals is challenging; therefore, it is advantageous to describe the chemical dynamics in different environmental compartments using less complex multimedia fugacity models [39]. In fact, fugacity-based models have been widely used for chemical risk assessment purposes such as assessment of persistent organic pollutants (POPs) and emerging contaminants [41, 42], playing a key role in science [40]. Thus, such multimedia models could provide a good understanding of key transport processes, fates, and sinks of synthetic pyrethroids used in aquaculture after their release into the marine ecosystem. Ng et al. [55] reported a first approach, developing a fugacity-based dynamic one-compartment mass balance model, which was used to assess polybrominated diphenyl ether (PBDE) uptake on an individual salmon farm during a complete sea-cage production period.

A primary objective in environmental fate studies is to predict the concentrations of synthetic pyrethroids released into the environment, with respect to space and time variables. Our knowledge of the behavior of antiparasitic pyrethroids can be used to model the space and time domains once emissions are known or estimated. Each of the levels in a fugacity-based model allows different kinds of information to be obtained. Level I can indicate the major environmental compartments where the chemical goes and Level II gives an indication of the main loss process occurring in the chemical agent in the simulated environment and provides some insights into persistence and residence time, since time is involved. Level III gives an indication of the most important transfer process within the different environmental compartments, since a non-equilibrium condition is imposed. For environmental risk purposes, this chapter argues that multimedia fugacity-based models (Level III) could play a key role in helping determine the potential effects of synthetic pyrethroids within a risk assessment perspective.

2.1.1 Description Fugacity-Based Model

A multimedia fugacity-based box model for synthetic pyrethroids was developed to predict the dynamics and fate of typical bath treatments for salmon. Our fugacity model considers a distribution-based model incorporating all environmental compartments of interest (water, sediment, and fish), based on steady-state and non-equilibrium condition fluxes during treatments. Environmental data inputs and typical characteristics of salmon farms located in the Southern Chile are shown in Table 2.

Chemical partitioning was described by the thermodynamic criterion of fugacity (f). Theoretically, fugacity is related to environmental concentration (C, mol m^{-3}) by the equation $C = f\ Z$, with Z the fugacity capacity of chemicals for each

Table 2 Environmental data used in multimedia fugacity-based model for pyrethroids

Parameters[a]	Units	Value
Salmon farm scenario		
Maximum production	kg	~5,900,000
Number of salmon produced	–	1,550,000
Salmon mortality rate (productive cycle)	%	15
Salmon weight (e.g., *Salmo salar*)	kg	4.5
Salmon lipid fraction	%	10–15
Salmon excretion rate (k_E)	d^{-1}	0.0025
Salmon growth rate (k_C)	d^{-1}	0.003
Cages treated	–	20
Salmon cage volume (with tarpaulin)	m	30 × 30 × 7
Total salmon in 20 cages (salmon density)[b]	–	964,678 (~16 kg/m^3)
Environmental data		
Water volume	m^3	61,000,000
Water density (seawater)	kg m^{-3}	1,027
Velocity current (average)	cm s^{-1}	6.2
Depth (average)	m	61
Sediment volume	m^3	50,000
Sediment density	kg m^{-3}	1,500
Organic carbon fraction	–	0.91
Suspended particle concentration (average)	mg L^{-1}	1.1
Suspended particle volume	m^3	45
Suspended particle density	kg m^{-3}	1,500
Suspended particle fraction	–	7.3E-7
Resuspension rate	m^3 m^{-2} d^{-1}	2.6E-7
Deposition rate	m^3 m^{-2} d^{-1}	1.1E-6

[a]Data collected from sampled salmon farms located in Southern Chile, Los Lagos Region
[b]This parameter included the salmon mortality rate

compartment (mol m^{-3} Pa^{-1}). The diffusive fluxes (N, mol d^{-1}) between compartments are described by Eq. (1):

$$N = D\left(f_i - f_j\right) \tag{1}$$

where D is the transfer coefficient (mol h^{-1} Pa^{-1}) and f_i and f_j are the fugacities of compartments i and j, respectively. The differences between fugacities determine the direction of diffusive fluxes of pyrethroids in the marine environment. Meanwhile, non-diffusive transfer processes were calculated through Eq. (2):

$$N = \mathrm{GC} = \mathrm{GZ}f = Df \tag{2}$$

where G is the volumetric flow rate (m^3 h^{-1}) of the transported material. Diffusive and non-diffusive D values were summed for all transfer processes from

compartment i to j (D_{ij}) and compartment j to i (D_{ji}); the net flux from i to j then becomes, as shown in Eq. (3):

$$N = D_{ij}\, f_i - D_{ji} f_j \tag{3}$$

Reaction processes (D_r) in a compartment were described by Eq. (4):

$$Dr = k_i VC = k_i V_i Z_i f = D_r f \tag{4}$$

where k_i is the reaction rate constant (h^{-1}), which was calculated from pyrethroid half-life ($t_{1/2}$) for a specific compartment through the equation $k_i = 0.693/t_{1/2}$. V_i is the defined volume for each compartment i (m^3) in the area where the salmon farm is located.

The fugacities were calculated from D values defined for each environmental compartment [39]. Equations (5) to (7) were used to calculate fugacities in water (subscript 1), sediment (subscript 2), and fish (subscript 3), respectively.

$$\textbf{Water}: G_{a1}C_{b1} + f_2\, D_{21} + f_3\, (D_{31} + D_{e3}) = f_1\, (D_{12} + D_{r2} + D_{a2}) \tag{5}$$

$$\textbf{Sediment}: E_2 + f_1\, D_{12} = f_2\, (D_{21} + D_{r2} + D_{b2}) \tag{6}$$

$$\textbf{Fish}: E_3 + f_1\, D_{13} = f_3\, \left(D_{31} + D_{r3} + D_{g3} + D_{e3}\right) \tag{7}$$

where E is the emission rate (mol h^{-1}), G_a is the advection inflow rate ($m^3\ h^{-1}$), C_b is the advection inflow concentration (mol m^{-3}), and D_r, D_a, D_b, D_g, and D_e are the reaction rate, advection outflow rate, sediment burial rate, fish growth rate, and fish excretion rate, respectively (mol h^{-1}). The model assumed bath treatments with direct release of pyrethroids into a marine system.

Monte Carlo simulation was used to test the sensitivity and contribution to variance in the multimedia model, in which the most influential parameters were identified. The simulation was carried out to assess the uncertainty of predictions based on the probability distributions for input parameters such as salmon density in cages (15–17 kg m^{-3}), current velocity (6.2 ± 3.0 cm s^{-1}), organic fraction in sediment (0.03 ± 0.54), concentration of suspended particles (4.7 ± 2.3 mg L^{-1}), and depth of the study area (40–80 m). The simulations were run for 100,000 trials using Crystal Ball 11.1.1 software [56].

2.1.2 Mass–Balance Model on Salmon Farms

The use of multimedia fugacity-based models has proven to be a good approach according to measured environmental concentrations [44]. Figure 2 shows a comparison between predicted and measured concentrations in water and sediment compartments. Our estimations show that predicted water concentrations (4.5–8.8 ng L^{-1} and 2.5–5.9 ng L^{-1} for cypermethrin and deltamethrin,

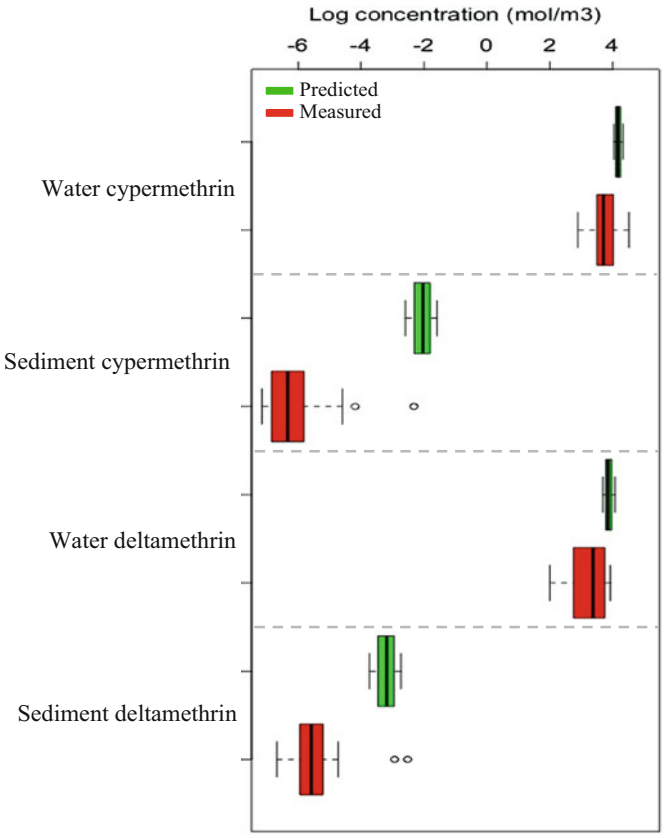


Fig. 2 Measured and modeled concentrations of cypermethrin and deltamethrin used to treat salmon

respectively) were consistent with pyrethroid concentrations measured after treatment of salmon. Detected water concentrations around salmon cages were quantified using an ethylene–vinyl acetate (EVA) copolymer passive sampler. Water concentrations ranged from 0.3 to 13.6 for cypermethrin and 0.1 to 4.3 for deltamethrin (see Sect. 2.2.1).

In contrast, estimated sediment concentrations were slightly overestimated relative to measured concentrations under salmon cages; however, they were close to the higher sediment levels detected in Southern Chile: 1,323 ng g^{-1} and 1,020 ng g^{-1} for cypermethrin and deltamethrin, respectively (see Sect. 2.2.2).

For all predicted water and sediment concentrations, the model always presented a greater concentration than the measured concentration. This could suggest that the model predicts the worst-case scenario, essential for assessing the environmental risks of chemicals used in the salmon industry.

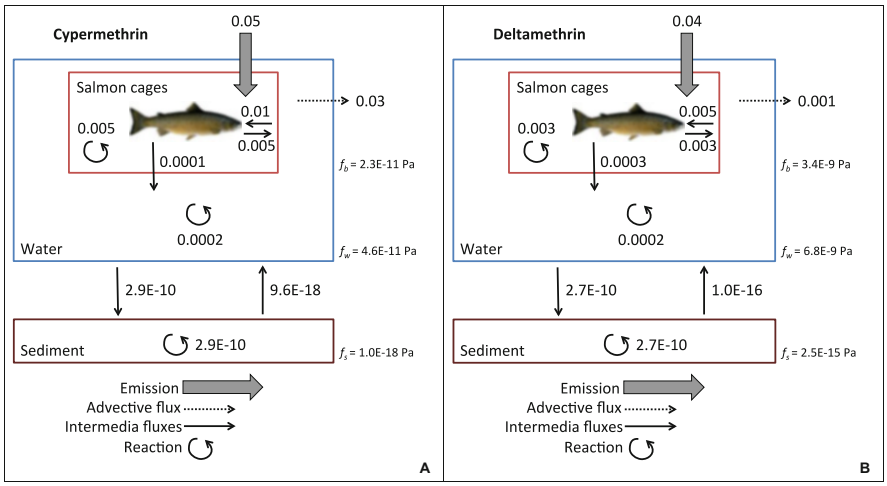


Fig. 3 Estimated transport and reaction rates (mol h^{-1}) of cypermethrin (**a**) and deltamethrin (**b**) to be released into marine ecosystems. Fugacities in fish (f_b), water (f_w), and sediment (f_s) are also reported in this multimedia fugacity-based model

The mass–balance model reported intercompartmental transport and reaction rates for pyrethroid dominated by advective flux (Fig. 3). Once pyrethroids are transported in the water column, because of their high affinity for suspended solids, they are deposited in the bottom sediment, suggesting a little mobility in sediment (i.e., low fugacity). However, in salmon synthetic pyrethroids appear to be rapidly metabolized and thus eliminated by excretion [7, 8].

2.2 *Sampling of Pyrethroids on Salmon Farms*

From November to December 2014 (spring–summer) and April to July 2015 (autumn–winter), monitoring campaigns were carried out on four salmon farms located in the northern Patagonia of Chile, specifically Chiloé Island (Fig. 4). For each monitoring campaign, salmon farms were treated with specific synthetic pyrethroids, and sediment samples were taken. More details on sampling and environmental characteristics of salmon farms are shown in Table 3. In the study areas, sediment samples were collected using a Van Veen Grab Sampler (462 cm^2) at distances of 0, 100, and 500 m in a cross design. Control samples without salmon farm treatments were also collected. In addition, passive samplers in water were deployed around salmon cages to detect the dissolved concentration of pyrethroids. More details on water and sediment sampling around salmon cages are presented in Sects. 2.2.1 and 2.2.2, respectively.

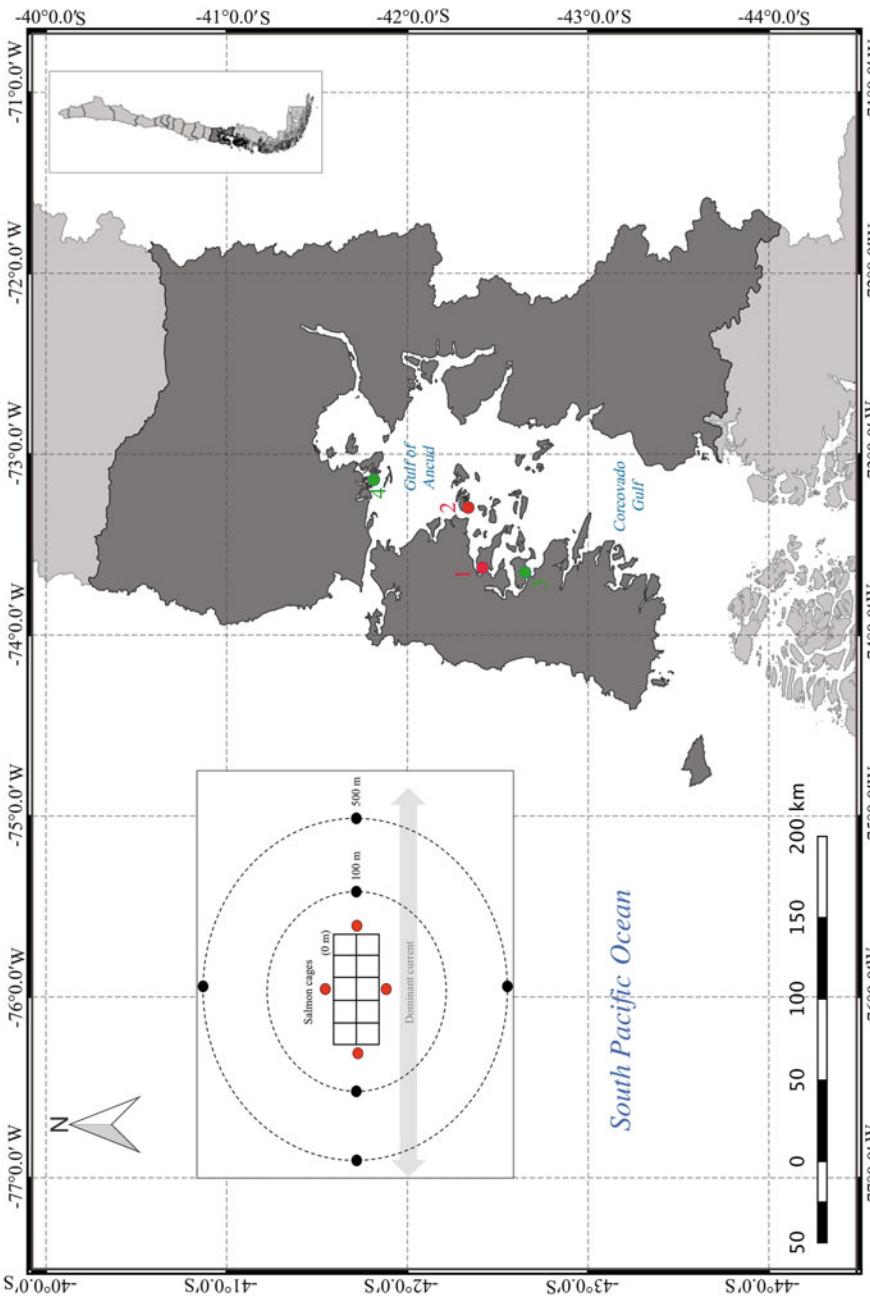


Fig. 4 Locations of salmon farms (1–4) and monitoring strategy used during 2014 (red numbers) and 2015 campaigns (green numbers) in the northern Chilean Patagonia

Table 3 Environmental characteristics and specific pyrethroid treatments used by salmon farms located in the northern Chilean Patagonia

Data	First campaign November–December 2014		Second campaign April–July 2015	
	Salmon farm 1 (SF-1)	Salmon farm 2 (SF-2)	Salmon farm 3 (SF-3)	Salmon farm 4 (SF-4)
Environmental conditions				
Water temperature (°C)	11.3 ± 0.2	11.0 ± 0.1	11.7 ± 0.1	11.4 ± 0.03
Salinity (PSU)	31.8 ± 3.1	32.5 ± 0.1	33.5 ± 0.03	32.3 ± 0.06
Dissolved oxygen (mg L^{-1})	8.2 ± 0.7	8.5 ± 0.5	4.2 ± 0.2	5.8 ± 0.5
pH	8.4 ± 0.03	8.4 ± 0.02	8.0 ± 0.01	9.7 ± 0.0
Depth (m)	40	80	80	30
Current velocity (cm s^{-1})	7.6 ± 2.9	8.1 ± 6.4	2.9 ± 1.4	9.0 ± 3.8
Sediment grain size	Very fine sand to fine sand (62.5–250 μm)	Fine sand (125–250 μm)	Fine sand to medium sand (125–500 μm)	Medium sand (250–500 μm)
Organic matter (%)	1.2 ± 0.2	1.5 ± 0.3	1.6 ± 0.4	1.1 ± 0.3
Salmon farming treatments				
Location	42° 25′ 42.54″S 73° 37′ 19.08″W	42° 18′ 25.36″S 73° 18′ 25.63″W	42° 39′ 59.23″S 73° 37′ 50.16″W	41° 48′ 35.32″S 73° 09′ 56.94″W
Species farmed	Atlantic salmon	Atlantic salmon	Coho salmon/rainbow trout	Atlantic salmon
Formulation used	Deltafav®	Betamax®	Deltafav®	Betamax®
Active ingredient	Deltamethrin	Cypermethrin	Deltamethrin	Cypermethrin
Doses (μg L^{-1})	3	15	3	15

2.2.1 Passive Sampling in Water

Passive sampling devices (PSDs) in water may play a key role in regulatory management as water quality monitoring tools [57, 58]. For decades many PSDs have been designed to detect pollutants in water (e.g., [57, 59–62]), sediment (e.g., [63, 64]), and air (e.g., [65–67]), with these studies focusing on the detection of legacy POPs and contaminants of emerging concern [68]. Time-integrative sampling with PSDs deployed in the field may be a useful and cost-effective method to determine the bioavailability of pollutants in different places [69, 70]. Some

hydrophobic organic pollutants are often hardly detected with conventional methods such as grab sampling, making it difficult to detect trace levels of pollutants. Robust data obtained from PSDs, with previous calibration and analytic methods performed in the laboratory, allows trace levels to be determined. These procedures allow the passive sampling method to be validated and increase confidence in the field sampling.

In theory, passive samplers are devices that are based on the initial uptake of dissolved pollutants (dissolved free fraction) to the receiver medium (passive sampler), given the different concentrations in the water and sampler, called the *kinetic phase*. The linear uptake continues until the *curvilinear phase* is reached. Finally, as exposure time increases, the net flow of analytes from the water to the sampler continues until equilibrium – called the *equilibrium phase* – is reached.

The kinetic exchange between the passive sampler and the sampled medium can be described by a first-order Eq. (8):

$$C_{s(t)} = C_{\mathrm{W}}\, K_{\mathrm{SW}} \left(1 - \mathrm{e}^{-k\, t}\right) \tag{8}$$

where $C_{s(t)}$ is the concentration of the chemical in the sampler at exposure time t, C_{W} is the chemical concentration in the aqueous phase, and K_{SW} is the sampler–water partition coefficient. Once the equilibrium between the sampler and water phases is reached, C_{W} is estimated by Eq. (9):

$$C_{\mathrm{W}} = \frac{C_s}{K_{\mathrm{EVA-W}}} \tag{9}$$

PSDs in the kinetic phase can be often affected by diverse environmental factors during their exposure in water, interrupting the sampler's contaminant uptake rate. Environmental factors such as temperature, salinity, pH, hydrodynamics, and biofouling may influence uptake and the equilibrium between the sampler and the aquatic medium [60, 68, 71]. For instance, the presence of biofouling in the aquatic system (bacterial and/or algal biofilm) could be critical in the uptake of the contaminants by the sampler. Biofouling could interrupt the uptake kinetics of organic compounds to passive samplers due to (1) increased mass transfer resistance, (2) increased thickness, or (3) damage to the passive sampler surface [60].

A good alternative for PSDs in water is the copolymer ethylene–vinyl acetate (EVA). EVA has been identified as effective at measuring bioavailable pollutant fractions and has been used to monitor different environmental matrices [63, 66, 72–74]. It is a flexible thin-film copolymer, which can be easily processed in the laboratory, as it is adapted to different substrates (e.g., glass fiber filters or glass marbles). Additionally, it is resistant to high pressures, temperatures, and UV radiation and is also waterproof, making it an efficient polymer for capturing pollutants in the aquatic environment [72]. In Chile, few studies have used PSDs to detect hydrophobic pollutants in marine ecosystems, one of which is our study on the occurrence of cypermethrin after treatments in salmon cages [73].

Pyrethroid Occurrence in Seawater

Based on the theory mentioned above and in order to ascertain the concentration of the dissolved fraction of pyrethroids, a study using passive water samplers in the field was carried out in Southern Chile. Thin-film (~7–10 μm) EVA samplers were deployed (~4 m distance) around four different salmon farms located in the northern Chilean Patagonia, as shown in Fig. 4. The study was based on the laboratory methodology and field deployment strategy previously reported by Tucca et al. [73]. As a first approach, EVA–water partition coefficients ($K_{EVA\text{-}W}$) for cypermethrin and deltamethrin were estimated according to the plot constructed by St. George et al. [72], as shown in Eq. (10):

$$\text{Log } K_{EVA-W} = 1.04 \text{ Log } K_{OW} + 0.22 \tag{10}$$

with this plot constructed under laboratory conditions. The EVA sampler presented a good relationship with the octanol–water partition coefficient (K_{OW}) using pesticides and PCBs [72, 75]. The estimation of C_W in the field was calculated using the following Eq. (11):

$$N = K_{EVA-W}\, V_{EVA}\, C_W \left[1 - \exp\left(\frac{R_s\, t}{K_{EVA-W}\, V_{EVA}}\right)\right] \tag{11}$$

where N is the amount of pyrethroids in the sampler, V_{EVA} is the volume of the EVA copolymer, and R_S is the sampling rate of the EVA for time t deployed in the field (7 days). Based on laboratory calibration, an R_S of 0.72 L d^{-1} was used in this study. More adjustment conditions through in situ calibrations (i.e., flow, salinity, and temperature conditions) should be considered for future studies to establish sampling parameters for the EVA sampler [76].

The cypermethrin and deltamethrin concentrations in water detected by passive samplers in salmon cages are shown in Table 4. Pyrethroid concentrations in seawater ranged between 0.05 and 13.62 ng L^{-1}. Deltamethrin means of 1.11

Table 4 Water concentrations of synthetic pyrethroids after treatments on salmon farms located in Southern Chile

Salmon farm	Treatment	Water concentration (ng L^{-1})		
		Mean	SD (n)	Range
SF-1	Deltamethrin	1.11	± 0.88 (5)	0.05–2.43
SF-2	Cypermethrin	3.40	± 4.75 (7)	0.33–13.62
SF-3	Deltamethrin	2.54	± 2.16 (3)	0.12–4.28
SF-4	Cypermethrin	ND	ND	ND

ND not detected, *SF* salmon farm, *n* number of passive samplers with detected pyrethroids after deployment in the field

(±0.9) ng L^{-1} and 2.54 (±2.2) ng L^{-1} at salmon farms 1 and 2 were observed, while a cypermethrin mean of 3.40 (±4.8) ng L^{-1} was recorded at salmon farm 3. No cypermethrin concentration in seawater was detected at salmon farm 4. These levels were within an order of magnitude (ng L^{-1}) of those detected using grab sampling at several times during cypermethrin and deltamethrin treatments on salmon farms [8, 34, 35]. It has been reported – and was observed in this study – that pyrethroids decrease rapidly once released from a cage site after treatment. Due to their low water persistence and rapid dispersion in seawater, passive samplers may be useful time-integrative tools to detect the dissolved fraction of organic chemicals bioavailable in seawater after treatment of fish.

2.2.2 Pyrethroid Occurrence in Sediment

The northern Chilean Patagonia (Los Lagos Region, 41° 28′ 18″S; 72° 56′ 18″W) is characterized by the presence of active aquaculture. However, there are few reports on the environmental occurrence of pyrethroids in the northern Chilean Patagonia originating in the salmon farming industry [77, 78]. Tucca et al. [77] reported cypermethrin concentrations in sediment (dry weight, d.w.) based on a sampling strategy around salmon cages (radius <100 m) in accord with dominant currents and tidal influences. Cypermethrin concentrations ranged between 18.0 and 1,323.7 ng g^{-1}, while deltamethrin was not detected on the sampled salmon farm. In addition, Placencia et al. [78] reported deltamethrin concentrations in surface sediment samples collected on a cruise among 12 stations distributed throughout the continuous waterways of the fjords–Chiloé inner sea. Detected deltamethrin concentrations in sediment (d.w.) proved to be higher than those reported around salmon farms, with ranges between 390 and 1,020 ng g^{-1}. These results suggest that deltamethrin-accumulating areas are dominated by the hydrodynamics in the study area, which act as a long-distance transport pathway for this antiparasitic medicine.

Pyrethroids in sediment samples (1 g, d.w.) were extracted using the methodology described by Feo et al. [79]. Briefly, sediment mixed with powdered copper (0.5 g) was extracted twice with *n*-hexane/dichloromethane (2:1, 20 mL) in an ultrasonic bath for 15 min at room temperature. Then, extracts were cleaned using a Florisil cartridge (2 g/15 mL). Previously, the cartridges were conditioned with ethyl acetate/dichloromethane (2:1). The cartridges were eluted with 50 mL of ethyl acetate until reaching full concentration. The samples were concentrated using a rotary evaporator and reconstituted with 250 μL of ethyl acetate. Pyrethroids were quantified by GC-NCI-MS [79].

Pyrethroid concentrations detected in the sediment of four salmon farms located in the northern Chilean Patagonia are shown in Table 5. On salmon farms 1 (SF-1) and 3 (SF-3), where deltamethrin was used as a treatment of fish, mean levels of 1.55 (±1.19, $n = 21$) ng g^{-1} and 1.12 (±1.69, $n = 24$) ng g^{-1}, respectively, were found. Lower levels of cypermethrin were observed, with mean concentrations of 0.70 (±1.06, $n = 15$) and 0.08 (±0.09, $n = 18$) on SF-2 and SF-4, respectively. Higher concentrations of both pyrethroids were measured within a radius of 500 m, with the

Table 5 Pyrethroid concentrations (ng g^{-1}, d.w.) in sediment from the northern Chilean Patagonia

Farm	Treatment	Distance (m)	Mean ± SD (*n*)	Range
SF-1	DE	0	2.15 ± 1.21 (4)	1.16–3.83
		100	2.23 ± 1.56 (6)	0.46–4.67
		500	0.96 ± 0.59 (11)	0.37–2.41
		0–500	1.55 ± 1.19	0.37–4.67
SF-2	CP	0	1.48 ± 2.28 (3)	0.13–4.11
		100	0.52 ± 0.77 (6)	0.13–2.09
		500	0.49 ± 0.12 (6)	0.36–0.71
		0–500	0.70 ± 1.06	0.13–4.11
SF-3	DE	0	0.72 ± 0.78 (6)	0.07–2.04
		100	0.71 ± 0.65 (7)	0.09–1.98
		500	1.60 ± 2.35 (11)	0.15–6.24
		0–500	1.12 ± 1.69	0.07–6.24
SF-4	CP	0	0.06 ± 0.05 (4)	0.02–0.13
		100	0.06 ± 0.04 (3)	0.03–0.10
		500	0.10 ± 0.11 (11)	0.02–0.37
		0–500	0.08 ± 0.09	0.02–0.37

Table 6 Comparative analysis of pyrethroid concentration levels detected in this study and those in previous published reports

Pyrethroid	Country	Concentration (ng g^{-1}, d.w.)	Reference
Cypermethrin	Scotland	0.03–7.20	[80, 81]
	Norway	<15.00	[29]
	Chile	18.00 to 1,323.70	[77]
	Chile	**0.02–4.11**	**This study**
Deltamethrin	Norway	<15.00	[29]
	Chile	<10.40	[77]
	Chile	390.00–1,020.00	[78]
	Chile	**0.07–6.24**	**This study**

Bold values are the data obtained in the frame of this research, while the other data are from the literature search

pyrethroid deposition under cages dominated mainly by the hydrodynamics of each study site. These pyrethroid concentrations were comparable with those in previous studies (Table 6), with cypermethrin ranges close to those reported in Scotland [80, 81], but lower than those reported in Chile.

3 Effect Assessment: Nontarget Marine Species Sensitivity

3.1 Studies on the Effects of Pyrethroid Insecticides on Native Organisms

Effects of pyrethroids on nontarget marine species have been widely reported in literature (e.g., [7, 8, 13]), including lethal and chronic copepod [9, 10], crustacean [12, 82], and bivalve responses [83, 84]. Furthermore, it is known that low doses of pyrethroids can be highly effective on aquatic organisms, with crustaceans the group that is most vulnerable to the action of these chemicals. Pyrethroids are recognized as slightly toxic to birds and mammals [32]. Moreover, pyrethroids are unlikely to be accumulated in fish and aquatic food chains since they are rapidly metabolized [7, 8].

Synthetic pyrethroids used as chemotherapeutic treatments on farmed fish can lead to potential negative environmental effects and harm nontarget organisms (e.g., [7, 8, 12, 13, 77, 78]), including even commercially important crab species in larval stages [82]. In Chile, there was a discussion on the impacts of pyrethroids on mussel physiology, since salmon and shellfish farms are established in the same areas, meaning that cultured shellfish are potential nontarget receptors of pyrethroid treatments.

4 Risk Assessment

4.1 Assessing the Risks of the Use of Pyrethroids in the Chilean Marine Environment

To conduct a risk assessment, two methodological schemes are proposed, the first a deterministic approach, as required by Chilean authorities, and the second a probabilistic approach. Risk assessment procedures basically consist of both an exposure assessment, that is, a determination of the Predicted Environmental Concentrations (PEC, predictive model) or Measured Environmental Concentration (MEC, experimental field measurements) of pyrethroids and a comparison these data with ecotoxicological thresholds such as the predicted no-effect concentration (PNEC) for different species from different trophic levels by calculating a risk quotient (RQ) shown in the following Eq. (12)

$$\mathrm{RQ} = \frac{\mathrm{PEC\ (or\ MEC)}}{\mathrm{PNEC}} \tag{12}$$

PNEC may be derived from the experimental no-observed effect concentration divided by an application factor (AF). This AF depends on the quality of the information used to predict the PNEC and is usually a value ranging from 10 to 1,000 [85]. If the RQ is higher than 1, there is environmental risk, while if it is

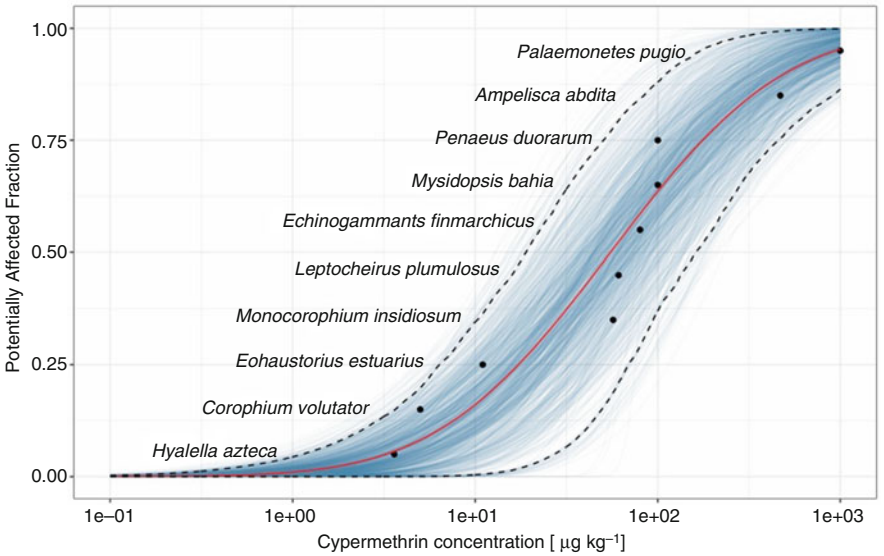


Fig. 5 Sensitivity species distribution (SSD) of benthic invertebrate organisms exposed to cypermethrin pyrethroid

below 1, there is no environmental risk. By tradition, the ecotoxicological threshold used is the no-observed effect concentration (NOEC) value corresponding to the species most sensitive to the assessed chemical based on laboratory tests.

A second approach that may be used is the probabilistic estimation of risk is based on a species sensitivity distribution (SSD). In our view, this is a more robust approach since it is based on the complete distribution of the sensitivity of different species to the chemical and the calculation, within the distribution, of the concentration impacting 5% of the total species considered in the analysis. Therefore, a new way of calculating the risk quotient is the same as that in the Eq. (12), but here PNEC is the predicted value affecting the 5% of the all species tested, including native organisms. SSD follows a log logistic curve, as shown in Fig. 5. Meanwhile, Table 7 presents a comparison of the RQs calculated using the two methods for both synthetic pyrethroids. It can be observed that the probabilistic method provides higher-risk values (i.e., RQ $>$ 1,000), but in both cases, risk is predicted for nontarget marine organisms, especially invertebrates.

In Chile, there is no maximum allowable concentration (MAC) of these antiparasitic chemicals used by salmon farms; therefore, the risk values estimated with both methods have no regulatory effects. There is only a SAG requirement (i.e., SAG Decree 665, 2010) that field studies be conducted on a case-by-case basis when the deterministic RQ presents values greater than or equal to 1,000.

Table 7 Comparative analysis of deterministic and probabilistic methods used for the risk assessment procedures

	MEC_{max}[a]		PNEC[b]				Risk characterization[c]			
			Water (ng L^{-1})		Sediment (ng g^{-1})		Water		Sediment	
Pyrethroid	Water (ng L^{-1})	Sediment (ng g^{-1})	Determ.	Prob.	Determ.	Prob.	RQ_{determ}	RQ_{prob}	RQ_{determ}	RQ_{prob}
Cypermethrin	13.62	4.11	5	2.15	5	3.25	**2,724**	**6,335**	**822**	**1,265**
Deltamethrin	4.28	6.24	15	12.80	0.54	0.39	**285**	**334**	**11,556**	**16,000**

Bold values are the data obtained in the frame of this research, while the other data are from the literature search

[a]Maximum measured water and sediment concentrations recorded in the field after treatment of fish (see Sect. 2.2)

[b]Ecotoxicological data were obtained from open literature and local species tested in the laboratory under controlled conditions. For the deterministic method, the most sensitive species exposed to pyrethroids in water ($LC_{50\text{-}96h}$) were crustaceans such as *Acartia tonsa* (cypermethrin) [9] and *Tisbe battagliai* (deltamethrin) [10], while in sediment they were *Corophium volutator* (cypermethrin) [86] and *Eohaustorius estuarius* (deltamethrin) [11]. For the probabilistic method, the hazardous concentration (5th percentile or HC_5) was derived from species sensitivity distribution (SSD) curves

[c]An application factor of 1,000 was used to calculate RQ values (all acute responses)

5 Concluding Remarks

While the principles of risk assessment have long been a part of international environmental regulations, chemical risk assessment in Chile is still in its early stages of development. Southern Chile is an area with an actively developing salmon farming industry, and the country is the world's second largest producer, after Norway. One of the challenges posed by the rapid growth of the salmon farming industry is the presence of diseases and parasites that affect salmon production such as the copepod *Caligus rogercresseyi*, commonly known as "sea lice." To combat sea lice, a series of pesticide chemicals such as cypermethrin and deltamethrin pyrethroids are used, which are applied through bath treatments. The use of chemicals in salmon farming is subject to a risk assessment procedure based on VICH regulations. This procedure must include the determination of a risk quotient (RQ) between predicted environmental concentrations and no-observed effect concentrations (NOECs) for local marine species. Thus, a comprehensive approach adapted to Chilean conditions for an adequate risk assessment of such chemicals is required.

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Bioavailability and Bioaccumulation of Pyrethroid Insecticides in Wildlife and Humans

Ò. Aznar-Alemany and E. Eljarrat

Contents

Abstract Despite the initial assumption that pyrethroid insecticides are "ideal" because they do not bioaccumulate and because they are able to be metabolized by mammals, recent studies have showed the opposite. Based on desorption kinetics from sediment, cyfluthrin has been reported as the most bioavailable compound, while λ-cyhalothrin was the less bioavailable. Bioaccumulation has been reported for several species. Franciscana dolphins from Brazil showed pyrethroid levels of 7.04–68.4 ng/g lw. A trend of levels connected to the age of dolphins was observed. Striped dolphins from the Spanish Mediterranean had a mean total concentration of 300 $\pm$ 932 ng/g lw. Pyrethroid levels in wild Iberian river fish were 12–4,940 ng/g lw. Pyrethroid profiles possibly reflected the local use of pesticides, and interspecies profile variation for fish was reported. While bioavailability of pyrethroids seemed considerably lower than that of POPs, concentrations of pyrethroids in striped dolphins and Iberian fish were comparable or higher than those of some POPs such as flame retardants. Mean total pyrethroid levels in unhatched eggs from wild birds collected in Spain were 1.93–162 ng/g lw,

Ò. Aznar-Alemany and E. Eljarrat (✉)
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain
e-mail: eeeqam@cid.csic.es

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depending on the species and their feeding habits. Pyrethroid levels in human milk samples were 87–1,200 ng/g lw for a rural area in Mozambique, where they are used against the malaria vector, and 1.45–24.2 ng/g lw for urban and rural areas of Colombia, Spain and Brazil. The contamination in milk decreased exponentially with parity, supporting the hypothesis of maternal transfer of pyrethroids. The maternal transfer of pyrethroids has been observed using several tissues from mothers and foetuses of dolphins. Isomer-specific accumulation or metabolization of pyrethroids has been assessed with somewhat consistent results, although analysing environmental samples from the areas where biological samples are collected would allow more accurate observations.

Keywords Bioaccumulation, Bioavailability, Maternal transfer, Metabolization, Pesticides, Pyrethroids

Abbreviations

ADI	Acceptable daily intake
DDT	Dichlorodiphenyltrichloroetane
EDI	Estimated daily intake
EF	Enantiomeric factor
EPA	Environmental Protection Agency
f/m	Foetus-to-mother
HBB	Hexabromobenzene
HBCD	Hexabromocyclododecane
K_{ow}	Octanol-water partition coefficient
lw	Lipid weight
OPFR	Organophosphorus flame retardant
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
R	Diastereoisomeric factor
WHO	World Health Organization
ww	Wet weight

1 Introduction

Pyrethroids are commonly and extensively used in agronomics, on pets and cattle, as domestic insecticides and for health purposes against lice, scabies or vectors of diseases such as typhus or malaria [1].

Pyrethroids were the alternative to organochlorines and organophosphates because of their low toxicity and persistence, usually lower than 90 days [2]. However, they are found in environmental samples, such as sediments and water [3, 4], food [5, 6], mammals [7, 8] and humans [9, 10].

Agronomics should be an important source for the introduction of pyrethroids in the environment. Conversely, it has been reported that the occurrence of pyrethroids in rivers caused by agronomics fluctuates depending on their application [3]. Moreover, their use in agronomics has been banned in some countries with legislation such as the Council Directive 91/414/EEC. On the other hand, they are commonly used in industrial and domestic sectors. The United States Environmental Protection Agency (EPA) Pesticides Industry Sales and Usage 2008–2012 Market Estimates estimated that in 2012 between 450 and 1,360 t of pyrethroid active ingredient were used only in the US home and garden market sector. Hence, domestic and urban applications may be an important source [11].

Benthic organisms can be exposed to pyrethroids via ingestion or contact with contaminated sediment particles or from interstitial water [7]. Fish can absorb pyrethroids either through their gills due to their lipophilicity or through food webs.

All things considered, pyrethroids are still generally regarded as safe as fish can oxidate them and mammals can hydrolyse them into non-toxic metabolites [12, 13]. Most studies on exposure have been based on the analysis of these metabolites in urine samples. This chapter reports data of the actual pyrethroids accumulated in biota samples, including humans.

2 Bioavailability

Pyrethroids are applied for pest control in agricultural and urban areas. They are easily adsorbed to sediment due to their very low water solubility (of a few μg/L) and high hydrophobicity (with logarithms of their octanol-water partition coefficient (K_{ow}) ranging from 5.7 to 7.6) [14].

Bioavailability plays a key role in sediment toxicity [15, 16]. Desorption of chemicals from sediment occurs in different kinetic stages [17, 18]. There is a simple method to assess the availability of contaminants associated with sediment and, therefore, the fraction of them that is bioavailable [19]. This method uses Tenax, a polymeric sorbent, in solid-phase extraction to measure the rate of mass transfer from the sediment to the Tenax. Tenax has been applied to determine desorption of contaminants like dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) [20, 21].

Many publications report that bioaccumulation levels are not a good estimate of bioavailability for organic compounds that can be metabolized [22]. Additionally, toxic compounds would kill sensitive species after exposure [23, 24]; thus toxicity endpoints have been used with sensitive species to assess the bioavailable pyrethroids in sediment samples [25].

A few studies have used Tenax to evaluate the bioavailability of pyrethroids [25–27]. Pyrethroids were found not to be very bioavailable to sediment-dwelling organisms such as *Lumbriculus variegatus* and to have a low toxicity to *Hyalella azteca*. Additionally, ageing time showed no significant influence on bioavailability; for instance, desorption decreased quickly over contact time meaning bioavailability diminished accordingly. However, these studies were mostly limited to chemical analyses. Combining Tenax desorption kinetics with toxicity response could shed a light on the pyrethroid fraction that is bioavailable and can affect the organisms [25, 28]. The toxicity of sampled sediments appears to be better predicted when confronted with measured residue levels from Tenax extracts [25].

A different study evaluated the bioavailability of pyrethroids in sediments with different organic carbon contents using Tenax extractions [29]. Toxicity experiments were performed using *Daphnia magna*, which is extremely sensitive to λ-cyhalothrin and deltamethrin, as a surrogate sediment toxicity test organism [30, 31]. As a planktonic organism that lives on the water column, *Daphnia magna* is exposed to the bioavailable pyrethroid fraction in the water phase rather than the fraction bound to sediment particles.

Two sediment samples free of pyrethroids were collected along the Ebro River (north-east of Spain) far away from agricultural, industrial and highly urbanized areas, and toxicity experiments were performed on sediment from a pristine water reservoir located in Huesca (north-east of Spain) with no sources of pollution [29]. Rapid desorption took place in the first 30 h of desorption, while slow desorption was observed from hour 72 to 432. The slow desorption would represent the fraction of contaminant that is strongly bound to the sediment's organic matter. Bioavailability increased with the decrease in organic carbon content, which had previously been reported for cypermethrin [32]. The percentage of desorption was 10–20% for sediment I (5.8% of organic content) and 15–40% for sediment II (2% of organic content). The percentages of desorption were ranged between 4 and 17% for cyfluthrin, cypermethrin, fluvalinate and phenothrin in sediment I and between 7 and 36% in sediment II [29]. Furthermore, the kinetic constant for the first 6 h of desorption was also greater for sediment II. Conversely, the desorptions of bifenthrin, λ-cyhalothrin and deltamethrin were very similar for both sediments, 3–22%.

Coincidentally, cyfluthrin, with the second highest log K_{ow} of the selected pyrethroids, was observed to be the most bioavailable compound, while λ-cyhalothrin, with the lowest log K_{ow}, was the less bioavailable of the assessed pyrethroids [29]. However, this correlation was not observed for the rest of the pyrethroids. The order of the other pyrethroids was not the same in both sediments, but cypermethrin, esfenvalerate and permethrin were always in the most bioavailable half, fenpropathrin and fluvalinate were around the centre of the list, and bifenthrin and tetramethrin were in the less bioavailable half.

Another publication reported that the Tenax method is better than using solid-phase microextraction fibres because of its capacity to remove a larger fraction of the contaminant from the matrix [33]. Their calculated percentages of desorption were greater than those listed above as the organic carbon content of the sediment was lower.

The literature indicates that the bioavailability of pyrethroids is considerably lower than that of persistent organic pollutants (POPs) such as PBDEs (5–85% percentage of desorption), DDT (70–90%) and hexabromocyclododecane (HBCD) (around 90%) [20].

3 Bioaccumulation

The capacity of mammals of metabolizing pyrethroids has been regarded as one of the best qualities of these pesticides. However, evidence of their bioaccumulation has been reported in several publications.

3.1 Aquatic Organisms

Evidence of bioaccumulation in marine mammals was first found in 23 liver samples of male Franciscana dolphins (*Pontoporia blainvillei*) from the Brazilian coast: São Paulo (SP), $n = 12$, urban area, and Rio Grande do Sul (RS), $n = 11$, agricultural area [7]. In order to avoid the high variation in the levels of lipophilic pollutants of females (see Sect. 4), only male dolphins were included to assess the concentrations of pyrethroid in different locations and through the life cycle of the dolphins [34, 35].

All targeted pyrethroids but resmethrin were detected in liver samples [7]. Cyfluthrin, deltamethrin and tralomethrin were found in 73–83% of the samples. In RS, λ-cyhalothrin and tetramethrin were found in 82% of the samples, and bifenthrin and fluvalinate were found in 91%. Total pyrethroid concentrations ranged from 7.04 ng/g lw (adult, SP) to 68.4 ng/g lw (calf, SP). Permethrin showed the highest concentrations, from 4.48 ng/g lw (adult, SP) to 54.6 ng/g lw (calf, SP). The other compounds in decreasing order of concentrations were cypermethrin (<25 ng/g lw); tetramethrin (<16 ng/g lw); deltamethrin, fluvalinate and λ-cyhalothrin (<6 ng/g lw); fenvalerate and cyfluthrin (<4 ng/g lw); and bifenthrin (<3 ng/g lw).

Greater pyrethroid concentrations were reported in urban areas (SP). This was also true for individual bifenthrin and permethrin. It had been previously observed in California that the urban run-off supposed a greater pyrethroid input than the irrigation run-off [11, 36]. Conversely, deltamethrin levels were significantly higher in calves from RS. Deltamethrin had been used in RS since the 1980s to control stored grain insects [37].

A trend of pyrethroid concentrations according to the dolphins' length was suggested (Fig. 1) [7]. High concentrations were found in calves due to pyrethroids accumulated via maternal transfer. Concentrations could dilute with the dolphins growth and rise again from dietary intake. Finally, when individuals reach maturity, that is, for adult individuals, they would be able to metabolize pyrethroids decreasing their concentration.

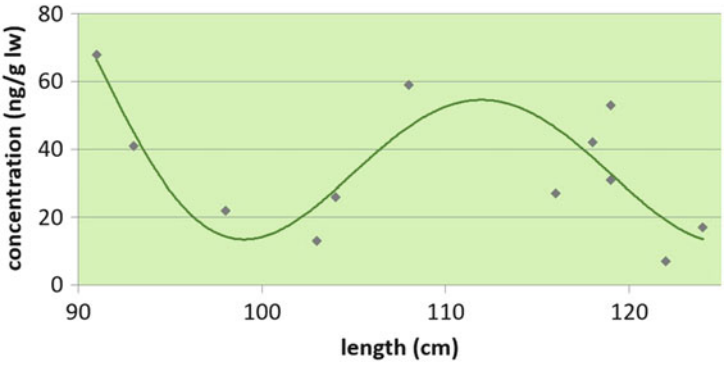

Fig. 1 Concentration of pyrethroids in Franciscana dolphins according to dolphin length (*adapted from* [7])

A similar study was performed with dolphin liver (27 males and 10 females) of striped dolphin (*Stenella coeruleoalba*) from the Mediterranean coast of southern Spain [8]. Pyrethroids were detected in 87% of the samples, including bifenthrin, cyhalothrin, deltamethrin, permethrin and tetramethrin. Mean total pyrethroid concentration was 300 ± 932 ng/g lw (range 2.70–5,200 ng/g lw).

While there were not enough samples for a life trend assessment, the results were not dissimilar from the ones reported for the Brazilian dolphins. Concentrations seemed to increase somewhat from calves to juveniles due to dietary intake. After achieving sexual maturity, the levels stayed stable as metabolization of pyrethroids would compete with further bioaccumulation.

No statistical differences were found with the Brazilian levels. Franciscana dolphins are live in coastal waters, while striped dolphins are found further offshore. The smaller size of the Mediterranean Sea, which is surrounded by human population, as opposed to the big and open Atlantic Ocean, might compensate for the distance of the striped dolphins to the source of contamination. Additionally, the western Mediterranean Sea has been identified as a global PCB hotspot for marine mammals [38], which might also be true for other pollutants like pyrethroids.

Other organic contaminants have been analysed in striped dolphin from the Mediterranean Sea, including PBDEs, decloranes, hexabromobenzene (HBB), PCBs and DDT (Fig. 2) [39–42]. Striped dolphins from the same area and years showed a higher mean for PBDEs (940 ng/g lw) albeit in the lower half of the reported pyrethroid range. Ranges of dechloranes and HBB in those dolphins were detected in the same low area (<380 and <9 ng/g lw, respectively). In other regions of the Mediterranean Sea, PCBs and DDT were present in striped dolphins at much higher concentrations (2.1–170 and 1.1–260 μg/g lw, respectively), whereas PBDEs (12–290 ng/g lw) and PAH (200 ng/g lw) were included in the pyrethroid range.

Regarding fish, the bioaccumulation of pyrethroids in wild river fish was first reported using 42 pooled edible fish samples from four Iberian river basins [43]. One of the sampling points corresponded to a reservoir. The selected species

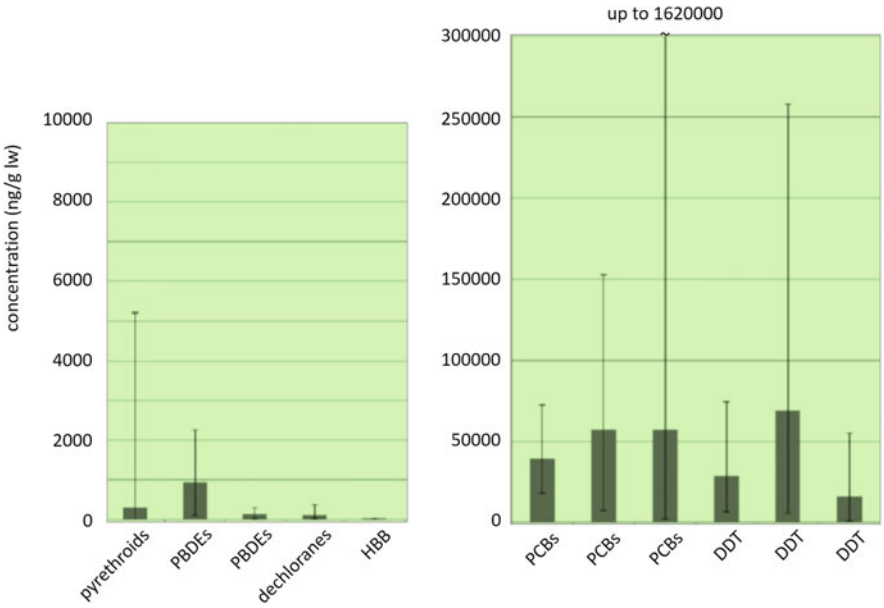

Fig. 2 Mean concentrations and range of organic contaminants in striped dolphins from the Mediterranean Sea

included barbel and carp, when possible, or catfish, gudgeon or trout. Pyrethroids were found in all the samples. Total concentrations ranged from 12 to 4,940 ng/g lw. The most contaminated sample was reservoir trout. However, carp appeared to be the species with a higher pyrethroid bioaccumulation capacity. Previously reported levels in exposed rainbow trout were in the same order of magnitude: 30–40 ng/g wet weight (ww) of *cis*-cypermethrin, deltamethrin, fenvalerate and *cis*-permethrin [44].

Bifenthrin, cyhalothrin and cypermethrin were quantifiable in all the Iberian fish samples [43]. Fluvalinate, phenothrin and resmethrin were not detected. Fenvalerate, tetramethrin, permethrin were present in 80–90% of the samples, cyfluthrin in 57% of the samples and permethrin in 31% of them.

Permethrin dominated the pyrethroid profiles in the Ebro and Llobregat river basins, while cypermethrin and tetramethrin dominated the profiles in the Guadalquivir and Jucar basins. This could reflect the local use of pesticides as the closest rivers, Ebro and Llobregat, showed similar profiles. The presence of pyrethroids banned from agricultural uses by the Council Directive 91/414/EEC (e.g. bifenthrin) supports the hypothesis that non-agrarian sectors are an important source of pyrethroids in the environment. On the other hand, interspecies profile variation was reported within a sampling point. The authors suggested different bioaccumulations depending on the species due to differences in their metabolism or dietary habits.

Flame retardants, personal care products, hormones and pharmaceuticals were analysed in the same Iberian fish samples (Fig. 3). PBDEs and dechloranes showed frequencies of detection close to the 100% observed for pyrethroids [45]. The other contaminants occurred in less than 50% of the samples [46, 47]. Pyrethroid showed the highest concentrations, followed by parabens (levels below a third of the pyrethroids' maximum) and organophosphorus flame retardants (OPFRs) (levels below a sixth of the pyrethroids' maximum).

Pyrethroids have also been reported to accumulate in salmon in a study on the effects of the pyrethroid treatment against sea lice in fish farms [48]. The study compared the pyrethroid levels and profiles from salmon farmed in several European countries and the Pacific Ocean with wild salmon from Alaska. The pyrethroid concentrations in farmed salmon (1.31 ± 1.39 ng/g ww) were higher than in wild salmon (0.02 ± 0.03 ng/g ww), supposedly as a result of the pyrethroid baths. The pyrethroid profiles supported this hypothesis as cypermethrin and deltamethrin, the active ingredients of anti-lice formulations, contributed to 77% of the farmed salmons' profiles, whereas no individual pyrethroid showed predominance in the wild salmon samples.

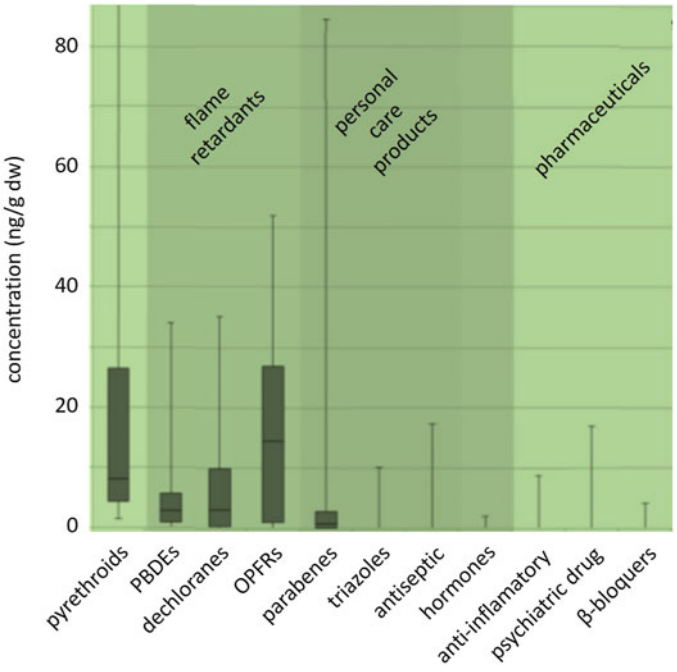

Fig. 3 Box plots of the concentrations of organic contaminants in wild fish from Iberian rivers (*adapted from* [43])

3.2 Terrestrial Organisms

A few studies have analysed pyrethroids in poultry eggs, which are not environmentally representative [49, 50]. Low levels of λ-cyhalothrin [51], tefluthrin, cyfluthrin and cypermethrin [52] have been reported in eggs and carcasses of wild grey partridges.

A more in-depth study assessed the presence of pyrethroids in 123 unhatched eggs from 16 species of wild birds from Doñana (nature reserve in southwestern Spain) and surrounding areas [53]. Pyrethroids were detected in 93% of the eggs. The highest mean values of contamination belonged to samples of black-headed gull (162 ng/g lw), gull-billed tern (61.5 ng/g lw) and black kite (48.5 ng/g lw). The lowest means were found for purple heron (1.49 ng/g lw), glossy ibis (1.59 ng/g lw) and black-winged kite (1.93 ng/g lw). The total range was between not detected and 324 ng/g lw. The many factors for this variation could include the feeding habits, as previously described for other lipophilic contaminants [54, 55], body condition, age, habitat and migratory behaviour [56].

Although comparing these levels to the studies on poultry eggs could be far-fetched, it is interesting to notice the difference in the pyrethroid profiles. Only bifenthrin or cypermethrin were detected in the poultry eggs [49, 50] as a result of a specific pesticide treatment. However, wild bird eggs should reflect the variety of pyrethroids in the environment. The samples from Doñana showed 12 individual pyrethroids [53], eggs of wild grey partridges contained λ-cyhalothrin [51], and the carcasses of the same species contained cyfluthrin, cypermethrin and tefluthrin [52].

The eggs from Doñana were also tested for halogenated flame retardants [54]. Flame retardants were found in 100% of the samples, similar to the 93% for pyrethroids. Unlike POPs, pyrethroids have low environmental persistence ($\leq$90 days) [2], but their massive use makes them constantly present. PBDE and dechlorane concentrations were in the same range as pyrethroids.

Fenpropathrin, fluvalinate and resmethrin were not detected in the eggs from Doñana [53]. Cypermethrin, λ-cyhalothrin and bifenthrin were found in over 75% of the samples. Tetramethrin, permethrin, fenvalerate and cyfluthrin occurred in about half of the samples. Deltamethrin and phenothrin were detected in about a quarter of them.

The species were divided into four categories according to their diet: terrestrial, feeding from terrestrial ecosystems; anthropogenic, including a proportion of food from human sources; aquatic, feeding from aquatic ecosystems; and herbivorous, feeding on plants and algae [53]. Eggs of species with anthropogenic feeding habits were more contaminated, followed by eggs of birds of the aquatic feeding category. This suggests that dietary intake might play an important role in the bioaccumulation of pyrethroids. It is also consistent with the higher contamination in urban areas and the aquatic ecosystems being easily contaminated via run-off.

3.3 Humans

A limited number of studies have considered the accumulation of pyrethroids in human milk. In 2017, 206–258 million people were estimated to have contracted malaria and 0.41 million died (94% in Africa and 67% children under 5 years) [57]. The World Health Organization (WHO) supports the use of specific pesticides for malaria control. In tropical Africa, pyrethroids were used on mosquito nets and as indoor sprays [58].

Pyrethroid exposure in a rural area at the south of Mozambique was assessed using 22 breast milk samples from 2002 [59]. Indoor thatch samples were also collected from walls to determine potential exposure. Permethrin and λ-cyhalothrin, esfenvalerate, cypermethrin, tetramethrin and bifenthrin were found in 19 samples, while cyfluthrin was detected in only 9 samples. Deltamethrin, phenothrin and resmethrin were never detected. Individual concentrations went up to 36 pg/g lw for bifenthrin; 160–230 pg/g lw for cypermethrin, esfenvalerate, tetramethrin, cyfluthrin and permethrin; and 440 pg/g lw for λ-cyhalothrin. Total pyrethroid levels were between 87 and 1,200 ng/g lw. The main contributor to the pyrethroid profiles was λ-cyhalothrin (35%) followed by permethrin (21%) and cypermethrin, esfenvalerate and tetramethrin (14%).

Pyrethroids had previously been found in human breast milk from Switzerland with median total concentrations between 15 and 31 ng/g lw [60]. The difference to the levels from Mozambique can be related to a more limited use for agricultural and domestic applications in Switzerland [60].

Conversely, a mean value of 1,200 ng/g lw of permethrin and lower levels of cypermethrin and cyfluthrin had been reported in samples from South Africa [61]. These pyrethroids were also detected in other samples from the same region at 14,500, 4,200 and 42,000 ng/l, respectively [9]. The authors suggested a domestic source of contamination for the first group of samples and an agricultural source for the latter. Converting these results according to their reported fat content of 4%, total concentrations ranged from 110 to 1,050 ng/g lw, which is similar to the milk samples from Mozambique.

The fact that mothers accumulate pyrethroids implies that they could be transferred to their offspring through lactancy. Estimated daily intake (EDI) values for the babies in Mozambique went from 0.12 μg/kg of body weight and per day for cypermethrin to 3.4 μg/kg of body weight and per day for cyhalothrin [59]. The acceptable daily intake (ADI) values for individual pyrethroids are set between 10 and 50 μg/kg bw/day [62], which means that pyrethroid intake should not pose a threat to the babies.

A bigger and more recent study included 56 human milk samples from Colombia, Spain and Brazil, including urban and rural areas [10]. Pyrethroids occurred in all the samples at concentrations from 1.45 to 24.2 ng/g lw in the three countries. Their results were also similar to the ones reported for the Swiss samples [60]. Urban samples from Spain and Brazil showed a mean around 5 ng/g lw, and rural samples from Brazil and Colombia had a mean value just above 9 ng/g lw.

Cypermethrin, permethrin, λ-cyhalothrin and fenvalerate were detected in all or almost all the samples. Cyfluthrin, fluvalinate, phenothrin and resmethrin were never detected. Individual concentrations decreased from cypermethrin (up to 16.4 ng/g lw) through tetramethrin, λ-cyhalothrin, bifenthrin, permethrin, fenvalerate to deltamethrin (up to 1.86 ng/g lw).

Pyrethroid profiles suggested usage of different pyrethroids in different areas. For example, bifenthrin had a great contribution in the Brazilian samples, but not so much in the others. Cypermethrin was the biggest contributor to the Colombian profiles, which is consistent with the pesticide use in that country [10]. Finally, permethrin dominated the Spanish samples, which agrees with the results of the aforementioned striped dolphin livers from southern Spain [8].

The authors reported no correlation between pyrethroid levels and the age of the mother or between the domestic use of pesticides and levels in breast milk. On the other hand, the contamination in milk decreased exponentially with parity (number of children of a mother), supporting the hypothesis of maternal transfer of pyrethroids. All samples presented EDI values below the ADI values. However, cypermethrin was very close to its 50 μg/kg bw/day ADI with occasional EDI values of 48.8 and 44.2 μg/kg bw/day for samples from 2003 to 2004, respectively. Cypermethrin had been used to control dengue.

3.4 Isomer-Specific Accumulation

Pyrethroids have two or three chiral centres in their structures. This means that they have two or four diastereomers and four or eight enantiomers. Many of the works referenced in this chapter performed isomeric analysis with a chiral chromatographic column after the corresponding quantitative analysis. The enantiomeric factors (EF) for each enantiomeric pair were calculated dividing the chromatographic area of the first eluting enantiomer by the sum of the areas of both enantiomers [63]. A racemic mixture, containing equal amounts of each, corresponds to EF = 0.5. As type I pyrethroids present a *cis* and a *trans* enantiomeric pairs, EF_{cis} and EF_{trans} are defined. Type II pyrethroids present two of each, defined as EF_{cis1}, EF_{cis2}, EF_{trans1} and EF_{trans2}. Diastereoisomeric factors (R) were also calculated [63]. $R_{cis/trans}$ represents the ratio between *cis* and *trans* isomers of an individual pyrethroid; meaning *cis*1 + *cis*2 and *trans*1 + *trans*2 for type II pyrethroids. $R_{cis1/cis2}$ and $R_{trans1/trans2}$ were also assessed for type II pyrethroids.

These factors can help determine isomer-specific accumulation or metabolization in mammals. R values for esfenvalerate, permethrin and cypermethrin for calves, juvenile and adult Franciscana dolphins were calculated [7]. While esfenvalerate and cypermethrin showed no differences with age group (R_{esfen} = 0.48–0.67, R_{cyp} = 0.28–0.49), some differences were reported for permethrin. The mean R_{perm} value in calves was 0.84, showing a higher contribution of the first isomer. However, the mean R_{perm} values for juveniles and adults were 0.60 and 0.69, suggesting either a selective bioaccumulation of the *trans* isomer in the first years

of life of the dolphins or a selective metabolization of the *cis* isomer after they reach sexual maturity. An enrichment on the *trans* isomer of permethrin was also observed in the samples of human milk from Mozambique [59].

The Mediterranean striped dolphins had $EF_{cis} = 0.51 \pm 0.17$ and $EF_{trans} = 0.47 \pm 0.10$ for tetramethrin [8]. These values, corresponding to a racemic mixture, agreed with the values reported for household insecticides purchased in Spanish supermarkets [63], indicating no enantiomer-specific accumulation of tetramethrin in the liver of the individuals. However, the EF_{cis} for permethrin in dolphins was 0.42 ± 0.05, which was statistically lower than 0.35 ± 0.04 in commercial pesticides, suggesting enantiomer-specific bioaccumulation of (1*S*,3*S*)-permethrin rather than (1*R*,3*R*)-permethrin in dolphin liver.

On the other hand, whereas the $R_{cis/trans}$ values of permethrin in dolphins and commercial pesticides were similar, tetramethrin showed higher values in dolphin liver ($1.88 \pm 0.52 > 0.32 \pm 0.09$). These results suggest no selective bioaccumulation of the permethrin enantiomers, contrary to what had been reported for the Franciscana species in Brazil, and selective bioaccumulation of *cis*-tetramethrin.

Moving on to fish, almost all the samples of Iberian river fish showed a preference to bioaccumulate the *cis* isomers of pyrethroids, with maximum $R_{cis/trans}$ values of 30 for cypermethrin, 1.3 for cyfluthrin and 11 for permethrin [43]. Conversely, tetramethrin showed the opposed trend. The authors argued that commercial mixtures might be rich in *trans*-tetramethrin as its 1*R*,3*S* isomer has a stronger pesticide activity. Thus a low $R_{cis/trans}$ value could reflect the environmental levels. The preference for *cis*-permethrin is a new situation after seeing the preference for the *trans* isomer in Franciscana dolphins and human milk from Mozambique and the lack of selectivity in the striped dolphins. However, the *cis* preference was also reported for the samples of human milk [10]. Some studies on mice found that *cis*-permethrin was less metabolized than *trans*-permethrin and more accumulative and toxic [64, 65].

It is important to note that commercial mixtures are rich in some *cis* isomers [63]; thus $R_{cis/trans} > 1$ might just represent the environmental contamination. That is why analysing environmental samples from the areas where biological samples are collected would allow more accurate observations.

The EF_{cis1} for cyfluthrin was always below 0.39. On the other hand, the EF_{cis1} for cypermethrin was always below 0.5, except for the catfish samples [43]. This might suggest that enantiomer-selective bioaccumulation could depend on species, as catfish samples were collected in the same locations as the rest [63].

Permethrin EF_{cis} values were very different among the Iberian fish samples and the sampling points [43]. For example, samples of catfish and barbel presented higher content of (1*R*,3*R*,α*R*)-cyhalothrin in the first *cis* enantiomeric pair, while the second *cis* pair appeared as a racemic mixture. However, the gudgeon samples, which were not collected in the same location, presented a racemic mixture for the first *cis* pair, but higher accumulation of (1*R*,3*R*,α*S*)-cyhalothrin in the second pair. These variations could support the hypothesis of species-specific selectivity or indicate different uses of commercial mixtures in the targeted locations.

The study on the effect of the pyrethroid treatment against sea lice in farmed salmon reported no difference in the EF and *R* values obtained for a given species farmed in several European countries and the Pacific Ocean [48]. However, *Salmo salar* and *Oncorhynchus mykiss* showed selective bioaccumulation of the opposed *cis*-cypermethrin enantiomer of the first *cis* pair eluted and different degrees of preference for the same *cis* enantiomer of the second pair. These species also showed opposing preferences for $R_{cis/trans}$ and $R_{cis1/cis2}$. These data reinforce the hypothesis of species-depending selectivity in fish.

A third of the unhatched eggs from the wild birds from the Doñana region were also analysed and showed $R_{cis/trans} > 1$ [53] agreeing with the already mentioned results for river fish and some human milk from Spain, Colombia and Brazil [10, 43]. However, tetramethrin contradicted this trend, also agreeing with the other studies.

The study showed equal accumulation for both *cis* enantiomers of cyhalothrin, cypermethrin, permethrin and tetramethrin [53]. Gadwall eggs were an exception for cyhalothrin and cypermethrin, and black kites, black-headed gulls and glossy ibis were exceptions for permethrin. The EF_{trans} of tetramethrin revealed selective accumulation of *trans*-(1*S*,1*R*)-tetramethrin. This had also been observed in Spanish human breast milk samples [63].

The analysis of human milk samples from Brazil, Colombia and Spain showed higher accumulation of the *cis* isomer for esfenvalerate and permethrin and the contrary for cyfluthrin, cypermethrin and tetramethrin [10]. It has already been mentioned that the commercial mixtures available in Spanish markets are usually rich in *cis* isomers [63]. This might imply that *trans*-cyfluthrin and *trans*-cypermethrin were selectively accumulated in human milk. The cypermethrin trend was stronger in the Brazilian and Spanish samples than in the Colombian ones, indicating possible different exposures on those locations.

The human milk samples from Mozambique were compared to a commercial mixture and the thatch material from indoor walls [59]. For permethrin, the calculated EF values were 0.84 for commercial mixtures, 0.69 for thatch material and 0.52 for human milk. This enrichment in *trans*-permethrin may reflect a higher bioaccumulation of this isomer or a better metabolization of *cis*-permethrin. This finding contradicts the distribution reported in the study of the previous paragraph.

In 2009, seven human breast milk samples from the metropolitan area of Barcelona (Spain), as well as eight domestic pesticides, one pet pesticide solution and one human skin cream against crabs and scabies, were analysed [63].

The domestic pesticides contained racemic mixtures of the *cis*-tetramethrin pair, the *trans*-tetramethrin pair and the *cis*-cypermethrin pair, while the *cis*-permethrin pair was rich in (1*S*,3*S*)-permethrin ($EF_{cis} = 0.35$). The human milk samples also showed a racemic mixture of *cis*-tetramethrin. However, they presented a higher contribution, hence accumulation, of *trans*-tetramethrin ($EF_{trans} = 0.32$) and (1*R*,3*R*)-permethrin ($EF_{cis} = 0.43$) than the domestic pesticides. Human samples showed greater contribution of (1*S*,3*S*,α*R*)-cyhalothrin than the pesticides ($EF_{cis2} = 0.20$). Although the first *cis* pair of cypermethrin was present as a racemic

mixture, the second pair differed from this behaviour with a potential selective accumulation of (1*S*,3*S*,α*R*)-cypermethrin ($EF_{cis2} < 0.35$) as the commercial pesticides had racemic mixtures.

While $R_{cis/trans}$ values for tetramethrin and cypermethrin were similar for human milk and the commercial pesticides, suggesting no specific bioaccumulation, *cis*-permethrin seemed to be selectively accumulated in human samples [63]. A great preference for the second *cis* isomer of cypermethrin eluted was also observed when compared to the commercial pesticides.

4 Maternal Transfer

Maternal transfer occurs during gestation and lactation [66, 67]. Therefore, the exposition of pregnant individuals to contaminants might threaten their offspring, increasing their susceptibility to disease in adulthood [68, 69].

Samples of breast milk and placenta from three pregnant and lactating dead Franciscana dolphins (*Pontoporia blainvillei*) were collected in Brazil and analysed in a first attempt to study maternal transfer [7]. The placenta provides an indication of prenatal exposure to pyrethroids, while breast milk indicates the postnatal transfer to calves. Pyrethroids were found in both milk (2.5–4.8 ng/g lw) and placenta (331–1812 ng/g lw), suggesting the maternal transfer of pyrethroids by both gestational and lactation pathways.

The same authors added data to the literature with samples of several tissues from five mother-foetus pairs of Franciscana dolphins and from three mother-foetus pairs of Guiana dolphins (*Sotalia guianensis*) [70]. Muscle and blubber from mother and foetus were taken from both species, as well as placenta, umbilical cord and milk from Franciscana dolphins.

Pyrethroids were found in all the samples. Foetus-to-mother ratios (f/m) of total pyrethroid concentrations in Franciscana dolphins were 1.43, 2.67, 4.13 and 19.5 in blubber and 0.28 and 30 in muscle. A f/m value higher than 1 indicated higher pyrethroid burden on the foetus than their mother. A higher bioaccumulation in foetuses than mothers had already been observed for hexachlorobenzene in long-finned pilot whales (*Globicephala melas*) from Australia and in beluga whales (*Delphinapterus leucas*) from Alaska [71, 72]. A tendency to transfer low-chlorinated contaminants and with lower log K_{ow} from cetacean mothers to foetuses had also been reported [68, 71]. Accordingly, the predominant pyrethroids in foetal blubber of the Franciscana dolphins were two-chlorinated cypermethrin and permethrin (log K_{ow} 6.6 and 6.5), suggesting a tendency similar to low-chlorinated pesticides [70].

The f/m values of total pyrethroid concentrations in Guiana dolphins were 0.42, 1.39 and 1.47 in blubber and 0.09, 0.12 and 0.35 in muscle. The two different tissues showed different patterns, with a higher burden in the foetuses' blubber, but higher

burden in the mothers' muscle. While the limited number of samples prevents an in-depth argumentation for that, the evidence show that pyrethroids penetrated the placental membrane and bioaccumulated in the developing foetus in every pair.

In a previous section of this chapter (Sect. 3.3), it has been shown that pyrethroids are also found in human milk, thus transferred to lactating babies judging by what has been proved for dolphins.

5 Final Remarks

Bioavailability is the first step to bioaccumulation. Based on desorption kinetic from sediment, cyfluthrin was the most bioavailable compound, while λ-cyhalothrin was the less bioavailable of the assessed pyrethroids [29]. Cypermethrin, esfenvalerate and permethrin were in the most bioavailable half of the list, fenpropathrin and fluvalinate were around the centre, and bifenthrin and tetramethrin were in the less bioavailable half. The literature indicates that the bioavailability of pyrethroids, with percentages of desorption between 9 and 36%, is considerably lower than that of POPs such as PBDEs, DDT and HBCD, with values over 70%.

Bioaccumulation of pyrethroids had been disregarded in the past to the mammalian capacity of metabolizing them. However, evidence of their bioaccumulation has been reported in several publications. Evidence of bioaccumulation in marine mammals was first found in samples of Franciscana dolphins from Brazil [7]. Total pyrethroid concentrations ranged from 7.04 to 68.4 ng/g lw. Permethrin showed the highest concentrations, reaching up to 54.6 ng/g lw. The other compounds in decreasing order of concentrations were cypermethrin, tetramethrin, deltamethrin, fluvalinate, λ-cyhalothrin, fenvalerate, cyfluthrin and bifenthrin, the latter at levels below 3 ng/g lw.

A trend of pyrethroid concentrations according to the dolphins' age was suggested. High concentrations were found in calves due to pyrethroids accumulated via maternal transfer. Concentrations could dilute with the dolphins growth and rise again from dietary intake. Finally, adult individuals would metabolize pyrethroids decreasing their concentration. Greater pyrethroid concentrations were reported in urban areas, except for deltamethrin, which had been used in rural areas to control stored grain insects.

A similar study with striped dolphins from the Spanish Mediterranean detected bifenthrin, cyhalothrin, deltamethrin, permethrin and tetramethrin in most samples [8]. Mean total pyrethroid concentration was 300 ± 932 ng/g lw. The environmental differences between the Mediterranean Sea and the Atlantic Ocean might account for the levels in striped dolphin compared to the Brazilian ones. The western Mediterranean Sea had already been identified as a contamination hotspot for marine mammals [38]. Concentrations of pyrethroids in Mediterranean striped dolphins were comparable to those of PBDEs and PAH and higher than those of HBB and dechloranes, but lower than PCB and DDT levels [39–42].

Pyrethroids were found in all the samples of wild Iberian river fish at concentrations ranging from 12 to 4,940 ng/g lw [43]. Bifenthrin, cyhalothrin, cypermethrin, fenvalerate, tetramethrin and permethrin were present in all the samples, while fluvalinate, phenothrin and resmethrin were not detected. Because of the similitudes among samples from closer river basins, pyrethroid profiles possibly reflected the local use of pesticides. On the other hand, interspecies profile variation was reported within a sampling point. Comparted to the levels of flame retardants, personal care products, hormones and pharmaceuticals in the same Iberian fish samples, pyrethroids were found as frequently as PBDEs and dechloranes [45] and more than twice as frequently as the rest [46, 47]. Pyrethroid showed the highest concentrations, followed by parabens and OPFRs. Pyrethroids have also been reported to accumulate in farmed salmon [48].

Pyrethroids were detected in most unhatched eggs from wild birds from Doñana (Spain), with mean total values from 1.93 to 162 ng/g lw depending on the species [53]. The many factors for this variation could include the feeding habits, body condition, age, habitat and migratory behaviour. Fenpropathrin, fluvalinate and resmethrin were not detected. Cypermethrin, λ-cyhalothrin and bifenthrin were found in over 75% of the samples. Eggs of species with anthropogenic feeding habits were more contaminated, followed by eggs of birds of the aquatic feeding category. This suggests that dietary intake might play an important role in the bioaccumulation of pyrethroids. It is also consistent with the higher contamination in urban areas and the aquatic ecosystems being easily contaminated via run-off.

A limited number of studies have considered the accumulation of pyrethroids in human milk. Permethrin, λ-cyhalothrin, esfenvalerate, cypermethrin, tetramethrin and bifenthrin were found in most milk samples from a rural area in Mozambique [59]. Total pyrethroid levels were between 87 and 1,200 ng/g lw. The main contributor to the pyrethroid profiles was λ-cyhalothrin, followed by permethrin and then cypermethrin, esfenvalerate and tetramethrin. The difference with the levels previously found in human breast milk from Switzerland (median 15–31 ng/g lw) can be related to a more limited use for agricultural and domestic applications in Switzerland [60]. Conversely, levels of pyrethroids similar to the samples from Mozambique had been found in South Africa [9, 61]. The authors suggested a domestic source of contamination for the Swiss samples and an agricultural source for the South African ones.

A bigger and more recent study including human milk samples from urban and rural areas of Colombia, Spain and Brazil reported concentrations from 1.45 to 24.2 ng/g lw [10]. These results were similar to the ones reported for the Swiss samples. Individual concentrations decreased from cypermethrin (up to 16.4 ng/g lw) through tetramethrin, λ-cyhalothrin, bifenthrin, permethrin, fenvalerate to deltamethrin (up to 1.86 ng/g lw). As reported in several studies with animal samples, the pyrethroid profiles suggested usage of different pyrethroids in different areas, supported by knowledge of local usage (cypermethrin in Colombia) [10] or agreement with other studies (permethrin in striped dolphins from southern Spain) [8]. The contamination in milk decreased exponentially with parity, supporting the hypothesis of maternal transfer of pyrethroids.

Many of the cited works performed isomeric analysis to assess the isomer-specific accumulation or metabolization in mammals. The Franciscana dolphins seemed to either bioaccumulate *trans*-permethrin selectively in their first years of life or metabolize *cis* isomer better after they reached sexual maturity [7]. An enrichment on the *trans* isomer of permethrin was also observed in the samples of human milk from Mozambique [59]. The isomeric data of Mediterranean striped dolphins [8] showed no enantiomer-specific accumulation of tetramethrin. However, enantiomer-specific accumulation of (1*S*,3*S*)-permethrin over (1*R*,3*R*)-permethrin was suggested.

Almost all the samples of Iberian river fish showed a preference to bioaccumulate the *cis* isomers of pyrethroids, with only tetramethrin opposing that trend [43]. The authors argued that this could mirror the environmental contamination as commercial mixtures might be rich in *trans*-tetramethrin and a dominance of *cis*-permethrin was also observed for the samples of Spanish human milk [10] and the unhatched eggs from Doñana [53]. Isomer-selective bioaccumulation depending on fish species was also hinted at in this study [43] and on the one on farmed salmon [48].

The analysis of human milk samples from Brazil, Colombia and Spain showed higher accumulation of the *cis* isomer for esfenvalerate and permethrin and the contrary for cyfluthrin, cypermethrin and tetramethrin [10]. Cyfluthrin and cypermethrin disagree with the *cis*-dominating trend of other studies, which might imply that their *trans* isomers were selectively accumulated in human milk. The human milk samples from Mozambique contradicted the *cis*-dominating trend for permethrin, which might reflect a higher bioaccumulation of the *trans* isomer or a better metabolization of *cis*-permethrin [59].

Analysing environmental samples from the areas where biological samples are collected would allow more accurate observations regarding isomer selectivity.

Related to breast milk, lactation and gestation result in the maternal transfer of contaminants [66, 67]. The maternal transfer of pyrethroids has been observed using samples of breast milk and placenta from pregnant and lactating Franciscana dolphins [7] and several tissues from mother-foetus pairs of Franciscana dolphins and Guiana dolphins [70]. Foetuses showed a higher pyrethroid burden in blubber than their mothers, but the contrary happened for muscle tissue. As pyrethroids are also found in human milk, they must be transferred to lactating babies; however, the EDI values reported by the corresponding studies for each pyrethroid were below their corresponding ADI [10].

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Indoor and Outdoor Pyrethroid Air Concentrations

Clifford P. Weisel

Contents

Abstract Pyrethroids are used throughout the world in agricultural settings and inside and outside of residences to control pests. This has resulted in their increase in air concentration leading to inhalation, and to a lesser extent dermal, exposures to applicators, their families, and the general public. Applicators need to wear appropriate personal protection equipment (PPE) to avoid high exposures during or after spraying of crops. The various uses of the pyrethroids and pyrethrins are regulated and education often mandated to minimize potential exposures. Outdoor levels are predominantly influenced by agricultural applications which can result in drift of the pesticides to the surrounding residential communities. Drift contributions decrease with distance from application and depend upon wind conditions, temperature, and precipitation. Only a limited number of studies have directly measured pyrethroid air concentrations due to the effort involved. Rather, air concentrations and the resulting exposure estimates rely on mathematical modeling to predict the transport and distribution of pyrethroids and on biomarker measurements to determine uptake in individuals. Urinary metabolites are the most common biomarkers. However, most of the metabolites are not specific to individual pyrethroids; rather, they provide

C. P. Weisel (✉)
Environmental and Occupational Health Sciences Institute, School of Public Health, Rutgers, The State University of New Jersey, Piscataway, NJ, USA
e-mail: weisel@eohsi.rutgers.edu

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evidence that an individual or population were exposed to one or more pyrethroid pesticide. Recently, silicone bracelets have been deployed to evaluate relative personal inhalation exposures to pyrethroids as part of a scan for multiple semi-volatile organic compounds. When pyrethroids are sprayed indoors, they are absorbed onto surfaces and by house dust. The absorbed pyrethroids subsequently equilibrate with the indoor air and are distributed throughout the home resulting in multiple exposures over an extended time period. Inhalation of pyrethroids usually contributes only a small portion (<10%) of the total exposure in the general population, with ingestion of foods grown or stored with pesticides to increase crop yield having the largest contribution. Inhalation exposures can be significant though following the use pesticide application devices that release larger amounts into the air or if individuals enter a treated area without adequate ventilation or prior to the pyrethroid air concentration declining sufficiently.

Keywords Chromatography, Metabolites, Reapplication timing, Silicone wristband, Volatilization

1 Introduction

Insecticide use extends beyond the agricultural settings into residential houses and gardens for pest control, with 82 million households in the USA applying insecticides annually [1]. Pyrethroids and pyrethrins are among the most frequently used insecticides in the USA and globally [2, 5]. Their use contaminates the personal air of applicators and causes elevated air concentrations, both indoors and outdoors, exposing the general public to these compounds. Pyrethrins are derived from chrysanthemum flowers. Pyrethroids are synthetic chemical insecticides whose chemical structure is based on pyrethrins. Pyrethroids' chemical structures have been modified from the naturally occurring pyrethrins to increase their stability in sunlight while retaining similar effects on the nerve functions of the target insect pests. These insecticides are effective against a wide range of insect pests including ants, mosquitoes, flies, fleas, and moths, which has led to their widespread use and presence in a variety of commercial and consumer products that are sprayed or released into the air. Pyrethroids affect the neurological system of the insect rapidly (within minutes) after contact leading to a knockdown of the insect, though the effect may not always be fatal. Their usage has increased extensively over the last several decades. According to the US EPA [6], approximately 1–3 million pounds of permethrin, a commonly used pyrethroid, is applied annually to residential homes and garden sites. Studies in Northern California involving 259 residential households found that 77% of the insecticides used were pyrethroids [7]. There are over 3,500 registered products that contain at least one pyrethroid as an active ingredient, many of which are formulated for use in and around households, on pets, in treated

clothing, for mosquito control, and in agricultural settings. The formulations include products that have a direct application on the skin of pets for lice treatment, impregnation of cloth, in sprays, and as part of aerosolizing devices for treatments of larger areas. Since many pyrethroids are semi-volatile compounds, even applications onto surfaces can result in elevated air concentrations as they volatilize. Whether these products are used by professionals or purchased for direct use by the consumer, the result is the same: elevated air concentrations, both indoors and outdoors, exposing a wide section of the population [8, 9].

A growing application of pyrethroid use is as a mosquito repellent, which can be applied topically but also released into the air from mosquito coils, electric vaporizers, or aerosol sprays resulting in elevated air levels [10, 11]. These devices, along with other applications, such as foggers implemented to kill swarming flying insects, can produce transient high indoor air concentrations ($\mu g/m^3$) during their application. Many formulations of pyrethrins also include agents that act as synergists, such as piperonyl butoxide, piperonyl sulfoxide, and sesamex, whose role is to interfere with the insects' enzymatic system by degrading pyrethrins, thereby improving the pyrethroid or permethrin's effectiveness.

2 Regulation of Pyrethroids

The production and use of pyrethroids, as well as all pesticides, in the USA are regulated by the US EPA Office of Pesticide Programs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Food Quality Protection Act (FQPA). Within Europe regulation of pesticides, also referred to as plant protection products (PPP), can be regulated by the European Union and for food by the European Food Safety Authority. Regulation can also be implemented through individual countries that are part of the European Union. Countries and regions throughout the world have generally developed regulations for pesticide use and acceptable residual amounts [12]. The regulations are designed to minimize harmful exposure through the air and other exposure routes by providing direction on (1) where and how a pesticide can be used and (2) what specific details need to be included on their labels for their use, storage, and disposal. The label provides guidance to professional applicators to protect themselves and the general public. Labels are also required for consumer products to indicate how they can be used safely. Pyrethroids are permitted pesticides for multiple applications in agricultural and residential settings. Regulations are based on human health risk assessments and ecological risk assessments and have been released or are in draft form for many pyrethroids (i.e., allethrins, bifenthrin, cyfluthrins, cypermethrin, cyphenothrin, d-phenothrin, deltamethrin, esfenvalerate, etofenprox, fenpropathrin, flumethrin, gamma-cyhalothrin, imiprothrin, lambda-cyhalothrin, momfluorothrin, permethrin, prallethrin, pyrethrins, resmethrin, tau-fluvalinate, tefluthrin, tetramethrin, and tralomethrin and for two synergists often added to the pesticide formulation piperonyl butoxide and MGK-264) [9]. The regulation for each pyrethroid provides information on its type (e.g., synthetic pyrethroid, knockdown agent), target

organism, mode of action, permissible/expected use site (e.g., agricultural setting, home, institutional sites, nonfood or food plant, indoors, outdoors, on pets), use classification (e.g., general use or pest control operators only), formation type (liquids, concentrates, coils, mats), application method (e.g., power, mechanical, commercial spray, aerosol can, fogger), application rate (includes percent active agent), and application timing (e.g., reapplication timing) [13].

3 Pyrethroids Associated with Agricultural Activities

Pyrethroids are currently among the most commonly used pesticides in agricultural settings to control insects on crops; in forestry, horticulture, and gardens; and for flying insects on livestock and pets [14]. They can be sprayed aerially, from trucks, tractors, or handheld devices onto crops, all of which potentially increase outdoor air concentrations and expose applicators, farm workers in the fields, and residents in nearby homes. Most recent studies of exposure to workers and to individuals exposed to drift from agricultural applications have evaluated urinary levels of pyrethroid metabolites rather than measuring air concentrations to assess inhalation exposures [14]. Thus, few recent studies have reported worker's exposure to air concentrations. The current US occupational exposure limit for pyrethroids for an 8-h workday, 40-h workweek is 5 mg per cubic meter (mg/m^3) [14]. The most extensive study of exposure to pesticides in the USA is the Agricultural Health Study, initiated in 1993 when the participants used chlorinated and organophosphate insecticides. More recently, pyrethroid exposures are being examined in a subset of the participants in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study [6] with cyfluthrin and permethrin being reported to be used in 2010 by 13% and 12%, respectively, of the 1,223 participants. While the applicator is expected to encounter the highest air concentrations, workers are supposed to be supplied with personal protective equipment (PPE), which if properly used reduces the inhaled pesticide levels and skin contact [15, 16]. The use of PPE is part of the EPA's Agricultural Worker Protection Standard (WPS) [17]. The WPS provides guidance on procedures to reduce worker exposure to pesticides and therefore the risk of pesticide poisoning and injury among over the two million agricultural workers, pesticide handlers, and their families in the United States. This is done through informing (safety training, written safety information, labeling, notification about treated areas to avoid), protecting (avoiding treated areas, suspending application when others are near, reentry guidelines, monitoring, proper personal protective equipment – including respirators), and mitigation of adverse events (availability of decontamination supplies – routinely and for emergencies, emergency transportation to medical facilities) [17].

4 Spray Drift Contribution of Air Concentrations

The spraying of crops is often done over large areas and uses significant amounts of pyrethroids which can result in spray (aerosol) drift or vapor phase (volatilization) transport of the pyrethroids reaching residences several hundred meters or more away [18]. The degree that the spray drift may impact air concentrations at surrounding residences is dependent upon the distance from the application to the receptor residence, the meteorological conditions (e.g., wind speed, wind direction, temperature, precipitation), application method, nozzle type, and the height the spray is released from [19]. Field measurements of spray drift can be difficult and expensive. Therefore, mathematical modeling of the drift has been used to predict the extent of the impacted areas and the concentration gradient for different scenarios, which can help guide the US EPA minimize the impact of spray applications on the surrounding environment and residences [20]. Recently, remote sensing instruments have been deployed to estimate the relative amount of deposition and spatial/temporal air concentrations and are used for model evaluation [21, 22]. Drift has been found to occur during every application and can account for approximately 2–25% of the pesticide loss during application with the drift spreading from a few yards to several hundred miles [23]. Various mathematical models of the drift have been developed. One computational fluid dynamic (CFD) model of spray droplets suggests that the air pesticide droplet concentration would decrease by two orders magnitude from 100 to 1 μg/L (1,000 μg/m^3) over a 200 m distance from its release [24]. A study based on samples collected between 1995 and 2015 looking at the variations in pesticide levels in house dust with distance from agricultural fields in North America showed, that the amount of pesticide drift decreased sharply and nonlinearly with distance from the source [25]. They reported that the geometric mean pesticide dust levels were 64% lower in homes 250 m from fields compared to homes only 23 m away and that homes near farms in which the pesticides were applied more recently or frequently were 2.3 higher than other homes near fields without recent pesticide applications.

5 Outdoor Air Levels

Few recent studies that have measured outdoor or personal worker pyrethroid air concentrations. This is due to sampling and analytical challenges and the need to evaluate the exchange between the vapor and particle phase for the semi-volatile pyrethroids. Current studies more commonly use biomarker measurements to assess exposure rather than air monitoring. As discussed below, biomarkers do not differentiate inhalation exposure from other exposure routes, and several biomarkers are non-specific, reflecting exposures to multiple pyrethroids and pyrethrins. A method developed within the last few years measures semi-volatile organic compounds (SVOCs) by having participants wear a silicone wristband for several days which

passively collects SVOCs including pyrethroids [26]. The silicone wristband is extracted and analyzed by gas chromatography/mass spectrometry or by gas chromatography/electron capture detection for the pyrethroids. The latter, while not positively confirming the compound's identity, often has a lower detection limit. The silicone wristband has the benefit of requiring little field effort and wearing it presents relatively low participant burden that is often acceptable to individuals across both genders and over a wide range of ages and ethnic groups. However, the amount collected on the wristband may have contributions from dermal contact and is dependent upon the compound's diffusion rate which varies based on if the wristband is opened to the air or covered by clothing and the time period it is worn. Therefore, the amount collected does not readily translate to average personal air concentrations [27] but rather provides a relative measure of the air concentration encountered and provides confirmation that inhalation exposure occurred. Table 1 lists a number of studies conducted in different countries using this method and the pyrethroids detected along with the mass collected on the silicone wristband. In addition to detecting several pyrethroids in multiple settings, piperonyl butoxide, a synergist added to pyrethroid application mixtures, was found in wristbands for more than half of the participants.

6 Indoor Air Levels

While the quantity of pesticides sprayed outdoors during a single application in agricultural settings is typically much higher than the amount sprayed indoors, indoor pyrethroid air concentrations and the associated exposures can be higher than outdoors. Spraying indoors may occur in close proximity to individuals not wearing personal protective equipment (PPE) such as a respirator or gloves. Further, spraying indoors is done within a confined space, while spraying outdoors has significantly more dilution due to the open area and wind. A variety of factors that degrade pyrethroid residue outdoors, e.g., sunshine, precipitation, and temperature extremes, are either not present or less extreme indoors extending their residence time in indoor settings compared to outdoors [32]. Elevated pyrethroid air concentrations can also occur indoors from the redistribution of these compounds from sprayed surfaces when the pyrethroids volatilize, redistribute onto dust, and become resuspended [33]. Pyrethroid pesticides are SVOCs with low vapor pressures and high octanol/water and water/organic carbon partitioning coefficients facilitating their absorption onto the organic component of house dust. House dust has been shown to be a reservoir for many SVOCs, including pesticides [34]. An additional concern for pyrethroids present in indoor air is that they are breathed by a range of individuals, including those potentially more sensitive to adverse health effects than the healthy workers including children, the elderly, and individuals with preexisting health conditions. Further, the amount of time that people spend indoors (>90%) exceeds that spent outdoors, which for the general population is <10% and for workers 20–50% (35–40 h/week). This results in more exposure to indoor contaminants.

Table 1 Personal air samples collected using silicone wristbands

Study and location	Pyrethroids measured	Amount per band and frequency of detection
Donald et al. [28] (West Africa) $n = 70$	Deltamethrin	530 ng per ng band (frequency 99%)
	Cypermethrin	293 ng per ng band (frequency 94%)
	Esfenvalerate	14.6 ng per ng band (frequency 40%)
	trans-Permethrin	48.8 ng per ng band (frequency 27%)
	cis-Permethrin	19.5 ng per ng band (frequency 17%)
Bergmann et al. [29] (Peru) $n = 65$	Cypermethrin	77–7,700 ng per ng band (frequency 71%)
	Bioallethrin	Only in single sample
	Cyhalothrin gamma	Only in single sample
	Cyhalothrin lambda	Only in single sample
	Cypermethrin I	Only in single sample [28]
	Cypermethrin II	Only in three samples
	Cypermethrin III	Only in single sample 7
	cis-Cyphenothrin	Only in single sample
	trans-Cyphenothrin	Only in single sample
	Fipronil	Only in single sample
	cis-Permethrin	Only in two samples
	trans-Permethrin	Only in single sample
	Piperonyl butoxide	Only in nine samples
Harley et al. [30] (California, USA) $n = 97$		Only frequency reported
	Cypermethrin	56%
	trans-Permethrin	52%
	cis-Permethrin	49%
	Esfenvalerate	41%
	Piperonyl butoxide	19%
	Fipronil	10%
	Fipronil sulfide (breakdown product)	87%
	Fipronil sulfone (breakdown product)	45%
Aerts et al. [31] (Belgium) $n = 30$	Fipronil	0.8–90 ng per ng band (frequency 33%)
	Fipronil-desulfinyl	0.4–47 ng per ng band (frequency 10%)
	Fipronil sulfone	0.4–2.0 ng per ng band (frequency 27%)
	Mepanipyrim	0.8 ng per ng band (frequency 3.3%)
	Pyrimethanil	2.9–8.7 ng per ng band (frequency 10%)
	Pyriproxyfen	3 ng per ng band (frequency 3.3%)
	Piperonyl butoxide	0.9–55 ng per ng band (frequency 63%)

Pyrethroid sources to indoor air include applications by exterminators and residents within buildings, penetration of pyrethroids from outdoors associated with drift from agricultural settings and treatment of surrounding outdoor settings or neighboring apartments/buildings, and resuspension of dust that absorbed pyrethroids or volatilization from dust and indoor surfaces. Indoor pesticide application equipment that directly increases air concentrations include ready-to-use products with a trigger pump spray, pressurized aerosol cans, compressed air sprayers, broadcast applications, coils, and vaporizers.

Li et al. [10] evaluated the indoor air levels during and post-application for a series of controlled mosquito control applications using four different application methods (mosquito coil, liquid vaporizer, vaporizing mat, and aerosol spray). They measured sub-μg/m^3 levels of several pyrethroids during the application with air concentrations decreasing 1–2 orders of magnitude within 12 h following the application (Table 2). They also observed lower air levels when windows were opened as opposed to closed, which is consistent with higher ventilation rates reducing air concentrations. The percentage of pyrethroids in the particulate phase varied from 40 to >95% for dimefluthrin, allethrin, cypermethrin, and tetramethrin, with compounds having lower vapor pressure being more associated with the particulate phase [10]. An older study quoted by Li et al. reported ppm air concentrations of various pyrethroids in residue over very short-time intervals of minutes [40]. Li et al. suggested that the apparent higher levels measured previously reflected the timing between the application and the sample collection and the sampling duration [41]. Multiple sample collection indicates, not surprisingly, that the peak air concentrations are during the pyrethroid application. To avoid unnecessary pesticide exposure, typical labels caution against vulnerable individuals, such as children, being in the room when spraying is done, and the sprayed area should be adequately ventilated before it is reoccupied. Nazimek et al. measured 1.3–5.2 μg/m^3 of transfluthrin in the indoor air after application of gel and liquid formulas in an electro-vaporizer application, though the levels were below detection 18–24 h after the application [41]. An evaluation of multiple pyrethroids in residences in South Korea found that the air concentration of the sum of pyrethroids present (Table 3) was inversely related to the time since it was last sprayed but not to frequency of use, room sprayed, or if products were stored indoors [44]. Vesin et al. [46] used a high sensitivity proton-transfer-reaction mass spectrometer (HS-PTRMS) to measure time-resolved gas-phase air concentrations of transfluthrin emitted during an electric vaporizer application and reported a constant increase until the unit was unplugged, then reaching 4.9 μg/m^3 after 8 h at a room air exchange rate (AER) of 0.35 h^{-1} and 8.5 μg/m^3 at an AER of 0.14 h^{-1}. Once the vaporizer was unplugged, the air concentration decreased exponentially at a rate based on the AER. They also reported that the air concentrations continued to rise reaching a steady-state concentration of 16 μg/m^3 after 33 h for the lowest AER (0.14 h^{-1}) examined.

Pyrethroid exposure of children and pregnant women is of particular concern since pyrethroids can affect the neurological system and potentially other organs [47–49]. Ingestion of food contaminated with pyrethroids and inadvertent ingestion of household dust in treated residences are generally larger exposure routes than

Table 2 Outdoor pyrethroid air concentrations

Study and location	Pyrethroids measured	Range detected and frequency of detection
Blanchard et al. [35] (France)	Bifenthrin	ND–2.9 ng/m^3
	cis-Permethrin	ND–8.0 ng/m^3
Morgan et al. [36] (Ohio, USA)	Permethrin	In 18% of samples >1 ng ng/m^3
Li et al. [10] (China)	Cypermethrin particles	0.218 ± 0.369 ng/m^3 (1.3 max)
	Cypermethrin vapor	0.010 ± 0.003 ng/m^3 (0.015 max)
	Total pyrethroid particulate	0.352 ± 0.443 ng/m^3 (1.8 max)
	Total pyrethroid vapor	0.061 ± 0.051 ng/m^3 (0.1 max)
Bradman et al. [37] (CA, USA)	*cis*-Allethrin	<21
	trans-Allethrin	<2
	Bifenthrin	<1
	Cyfluthrin	<100
	λ-Cyhalothrin	<10
	Cypermethrin	<100
	Deltamethrin	<50
	Esfenvalerate	<25
	cis-Permethrin	ND–8.0 ng/m^3 (frequency 30%)
	trans-Permethrin	<2
	Resmethrin	<2
	Sumithrin	<2
	Tetramethrin	<4
Morgan [38] (North Carolina, USA)	Cyfluthrin	ND
	cis-Permethrin	ND–1.62 ng/m^3 (frequency 16%)
	trans-Permethrin	ND–1.01 ng/m^3 (frequency 16%)
	Cyfluthrin	ND
	cis-Permethrin	ND–0.45 ng/m^3 (frequency 39%)
	trans-Permethrin	ND–0.34 ng/m^3 (frequency 39%)
Tulve et al. [39] (USA)	*cis*-Allethrin	ND
	trans-Allethrin	ND
	Bifenthrin	ND
	λ-Cyhalothrin	ND
	Cyfluthrin	ND
	Cypermethrin	ND–19 ng/m^3 (frequency 22%)
	Deltamethrin	ND
	Esfenvalerate	ND
	cis-Permethrin	ND–2.3 ng/m^3 (frequency 100%)
	trans-Permethrin	ND–10 ng/m^3 (frequency 100%)
	Pyrethrin I	ND
	Pyrethrin II	ND
	Sumithrin	ND
	Tetramethrin	ND–0.15 ng/m^3 (frequency 33%)
	Piperonyl butoxide	ND–3.1 ng/m^3 (frequency 100%)

Table 3 Indoor pyrethroid air concentrations

Study and location	Pyrethroids measured	Range detected and frequency of detection	
Yoshida et al. [42] (Japan)	*Sampled after mosquito clothes repellent used*		
	Empenthrin	2.3 ng/m^3	
	Profluthrin	1 ng/m^3	
	Sampled after mosquito electrical repellent used	Day	Night
	Prallethrin	34 ng/m^3	37 ng/m^3
	Furamethrin	39 ng/m^3	24 ng/m^3
	Allethrin	148 ng/m^3	122 ng/m^3
	Furamethrin	5 ng/m^3	4.1 ng/m^3
	Transfluthrin	12 ng/m^3	9.2 ng/m^3
	Prallethrin	69 ng/m^3	23 ng/m^3
	Metofluthrin	0.24 ng/m^3	0.15 ng/m^3
Leng et al. [43] (Germany)	Cypermethrin	ND–934 ng/m^3 (frequency 9.4%)	
Bradman et al. [37] (California, USA)	*cis*-Allethrin	<21–63 ng/m^3 (frequency 15%)	
	trans-Allethrin	<2–61 ng/m^3 (frequency 15%)	
	Bifenthrin	1–3.1 ng/m^3 (frequency 5%)	
	Cyfluthrin	<100	
	λ-Cyhalothrin	<10	
	Cypermethrin	<100–310 ng/m^3 (frequency 5%)	
	Deltamethrin	<50	
	Esfenvalerate	<25	
	cis-Permethrin	<2–8.2 ng/m^3 (frequency 40%)	
	trans-Permethrin	<2–11 ng/m^3 (frequency 16%)	
	Resmethrin	<2	
	Sumithrin	<2–96 ng/m^3 (frequency 10%)	
	Tetramethrin	<4	
Le et al. [10] (Korea)	*After mosquito coil used*	During application	12 h post
	Dimefluthrin windows open	503–549 ng/m^3	0.1–1. Ng/m^3
	Dimefluthrin windows closed	454–781 ng/m^3	34–46 ng/m^3
	After mosquito liquid vaporizer		
	Dimefluthrin	193–346 ng/m^3	116–181 ng/m^3
	After mosquito vaporizing mat		
	Allethrin	15,100–24,300 ng/m^3	310–1,570 ng/m^3
	After mosquito aerosol spray		
	Allethrin	170–270 ng/m^3	21–74 ng/m^3
	Cypermethrin	21.7–36 ng/m^3	0.5–0.6 ng/m^3
	Transfluthrin	16.5–48.3 ng/m^3	3.5–9.5 ng/m^3

(continued)

Table 3 (continued)

Study and location	Pyrethroids measured	Range detected and frequency of detection	
Blanchard et al. [35] (France)		Particles	
	Cypermethrin	<0.2–0.28 ng/m^3 (frequency 3%)	
	Permethrin	<0.002–1.5 ng/m^3 (frequency 40%)	
	Tetramethrin	<0.002–85.0 ng/m^3 (frequency 27%) Vapor phase <0.6 ng/m^3 (not detected)	
Nazimek et al. [41] (Poland)	Transfluthrin	1.3–2.4 ng/m^3 gel formulation 3.8–5.2 ng/m^3 liquid formulation ND 28–18 h later	
Pentamwa et al. [44] (Bangkok, Thailand)	Sum pyrethroids		
	Home sprayed 1 per week	0.09–2.0 ng/m^3	
	Home sprayed 1 per month	0.01–0.04 ng/m^3	
	Home sprayed 1 per 6 months	ND	
Wyatt et al. [45] (New York City, USA)		Indoor air	Personal air
	Piperonyl butoxide	<0.2–608 ng/m^3 (46%)	0.2–98.2 ng/m^3 (61%)
	trans-Permethrin	<0.1–164 ng/m^3 (14%)	<0.1–7.5 ng/m^3 (15%)
	cis-Permethrin	<0.4–125 ng/m^3 (17%)	<0.4–9.4 ng/m^3 (13%)
Tulve et al. [39] (USA)	*cis*-Allethrin	ND–74 ng/m^3 (frequency 33%)	
	trans-Allethrin	ND–38 ng/m^3 (frequency 33%)	
	Bifenthrin	ND–4 ng/m^3 (frequency 11%)	
	λ-Cyhalothrin	ND–5.5 ng/m^3 (frequency 11%)	
	Cyfluthrin	ND	
	Cypermethrin	ND–100 ng/m^3 (frequency 22%)	
	Deltamethrin	ND	
	Esfenvalerate	ND–0.32	
	cis-Permethrin	ND–92 ng/m^3 (frequency 89%-median 2.0)	
	trans-Permethrin	ND–130 ng/m^3 (frequency 89%-median 3.1)	
	Pyrethrin I	ND–12 ng/m^3 (frequency 44%)	
	Pyrethrin II	ND–0.91 ng/m^3 (frequency 11%)	
	Sumithrin (d-phenothrin)	ND–4.2 ng/m^3 (frequency 11%)	
	Tetramethrin	ND–63 ng/m^3 (frequency 22%)	
	Piperonyl butoxide	ND–378 ng/m^3 (frequency 89%-median 7.4)	

inhalation exposure at typical air levels, with inhalation contributing 5–10% of the total exposure [50]. Pyrethroid air concentration is in steady state with household dust levels. Bradman et al. measured air concentrations indoors and outdoors along with house dust levels in the homes of 20 children and only found measurable levels of *cis*-permethrin in the air, while several other pyrethroids were present in the house dust [37]. Tulve et al. measured indoor and outdoor air, wipe samples from play areas, levels on socks, and in food for 14 pyrethroids, piperonyl butoxide, and 2 other pesticides in the homes of 9 children (Table 3) [39]. Most pyrethroids were detected more frequently in indoor air than outdoor air, and the median and maximum concentrations were higher [39] in the indoor air samples. They also found correlations between the wipe samples and the indoor air levels for multiple pyrethroids across the homes.

Since dust can be resuspended by movement in a home, Zhou et al. used a robot to simulate a toddler's movement and observed that the movement increased particulate pyrethroid air concentrations [51]. They measured twice the permethrin air concentrations near the moving robot at a toddler's breathing zone height compared to levels at an indoor stationary sampler collected simultaneously. They also found differences in the air concentration when the robot resuspended dust from a vinyl floor (65 and 143 ng/m^3, stationary and robot sample, respectively) compared to a carpeted floor (34 and 61 ng/m^3, stationary and robot sample, respectively). This study demonstrated the need for caution when using indoor air concentrations rather than personal air concentration measurements to estimate pyrethroid inhalation exposure.

7 Urinary Metabolites of Pyrethroids

Once inhaled, pyrethroids are metabolized in the body and excreted with many compounds having half-lives of just hours. A list of common pyrethroids and their metabolites is given in Table 4 [48, 52]. Several pyrethroids have the same metabolites, e.g., 3-BPA, *cis*-DCCA, and *trans*-DCCA, so while the presence of these metabolites in urine indicates that there was likely an exposure to a pyrethroid, it does not confirm which specific pyrethroid was present nor the exposure route. The metabolites are predominantly excreted as sulfate and glucuronide conjugates in the urine. The urinary metabolite levels have been used to evaluate exposure models. Several studies have used the US EPA Stochastic Human Exposure and Dose Simulation (SHEDS)-Multimedia model to predict the relative contributions of pyrethroid exposures across all routes and compare the results to urinary 3-PBA levels [50, 53, 54]. While there was a strong correlation between the total exposure predicted and the urinary 3-PBA levels, only a small percentage of the cumulative exposure was calculated to be via inhalation, and the inhalation exposure was not correlated to the urinary levels across the entire population studies.

Time series changes in urinary levels of *trans*-DCAA and 3-PBA were shown to be related application of permethrin in an agricultural settings to workers exposed

Table 4 Selected metabolites of commonly used pyrethroids and pyrethrins

Pyrethroid	3-Phenoxybenzoic acid (3-PBA)
Allethrin	3-Phenoxybenzoic acid (3-PBA)
Cypermethrin	3-Phenoxybenzoic acid (3-PBA) *cis*-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*cis*-DCCA) *trans*-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*trans*-DCCA)
Cyfluthrin	*cis*-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*cis*-DCCA) *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*trans*-DCCA) 4-Fluoro-3-phenoxybenzoic acid (*FPBA*)
λ-Cyhalothrin	3-Phenoxybenzoic acid (3-PBA) *cis*-3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid (CFMP)
Deltamethrin	3-Phenoxybenzoic acid (3-PBA) *cis*-3-(2,2-Dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA)
Esfenvalerate	3-Phenoxybenzoic acid (3-PBA) 2′-(4′-Hydroxyphenoxy)-benzoic acid or 3′-(4′-Hydroxyphenoxy)-benzoic acid
Fenvalerate	3-Phenoxybenzoic acid (3-PBA)
Flumethrin	3-(2-Chloro-2-(4-chlorophenyl)ethenyl)-2,2-dimethylcyclopropanecarboxylic acid (flumethrin acid) 4-Fluoro-3-phenoxybenzoic acid (FPB acid), 4′-OH-FPB acid
Permethrin	3-Phenoxybenzoic acid (3-PBA) *cis*-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*cis*-DCCA) *trans*-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (*trans*-DCCA)
Phenothrin	3-Phenoxybenzoic acid (3-PBA)
Resmethrin	3-Phenoxybenzoic acid (3-PBA)
Tetramethrin	3-Hydroxy-cyclohexane-1,2-dicarboximide

through inhalation and dermally and provided information on the kinetics of permethrin and its metabolites [55]. Physiologically based pharmacokinetic (PBPK) models predict how a compound is taken up, distributed, metabolized, and excreted by the body. PBPK models have been used to evaluate urinary metabolite data and to estimate the corresponding inhalation exposure and other exposure routes and back-calculate the pyrethroid air concentrations associated with the measured urinary metabolite levels [56–59]. These studies have also suggested that for typical household indoor air concentration, and the corresponding inhalation exposure is not the major source of pyrethroids exposures to individuals. However, estimates related to peak, shorter-term exposures that can occur during pesticide applications and corresponding health effects have not been adequately evaluated using PBPK modeling.

8 Summary

Peak pyrethroid air concentrations can occur during application of pesticides both outdoors and indoors. Professional applicators should be deploying appropriate personal protective equipment to reduce their internal pyrethroid exposure and protect their health. Further, pyrethroids should not be sprayed when it may cause others to encounter elevated air levels. Indoor pyrethroid air concentration is impacted by drift from agricultural uses, exterior spraying of nearby areas, spraying indoors, and resuspension or volatilization of pyrethroids on house dust. Indoor pyrethroid air concentrations can exceed outdoor levels and expose vulnerable populations. Current studies typically measure urinary metabolites of pyrethroids rather than air concentrations to evaluate exposure to these compounds and have found that for cumulative exposure inhalation of air generally contributes $<10\%$ of the total dose that is received to the general population. Air concentration present during or shortly after applications such as spraying, use of mosquito repellent coil or vaporizers and foggers can result in higher air concentration and more significant inhalation exposures if proper precautions are not taken.

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Risk Assessment of Human Exposure to Pyrethroids Through Food

Tânia Mara Pizzolato and Alexsandro Dallegrave

Contents

Abstract For decades, the global demand for food has been increasing as a result of population growth and changes in diets. Together with this demand, the ample use of pesticides and insecticides in every step of the production chain has grown. Pyrethroids are systemic pesticides widely used in both agriculture and veterinary. They are often found on the surface of fruits and leafy vegetables or deposited on the lipid bilayer in products of animal origin. Considering the high use of pyrethroids all around the world, the potential risks of human exposure to residues in food products are a matter of great concern. Risk assessment is the scientific basis for risk management according to various international agencies. The vast majority of pesticide residue risk assessments in food are based on the toxicological evaluation of individual compounds, but assessments of cumulative exposure to multiple residues have gained notoriety. The evaluation of the "daily intake" is of great importance for human and environment safety.

Keywords Food, Pyrethroids, Risk assessment

T. M. Pizzolato (✉) and A. Dallegrave
Chemical Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
e-mail: tania.pizzolato@ufrgs.br; adallegrave@iq.ufrgs.br

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According to Paracelsus, pioneer of the medical revolution of the sixteenth century, "Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy." Paracelsus' quote remains valid nowadays. Humans are subjected to high chemical daily exposure levels, thus making risk assessment of the utmost importance. Food safety is an important means to promote public health, emerging as an extremely relevant research area. Still, the dissemination of scientific information regarding food safety is not widely explored, leading us to further investigate its specifics and preferred methods of assessment. For decades, the global demand for food has been increasing as a result of population growth and changes in diets. Land for agriculture and storage options are scarce, justifying the ample use of pesticides and insecticides in every step of the production chain.

Pyrethroids constitute the majority of agricultural and veterinary pesticides and commercial household insecticides. Residues of pyrethroids are the main source of agricultural pollution and are potentially hazardous, becoming a public health concern [1].

Pyrethroids are systemic pesticides with a regulated use in food products, livestock, and livestock feed. They are often found on the surface of fruits and leafy vegetables [2] or deposited on the lipid bilayer in products of animal origin [3]. In this chapter, we will explore topics concerning the potential risks of human exposure to pyrethroid residues in food products, considering the role of population's diet in the risk assessment.

Risk assessment is the scientific basis for risk management according to various international agencies. The US Environmental Protection Agency defines the evaluation of potential outcomes of pesticides in food products through human health risk assessment as the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/overview-risk-assessment-pesticide-program). Risk assessment is also the basis of the Codex Alimentarius Commission, which through the Joint Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) establishes international guidelines for pesticide residues in specific food items [4].

Most international environmental protection agencies use a four-step process for human health risk assessments:

1. *Hazard identification* – aims to analyze available data on toxicity and mode of action of agents present in a particular food or group of foods which are capable of causing adverse health effects. Hazard identification is traditionally performed through observation of the effects of pesticide residues in humans and animals (domesticated and laboratory) and in vitro and structure-activity relationship analyses.
2. *Hazard characterization* – is the description of the relationship between levels or dose of the consumed residue of pesticide and the probability of development and severity of an adverse health outcome. Hazard characterization of threshold toxic effects usually constitutes reference data, such as the acceptable daily intake (ADI), for example, for a residue of a pesticide in food products.

3. *Exposure assessment* – examines the levels of pesticides in human diet, analyzing frequency and timing of contact with or consumption of food products with residues of pesticides. It estimates various factors such as age, gender, and pre-existing health conditions.
4. *Risk characterization* – examines the nature and extent of human health risks from exposure to pesticides. It indicates the overall degree of confidence in the assessment and information about populations more likely to be susceptible to pesticides.

The vast majority of pesticide residue risk assessments in food are based on the toxicological evaluation of individual compounds, but assessments of cumulative exposure to multiple residues have gained notoriety [5].

1 Human Exposure to Pyrethroids

Exposure to pyrethroids can be either occupational or nonoccupational and can occur in several ways, such as inhalation and oral and dermal routes. The majority of the population is not substantially exposed to pyrethroids via inhalation and dermal routes, as the uptake is mostly caused by manipulation of household products with pyrethroids in their formula. On the other hand, they are the major routes of exposure for agriculturists working with pesticides. Oral exposure is the primary contamination route in general population due to ingestion of food products containing pyrethroid residues [1, 6].

Ingestion of food products of vegetal origin such as fruits and vegetables usually causes more human health damage since their consumption is in a raw or a semi-processed form. Conversely, cereals and animal products are heavily processed, oftentimes through high-temperature and pasteurization processes, leading to degradation of pyrethroids [7].

Deterministic and probabilistic approaches are often employed to analyze data on food consumption and to quantitatively assess exposure [8, 9]. The deterministic model utilizes available data and does not require evaluation of uncertainty components, expressing results which can be easily elucidated. Based on results from previous studies (REFs) performed in Spain in 2016, Quijano et al. [7] a mean-estimated chronic cumulative risk assessment determined by multiplying the mean pesticide concentration in a food product by the mean or the 95th percentile of the food consumption, thus defining lower-bound and upper-bound scenarios, respectively.

The probabilistic approach quantifies variation and uncertainty, representing the data as a distribution instead of fixed values, including variance parameters. Parameters such as food consumption data, pesticide levels, body weight, and susceptible population groups (infants, expecting and breastfeeding mothers, individuals with kidney or liver disorders) are used in the probabilistic approach for higher accuracy. Monte Carlo simulation is the most commonly used approach to estimate exposure, taking into account probability distributions. Risk assessment requires an exact and systematic quantitative data analysis model, particularly

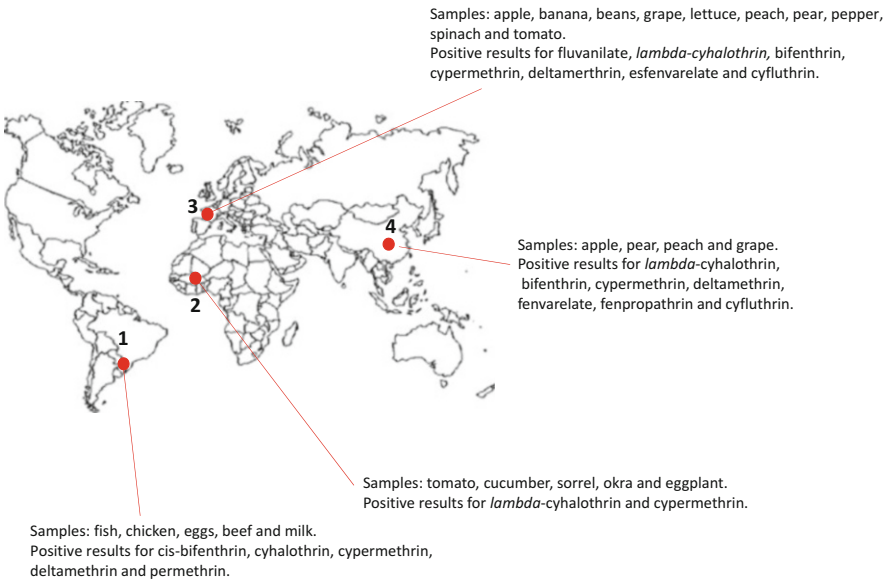

Fig. 1 Detection of pyrethroid residues in food from several continents: *1* South America [3], *2* Africa [11], *3* Europe [7] and *4* Asia [12]

when the calculated risk exceeds the acceptable values. Thus, the probabilistic model is expected to surpass the deterministic model in the near future [10] (https://www.epa.gov/expobox/exposure-assessment-tools-tiers-and-types-deterministic-and-probabilistic-assessments. Accessed 18 Apr 2019).

Global exposure to pyrethroids through food consumption is reaching alarming levels. Several studies performed in different countries reveal cases in which pyrethroids were found in food products: Dallegrave et al. [3] analyzed the presence of pyrethroid residues in food products of animal origin, finding approximately 10% of milk samples contaminated with at least five different pyrethroids. Lehmann et al. [11] analyzed food products of vegetal origin, and 8.5% of the samples had residue levels higher than the MRL for lambda-cyhalothrin, and even the acute hazard quotient (HQacute) was greater than 1, indicating risk. Quijano et al. [7] detected lambda-cyhalothrin, cypermethrin, and bifenthrin in 9, 5 and 4% of the vegetal food product samples, respectively. Zhixia Li et al. [12] reported that 30% of food products of vegetal origin showed 2, 3 or 4 different pyrethroid residues, 3% in levels higher than the MRLs. The authors also identified cypermethrin, bifenthrin, and lambda-cyhalothrin with the highest acute and chronic hazard index values (Fig. 1).

2 In Vivo Toxicity

Pyrethroids are classified in two distinct groups according to the absence (type I) or presence (type II) of a cyano group bound to the alpha-carbon in the molecule. Figure 2 depicts structures of the main type I and type II pyrethroids.

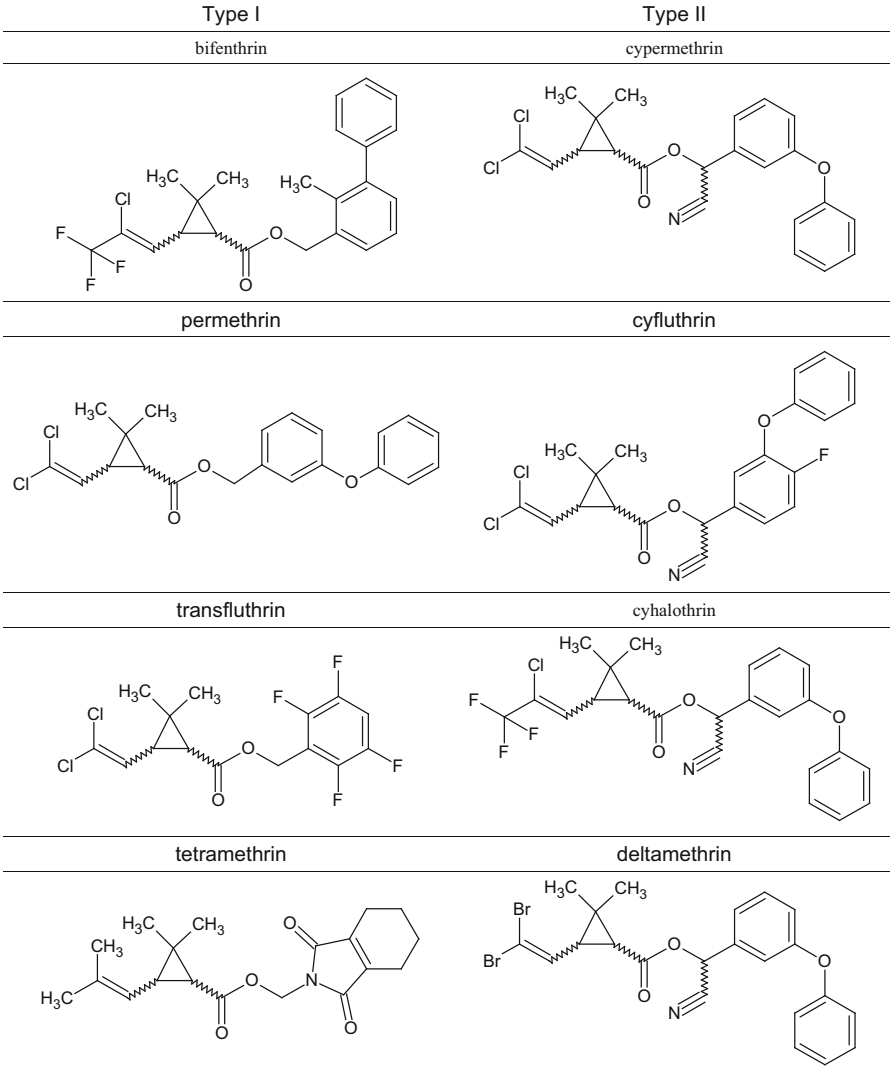

Fig. 2 Chemical structure of the type I pyrethroids (bifenthrin, permethrin, transfluthrin, and tetramethrin) and type II pyrethroids (cypermethrin, cyfluthrin, cyhalothrin, and deltamethrin)

Toxicity tests in laboratory animals revealed the occurrence of two syndromes, namely, T and CS syndromes, related to type I and type II pyrethroids, respectively. Neurotoxic symptoms caused by type I pyrethroids include shivering, irritability, high fever, comatosis, and death. Type II pyrethroids may cause salivation, involuntary movements, violent trembling, comatosis, and death. Exposure to certain pyrethroids, e.g., fempropatrin and esfenvalerate, leads to both T and CS syndromes. Mammalian toxicity is low, and specific enzymatic systems allow mammals to recover from contamination by pyrethroids in 24–48 h. Conversely, such degradation route is not present in insects, causing a higher insect toxicity [13].

3 Human Contamination

Recent research unanimously identifies ingestion of contaminated food products as the most relevant factor of human health damages caused by pyrethroids. When ingested, pyrethroids are immediately metabolized via hydrolysis of the ester, forming the corresponding carboxylic acids, oxidation and glucuronidation, and expelled in urine as conjugates. The main metabolites of pyrethroids in urine are the *cis*- and *trans*-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DCCA and *trans*-DCCA) and 3-phenoxybenzoic acid (3-PBA). 3-PBA is a metabolite of various pyrethroids including fenvalerate, sumithrin, deltamethrin, permethrin, cyhalothrin, and cypermethrin. DCCA is a metabolite of permethrin, cyfluthrin, and cypermethrin. DBCA (*cis*-dibromo dimethyl vinyl cyclopropane carboxylic acid) is a metabolite of deltamethrin. 4F3PBA (4-fluoro-3-phenoxybenzoic acid) is a metabolite of cyfluthrin [14–16]. Structures of those metabolites are depicted in Fig. 3. The rapid metabolism prevents the accumulation of intact pyrethroids in plasma and blood serum; therefore, urine samples are preferred for intoxication monitoring.

Analysis of metabolites of pyrethroids in human urine has been widely used to assess the real human exposure to pyrethroids. Several studies reported the presence of metabolites of pyrethroids in human urine: 3-BPA and *cis*- and *trans*-DCCA were found in the urine of children in China [17], 3-BPA, *cis*- and *trans*-DCCA, and DBCA were found in the urine of children in Poland [18] and in Japan [19], and 3-BPA was found in the urine of children and expectant mothers in the USA [20], which was also found in the urine of expectant mothers in Japan [21].

Despite the fact that pyrethroids undergo a rapid metabolism in humans, due to its lipophilic nature, it is possible to find non-metabolized pyrethroids in breast milk. Corcellas et al. [22] reported tetramethrin, bifenthrin, λ-cyhalothrin, deltamethrin, fenvalerate, permethrin, and cypermethrin in breast milk samples in Brazil,

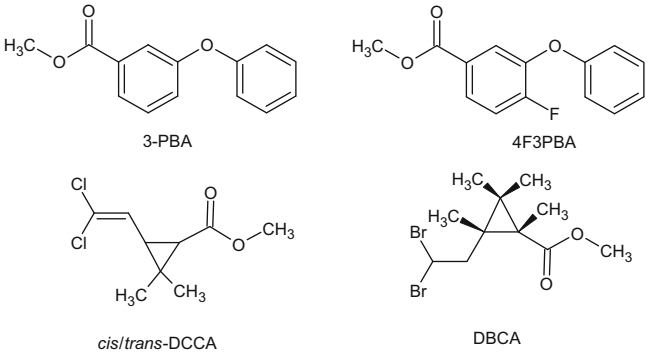

Fig. 3 Chemical structure of the pyrethroid metabolites: 3-Phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenzoic acid (4F3PBA), *cis*- and *trans*-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-/*trans*-DCCA), and *cis*-2,2 dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA)

Colombia, and Spain. The presence of pyrethroids in breast milk samples is an alarming evidence of the harmful effects of pyrethroids to human health. Newborn children are the most affected by the exposure to pyrethroids due to the high dosage/body weight ratio and developing immunological system.

4 Pyrethroids and Human Health

Human health effects caused by pyrethroids can be classified as local or systemic, depending on the route of contamination and levels of exposure. Acute symptoms may include irritation of the respiratory tract (coughing and lung irritation due to inhalation of dust or aerosol particles), vertigo and headaches, nausea and vomiting, eye irritation and inflammation, and paresthesia. Studies on chronic symptoms are still very limited and oftentimes controversial [1, 14].

Epidemiological studies in men showed the impacts in male fertility related to quality the DNA of sperm and reproductive hormones. Ji et al. [23] analyzed urine and semen samples of 240 males and observed a correlation between 3-BPA metabolite levels, low concentration of sperm, and DNA damage. Toshima et al. [24] inspected urine and sperm samples of 42 males, finding a correlation between the presence of the 3-BPA metabolite and low sperm mobility. Jurewicz et al. [25] found a positive association between *cis*-DCCA and DNA damage, as well as a correlation between 3-BPA levels and sperm DNA damage in urine and semen samples of 286 males.

In women, epidemiological studies analyzed pyrethroid exposure during pregnancy. Shelton et al. [26] correlated exposure to pyrethroids during pregnancy and neurobehavioral disorders, such as autism spectrum disorders in children. Reardon et al. [27] suggested there could be an association between respiratory problems in infants and exposure of mothers to pyrethroids during pregnancy.

Research on the correlation between pyrethroid exposure and cancer are still in its infancy, and current data is still inconclusive. Nonetheless, the US EPA classified permethrin, a common insecticide and insect repellent, also used to treat lice, as "probably cancerogenic to humans" when ingested, and the International Agency for Research on Cancer (IARC) recognized potential cancerogenic risks, including permethrin, in a high-priority review list for the 2015–2019 review period (https://monographs.iarc.fr/wp-content/uploads/2018/08/14-002.pdf. Accessed 3 Mar 2019).

5 Pyrethroid Risk Assessment

The presence of pyrethroid residues on food products is a substantial risk to human health. Therefore, the levels of pesticide residues are established according to parameters such as the MRL, maximum residue limit; the ADI, acceptable daily intake; and the ARfD, acute reference dose. Those limits are determined by national and international regulatory agencies and vary according to those agencies. The

Codex Alimentarius (WHO/FAO), the US Environmental Protection Agency (US EPA), European pesticides database, Japan Food Chemical Research Foundation (JFCRF), and Agência Nacional de Vigilância Sanitária (ANVISA) are the main pesticide regulatory agencies worldwide; however, a unanimous decision regarding acceptable pesticide levels has not been reached yet. The MRL values for bifenthrin in tomatoes can range from 0.02 to 0.5 mg kg^{-1}; according to the regulatory agencies, Codex Alimentarius and European pesticides database, MRL is 0.3 mg kg^{-1}, JFCRF is 0.5 mg kg^{-1}, ANVISA is 0.02 mg kg^{-1}, and EPA is 0.15 mg kg^{-1}.

For decades, developed countries have been monitoring the levels of pesticide residues on food products. Conversely, such effort is practically nonexistent in developing countries, mainly because of the high cost involved in the analysis. Analysis of pesticide residues in food produced in Togo (Africa) [28], in Ghana (Africa) [29], and in Bolívia (América do Sul) [30] reported data on pesticide residues exceeding the MRL and ADI values, increasing the potential risks to consumers, and thus confirming the urgency on guaranteeing food safety through effective pesticide monitoring programs [11].

ADI values are estimate according to Eq. (1)

$$\mathrm{EDI}x = \frac{\sum(\mathrm{C}xy * \mathrm{FC}y)}{\mathrm{bw}} \tag{1}$$

in which

- EDIx is the estimated daily intake of pesticide x
- Cxy is the concentration of pesticide x on food item y
- bw is the body weight of the individual
- FCy is the food processing factor of food item y, as utilized by Lehman et al. [11]. The significance of FCy depends on the combination of pesticides, crops, and processes.

Diet plays an important role in pesticide risk assessment. In order to assess pesticide risks to human health, a dietary assessment method factoring history and frequency of ingestion of certain food items should be used. Moreover, regional and cultural factors should be taken into account, particularly when using national averages to estimate exposure in large countries. A wide variety of dietary survey methods exists, with each one presenting a series of advantages and disadvantages The 24-h recall method proposed by Gibson and Ferguson in 1999 [31] is an example of dietary assessment method which quantifies all food items and drinks ingested during a period of 24 h prior to the interview. Quality of data thus depends on both good memory and cooperation of the interviewee, as well as the interviewer's ability to maintain an open communication channel. The 24-h recall method is noninvasive, quick, and practical for both interviewer and interviewee.

Acute and chronic pesticide risks can be evaluated using a hazard quotient – HQ. In the case of exposure to pesticides, an HQ is defined as the ratio of the amount

of pesticide ingested and the ADI or ARfD for acute and chronic risks, respectively, as shown in Eqs. (2) and (3).

$$\text{HQacute} = \frac{\text{EDI}}{\text{ARfD}} \tag{2}$$

$$\text{HQchronic} = \frac{\text{EDI}}{\text{ADI}} \tag{3}$$

Since ADI and ARfD express the level at which no adverse effects are expected following ingestion of pesticide residues, if HQ is calculated to be less than 1, then no adverse health effects are expected as a result of exposure.

The vast majority of the studies performed in the last decade only consider individual data, to the detriment of the understanding of cumulative risks of pesticides. Daily exposure is not limited to one specific pesticide. On the contrary, people are exposed to a variety of pesticide residues via ingestion of multiple food items containing a combination of pesticide residues. Dallegrave et al. [3] found several pyrethroid residues in samples of milk, eggs, fish, chicken, and beef. In milk, there were found as many as five different pyrethroid residues. Li et al. [12] analyzed 1,450 samples of fruit, including apples, grapes, pears, and peaches. At least two and as many as four different pyrethroids of the same chemical class were detected on approximately 30% of the samples. In those cases, a simultaneous assessment including cumulative risk would therefore be preferred [7].

Pyrethroid residues of the same chemical class present similar mechanisms of action. Thus, the exposure effects and human health risks are cumulative, and a cumulative risk approach is crucial [7, 10]. Current reports referring to cumulative risk assessment of pesticide residue mostly focus on two methods, the HI and the RPF methods. Boobis et al. [32] reported data utilizing the hazard index (HI) (Eq. 4) defined by Teuschler and Hertzberg [33] as is the sum of HQs of pesticides of similar toxic effects.

$$\text{HI} = \sum\nolimits_{i}^{n} \text{HQ}i \tag{4}$$

As HI values are dependent on HQ values, HIs larger than 1.0 are not considered acceptable.

In the relative potency factor (RPF) approach, the toxic potency of each pesticide residue in the mixture is compared to that of an index chemical generating a relative measure of potency for each residue. For pyrethroids, the RPF approach is usually combined to dose additivity (when the effect of the combination is the effect expected from the equivalent dose of an index chemical) as pyrethroid, carbamate, and organophosphate pesticides present similar neurotoxicity [10, 34]. Thus, the cumulative risk is assessed as an equivalent dose or the sum of pesticide residue doses scaled by their potency relative to the index chemical [35]. The equivalent dose is then compared to reference values for ADI and ARfD. Those methods are used to assess cumulative risks related to ingestion of a food product containing

residues of different pesticides, ingestion of different food items containing residues of one specific pesticide, or ingestion of several food items containing residues of different pesticides. Other approaches can estimate cumulative risk, such as margin of exposure (MoE), the ratio of no-observed-adverse-effect level (NOAEL) obtained from animal toxicology studies to the predicted and estimated exposure dose, and cumulative risk index (CRI), the reciprocal of the HI because both are based on reference values [5, 32, 36].

Evans et al. [36] calculated cumulative risk HIs and individual risk HQs of 67 pesticides in 5-year cumulative data provided by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for 13 different regions (Global Environment Monitoring System – Food Contamination and Assessment Programme) [37]. Presence of isomers was considered. Individual risk assessment showed an HQ larger than 1 twice only for chlorpyrifos-methyl. Cumulative risk assessment showed HIs larger than 1 for all regions. Region B, comprising Africa, Europe, and Middle East, showed a surprising HI larger than 10. Calculated HIs suggest a great contamination risk and call for broader collection and more refined treatment of data. When HI values exceed 1, HQ distributions can help in identifying the compounds with more significance to the cumulative risk and how the risk assessment model can be adjusted to incorporate those effects [36].

The European Food Safety Authority (EFSA) devised a methodology to classify pesticides into cumulative assessment groups, or CAGs. The methodology rests on the assumption that pesticides causing the same specific effects can produce cumulative toxicity – even if they do not have similar modes of action. CAGs are defined according to pesticides' chemical structure, toxicity mechanisms in mammals, and common toxic effects [38]. Cumulative risk assessment is then defined from CAG data based on hazard identification (effects specific to vulnerable populations and effects from stressor interactions) for further determination of the dose-response assessment (dose-response for sensitive populations, toxicological interactions, and combined doses of multiple stressors) and exposure assessment (multiple exposure routes and pathways, social, cultural, and economic factors that influence exposure) concluding with risk characterization (uncertainties associated with combining risks and qualitative factors affecting risk outcomes) [38]. The US Environmental Protection Agency (EPA) defined the CRA for five different classes of pesticides: organophosphates, N-methylcarbamates, s-triazines, chloroacetanilides, and pyrethrins/pyrethroids. The most recent CRA regarding pyrethroids was published in 2011 and includes a class of pyrethroids which trigger neurotoxicological effects via voltage-gated sodium ion channel through the cell membrane. All pyrethroids were classified under only one CAG, with deltamethrin as index compound (IC). The IC is selected to model the associated risk and extrapolate the estimated exposure levels in the population, thus decreasing errors and uncertainties in the risk assessment estimates. Pyrethroids with toxic potential significantly lower than IC and those with no detectable residues in monitoring were disregarded.

According to the EFSA, pesticides may cause toxic effects at multiple sites by a single mode of action. Therefore, substances can be grouped in more than one CAG. The effects considered for the establishment of reference values (ADI and ARfD) are

not necessarily representative for the CAGs, i.e., an effect observed at higher dose levels may be the specific effect relevant for grouping.

Risk assessment should consider vulnerability factors such as genetics, lifestyle, differential exposure to pesticides (including diet and distance from place of application), manufacturing processes, and recovering capacity. Moreover, food products are exposed to a myriad of pesticides and chemicals, not only to pyrethroids. For that reason, a more complete analysis employing the mixture risk assessment (MRA) approach is necessary. Even though there might be a consensus regarding cumulative risks and exposure to pesticides, the pathway to the formulation of an adequate regulation is still vague.

6 Uncertainties Associated with Exposure Assessment

Dietary exposure assessment methods are strongly affected by scientific uncertainties related to the sampling procedure which should be taken into account when interpreting the results, for example, duration of exposure, sampling sites, body weight, concentration of pyrethroid in food samples and uncertainty of the analytical techniques utilized, whether a food item or a food group has been sampled, and food processing levels. Moreover, specific characteristics of the population, such as pregnancy, breastfeeding, age, kidney or liver disorders, and hypersensibility to pesticides, are extremely important and should be carefully considered when deciding on a sampling procedure [12].

7 Perspectives

Future research efforts on the assessment of the risks related with the exposure to pesticides should focus on the analysis of total cumulative intake, considering the specifics of different population groups. The constitution of a dependable database on pesticide residues in food, water, and air is crucial to the human health and environment risk assessment. Through dietary habits, the entryway of pesticide residues into the human body, we are exposed to multiple harmful chemical substances. It is imperative that a thorough cumulative risk assessment of mixtures of pesticides is performed, providing reliable data.

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Human Risk Associated with Long-Term Exposure to Pyrethroid Insecticides

Anne-Marie Saillenfait and Stéphane Malard

Contents

Abstract The aim of this review is to provide a broad summary of the latest state of knowledge about the potential long-term adverse effects of pyrethroids on human health. The oldest and recent epidemiological studies mainly addressed respiratory, neurological, hormonal, and reproductive outcomes in adults after environmental and occupational exposures. Although several of these studies have suggested negative effects, especially on male hormonal and sperm parameters, findings were often equivocal or inconsistent across studies, and no firm and reliable conclusions can be reached yet. Regarding developmental outcomes, there is increasing evidence that fetal exposure to pyrethroids may be associated with poorer children's neurodevelopment. Prevention measures should be considered to reduce exposure of pregnant women and children to these widely used insecticides.

A.-M. Saillenfait (✉)
Institut National de Recherche et de Sécurité, Vandoeuvre-les-Nancy, France
e-mail: anne-marie.saillenfait@inrs.fr

S. Malard
Institut National de Recherche et de Sécurité, Paris, France

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1 Introduction

The pyrethroid insecticides are widely and increasingly used worldwide, and their metabolites were omnipresent in the urine samples collected from the general population across different countries [1]. The general population is primarily exposed to pyrethroids by ingestion of contaminated food (e.g., residues on fruits and vegetables) and dust particles. Inhalation and dermal intakes can also occur via residential and/or occupational indoor and/or outdoor application for pest control. The possibility of adverse health effects of pyrethroids after short- or long-term exposure has emerged as a major public health concern. A number of human studies have suggested genotoxic, hormonal, and reproductive effects of pyrethroids in male adults [1, 2]. Prenatal and childhood exposures have also been associated to neurodevelopmental effects and different adverse birth outcomes in a few human studies. During the last years, the body of research has grown and the possible long-term effects of pyrethroids deserve further evaluation. The purpose of the present review is to evaluate the weight of evidence complied since earlier reviews on the relationship between pyrethroid exposure and health effects.

2 Materials and Methods

As previous reviews included epidemiological studies up to about 2014, we conducted an electronic search for recent articles published in peer-reviewed journals using PubMed, starting from 2014 to March 2019. As a first step, all articles containing the words *pyreth*∗, *permethrin*, *cypermethrin*, *fenvalerate*, *cyfluthrin*, *deltamethrin*, and *cyhalothrin* in combination with any of the following, *endocrin*∗, *reprod*∗, *pregnan*∗, *thyroid*, *hormone*, *genotox*∗, *tumor*, *cancer*, *immun*∗, *resp*∗, and *neuro*∗, in their title or abstract or as a keyword were collected. The references cited in identified publications were also searched to locate additional articles. Studies included in this paper were those written in English, pertaining to occupational or environmental exposure to a specific pyrethroid or to the class of pyrethroids in association with human health outcomes, and presenting original results. Because there is a limited body of research, and to better identify the potential areas of concern related to human pyrethroid exposure, all studies that met the above-described criteria were included in this review, regardless of reporting quality.

3 Results

The recent epidemiological studies on the influence of chronic exposure of adults to pyrethroids have mainly focused on four areas of health effects, providing new information on reproductive-, thyroid-, respiratory-, and neurological-related outcomes. In addition, a great and increasing number of studies have investigated the possible association between prenatal and/or postnatal childhood exposures and child health, especially neurodevelopment. Exposures could be occupational or environmental, and most studies analyzed many pesticides and/or insecticides at once (e.g., organophosphate and pyrethroid classes). Exposure assessment relied on survey data (e.g., residential proximity to pesticides agricultural applications using Californian Pesticide Use Reports-CPU), self-reported exposure mostly with a dichotomous answer (e.g., user/no user in occupational settings), or measurement of biomarkers, allowing possible evaluations of exposure-response patterns (e.g., by stratifying exposures into a few levels). The most commonly used biomarkers were pyrethroid urinary metabolites, primarily 3-phenoxybenzoic acid (3-PBA). It is a general metabolite of several pyrethroids, and associations with 3-PBA implied more the pyrethroid class than a specific parent chemical. Its detection frequency in urine was generally in a range from 70 to 100% of the studied populations.

3.1 Respiratory Outcomes

All identified studies on respiratory health used cross-sectional analyses (Table 1). Respiratory symptoms were assessed in agricultural or farm workers and their families following occupational exposures to specific or different subclasses of pesticides, including pyrethroid insecticides. They essentially consisted in self-reported rhinitis, wheeze, and asthma. Two large-scale studies have been conducted in the USA. In an updated analysis of the Agricultural Health Study data, Hoppin et al. [5] reported a slight increase in wheezing with exposure to three individual pyrethroids. Of the 22,134 male applicators who had been interviewed, 6% had both wheeze and allergy and 18% reported wheeze only. In the Farm and Ranch Safety Survey, 30.8% of an estimated 2.1 million farm operators reported lifetime allergic rhinitis, and 5.1% had current asthma [4]. A positive association was found between lifetime allergic rhinitis and pyrethroids and other insecticides. However, in both studies, pesticide uses and outcomes were self-reported. The potential relationship between asthma and the pyrethroid class has not been specifically analyzed [4].

Information on the impact of non-occupational exposure to pyrethroids on respiratory health is scarce. Lung function was examined in a single study which suggested association between urinary concentrations of pyrethroid metabolites and changes in lung functions in children and adolescents from the Canadian general population [10]. No association with respiratory symptoms and diseases was observed in this population-based study.

Table 1 Summary of epidemiological studies investigating potential association between exposure to pyrethroids and respiratory outcomes

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/metabolites	Outcomes of interest	Main pyrethroid findings
Respiratory symptoms and disease (asthma)						
Cherry et al., 2018 [3] Canada	Cross-sectional study 2002	2,422 grain farmers	Self-reported use	Pyrethroids (class) (ever use, total years of use)	Asthma (self-reported)	No association (authors' comment: use too infrequent for any strong conclusion)
Patel et al., 2018 [4] USA	Cross-sectional analyses Part of the Farm Ranch Safety Survey 2011	11,210 primary farm operators (84% males)	Self-reported use in the 12 months prior to the interview	Pyrethroids (class) (use/no use)	Lifetime allergic rhinitis (self-reported)	Significant association between lifetime allergic rhinitis and overall insecticide use and with use of pyrethroids (POR, adjusted prevalence odds ratio; 95% CI, confidence interval = 2.1; 1.6–2.7, and 2.4; 1.5–3.9, respectively) (2.6; 1.5–4.6 among operators with crop farms)
Hoppin et al., 2017 [5] USA	Cross-sectional analyses Part of the Agricultural Health Study (AHS) 2005–2010	22,134 male farm applicators	Self-reported current use	8 pyrethroids (permethrin, cyfluthrin, bifenthrin, esfenvalerate, lambda-cyhalothrin, zeta cypermethrin, tefluthrin, pyrethrins) (use/no use and days of use/year for permethrin and cyfluthrin-5 exposure categories)	Allergic and non-allergic wheeze (self-reported)	Two pyrethroids associated with both allergic and non-allergic wheeze: Permethrin OR; 95% CI = 1.38; 1.09–1.75, and 1.35; 1.17–1.55, respectively And pyrethrins OR; 95% CI = 1.70; 1.13–2.56, and 1.43; 1.10–1.85, respectively

						Highest odd ratio with the highest level of use for permethrin (13–365 days) Zeta cypermethrin associated with allergic wheeze (OR; 95% CI = 2.02; 1.24–3.30) Highest use of permethrin (13–365 days) associated with allergic and non-allergic wheeze, with the highest OR (OR; 95% CI = 1.79; 1.05–3.04, and 1.76; 1.30–2.39), respectively
Koureas et al., 2017 [6] Greece	Cross-sectional study Presumably 2010	Pesticide sprayers in farms ($n = 80$) and general population ($n = 90$)	Self-reported use	Pyrethroids (class) (two indicators I: number of applications/year and total years applying pesticides II: I + total area spread throughout the lifespan)	Allergic rhinitis and rheumatoid arthritis (self-reported)	Association between lifetime exposure to pyrethroids and allergic rhinitis
Quansah et al., 2016 [7] Ghana	Cross-sectional study Period not indicated	300 farmers (74% men)	Self-reported use ($n = 300$) and spot urine ($n = 100$)	Cypermethrin and lambda cyhalothrin in urine	Respiratory symptoms (chronic cough, wheezing, phlegm production, breathlessness) (self-reported)	No association
Lu et al., 2015	Part of a randomized	90 mother and child pairs	Users of insecticide-treated bed nets (ITN)		Rhinitis, cough (self-reported) (frequency of	No differences in the frequency and duration

(continued)

Table 1 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/metabolites	Outcomes of interest	Main pyrethroid findings
[8] Burkina Faso	controlled trial 2001–2002		(presumably with pyrethroids and deltamethrin in particular)		symptoms for different follow-up periods – first week, second to fourth weeks, total 18 weeks) 2 groups: ITN from birth (A) or from age 6 months	of side effects (including cough and rhinitis = most frequently mentioned symptoms in children), except the frequency of headache in mothers from group A
Others						
Baumert et al., 2018 [9] USA	Case-control study nested within AHS 2009–2013	1,596 male pesticide applicators (234 cases and 1,335 non-cases)	Self-reported use in their lifetimes	Bifenthrin, cyfluthrin, lambda cyhalothrin, permethrin, tefluthrin (use/no use)	Sleep apnea (self-report of doctor diagnosis plus treatment)	Association with bifenthrin but only exposure of 5 cases and 11 non-cases
Ye et al., 2016 [10] Canada	Cross-sectional study Subset of the Canadian Health Measures Survey (CHMS) 2007–2009	5,436 participants (6–79 years of age)	Spot urine	Pyrethroid metabolites in urine (3-PBA, 4-F-3-PBA, *cis*- and *trans*-DCCA, *cis*-DBCA)	Lung function (FEV1, FV)	Concentrations of total pyrethroid metabolites significantly associated with decreased in FEV1 (forced expiratory volume in 1s) in children, in forced vital capacity (FV) in adolescents, and a relatively higher FEV1/FV ratio with both reduction in FEV1 and FV in adults No association with

						respiratory symptoms or diseases (i.e., cough, asthma, chronic bronchitis)
Mwanga et al., 2016 [11] South Africa	Cross-sectional study Period not indicated	211 rural women	Spot urine and blood collected at the end of working day	Pyrethroid metabolites in urine (3-PBA, 4-F-3-PBA, *cis*- and *trans*-DCCA, *cis*-DBCA) ($n = 182$)	Serum cytokine related to allergic and non-allergic asthma pathways	Some metabolites positively associated with both Th2 and non-Th2 cytokines

3-PBA 3-phenoxybenzoic acid, *Cis-DCCA cis*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *TDCCA trans*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *Trans-DDCA trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *4-F-3-PBA* 4-fluoro-3-phenoxybenzoic acid, *DBCA cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid

3.2 Thyroid Function

The relationships between farm-related exposures to different classes of pesticides and thyroid disease and/or thyroid hormone disruption have been examined in a series of recent studies using the cohort of the US Agricultural Health Survey (Table 2) [13–16, 18]. Weak association between long-term use of permethrin applied to crops and an increased risk of hypothyroidism was found among female spouses of farmers aged over 60 years [14]. No significant effect was found for permethrin applicators. Although they were conducted with large sample sizes over long time periods, these studies presented several limitations, including self-report of pesticide uses and lack of doctor diagnosis confirmation.

Regarding environmental exposures, no association was found between thyroid hormone levels and the urinary pyrethroid metabolite 3-PBA, in a representative sample of individuals from the US National Health and Nutrition Examination Survey [17], and in Japanese pregnant women [19] and prenatally exposed neonates [18].

3.3 Reproductive Effects

Several cross-sectional studies have evaluated the impact of environmental exposure to pyrethroids on male reproductive health (Table 3). In all studies, assessment relied on a single semen or blood sample and on the single measure of one (3-PBA) or several pyrethroid metabolites in urine. A series of studies conducted in men recruited in fertility clinics showed associations between pyrethroid urinary metabolites (3-PBA, *cis*-DCCA, and/or *trans*-DCCA) and sperm quality [31], DNA damages [27–29], and testosterone levels [31]. However, it is not clear if these results may apply to the general population. In contrast to these findings, urinary concentrations of 3-PBA were not found to be associated with sexual hormones and semen parameters in men from the Japanese general population [30, 32] and/or occupationally exposed to pyrethroids [25].

Comparatively, a relatively small number of epidemiological studies have been published on the possible association of pyrethroid exposure and female reproductive health. Biomarkers of pyrethroids were primarily used to confirm exposure. A variety of outcomes of interest has been evaluated in single studies (Table 3). Although limited, data available suggest that more attention should be paid to time to pregnancy [21], female reproductive hormones [22], and girl puberty [23], especially at higher levels of pyrethroid exposure.

Table 2 Summary of epidemiological studies investigating potential association between exposure to pyrethroids and thyroid effects

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pyrethroid exposure analysis	Outcomes of interest	Main pyrethroid findings
Santos et al., 2019 [12] Brazil	Cross-sectional study 2017	122 male and female agricultural workers	Self-reported use	Pyrethroids (deltamethrin, lambda cyhalothrin) (lifetime and recent use)	Thyroid hormones (TSH, free and total T3 and T4) in serum	Significant association between recent use of lambda cyhalothrin and reduced total T4 (−7%; 95% CI = −20 to −9%) and free T3 (−11%; 95% CI = −19 to −1%) (9 participants exposed to lambda cyhalothrin, 14 to pyrethroids)
Shrestha et al., 2019 [13] USA	Subset of AHS study (Enrolment: 1993–1997. Follow-up interviews: 1999–2003, 2005–2010, 2013–2016)	35,150 pesticide male and female applicators	Self-reported use	Permethrin (ever/never use)	Hyperthyroidism (self-reported diagnoses)	No association with permethrin exposure
Shrestha et al., 2018 [14] USA	Subset of AHS study (Enrolment: 1993–1997. Follow-up interviews: 1999–2003, 2005–2010, 2013–2016)	24,092 female spouses of farmers	Self-reported use	Permethrin (ever/never use)	Hyper- and hypothyroidism (self-reported diagnoses)	Permethrin (applied to crops) associated with modestly increased hypothyroidism among those over 60 years of age (adjusted hazard ratio; 95% CI = 1.68; 1.01–2.82)
Shrestha et al., 2018 [15] USA	Subset of AHS study (Enrolment: 1993–1997. Follow-up interviews: 1999–2003, 2005–2010, 2013–2016)	35,150 pesticide male and female applicators	Self-reported use	Permethrin (ever/never use and intensity-weighted lifetime days of use)	Hypothyroidism (self-reported diagnoses)	Non-statistically significant association between permethrin (applied to animals or crops) and increased risk of hypothyroidism (adjusted hazard ratio; 95% CI = 1.20; 0.99–1.46, and 1.19; 0.98–1.46, respectively) Greatest with higher intensity-weighed lifetime days ($p = 0.06$ for >490 days)
Lerro et al., 2018 [16] USA	Subset of AHS study 2010–2013 (Enrolment: 1993–1997. Follow-up interviews: 1999–2003, 2005–2010)	679 pesticide male applicators	Self-reported use	Permethrin (intensity-weighted exposure days/years)	Thyroid hormones (TSH, total T3 and T4) in serum Subclinical hypothyroidism	No association with permethrin exposure
Jain, 2016 [17] USA	Cross-sectional study Data from the National Health and Nutrition	2015 participants ≥12 years	Spot urine	3-PBA	TSH, free and total T3 and T4, thyroglobulin in serum	No association with 3-PBA levels

(continued)

Table 2 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pyrethroid exposure analysis	Outcomes of interest	Main pyrethroid findings
	Examination Survey (NHANES) 2007–2008					
Zhang et al., 2014 [18] Japan	Longitudinal birth cohort study 2009–2011	147 pairs mother and their newborn baby	Spot urine from the pregnant women (10–12 weeks) (on the day of blood sampling)	3-PBA in urine from the pregnant women	Neonates: TSH, free T4 in blood Pregnant women: TSH, free T4 and thyroid binding globulin in serum, urinary iodine	No association between 3-PBA levels in maternal urine in the first trimester of pregnancy and neonatal thyroid hormones or with body size
Zhang et al., 2013 [19] Japan	Cross-sectional study 2009–2011	231 pregnant women (10–12 weeks)	Spot urine (on the day of blood sampling)	3-PBA	TSH, free T4 and thyroid binding globulin in serum, urinary iodine	No association with 3-PBA levels

Detection frequencies of DBCA and 4-F-3-PBA $< 7\%$, associations not analyzed [17]
3-PBA 3-phenoxybenzoic acid, *AHS* Agricultural Health Study, a prospective cohort study

Table 3 Summary of epidemiological studies investigating potential association between exposure to pyrethroids and reproductive effects in males and females

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
Women-related outcomes						
Al-Hussaini et al., 2018 [20] Egypt	Cross-sectional study 2010–2013	94 women who underwent intracytoplasmic sperm injection, for male factor infertility	Follicular fluid (sperm not analyzed)	β-Cyfluthrin and bioallethrin	Endometrial thickness, oocytes retrieved, early embryo cleavage	Negative correlation between β-cyfluthrin concentrations and endometrial thickness, number of oocytes retrieved, fertilization and early embryo cleavage rates
Hu et al., 2018 [21] China	Shanghai Birth Cohort Study 2013–2015	569 women planning a pregnancy	Preconception spot urine sample (partner not assessed)	3-PBA	Time to pregnancy (TTP)	Women with highest quartile of 3-PBA levels had longer TTP and increased odds infertility compared with the women in the lowest quartile, with significant associations in nulliparous women
Li et al., 2018 [22] China	Case-control study 2015–2017	172 women diagnosed with primary ovarian insufficiency (POI) and 247 controls	Spot urine sample	Metabolites of pyrethroids (3-PBA, 4-F-3-PBA) (33–34% 4-F-3-PBA> LOD)	Serum levels of FSH, LH, and AMH	Highest quartile of 3-PBA levels associated with increased risk of POI Positive trend for FSH and LH and negative trend for AMH, with increasing 3-PBA levels No association between POI and 4-F-3-PBA level
Ye et al., 2017 [23] China	Cross-sectional study	305 girls (9–15 years)	Spot urine sample	3-PBA	Questionnaire on current stage of puberty (breast and pubic hair stages, menarche status)	Association between 3-PBA levels and delayed puberty onset

(continued)

Table 3 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
	2014–2015					
Whitworth et al., 2015 [24] South Africa	Cross-sectional study 2010–2011	420 women	Questionnaire (self-reported of indoor residual spraying)		Plasma AMH	Spraying in homes with painted walls (considered indicative of exposure to pyrethroids) associated with decreased AMH
Men-related outcomes						
Santos et al., 2019 [12] Brazil	Cross-sectional study 2017	122 male agricultural workers	Self-reported use	Pyrethroids (deltamethrin, lambda cyhalothrin) (life-time and recent use)	Testosterone, estradiol, FSH, LH in serum	Significant association between recent use of lambda cyhalothrin and increased LH (59%; 95% CI = 13–129%) (9 participants exposed to lambda cyhalothrin, 14 to pyrethroids)
Panuwet et al., 2018 [25] Thailand	Cross-sectional study 2006	133 male farmers	Spot urine sample (on the day of blood sampling)	Metabolites of pyrethroids (3-PBA, *trans*-DCCA) (37.5% *trans*-DCCA > LOD)	Testosterone level in serum	No association between 3-PBA concentration and testosterone levels (total and free) No association with *trans*-DCCA (detection vs non-detection)
Ye et al., 2017 [26] China	Cross-sectional study	463 boys (9–16 years)	Spot urine sample	3-PBA	Urinary LH and FSH, and questionnaire on current stage of puberty (testis volume, genitalia stage)	Higher 3-PBA concentration associated with higher levels of LH and FSH Significant association

	2014–2015					between 3-PBA levels and earlier pubertal development
Jurewicz et al., 2016 [27] Poland	Cross-sectional study 2008–2011	194 men with normal semen parameters or with slight oligospermia attending a fertility clinic	Spot urine sample (on the day of semen sampling)	Urinary metabolites of pyrethroids (3-PBA, *cis*-DCCA, *trans*-DCCA)	Proportion of Y:X chromosome in sperm	Negative association between the proportion of *cis*-DCCA to *trans*-DCCA, and sperm chromosomal sex ratio
Jurewicz et al., 2015 [28] Poland	Cross-sectional study 2008–2011	286 men with normal semen parameters or with slight oligospermia attending a fertility clinic	Spot urine sample	Metabolites of pyrethroids (3-PBA, *cis*-DCCA, *trans*-DCCA, DBCA) (16.8% DBCA > LOD)	Sperm chromatin structure assay	Positive association between PBA level (>50th percentile) and the percentage of high DNA fragmentation index (DFI) Positive association between *cis*-DCCA level (>50th percentile) and the percentage of immature sperm (high DNA stainability) and of medium DFI No association with levels of *trans*-DCCA, DBCA, and total metabolites
Radwan et al., 2015 [29] Poland	Cross-sectional study 2008–2011	195 men with normal semen parameters or with slight oligospermia attending an infertility clinic	Single spot urine sample (on the day of semen sampling)	Metabolites of pyrethroids (3-PBA, *cis*-DCCA, *trans*-DCCA, DBCA) (16.8% DBCA > LOD)	Sperm aneuploidy analysis	Levels of *cis*-DCCA, *trans*-DCCA, and/or 3-PBA associated with sperm chromosome disomy of chromosome 18 (3-PBA, *cis*-DCCA, ≥50th percentile), XY (3-PBA, ≥50th percentile *trans*-DCCA), YY

(continued)

Table 3 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
						(3-PBA), 21 (3-PBA, ≥50th percentile *trans*-DCCA), and total disomy (3-PBA) No association with DBCA
Imai et al., 2014 [30] Japan	Cross-sectional study 2002–2003	323 healthy men	Spot urine sample (at the time of semen sampling)	3-PBA	Semen analysis	No association between 3-PBA levels and sperm parameters (semen volume, motility, concentration, total number of sperm and of motile sperm)
Radwan et al., 2014 [31] Poland	Cross-sectional study 2008–2011	334 men with normal semen parameters or with slight oligospermia attending an infertility clinic	Spot urine sample (on the day of semen and blood sampling)	Metabolites of pyrethroids (3-PBA, *cis*-DCCA, *trans*-DCCA, DBCA)	Semen analysis and plasma levels of testosterone, FSH, and estradiol	Levels of 3-PBA, TDCCA, and *cis*-DCCA associated with the % of sperm with abnormal morphology (≥50th percentile *cis*-DCCA, *trans*-DCCA, ≥50th percentile sum), decrease in sperm concentration (≥50th percentile TDCCA), level of testosterone (≥50th percentile *trans*-DCCA), and computer-aided semen analysis (CASA) parameters (≥50th percentile 3-PBA, DBCA)

Yoshinaga et al., 2014 [32] Japan	Cross-sectional study 1999–2000 and 2002–2003	322 males (subpopulation of a large cross-sectional multicenter study)	Spot urine sample (on the day of blood sampling)	3-PBA	Serum levels of free testosterone, FSH, LH, SHBG, inhibin B	No association with 3-PBA levels

Detection frequency of 4-F-3-PBA $< 5\%$ [26] and of *cis*- and *trans*-DCCA $< 75\%$ (14.1 and 60.9%, respectively, limit for analyses was $\geq 75\%$) [21], associations not analyzed

3-PBA 3-phenoxybenzoic acid, *Cis-DCCA* *cis*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *TDCCA* *trans*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *Trans-DDCA* *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *4-F-3-PBA* 4-fluoro-3-phenoxybenzoic acid, *DBCA* *cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid, *AMH* anti-Mullerian hormone, *CASA* computer-assisted sperm analysis, *LH* luteinizing hormone, *SHBG* sex hormone-binding globulin, *FSH* follicle stimulating hormone

3.4 Prenatal Exposure and Outcomes at Birth and in Childhood

Several birth cohort studies and a small number of large case-control studies have assessed the relationship between pyrethroid exposure during pregnancy and common outcomes at birth, i.e., gestation duration, preterm birth, birth weight and height, and head circumference (Table 4). They mainly provided no or modest evidence of potential effects on non-occupationally exposed populations. To notice, one of the largest case-control studies suggested that exposure to two or more pyrethroids during the first or second trimester of pregnancy may increase the risk of spontaneous preterm birth [36]. Pesticide exposure was based on the proximity of mother's residence with pesticide application sites. This finding was not replicated in a similar case-control study also based on California birth records [37]. In the VHEMBE South Africa birth cohort (Venda Health Examination of Mothers, Babies and their Environment), two urinary metabolites of pyrethroids (i.e., *cis*-DCCA and DBCA) measured at delivery were negatively associated with body weight and body mass index (BMI) in boys at 1 and 2 years of age [34]. Possible contribution of exposure to pyrethroids and other environmental factors during childhood (e.g., pesticide spray at home) was not controlled. Moreover, no effect was observed on these outcomes at birth [33, 35].

A few case-control studies, mostly by the same team, have examined potential association between risk of selected birth defects and residential proximity with agricultural pyrethroid applications during early pregnancy (Table 4). No association was found with the pyrethroid group, but there were elevated odd ratios for two specific pyrethroids, cyfluthrin (craniosynostosis) and lambda-cyhalothrin (heart defects) [39–41]. The authors considered that their studies added to the scant literature on this topic but that further verification and inquiry were needed before firm conclusions on individual chemicals teratogenicity.

3.5 Neurological Outcomes (Adult Exposure)

Several cross-sectional studies have examined the neurologic effects of long-term occupational exposure to pyrethroids in agricultural workers or pesticide applicators (Table 5). A broad spectrum of symptoms and functions has been assessed with different tools and indicators (e.g., medical diagnosis of symptoms, performance in neurobehavioral tests). Pyrethroid exposure was ascertained by history exposure questionnaires, except from one study which relied on urinary biomarkers [52]. Interpretation is limited due to the paucity of data and the small sample size in a number of these studies.

Table 4 Summary of epidemiological studies investigating potential association between prenatal exposure to pyrethroids and outcomes at birth and in childhood

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pyrethroid/metabolites	Outcomes of interest	Main pyrethroid findings
General pregnancy outcomes						
Chevrier et al., 2019 [33] South Africa	Longitudinal birth cohort study (VHEMBE cohort) 2012–2013	738 mother-child pairs (women recruited at early stage of labor)	Spot maternal urine samples collected either before or shortly after delivery	Pyrethroid metabolites (3-PBA, DCCA, DBCA)	Duration of gestation, birth weight and length, head circumference	No association with individual levels of pyrethroid metabolites
Coker et al., 2018 [34] South Africa	Longitudinal birth cohort study (VHEMBE cohort) 2012–2013	698 mother-child pairs (women recruited at early stage of labor)	Spot maternal urine samples collected just before or soon after delivery	Pyrethroid metabolites (3-PBA, DCCA, DBCA)	Infant body weight and length at 1 and 2 years of age	Levels of pyrethroid metabolites (i.e., *cis*-DBCA and *trans*-DCCA) associated with lower BMI-for-age and with weight-for-height; strongest and most consistent in boys Interaction between *cis*-DBCA and *p*, *p*′-DDE on body composition
Dalsager et al., 2018 [35] Denmark	Longitudinal birth cohort study 2010–2012	858 mother-child pairs	Three urine samples collected during pregnancy	3-PBA	Gestational length, birth outcomes (weight, head and abdominal circumference), AGD (measured at 3 months of age)	No significant association with 3-PBA levels (nonsignificant dose-related elongation of AGD in females, p of trend = 0.14)

(continued)

Table 4 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pyrethroid/metabolites	Outcomes of interest	Main pyrethroid findings
Ling et al., 2018 [36] USA	Case-control study 1998–2010	24,693 preterm births/ 220,297 term births and 4,412 term low birth weight/194,732 term normal birthweight infants	Residential exposure estimates based on the poundage of pesticides applied within 2 km of maternal address at birth	Pyrethroids (including permethrin) (any/none application)	Preterm birth and term low birth weight	Exposure to two or more pyrethroids associated with a small increase in the OR for preterm birth (OR; 95% CI = 1.06; 1.02–1.09, and 1.05; 1.01–1.08, first and second trimester exposure, respectively) Marginally elevated OR; 95% CI (1.05; 0.98–1.13, and 1.06; 0.99–1.13, first and second trimester exposure, respectively) for low birth weight in infants exposed to two or more pyrethroids
Shaw et al., 2018 [37] USA	Case-control study 1998–2011	27,913 preterm cases (20–36 weeks) and 197,461 term controls (37–41 weeks)	Residential exposure estimates based on the poundage of pesticides applied at proximity of maternal address	Pyrethroids (cypermethrin) (any/none application)	Preterm birth	No association with pyrethroids (chemical class)
Ding et al., 2015 [38] China	Longitudinal birth cohort study 2010–2012	454 mother-child pairs	Spot urine collected within 3 days before delivery	Metabolites of pyrethroids (3-PBA, *cis*- and *trans*-DCCA)	Birth outcomes (infant sex, preterm birth, length of gestation,	Negative association between total, but not individual, metabolite levels and birth

					body weight, length, head circumference)	weight (p for trend = 0.013) No association with other birth outcomes
Zhang et al., 2014 [18] Japan	Longitudinal birth cohort study 2009–2011	147 mother and child pairs	Blood and spot urine from the pregnant women (11–14 weeks)	Urinary 3-PBA from the pregnant women	Birth size (weight, length, chest, and head circumference of neonates) (maternal and neonatal thyroid hormones)	No association between 3-PBA levels in maternal urine in the first trimester of pregnancy and neonatal thyroid hormones or with body size
Birth defects						
Rappazzo et al., 2019 [39] USA	Case-control study 2003–2005	Cases ranged from 1,020 for atrial septal defects and patent ductus arteriosus to 39 for lower limb defects; 298,548 controls	Residential exposure estimates based on the poundage of pesticides applied within 0.5 km of maternal address	Cyhalothrin	Ten birth defects: three congenital heart defects and structural defects affecting the gastrointestinal, genitourinary, and musculoskeletal systems	Association of atrial septal defects with higher levels of exposure (OR; 95% CI = 1.81; 1.43–2.29 for >90th exposures)
Carmichael et al., 2016 [40] USA	Case-control study 1997–2006	367 unique cases (95 anotia/microtia, 77 anorectal atresia/stenosis, 59 transvers limb deficiency, 79 craniosynostosis, 62 diaphragmatic hernia) and 785 controls	Residential exposure estimates based on the poundage of pesticides applied within 0.5 km of maternal address	Group and individual (cyfluthrin only indicated) pyrethroid use during a 3-month periconceptional window (any/none application)	Anotia, microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, diaphragmatic hernia	Association between cyfluthrin and craniosynostosis (OR; 95% CI = 4.6; 1.5–14.0)
Carmichael et al., 2014 [41] USA	Case-control study 1997–2006	569 cases and 785 controls	Residential exposure estimates based on the poundage of pesticides applied within 0.5 km of maternal address	Group and individual (lambda-cyhalothrin, permethrin and cyfluthrin, only indicated) pyrethroid use during a 3-month periconceptional	Congenital heart defects	Association between lambda-cyhalothrin and atrial septum defect (OR; 95% CI = 2.9; 1.1–7.9)

(continued)

Table 4 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pyrethroid/metabolites	Outcomes of interest	Main pyrethroid findings
				window (any/none application)		
Shaw et al., 2014 [42] USA	Case-control study 1997–2006	156 cases and 785 controls	Residential exposure estimates based on the poundage of pesticides at proximity of maternal address	Group and individual (esfenvalerate only indicated) pyrethroid use during a 3-month periconceptional window (any/none application)	Gastroschisis	No association
Yang et al., 2014 [43] USA	Case-control study 1997–2006	589 unique cases (73 anencephaly, 123 spina bifida, 277 cleft lip with or without cleft palate, 117 cleft palate alone) and 785 controls	Residential exposure estimates based on the poundage of pesticides applied within 0.5 km of maternal address	Pyrethroid (group) use during a 3-month periconceptional window (any/none application)	Neural tube defects (NTD) and orofacial clefts	No association
Others						
Huang et al., 2018 [44] South Africa	Longitudinal birth cohort study (VHEMBE cohort) 2012–2013	666 mother-child pairs (women recruited at early stage of labor)	Maternal blood and spot urine samples collected either before or shortly after delivery	Pyrethroid metabolites (3-PBA, *cis*- and *trans*-DCCA, DBCA)	Childhood infection between 1 and 2 years of age (persistent fevers, otitis, severe sore throat) ascertained from maternal interviews	Limited evidence of associations between pyrethroid metabolite concentrations and outcomes of interest

Detection frequencies of *cis*-DBCA and 4-F-3-PBA < 4%, associations not analyzed [38]

VHEMBE Venda Health Examination of Mothers, Babies and their Environment, *3-PBA* 3-phenoxybenzoic acid, *Cis-DCCA cis*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *TDCCA trans*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *Trans-DDCA trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *DBCA cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid

Table 5 Summary of epidemiological studies investigating potential association between exposure to pyrethroids and neurological outcomes

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
Ismail et al., 2018 [45] Saudi Arabia	Cross-sectional study 2015	30 pesticides applicators in vector control units (men) and 30 administrative employees (males)	Applicators/non-applicators	Applicators presumably mostly exposed to a mixture of pyrethroids, i.e., bifenthrin, bioallethrin, deltamethrin, lambda cyhalothrin, cypermethrin, cyphenothrin	Neurologic symptoms (based on Q16 questionnaire) (self-reported) And neurobehavioral performance tests (Behavioral Assessment and Research System, BARS) (memory, attention/ short memory, executive, motor speed/ coordination, information processing speed functions)	Association between pesticide application and symptoms that represented different neurologic domains (e.g., behavioral, feeling anxious; autonomic, excessive sweating, loss of appetite; cognitive, difficulty of concentration; motor, shaking or trembling hands, etc.) Applicators performed significantly worse than non-applicators in two neurobehavioral functions: executive function (SDT latency measure) and motor speed/coordination function (tapping preferred and non-preferred hands)
Shreastha et al., 2018	Cross-sectional analyses Part of the	20,591 pesticide applicators (male farmers)	Self-reported exposure in 1993–1997	Permethrin (use/no use)	Dream-enacting behavior (DEB) during REM sleep	Modest association of permethrin with DEB (OR, 95% CI = 1.4,

(continued)

Table 5 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
[46] USA	Agricultural Health Study (AHS) 2013–2015				(specific prodromal symptom of Parkinson's disease) + motor (shake or tremble limbs) and non-motor (e.g., infrequent bowel movement) symptoms of Parkinson's disease (PD)	1.2–1.6 for poultry/ livestock; 1.2, 1.0–1.4 for crops; 1.3, 1.1–1.5 pooled)
Hansen et al., 2017 [47] Bolivia	Cross-sectional study 2012	58 spray men using only pyrethroids and 62 spray men using various pesticides	Self-reported exposure	Use of pyrethroids only Spraying duration (years), intensity (hours/week), cumulative spraying (total hours of exposure)	Subjective central nervous system (CNS) symptoms (self-reported) Neuromotor and neurocognitive performance tests (using computerized BARS and CATSYS)	No association with neuromotor performance High-level pyrethroid exposure associated with reduced cognitive performance and with reporting more CNS symptoms (e.g., headache, dizziness; not significant), compared with low level of exposure Workers only exposed to pyrethroids performed worse than workers also exposed to other pesticides

Huang et al., 2016 [48] China	Cross-sectional study 2012	218 farmers	Information recorded by the farmers Exposure estimates based on the amount (kg) of pesticide active ingredients applied by the farmers	Pyrethroids (class) Short-term exposure = 0.01 kg and medium-term exposure = 0.07 kg (means)	Peripheral nerve conduction (e.g., motor and sensory conduction velocity, upper and lower limbs)	Only association between decreased sensory nerve action potential amplitude of the ulna nerve and medium-term pyrethroid exposure Effects much slighter than those observed with organophosphates and were considered marginal [49]
Campos et al., 2016 [50] Brazil	Cross-sectional study 2011–2012	869 adults (89% working/worked at some time in agriculture)	Self-reported exposure	Pyrethroids (class) (use/no use, time of use: up to 5 years, or ≥6 years)	Common mental disorders (self-reporting questionnaire and self-reported depression prior diagnosis by a health professional)	Self-reported depression positively associated with use of pyrethroids (OR; 95% CI = 1.80; 1.01–4.70) No association when taking into account the time of use
Furlong et al., 2015 [51] USA	Case-control study (Farming and Movement Evaluation (FAME) study) Nested within the AHS 2002–2008	69 cases and 237 controls among pesticide applicators (94% males) (16 and 39 permethrin users, respectively)	Self-reported exposure during the 1980s/1990s	Permethrin Three categories: <50% glove use, ≥50% glove use, other personal protection	Parkinson's disease (PD) (self-reported or from state mortality files, neurologists examined living suspected cases and 5% of controls, remaining controls examined by trained technicians)	Association between permethrin and PD in nonusers of gloves (OR; 95% CI = 4.3; 1.2–15.6), but not among protective glove users
Motsoeneng et al., 2015		211 rural women (121 farm workers	Spot urine samples	Metabolites of pyrethroids (3-PBA, *cis*-		No association with 3-PBA and 4-F-3-PBA

(continued)

Table 5 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
[52] South Africa	Cross-sectional study 2009	and 90 town residents)		DBCA, *cis*- and *trans*-DCCA, 4-F-3-PBA)	Self-reported neurotoxic symptoms (Q16 questionnaire)	Three symptoms associated with *cis*-DCCA (OR; 95% CI = 3.03; 1.22–7.50 for buttoning), *trans*-DCCA (OR; 95% CI = 1.82; 1.00–3.32 for making notes), DBCA (OR; 95% CI = 8.93; 1.71–46.5 for buttoning, 2.95; 1.16–7.54 for reading, and 2.82; 1.04–7.63 for making notes) (Authors' comment: associations could be due to chance as 213 comparisons made, of which only 2.3% were significant at a $p < 0.05$ level), median exposure levels higher than in other countries

3-PBA 3-phenoxybenzoic acid, *Cis-DCCA cis*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *TDCCA trans*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *Trans-DDCA trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *4-F-3-PBA* 4-fluoro-3-phenoxybenzoic acid, *DBCA cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid, SDT latency test SDT-Symbol Digit-latency test, CATSYS Danish Product Development Ltd

3.6 Neurodevelopment After Prenatal and/or Childhood Exposure

Many of the epidemiological studies recently published pertain to children neurodevelopment after in utero or postnatal pyrethroid exposure (Table 6). All studies related to prenatal exposures were longitudinal birth cohort studies, while a cross-sectional design was used in all studies related to postnatal exposures. Cognitive, behavioral, and motor functions were examined in children and adolescents by using questionnaires filled by parents or health professionals and/or by individual or batteries of standardized and generally well-validated tests (e.g., Bayley Scales of Infant Development, Wechsler Intelligence Scale for Children). Most studies found evidence of an association between adverse neurodevelopmental outcomes in children (e.g., various early behavioral problems) and maternal urinary pyrethroid metabolites during pregnancy (i.e., 3-PBA, *cis*- and/or *trans*-DCCA). Age- and sex-specific associations were reported [53, 60]. However, there was no clear pattern of effects. This may be due to differences in the study designs, for example, in the exposure period (i.e., trimester of pregnancy) and the control of potential confounding variables (e.g., current child exposure to pyrethroids and other environmental agents such as neurodevelopmental toxic pesticides), as well as in the timing (3 months to 7 years of age), endpoints, and techniques/practices of child assessment. In addition, there was substantial variability in the urinary levels of the pyrethroid metabolites across studies (e.g., median 3-PBA level of 0.39 μg/L in [57] vs less than the limit of detection of 0.008 μg/L in [58]). On the other hand, the few studies available showed no consistent relationship between neurodevelopment and pyrethroid exposure during childhood, as assessed by metabolite measurements in the child urine.

Contrasting results have been reported regarding the association between childhood urinary 3-PBA and attention-deficit/hyperactivity disorder (ADHD) in subsets of the US NHANES cohort (National Health and Nutrition Survey) (Table 6). Methodological heterogeneity may have accounted for these differences (e.g., primary outcome definition, use of continuous 3-PBA levels vs dichotomized categorization detected/non-detected).

A couple of studies have focused on autism spectrum disorders (ASDs) and developmental delays, including two retrospective case-control studies from the Childhood Autism Risks from Genetics and Environment (CHARGE) [70, 73] (Table 6). They have established a link between ASDs risk and residential proximity to pyrethroid application, especially during the preconception and gestational periods. This suggested that exposure to these pesticides during critical periods of development may be a contributing factor to the likelihood of developing ASDs [74, 75].

Table 6 Summary of epidemiological studies investigating potential association between prenatal exposure to pyrethroids and neurodevelopment

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
Prenatal exposure						
Eskenazi et al., 2018 [53] South Africa	Longitudinal birth cohort study (VHEMBE cohort) 2012–2013	681 at 1 year and 671 at 2 years, mother-child pairs (women recruited at early stage of labor)	Maternal spot urine sample collected either before or shortly after delivery	Pyrethroid metabolites (3-PBA, *cis*- and *trans*-DCCA, DBCA)	Neurodevelopment at *1 and 2 years of age* (BSID-III, cognitive, fine and gross motor, receptive and expressive language subtests) (assessment by psychologists)	No association with child cognition Significant association between levels of pyrethroid metabolites (i.e., *cis*- and *trans*-DCCA and PBA) and decrement in social-emotional scores at 1 year of age Significant association between *cis*-DBCA and lower language composite score (especially expressive communication) at 2 years of age
Coker et al., 2017 [54] USA	Longitudinal birth cohort study (CHAMACOS cohort) 1999–2000	255 mother-child pairs	Residential exposure estimates based on the poundage of pesticides applied within 1 km of maternal address during pregnancy	Neurotoxic pesticides including four pyrethroids (cypermethrin, lambda cyhalothrin, permethrin, esfenvalerate) Identification of eight clusters of pesticide	Cognitive development at *7 years of age* (WISC-IV, 4 separate domains: verbal comprehension, perceptual reasoning, working memory, and processing speed, which were combined	Two clusters with the highest cumulative pesticide use levels were associated with deficits in FSIQ when compared with the cluster with the lowest level of pesticides use

				profiles based on individual pesticide uses (very high, moderately high, moderately low, very low)	to derive a full-scale intelligence quotient, FSIQ) (assessment by psychometrician)	
Furlong et al., 2017 [55] USA	Longitudinal birth cohort study 1998–2002	162 mother-child pairs	Maternal spot urine sample collected during the third trimester of pregnancy	Pyrethroid metabolites (3-PBA, *cis*- and *trans*-DCCA) (>LOD: PBA = 24%, *cis*-DCCA = 9%, *trans*-DCCA = 14%)	Children's adaptive and problem behaviors (Behavior Assessment System for Children, BASC) and children's problems with executive functioning (Behavior Rating Inventory of Executive Functioning, BRIEF) At *4–5, 6, and 7–9 years of age* (parent report assessment)	Detectable levels of 3-PBA associated with worse scores in Internalizing Behaviors from the BASC and in Behavioral Regulation from the BRIEF Detectable levels of *cis*-DCCA associated with a variety of behavioral and executive functioning deficits but detection frequencies low
Gunier et al., 2017 [56] USA	Longitudinal birth cohort study (CHAMACOS cohort) 1999–2000	283 mother-child pairs	Residential exposure estimates based on the poundage of pesticides applied within 1 km of maternal address during pregnancy	Pyrethroids (class)	Cognitive development *at 7 years of age* (WISC-IV, verbal comprehension, perceptual reasoning, working memory, and processing speed subtests) (assessment by psychometrician)	Association between increasing agricultural use of pyrethroids and decreased cognition (decrements in full-scale IQ, verbal comprehension, perceptual reasoning)
Hisada et al., 2017	Longitudinal birth cohort study 2009–2011	102 mother-child pairs	Maternal spot urine sample collected at	3-PBA	Children's behavior at *18 months of age* (Kinder Infant	Higher infant development score with higher 3-PBA levels,

(continued)

Table 6 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
[57] Japan			10–12 weeks of pregnancy		Development Scale (KIDS) questionnaire, 9 subscales, e.g., physical motor, social relationship with child, receptive and expressive language) (questionnaire completed by parents)	unknown confounding factor suspected to explain this beneficial effect
Viel et al., 2017 [58] France	Subset of the longitudinal mother-child cohort study (PELAGIE cohort) 2002–2006	287 mother-child pairs	Spot urine sample collected from the mother during early pregnancy (6–19 weeks) (205 samples) *and* from the child at 6 years of age (284 samples)	Pyrethroid metabolites (3-PBA, *cis*-DBCA, *cis*- and *trans*-DCCA, 4-F-3-PBA) (6–8% 4-F-3-PBA > LOD, detected vs not detected for this analysis)	Children's behavior at *6 years of age* (Strengths and Difficulties Questionnaire, SDQ subscales: prosocial behavior, internalizing and externalizing disorders) (questionnaire completed by parents)	Association between *prenatal cis*-DCCA levels and internalizing difficulties 3-PBA levels *at childhood* associated with externalizing difficulties and the median and high 3-PBA levels categories associated with abnormal and borderline social behavior compared to those with no detectable 3-PBA (OR; 95% CI = 2.93; 1.27–6.78 for 3-PBA in the range of

						0.008–0.037 μg/L, OR; 95% CI = 1.91; 0.80–4.57 for 3-PBA ≥ 0.038 μg/L) High *trans*-DCCA levels (≥0.134 μg/L) *at childhood* associated with reduced externalizing disorders (no current explanation)
Fluegge et al., 2016 [59] USA	Longitudinal birth cohort study 2002–2005	118 mother-child pairs	Spot urine sample collected from the mother during the second and third trimester of pregnancy *and* from the infant at 2 months of age	Metabolites of pyrethroids (3-PBA, *cis*- and *trans*-DCCA)	Neurodevelopment assessment at *3 months of age* (BSID-II, motor and mental assessments)	Significant association between levels of pyrethroid metabolites (i.e., 3-PBA and *trans*-DCCA) during the third trimester of pregnancy and infant mental development (opposite effects: negative and positive influences, respectively) Effects retained when controlling for concurrent infant exposure at 3 months of age
Watkins et al., 2016	Longitudinal birth cohort study	187 mother-child pairs	Maternal spot urine sample collected	3-PBA (46% > LOD)	Neurodevelopment assessment at *24 and 36 months of age*	No association between 3-PBA levels and PDI

(continued)

Table 6 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
[60] Mexico	(ELEMENT cohort) 1997–2001		during the third trimester of pregnancy		(BSID-IIS using mental and psychomotor developmental index – MDI and PDI) at *24 and 36 months of age* (assessments by research personnel)	scores At 24 months, lower MDI among participants in the median and high 3-PBA level categories compared to those with no detectable 3-PBA (only marginally significant, p for trend = 0.07), slightly stronger in girls
Viel et al., 2015 [61] France	Subset of the longitudinal mother-child cohort study (PELAGIE cohort) 2002–2006	287 mother-child pairs	Urine sample collected from the mothers during early pregnancy (6–19 weeks) (205 samples) *and* from the infant at 6 years of age (282 samples)	Metabolites of pyrethroids (3-PBA, *cis*- and *trans*-DCCA, *cis*-DBCA) (6–9% 4-F-3-PBA > LOD, detected vs not detected for this analysis)	Cognitive development at *6 years of age* (using verbal comprehension and working memory index from WISC-IV) (assessments by psychologist)	No association between *prenatal* metabolite levels and any children's cognitive scores Negative association between *childhood* 3-PBA and *cis*-DBCA levels and verbal and working memory scores No association with *childhood* DCCA (*cis* and *trans*) and 4-F-3-PBA levels

Postnatal exposure						
Van Wendel de Joode et al., 2016 [62] Costa Rica	Cross-sectional 2007	140 children (6–9 years of age)	Child spot urine sample on the day of tests ($n = 140$) Repeated urine samples from 40 of these children	3-PBA (207 samples)	Neurobehavioral tests by psychometricians (outcomes: intellectual ability with WISC-IV; behavioral problems including ADHD; sensory function by color discrimination; perception and memory, i.e., visuospatial construction and visual memory, verbal memory, and learning abilities; motor function, i.e., visual-motor coordination, fine motor functioning, and psychomotor speed)	Higher 3-PBA levels associated with poorer processing speed scores, particularly in girls No other association
Wang et al., 2016 [63] China	Cross-sectional Period not indicated	406 children (3–6 years of age)	Child spot urine sample (<1 week interval between urine collection and behavioral testing)	3-PBA (36% > LOD)	Neurobehavioral tests by trained technicians (outcomes: verbal discrimination, logical thinking ability, calculation, language and concentration abilities, some assessed with part of Wechsler preschool and primary scale of intelligence)	Negative association between multiple intellectual abilities and 3-PBA levels (affected abilities different when 3-PBA was used as continuous or dichotomous variable)

(continued)

Table 6 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
Fiedler et al., 2015 [64] Thailand	Cross-sectional study Period not indicated	53 children (6–8 years of age)	Child spot urine sample on the day of tests	Metabolites of pyrethroids (3-PBA and *cis*-/*trans*-DCCA) (high/low pesticide use season)	Neurobehavioral tests by trained examiner (computerized tests adapted from Behavioral Assessment and Research System (BARS) and Pediatric Environmental Neurobehavioral Test Battery) (outcomes: response speed and coordination, divided attention; dexterity, eye-hand coordination, memory and attention, recall and recognition memory, information processing speed, visual memory and sustained attention)	Metabolites not significant predictors of adverse neurobehavioral performance
Oulhote et al., 2013 [65] Canada	Cross-sectional Subset from the Canadian Health Measures Survey 2007–2009	1,030 children (6–11 years of age)	Child spot urine sample (within 2 weeks of questionnaire completion)	Metabolites of pyrethroids (3-PBA, *cis*- and *trans*-DCCA, *cis*-DBCA, 4-F-3-PBA)	Behavioral problems (Strengths and Difficulties Questionnaire SDQ subscales: emotional symptoms, conduct problems,	No association with levels of 3-PBA Association between levels of *cis*-DCCA and high scores of total difficulties (OR;

					peer problems, hyperactivity/inattention, total difficulties) (questionnaire completed by parents)	95% CI = 2.0; 1.1–3.6), stronger for boys. Nonsignificant association with *trans*-DCCA (OR = 1.6; 0.9–3.0) No association with high scores on dimension scales
Attention-deficit/hyperactivity disorder (ADHD)						
Richardson et al., 2015 [66] USA	Cross-sectional study Subset from the NHANES 1999–2002	2,123 children (6–15 years of age)	Child spot urine sample	3-PBA (detected vs not detected)	ADHD (parent-reported diagnosis)	The prevalence of ADHD was higher in children with detectable 3-PBA than in those with non-detectable level (adjusted OR; 95% CI = 2.3; 1.4–3.9)
Wagner-Schuman et al., 2015 [67] USA	Cross-sectional study Subset from the NHANES 2001–2002	687 children (8–15 years of age)	Child spot urine sample	3-PBA	ADHD (caregiver reports and/or meeting Diagnostic and Statistical Manual of Mental Disorders-IV)	The prevalence of ADHD was higher in children with detectable 3-PBA than in those with non-detectable level (adjusted OR; 95% CI = 2.42; 1.06–5.57) Higher 3-PBA levels associated with an increasing number of hyperactive-

(continued)

Table 6 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
						impulsive symptoms, mainly in boys
Quiros-Alcala et al., 2014 [68] USA	Cross-sectional study Subset from the NHANES 1999–2002	1,861 children (6–15 years of age) having urinary 3-PBA	Child spot urine sample	Metabolites of pyrethroids (3-PBA, *cis*- and *trans*-DCCA) (low number of children with both diseases and detectable *cis*- and *trans*-DCCA, use of detected vs not detected for these analyses)	Learning disability (LD) and ADHD (parent-reported)	No association of LD and/or ADHD with 3-PBA levels No significant association between any outcome and detection of *cis*- or *trans*-DCCA
Autism spectrum disorders (ASD)						
Von Ehrenstein et al., 2019 USA [69]	Case-control study 1998–2010	2,961 cases and 35,370 controls	Exposure estimates based on agricultural pesticide application near birth address *Prenatal and infant exposure*	Permethrin, bifenthrin	ASD (with or without intellectual disabilities)	Positive association between prenatal exposure to permethrin and risk of ASD (OR; 95% CI = 1.10; 1.01–1.20) and risk of ASD with intellectual disability (1.46; 1.20–1.78)
Schmidt et al., 2017 [70] USA	Case-control study Subset of the Childhood Autism Risks from	296 children (2–5 years of age) diagnosed with ASD	Exposure estimates based on the poundage of pesticides applied within	Pyrethroids (class) (use/no use)	ASD	Positive association between ASD and pyrethroid exposure (agricultural

	Genetics and Environment (CHARGE) study 2003–2011	and 220 controls (births 2000–2007)	1.75 km of maternal address, *preconception and prenatal periods*			use) + low maternal folic acid intake (FA, self-reported) in the 3 months prior or after conception (OR; 95% CI = 2.1; 0.9–4.8, the first month) (association attenuated among those with high FA vs low FA)
Hicks et al., 2017 [71] USA	Cross-sectional 2010–2015	19,073 children (<20 years of age) living in aerial exposed areas	*Child* exposure based on the aerial application of pyrethroids within 2.5 km of the residence	Pyrethroids (class) (exposed vs not exposed)	ASD and developmental delay (DV)	Significant association between pyrethroid child exposure and ASD/DD prevalence (RR; 95% CI = 1.37; 1.06–1.78)
Domingues et al., 2016 [72] Italy	Case-control study Period not indicated	21 children with ASD and 19 controls (5–12 years of age)	Child spot urine sample	3-PBA in urine	ASD	3-PBA level in ASD children higher than in control group, marginally significant ($p = 0.054$)
Shelton et al., 2014 [73] USA	Case-control study Subset of the CHARGE study 1997–2008	486 children (2–5 years of age) diagnosed with ASD, 168 with developmental delay (DV), and 316 controls (births 1999–2008)	Residential exposure estimates based on the poundage of pesticides applied within 1.75 km of maternal addresses during the *preconception and pregnancy periods*	Pyrethroids (class), (most commonly applied: esfenvalerate (24%), lambda cyhalothrin, permethrin, cypermethrin, tau-fluvalinate) (any/none application	ASD and DV	Positive association between ASD and pyrethroids in the 3 months prior conception and the third trimester of pregnancy (OR 1.64–1.87) Positive association

(continued)

Table 6 (continued)

Reference and study Location	Design Study dates	Population sample size	Exposure assessment	Pesticides/ metabolites	Outcomes of interest	Main pyrethroid findings
				within 1.25, 1.5, 1.75 km)		between DV and pyrethroids in the 3 months prior conception and the third trimester of pregnancy (OR 1.44–2.34, significant for the third trimester)

No or low detection frequencies of *cis*- and *trans*-DCCA [60], *cis*-DBCA [59, 63] and 4-F-3-PBA [53, 59, 61, 63]: Associations not analyzed
3-PBA 3-phenoxybenzoic acid, *Cis-DCCA cis*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *TDCCA trans*-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid, *Trans-DDCA trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *4-F-3-PBA* 4-fluoro-3-phenoxybenzoic acid, *DBCA cis*-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid, *VHEMBE* Venda Health Examination of Mothers, Babies and their Environment, *CHAMACOS* Center for Health Assessment of Mothers and Children of Salinas, *ELEMENT* Early life Exposures in Mexico to Environmental Toxicants, *PELAGIE* Perturbateurs Endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance, *BSID-III* Bayley Scales of Infant Development, third edition, *WISC-IV* Wechsler Intelligence Scale for Children, fourth edition, contains four domains: verbal comprehension, perceptual reasoning, work memory, processing speed, *CHARGE* Childhood Autism Risks from Genetics and Environment, *NHANES* National Health and Nutrition Examination Survey

3.7 Other Effects

Recent systematic reviews and meta-analyses have associated prenatal or childhood indoor exposure to insecticides, assessed as a class, to an increased risk of leukemia and brain tumors in children [76–82]. Possible association between the use of the common pyrethroid insecticide, permethrin, and various types of cancer has been more particularly evaluated in several epidemiological studies, mostly based on the US Agricultural Health Study cohort. Negative or inconclusive results were reported [77, 83–88]. Pooled analysis of large agricultural worker cohorts from France, the USA, and Norway found moderate association between chronic use of deltamethrin and elevations in risks of non-Hodgkin lymphoid malignancies (subtypes) [89].

The consequences of pyrethroid exposure on coronary heart disease [90], body mass index [91, 92], hematological parameters [93], genetic damage [94–97], rheumatoid arthritis [98], and prediabetes [99, 100] were infrequently examined.

4 Conclusions

A growing number of epidemiological studies have been carried out to investigate the health impact of long-term environmental and occupational exposures to pyrethroids.

Suggestive association between pyrethroid exposure and various respiratory and neurologic effects has been reported in adults in a few recent studies. Most of them addressed occupational exposures (e.g., agricultural workers), with self-reported uses. These exposure conditions may be specific and may not be readily generalizable to chronic environmental exposures of the adult population at relatively low levels. In addition, neurological findings were not consistent across studies and overall evidence remains limited yet. Regarding the thyroid function, no or weak changes in relation with pyrethroid exposure have been reported in the studies conducted so far.

Numerous earlier epidemiological studies have focused on the male endocrine and reproductive system, and some of them have suggested that pyrethroids may have potential deleterious effects on different male sexual characteristics [1, 101, 102]. In line with this hypothesis, several cross-sectional studies published in the last few years found an association between environmental pyrethroid exposure and decreased sperm quality and sperm DNA damages. However, the inconsistencies across all results available still prevent strong conclusions.

A great deal of attention has also been devoted to the consequences of environmental exposure to pyrethroids during pregnancy. Most existing studies, of which those currently reviewed, found weak evidence of adverse effects on general birth outcomes including birth weight and length and gestational age. Past and present studies on child neurodevelopment and behavior (i.e., infants to grade schoolers) were relatively consistent. The negative effects related to prenatal pyrethroid

exposure previously reported were further supported by a majority of the recent studies. Nevertheless, harmonization of the study designs would contribute to upgrade the confidence level of the evidence and identify the biological mechanisms potentially involved in the reported associations. There were fewer investigations on the risk of neurodevelopmental deficits following exposure to pyrethroids during childhood. The data were contradictory and evidence on a causal relationship is currently insufficient.

A major shortcoming of the available epidemiologic data is the lack of a detailed and consistent exposure assessment, capturing all the various sources and routes of pyrethroid exposure over long time periods. Many studies used urinary levels of nonspecific metabolites to quantitatively estimate individual pyrethroid exposure. When use and outcome were frequent enough, they could provide valuable exposure-response information, particularly regarding the lower environmental levels encountered by the general public. However, pyrethroids are nonpersistent chemicals which are rapidly metabolized and excreted, and a single measure of their urinary metabolites may only reflect current or recent exposures. Furthermore, the use of cross-sectional data in a majority of studies may not account for peak or duration of exposures. Characterization of extended and integrated exposure might be improved by combining reiterate urine sampling and specific pyrethroid biomarker measurements with other relevant information, for example, other indicators of long-term exposure (e.g., residential address history), occupational and domestic uses of pesticide compounds (e.g., frequency, intensity, duration, life period), use of protective equipment, diet and possible supplements intake, and occurrence of co-exposures [30, 51, 56, 70, 103].

In most studies statistical analyses included common potential confounding factors linked to the parameter of interest (e.g., maternal race/ethnicity, age, and smoking). Pesticides are often used as complex mixtures of chemicals belonging to the same or different classes (e.g., pyrethroid and organophosphate insecticides or pyrethroid and the synergist piperonyl butoxide). Workers and the general adult and child population are potentially exposed to multiple chemicals, with temporal and spatial variations. Co-exposure or use of pyrethroids with other pesticides was controlled in several studies (e.g., [61]). Although challenging, the possibility of join effects and interactions would deserve more consideration in future large epidemiological studies with longitudinal data collection [34, 43].

In conclusion, there is accumulating evidence that chronic exposure to the pyrethroids may have potential negative effects on human health, especially during pregnancy. Despite constant knowledge enhancement, this review also highlights the critical need of valid epidemiological studies for a broader and more reliable assessment of the risks associated with pyrethroids.

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Conclusions and Future Trends

E. Eljarrat

Contents

Abstract This chapter summarizes the main conclusions drawn from the 11 different chapters of this book, as well as the future trends in the field of research on pyrethroid insecticides. The chapter is divided into five sections. First of all, we discuss the different pyrethroid insecticides produced and used regularly, their various applications, and their physicochemical properties, with special attention to their stereochemistry, evaluating the different isomers and enantiomers for each pyrethroid. After that, we present the developments in analytical methodologies for pyrethroid determinations in environmental and food matrices, as well as the analysis of urinary metabolites. Then, the environmental fate in aquatic ecosystems, with special attention to salmon industry, was presented, as well as pyrethroid presence in other environmental compartments such as soil or air. Bioavailabilty and bioaccumulation in terrestrial and aquatic wildlife are also discussed. And finally, the human exposure to pyrethroid insecticides through inhalation and food ingestion and the risk associated to the long-term exposure are summarized.

Keywords Analytical approaches, Bioaccumulation, Food intake, Human exposure, Indoor and outdoor inhalation, Pyrethroid metabolites, Risk assessment

E. Eljarrat (✉)
Department of Environmental Chemistry, IDAEA, CSIC, Barcelona, Spain
e-mail: eeeqam@cid.csic.es

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1 Pyrethroid Insecticides

Chapter "Introduction to Pyrethroid Insecticides: Chemical Structures, Properties, Mode of Action and Use" summarizes information related to the chemical structures, properties, mode of action, and use of pyrethroid insecticides. They have been used worldwide since the 1980s because of their high level of effectiveness and low toxicity compared to other insecticides, such as organochlorine, organophosphorus, and carbamic ester compounds. Pyrethroids are the most widely used insecticides worldwide, accounting for about 25% of the pesticide use, and they are applied in households, in commercial products, and in medicine. Several desirable characteristics contribute to the commercial success of pyrethroids, including their efficacy against a broad range of insect pests and mites, low mammalian and avian toxicities, low potential to contaminate ground water, and relatively low application rates. They were believed to be the ideal pesticides, since they are not persistent and were thought to be metabolized and not bioaccumulated. In soils, most pyrethroids have half-lives ranging between 30 and 100 days, and their hydrolysis in the aquatic compartment occurs on the order of days to weeks [1]. The routes of degradation may be abiotic, hydrolysis, photolysis and oxidation, or mediated by bacteria and fungi. Therefore, they do not meet the requirements to be considered persistent organic pollutants (POPs). However, the continuous use of these insecticides in the different applications for which they are described makes them ubiquitous in the different environmental compartments. That because they are considered *pseudo*-POPs. In addition, different studies in both aquatic and terrestrial biota have shown the presence of pyrethroids in different tissues and at not negligible levels of concentration. Therefore, it is necessary to determine the relationship between pyrethroid metabolization and bioaccumulation. Something similar has been observed for humans. Finally, we must not forget the studies indicating the diverse toxicity of these compounds. All these data suggests reconsidering the theory that pyrethroids are the ideal insecticides (Fig. 1).

It is important to note that when we use the term pyrethroid insecticides, we are encompassing a large number of different compounds that, despite having a similar chemical structure, they have different physicochemical properties and, more important, different toxicological effects. As can be seen throughout the different chapters of this book, most studies focus on the same pyrethroids, such as bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, or permethrin. However, other pyrethroids such as allethrin, fluvalinate, imiprothrin, prallethrin, or resmethrin have been less studied, and it would be convenient to have more information also for these compounds.

The study of the different behavior of isomers and enantiomers of each pyrethroid is also crucial, as reflected throughout chapter "Stereoselectivity and Environmental Behaviour of Pyrethroids". A stereochemical approach is required to better understand the impacts of pyrethroids on the environment and on human health. Upon entering the environment, the chiral pyrethroids undergo selective enantiomeric bioaccumulation and degradation. And, as different toxicity has been

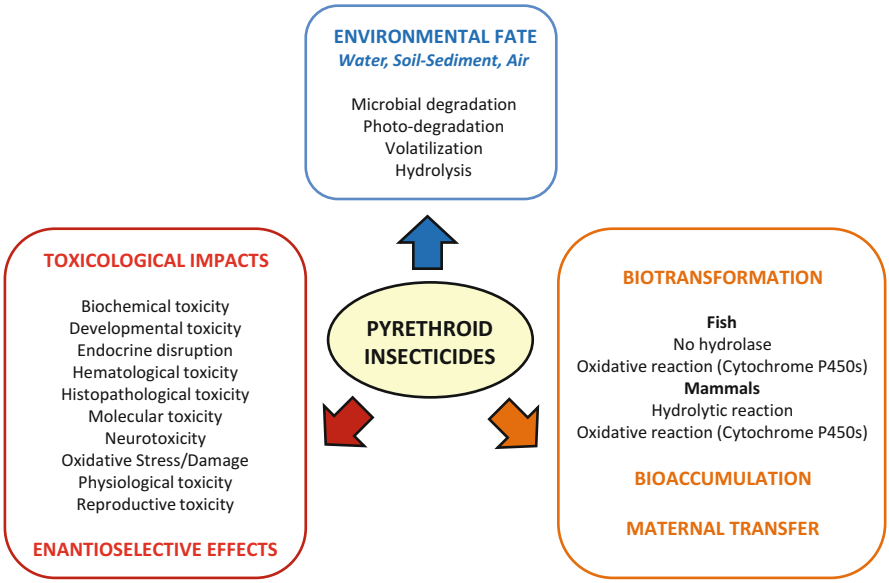

Fig. 1 Environmental fate, toxicological impacts, and biotransformation/bioaccumulation of pyrethroid insecticides

reported for specific enantiomers, an enantiomeric risk assessment is the way to obtain a more accurate and real evaluations. An achiral approach is able to only partially assess the potential adverse effects of pyrethroids in biological systems. Chiral approach is not the commonly used in published works. However, for future studies our recommendation would be to adopt a chiral approach in order to obtain more realistic and concise results.

2 Analytical Approaches

The continuous progress in analytical techniques has improved the capability of detecting chemicals and recognizing new substances and extended the list of detectable contaminants widespread in all environmental compartments by human activities. In the case of pyrethroid insecticides, these advances have been very useful for the detection of different pyrethroids in various matrices, both environmental and biological. However, analytical challenges include not only the analysis of pyrethroids, but also the determination of their metabolites in biological samples, as this gives important information about human exposure to these pollutants.

Chapter "Analytical Methods for Determining Pyrethroid Insecticides in Environmental and Food Matrices" compiles the different analytical approaches for the determination and quantification of pyrethroid insecticides in various matrices under study. Sample preparation procedure plays a fundamental role in developing

analytical methodologies. Extraction and cleanup steps have presented a high improved, especially in terms of automation reducing the sample manipulation and the time of analysis. Sample preparation methods for pyrethroids are well established for environmental and food samples, with acceptable recoveries and good reproducibilities. Regarding instrumental determination, developed methodologies are based on the use of gas chromatography (GC) coupled to mass spectrometry (MS). However, in order to achieve limits of detection adequate for the determination at environmentally relevant concentrations, the use of tandem mass spectrometry (MS-MS) seems mandatory. Moreover, it is important to guarantee the quality of analytical data in the analysis of pollutants, such as pyrethroids, in complex matrices. These quality parameters must be tested through the performance of interlaboratory tests and combined, if it is possible and available, with the use of reference materials. However, these have not yet been treated in the case of pyrethroids, and future works must be done in this sense.

Regarding enantiomeric separation, beta-cyclodextrin-based columns were usually applied due to its excellent enantioselectivity. However, the enantiomeric analysis is a complicated task. Different works achieved the separation of some enantiomeric pairs, especially for *cis* enantiomers. However, the separation of *trans* enantiomers still remains an unsolved task. Research is necessary into development of new chiral columns able to achieve this separation between *trans* enantiomers. Another challenge in chiral analysis is the lack of standards to enable quantification of individual enantiomers.

Chapter "Analytical Methods for Determination Urinary Metabolites of Synthetic Pyrethroids" summarizes the analytical work carried out for the analysis of pyrethroid metabolites in human samples. Urine, as a major route of elimination of pyrethroid metabolites, is considered the most appropriate matrix for the assessment of pyrethroid exposure. In general, sample preparation steps include a hydrolysis step before sample extraction. This step could be an acidic or an enzymatic hydrolysis. However, enzymatic hydrolysis has some disadvantages: is time consuming since it is usually performed overnight, and sample should be acidified before extraction. In contrast, after acidic hydrolysis, no pH adjustment is needed before extraction. New research is focused on the development of analytical methods for the metabolite determination of new pyrethroids and those less frequently used. The main problem is the lack of commercial availability of reference substances and relevant isotopically labeled internal standards.

The market of MS is extremely dynamic and manufacturers invest into the development of new technologies. Actually, ultrahigh resolving power analyzers ($>$100,000), such as Orbitrap-type systems, are increasing their use to identify non-target compounds. This opens the opportunity for the identification of new pyrethroids as well as their metabolites that are not currently included in traditional target methodologies. The analysis strategy is based on a "scan" in full-scan mode in an integrated (non-specific) way and with the help of software, such as SIEVE and ExactFinder, to identify the presence of potential *unknowns*. Once identified, and if commercial analytical standards are available, a definitive and unambiguous confirmation of the compounds could be done, as well as their quantitative analysis.

3 Environmental Fate

Environmental fate of pyrethroid insecticides occurs in different compartments, water, soil, and air. They have been widely detected at the global scale, with most reports being from China and the United States. In general, concentrations in soils and sediment are higher than those of air and water, being pyrethroid levels two orders of magnitude lower in water than in both soils and sediments [2]. In this book there are different chapters dealing with this topic. "Fate of Pyrethroids in Freshwater and Marine Environments", "The Ecological and Evolutionary Implications of Pyrethroid Exposure: A New Perspective on Aquatic Ecotoxicity" and "Environmental Risks of Synthetic Pyrethroids Used by the Salmon Industry in Chile" were more focused on the water-soil system, whereas chapter "Indoor and Outdoor Pyrethroid Air Concentrations" evaluates the impact in air.

Pyrethroids have high *n*-octanol-water partition coefficients (K_{ow}), with log K_{ow} values ranging from 4 to 7.5, indicating that these chemicals are much more likely to partition into the sediment and sorb to particulate organic matter than to remain in the water column [3]. However, and despite being highly lipophilic, pyrethroids may remain in the water column for days to weeks and can produce toxic effects at low concentrations. Generally, concentrations are under the 100 ng/L range for water samples, being cyhalothrin and bifenthrin those reaching the highest levels and also those more frequently exceeding regulatory threshold levels in surface freshwater. Acute mortality has been documented far below 1 μg/L range for fish and crustaceans [4], and acute toxicity has even been documented at levels below 1 ng/L [5]. The potential combined acute and chronic effects on aquatic ecosystems must be taken into account. It is also important to evaluate the possible synergistic or antisinergic effects between different pyrethroid insecticides, as well as among other different pollutants also present in aquatic ecosystems. Only taking these effects into account, we will be able to correctly assess the real effects of these compounds in aquatic media.

Most pyrethroids will be transported into sediments after entering water bodies, while some will evaporate into the atmosphere or enter the ocean. A recent review documented the occurrence of pyrethroids in sediments worldwide [6]. As expected and due to their lipophilicity, sediment concentration levels are higher than those found in water samples, being generally under the 100 ng/g range. Moreover, pyrethroid occurrence showed significant correlations with sediment toxicity. The frequent occurrence at high concentrations of pyrethroids in sediments from agricultural and residential areas constitutes a threat to freshwater ecosystems.

Historically, some pyrethroids were added to water directly as mosquito and black fly larvicides, but their toxicity, hydrophobicity, and sediment persistence have restricted their direct use in aquatic environments. However, in aquaculture, pyrethroids are still added directly to the water to remove parasites from farmed fish. Aquaculture is a locally direct source that likely constitutes an important environmental burden for seawater, which it is very poorly surveyed. Chapter "Environmental Risks of Synthetic Pyrethroids Used by the Salmon Industry in Chile" summarizes the effects of these applications, specifically the sea lice

treatments in salmon industry, in the marine environment. To combat sea lice, a series of pesticides such as cypermethrin and deltamethrin are used, which are applied by bath treatments. Concentration levels in marine waters around the fish farms are in the range of ng/L, but higher cypermethrin and deltamethrin concentrations in sediments were observed, reaching values in the range of 1,000 ng/g (1 μg/g). These levels are in the range of concentrations toxic to marine species, such as invertebrates. Given this high pollution as well as the increase in number of fish farms according to the fish world consumption, it is necessary to closely follow the pyrethroid treatment practices. Risk assessment studies must be done, and stricter regulations must include maximum concentration values allowed around the fish farms when these insecticides are applied.

Another very important consideration is the pyrethroid resistance in the aquatic environment. This fact can have far reaching implications that are important from a variety of different perspectives: human and animal health, ecological, evolutionary, and risk assessment. If the presence of pyrethroids is strong enough, some populations of sensitive taxa may evolve to resist pyrethroids. In addition, resistance in disease vectors can also threatens public health. Pyrethroid-impregnated mosquito nets have caused considerable reductions in morbidity and mortality associated with malaria in Africa. However, the intense selection pressure exerted by mosquito nets has precipitated widespread and increasing resistance to pyrethroids in African Anopheles populations, threatening to reverse the gains obtained from malaria control. A very recent study [7] shows pyrethroid resistance to *Anopheles gambiae*.

Since many pyrethroids are semi-volatile compounds even applications onto surfaces can result in elevated air concentrations as they volatilize. The recent recognition of pyrethroid occurrence in aerosols and in the gas phase opens a challenging view of their biogeochemical cycle and prompts further research to assess the relevance of atmospheric transport. Chapter “Indoor and Outdoor Pyrethroid Air Concentrations” summarizes scientific research done in this area.

4 Bioaccumulation in Wildlife

After entering the natural environment, pyrethroids circulate among the three phase of solid, liquid, and gas and enter organisms through food chains, resulting in substantial health risks. Pyrethroids are biotransformed easily by mammals through hydrolytic (esterase) and oxidative (cytochrome P450s) reactions. Therefore, they are less toxic to them. However, fish lack hydrolase and metabolize synthetic pyrethroids through oxidative (cytochrome P450s) reaction only. Therefore, they are highly toxic to fish and other aquatic organisms.

For many years, the scientific community ignored studies of pyrethroid accumulation in tissues of living beings and especially in mammals. This was due to the fact that mammals are able to metabolize pyrethroids, and, consequently, such contaminants would not be accumulated in the tissues but would be excreted. However, in recent years various studies have been published showing its presence

in tissues of different living things. Chapter "Bioavailability and Bioaccumulation of Pyrethroid Insecticides in Wildlife and Humans" shows a summary of these works. Currently, there are data of pyrethroids accumulated in aquatic biota, in different species of river fish, as well as in marine mammals such as dolphins. But pollution also occurs in terrestrial biota. Pyrethroids are detected in eggs of a large variety of birds. And finally, we must not forget that there are also studies in which the presence of pyrethroids accumulated in human breast milk is reported. Based on these new data, it is now necessary to evaluate the degrees of metabolization and accumulation in tissues. There are no studies in this sense, and it would be important to assess whether the degree of accumulation is important or can be considered negligible. In any case, the concentration values of accumulated pyrethroids in different organisms are similar to those found for other emerging pollutants. In addition, the detection frequency is also very high, with a detection percentage between 90 and 100%. All these remark the importance of including pyrethroids in environmental quality and monitoring studies, given that, even at nonlethal doses, pyrethroids are known as stressors and that the accumulation of pyrethroids in living tissues deserves further studies.

In any case, the study carried out by Alonso et al. [8] indicates that, in the case of marine mammals, pyrethroid metabolism could occur only when the individual reach sexual maturity. That means that throughout the early period of life, when growth and development is crucial, dolphins would be exposed to the accumulation of pyrethroids in their organisms. Taking into account the potential toxic effects of pyrethroids and the exposure to these pollutants in an early period of life, the need for further studies related to exposure to these compounds becomes clear. Likewise, the maternal transfer of pyrethroid insecticides has been also observed by both gestational and lactation pathways. Therefore, it would also be necessary to evaluate the impact of these pyrethroids during the development of the fetus.

5 Human Exposure

The use of pyrethroids has increased over the past three decades and correspondingly the opportunity for human exposure. Pyrethroids can enter the human body in different ways: food ingestion, residential environment, and various environmental media containing pyrethroid pesticides. Chapter "Indoor and Outdoor Pyrethroid Air Concentrations" is focused on indoor and outdoor pyrethroid levels and human exposure through inhalation, whereas chapter "Risk Assessment of Human Exposure to Pyrethroids Through Food" evaluates human exposure through food intake. Finally, chapter "Human Risk Associated with Long-term Exposure to Pyrethroid Insecticides" summarizes the human risk associated with long-term exposure to pyrethroid insecticides.

The evaluation of the exposure to environmentally significant and health-relevant compounds in indoor environments becomes a growing issue of concern since people spend on average more than 80% of their time indoors. Moreover,

pyrethroids are widely used indoors, accounting for more than 80% of the total market of public health insecticides. And, degradation rates of pyrethroids are much lower in indoor than outdoor environments, that is because pyrethroids have been detected at high levels in the indoor environments, with levels between the range of low ng/m^3 and low μg/m^3. Outdoor pyrethroid concentrations were much lower, with values between pg/m^3 and low ng/m^3 range. In indoor environments we must not forget either the contamination of house dust. Pyrethroids are semi-volatile compounds with low vapor pressures and high octanol/water and water/organic carbon partitioning coefficients facilitating their absorption onto the organic component of house dust.

Some studies carried out in different areas of the world showed the presence of pyrethroid insecticides in food products. The positive detection ranged between 10 and 30% of the samples analyzed and with the detection of between 2 and 5 different pyrethroids. In addition, in several cases the levels found exceed the established maximum residue limits (MRLs). Thus, exposure to pyrethroids through food consumption is reaching alarming levels. In any case, ingestion of food and household dust are generally larger exposure routes than inhalation, which contributes between 5 and 10% to the total exposure [9].

Numerous epidemiology studies have evaluated the association between health outcomes in humans and pyrethroid exposure. Absorbed pyrethroids are quickly metabolized and excreted, being the plasma half-life of pyrethroids in general less than 8 h [10]. Many studies used urinary levels of metabolites to quantitatively estimate pyrethroid exposure. However, pyrethroids are rapidly metabolized and excreted, and a single measure of their urinary metabolites may only reflect current or recent exposures, with misclassification of past exposures. In order to provide more robust data on potential health outcomes from exposure to pyrethroids, future epidemiological studies should quantify exposure over time.

Given the suspected effects of pesticides on the development of the fetus, exposure to pyrethroids during pregnancy is a major public health concern. Some studies suggest that environmental exposure to pyrethroids have adverse effects on pregnancy outcomes and infant health, including birth size, immune system, and neurodevelopment. One of the largest case-control studies suggested that exposure to two or more pyrethroids during the first or second trimester of pregnancy may increase the risk of spontaneous preterm birth [11]. Moreover, in case-control studies in China, the geometric mean concentrations of urinary pyrethroid metabolites of patients were higher than those of healthy children, indicating that exposure to pyrethroids may be associated with an increased risk of childhood brain tumors, childhood acute lymphocytic leukemia, and coronary heart disease [12–14].

Human contact to one or more pyrethroid insecticides is likely. That because future research works on risk assessment related with the exposure to pesticides should focus on the analysis of total cumulative intake.

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